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Zinc (II) Directed Triple-Stranded Helicate Incorporating Nine Membered Metallamacrocycle: Supramolecular Cylinders Mimicking P1 Nuclease

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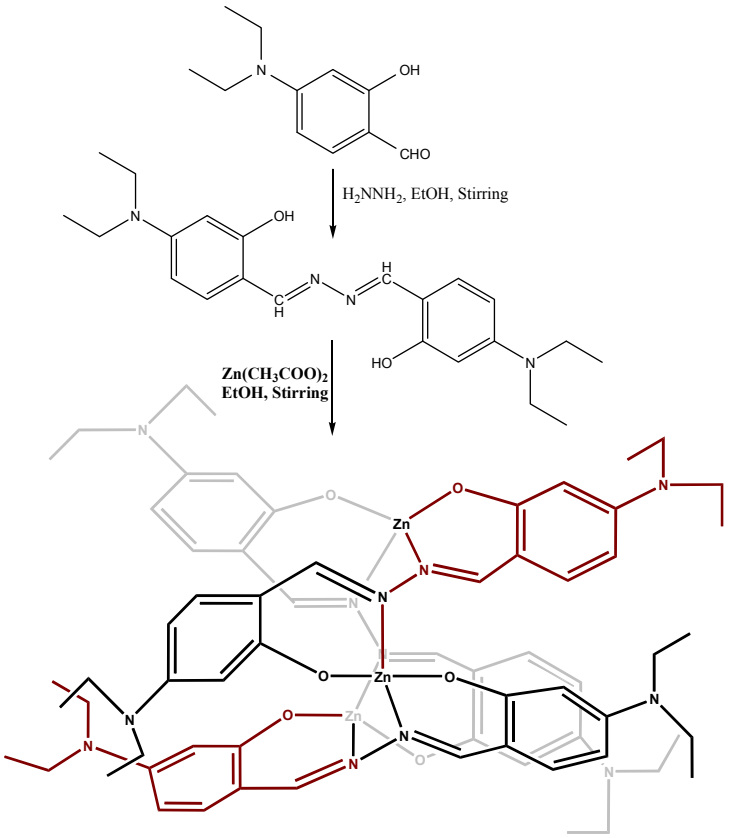
CONTENTS:

S1. SYNTHETIC SCHEME:



Scheme S1: Showing synthetic scheme for ligand and the complex

S2. SYNTHESIS AND CHARACTERIZATION OF LIGAND:

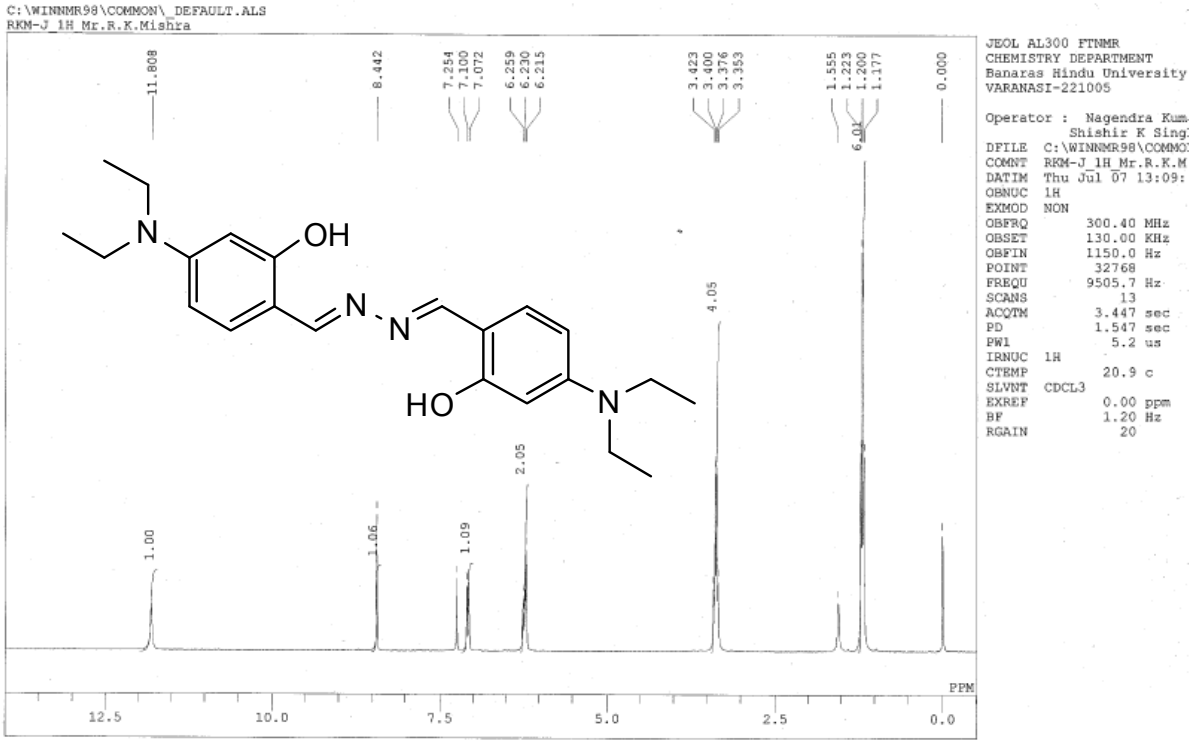
To a solution of 4-Diethylamino-2-hydroxy-benzaldehyde (0.965 g, 5 mMol) in EtOH (50 mL) the hydrazine hydrate was added slowly in 2:1 ratio and reaction mixture was left on stirring for 12 hrs. Yellow solid product was obtained, which was filtered and dried. Yield 95%, **IR** (cm^{-1}): 3438, 2971, 2928, 1630, 1591, 1513, 1410, 1348, 1297, 1226, 1130, 1078, 1014, 964, 826, 786, 761, 708, 558, 449; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.82(s, 2H, -OH), 8.45(s, 2H, CH=N), 7.10-7.08(d, 2H, ArH), 6.27-6.26 (d, 2H, ArH), 6.23-6.21(d, 2H, ArH), 3.42-3.36 (q, 8H, $-CH_2$), 1.23-1.18 (t, 12H, $-CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): 12.7, 44.5, 97.8, 103.9, 106.9, 133.2, 151.2, 160.9, 161.4. M+H: 383.2 au, calculated for $C_{22}H_{30}N_4O_2$ 382.5.

S3. SYNTHESIS AND CHARACTERIZATION OF COMPLEX:

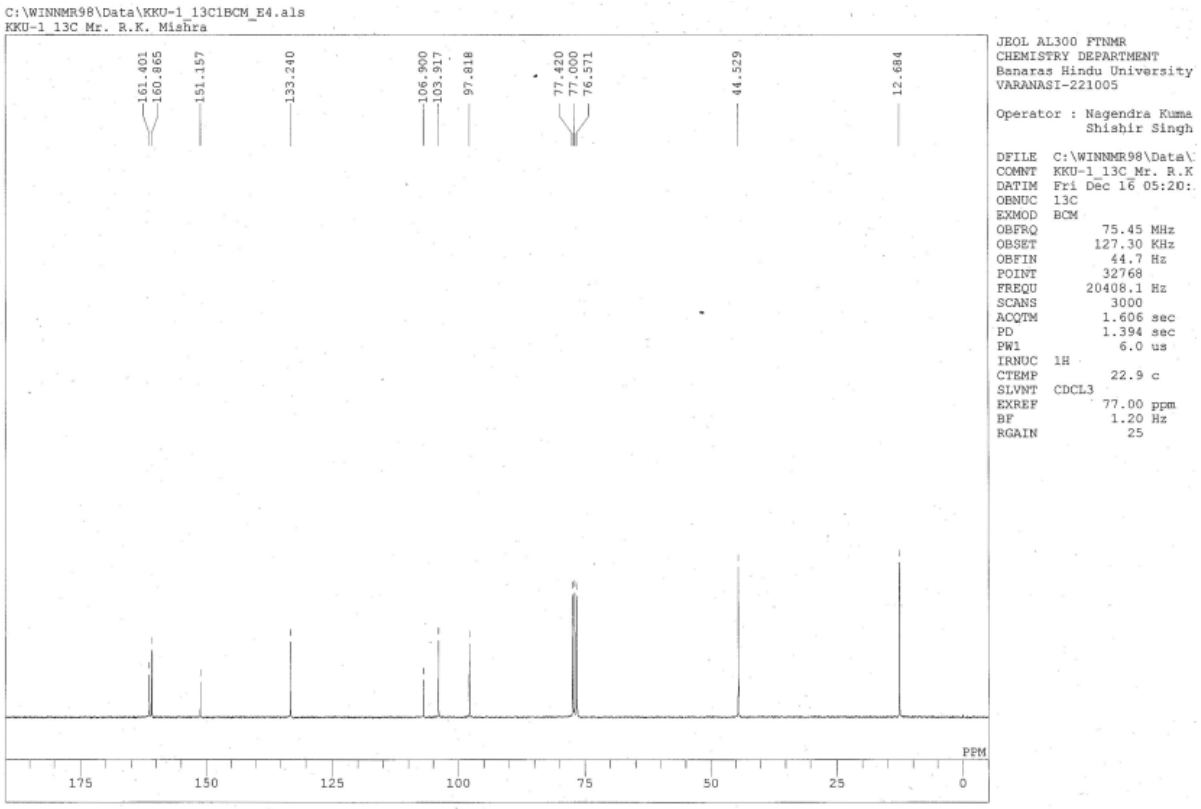
To a solution of ligand **L** (0.382 g, 1 mmol) in EtOH (25 mL), K_2CO_3 (5.0 mmol) was added and to this mixture a solution of $Zn(OAc)_2 \cdot 2H_2O$ (0.35 g, 1.59 mmol) in EtOH (10 mL). The yellow solution was then stirred for overnight and orange solid product was obtained which was filtered and washed with cold ethanol-water mixture (50/50 v/v) and dried to yield solid product (yield: 0.5g, ~90%,). MP 280 ˚C (decomposed), IR (cm^{-1}) 3447, 2967, 2928, 1585, 1503, 1425, 1397, 1347, 1243, 1213, 1137, 1075, 1014, 970, 824, 776, 708, 576; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95(s, 6H, CH=N), 6.15 (s, 6H, Ar-H), 5.95-5.92(d, 6H, Ar-H), 5.73-5.70 (d, 6H, Ar-H), 3.47-3.30 (m, 24H, -CH_2), 1.24-1.19 (t, 36H, -CH_3; ^{13}C NMR (75 MHz, $CDCl_3$): 170.4, 162.4, 153.2, 138.1, 107.7, 107.6, 102.6, 101.9, 44.7, 12.9. Mass (MALDI-MS): 1337.545; calculated for $C_{66}H_{84}N_{12}O_6Zn_3$ 1337.62.

S4. ^{1}H NMR SPECTRUM OF LIGAND:

All the ^{1}H and ^{13}C NMR spectra were recorded on JEOL AL 300 FT NMR spectrometer using TMS as internal reference standard.

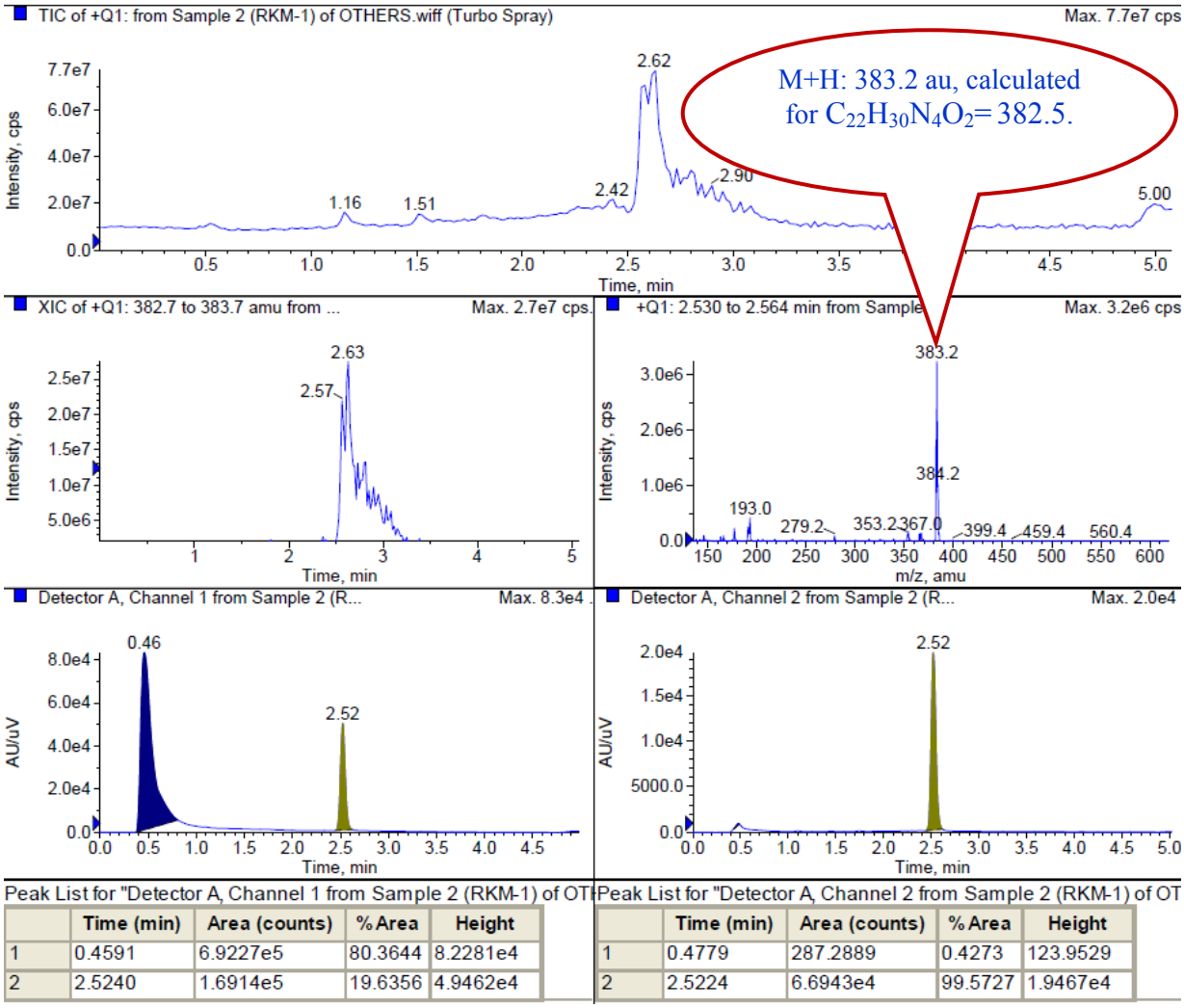


S5. ^{13}C NMR SPECTRUM OF LIGAND:



S6. LC-MS SPECTRUM OF LIGAND:

LC-MS and MALDI-MS Mass spectra were recorded on a MDS Sciex API 2000 LCMS spectrometer.



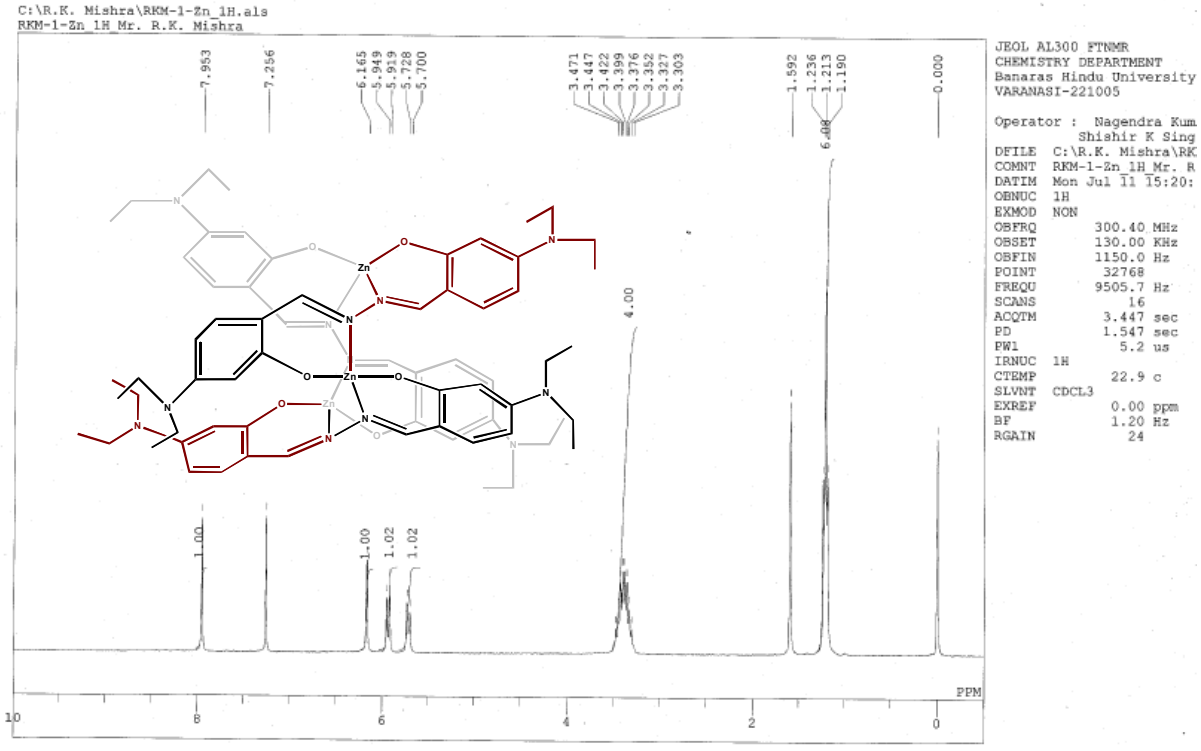
Peak List for "Detector A, Channel 1 from Sample 2 (RKM-1) of OT

	Time (min)	Area (counts)	% Area	Height
1	0.4591	6.9227e5	80.3644	8.2281e4
2	2.5240	1.6914e5	19.6356	4.9462e4

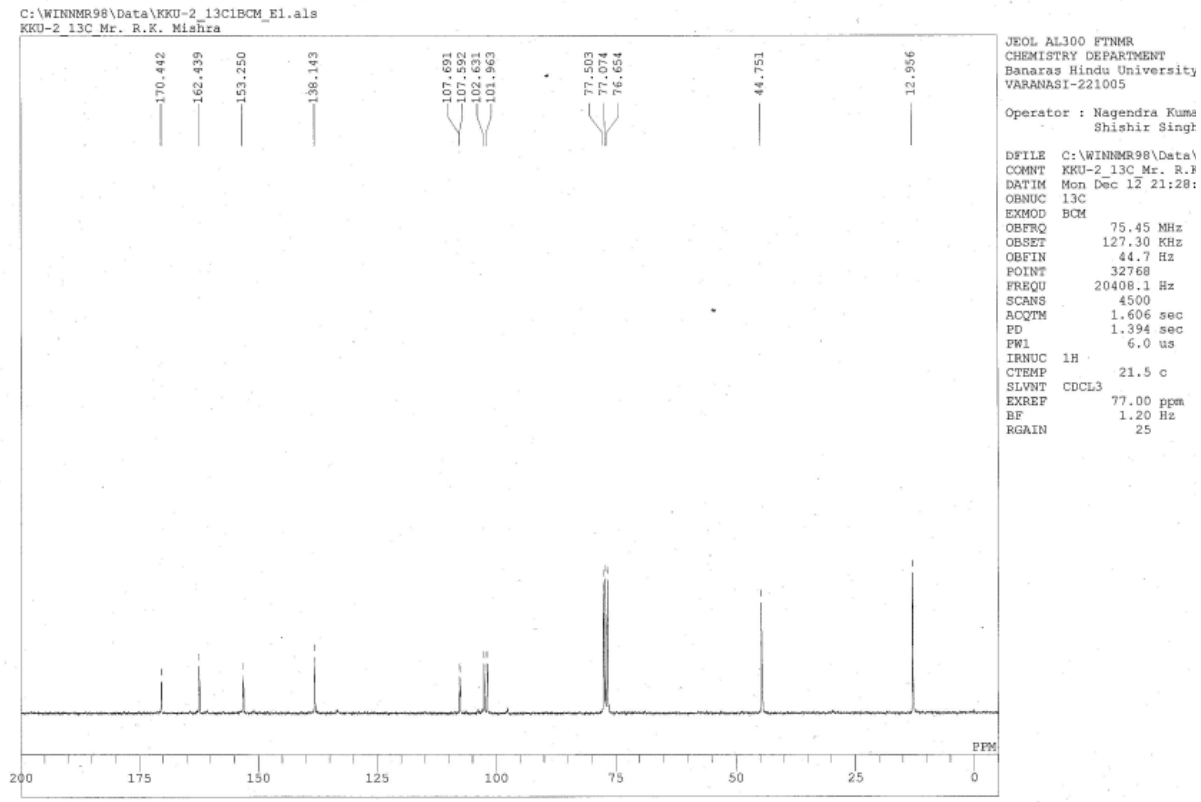
Peak List for "Detector A, Channel 2 from Sample 2 (RKM-1) of OT

	Time (min)	Area (counts)	% Area	Height
1	0.4779	287.2889	0.4273	123.9529
2	2.5224	6.6943e4	99.5727	1.9467e4

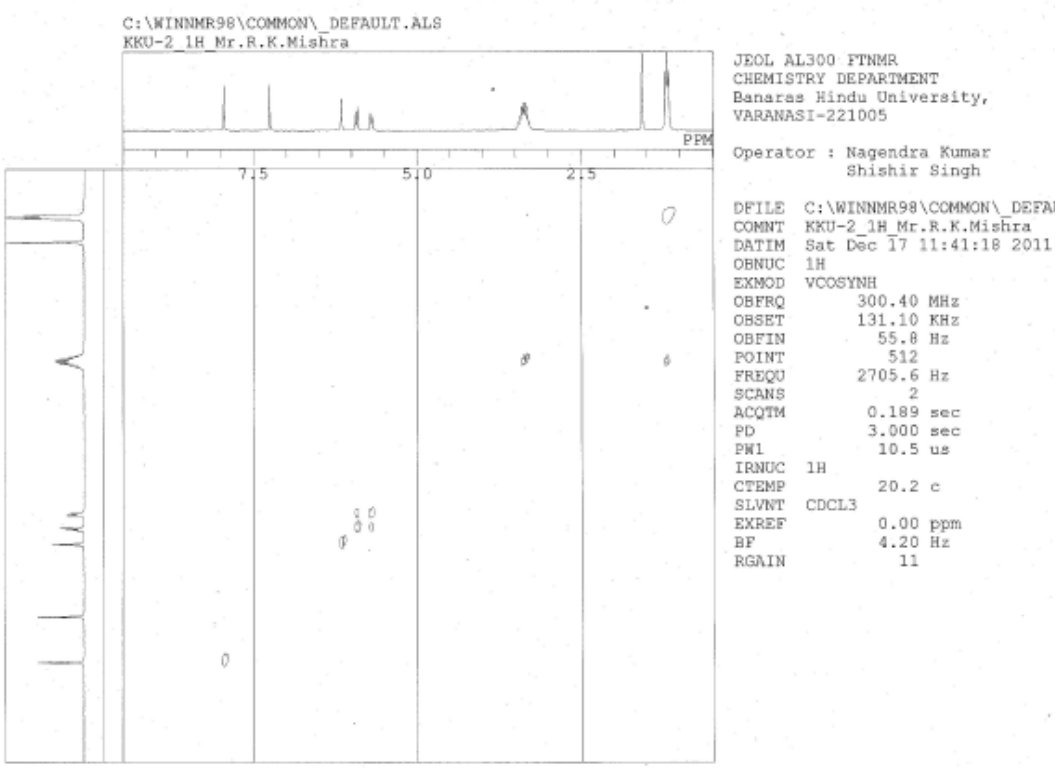
S7. ^{1}H NMR SPECTRUM OF COMPLEX:



S8. ^{13}C NMR SPECTRUM OF COMPLEX:



S9. ^{1}H-^{1}H COSY SPECTRUM OF THE COMPLEX



S10. MALDI-MS SPECTRUM OF COMPLEX:

Mass spectrum was recorded using a Bruker Daltonics Autoflex Speed MALDI TOF MS Mass Spectrometer.

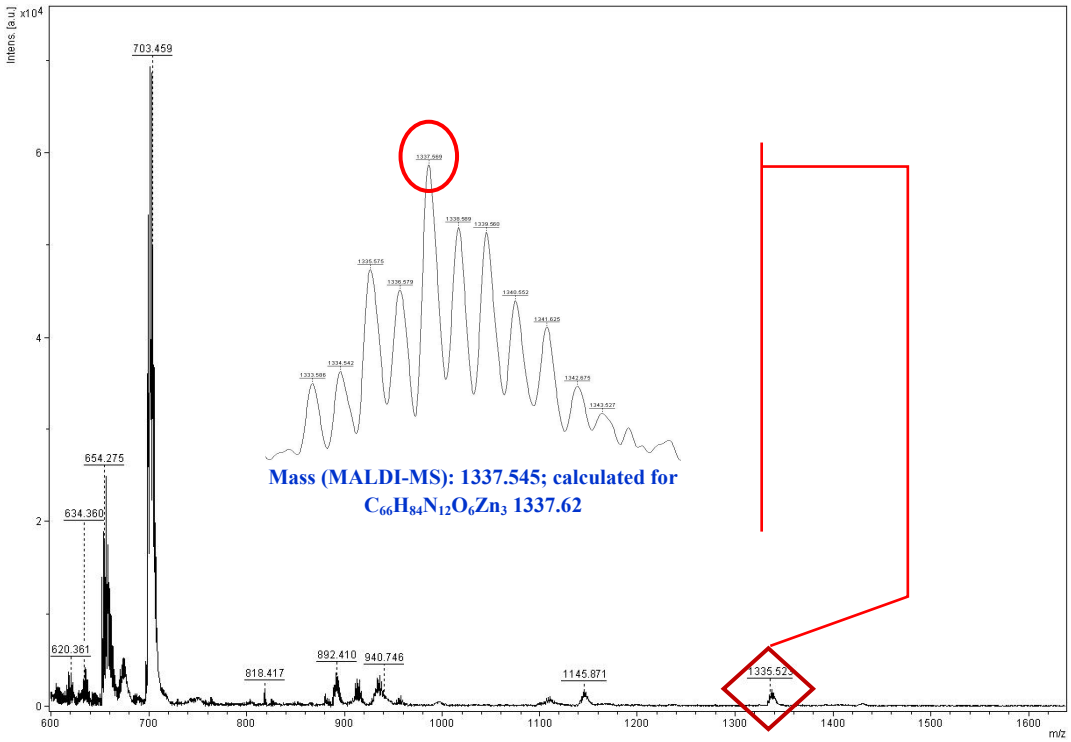


Figure S10: MALDI-MS spectrum of the Zn complex

S11. SCANNING ELECTRON MICROSCOPIC (SEM) STUDIES:

SEM pictures have been recorded on FEI Quanta 200 Scanning Electron Microscope.

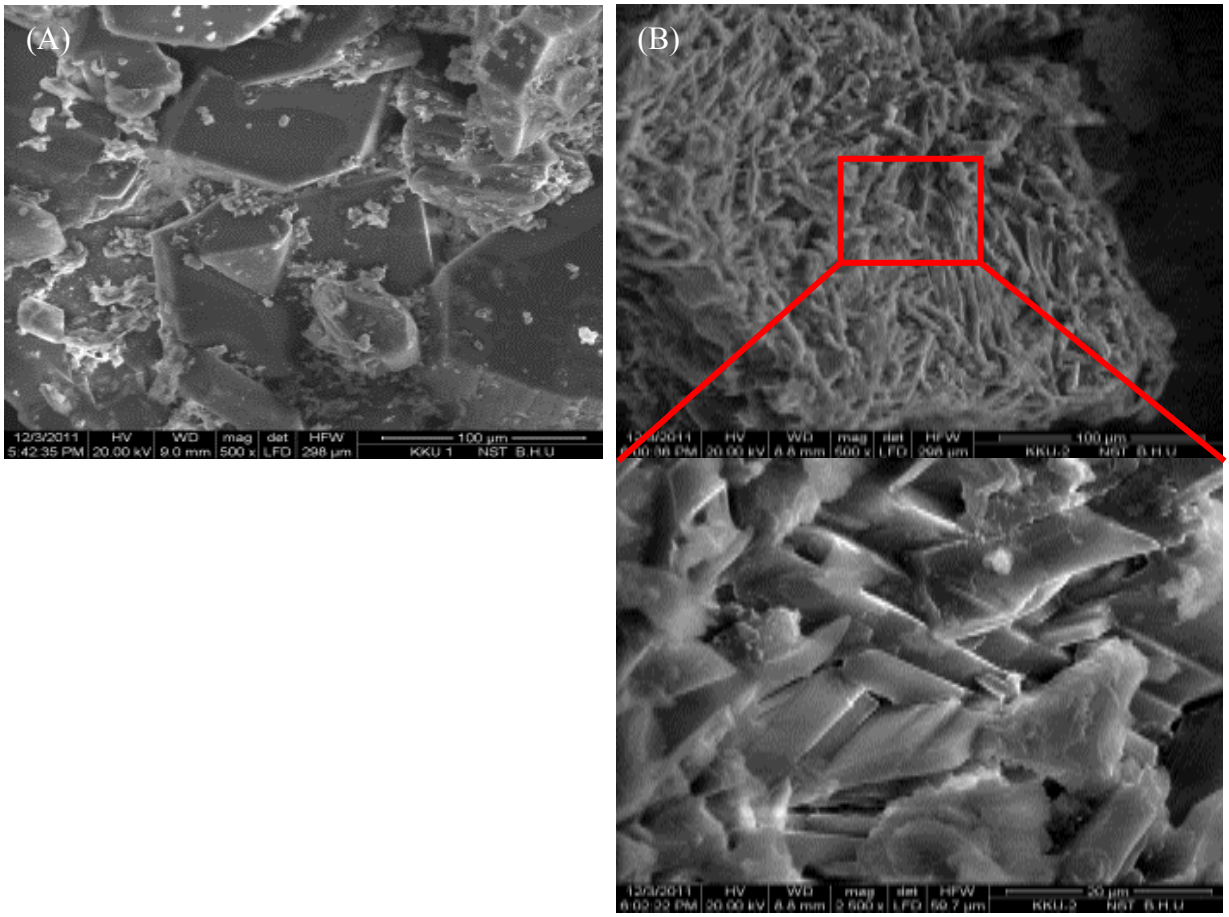


Figure S11: The SEM images of ligand **(A)** and the complex **(B)** respectively

S12. CRYSTAL REFINEMENT:

Single crystal X-ray data were collected on an Oxford Diffraction Xcalibur system with a Ruby CCD detector. All the determinations of unit cell and intensity data were performed with graphite-monochromated Mo-K_α radiation (λ= 0.71073 A˚). Data for the ligand was collected at room temperature while for the complex it was carried out at low temperature. The structures were solved by direct methods, using Fourier techniques, and refined on F^2 by a full-matrix least squares method. All the calculations were carried out with the SHELX-97 program.

S12a: IMPORTANT CRYSTAL DATA AND ORTEP PLOT FOR THE LIGAND:

Crystal data for Ligand: $C_{22}H_{30}N_4O_2$, M = 382.50, monoclinic, a = 12.0103(9)Å, b = 6.5415(4)Å, c = 13.3553(9)Å, α = 90.00°, β = 95.910(7)°, γ = 90.00°, V = 1043.69(12)Å^3, T = 293(2)K, space group *P121/*n1, Z = 2, 4218 reflections measured, 2124 independent reflections (R_{int} = 0.0285). The final R1 values were 0.0659 (I>2σ(I)). The final wR(F^2) values were 0.1532 (I>2σ(I)). The final R1 values were 0.1070 (all data). The final wR(F^2) values were 0.1827 (all data). The goodness of fit on F^2 was 1.035.

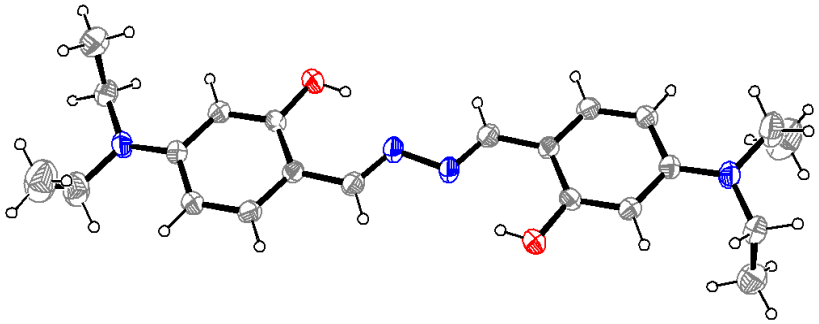


Figure S12a: Showing the ortep plot of Ligand with 30% ellipsoid probability

TABLE S12b: IMPORTANT BOND LENGTH AND BOND ANGLE FOR THE COMPLEX:

Zn1—O2b	1.891 (2)	Zn2—N2c	2.031 (4)
Zn1—O1a	1.907 (2)	Zn2—N3a	2.037 (3)
Zn1—N2a	2.024 (3)	Zn3—O2c	1.902 (2)
Zn1—N3b	2.050 (4)	Zn3—O1b	1.903 (3)
Zn2—O2a	1.897 (2)	Zn3—N3c	2.023 (3)
Zn2—O1c	1.900 (2)	Zn3—N2b	2.070 (3)
C11a—N2a— Zn1	118.3 (2)	O2b—Zn1—O1a	123.6 (1)
N3a—N2a—Zn1	126.1 (2)	O2b—Zn1—N2a	121.4 (1)
C11b—N2b—Zn3	116.4 (2)	O2b—Zn1—N3b	96.3 (1)
N3b—N2b—Zn3	125.4 (2)	O1a—Zn1—N2a	97.4 (1)
C11c—N2c—Zn2	117.9 (2)	O1a—Zn1—N3b	118.1 (1)
N3c—N2c—Zn2	125.9 (2)	N2a—Zn1—N3b	98.6 (1)
C12a—N3a—Zn2	117.0 (2)	O2a—Zn2—O1c	121.7 (1)
N2a—N3a—Zn2	125.2 (2)	O2a—Zn2—N2c	122.0 (1)
C12b—N3b—Zn1	117.4 (2)	O2a—Zn2—N3a	96.1 (1)
N2b—N3b—Zn1	126.1 (2)	O1c—Zn2—N2c	97.4 (1)
C12c—N3c—Zn3	119.8 (2)	O1c—Zn2—N3a	120.1 (1)
N2c—N3c—Zn3	124.8 (2)	N2c—Zn2—N3a	98.8 (1)
C7a—O1a—Zn1	123.4 (2)	O2c—Zn3—O1b	123.0 (1)
C7b—O1b—Zn3	123.6 (2)	O2c—Zn3—N3c	96.2 (1)
C7c—O1c—Zn2	123.3 (2)	O2c—Zn3—N2b	115.9 (1)
C14a—O2a—Zn2	122.6 (2)	O1b—Zn3—N3c	127.4 (1)
C14b—O2b—Zn1	124.1 (2)	O1b—Zn3—N2b	93.5 (1)
C14c—O2c—Zn3	124.8 (2)	N3c—Zn3—N2b	99.4 (1)

*esd's have been shown in parantheses.

S13. CRYSTAL PACKING DIAGRAM OF COMPLEX THROUGH DIFFERENT AXIS

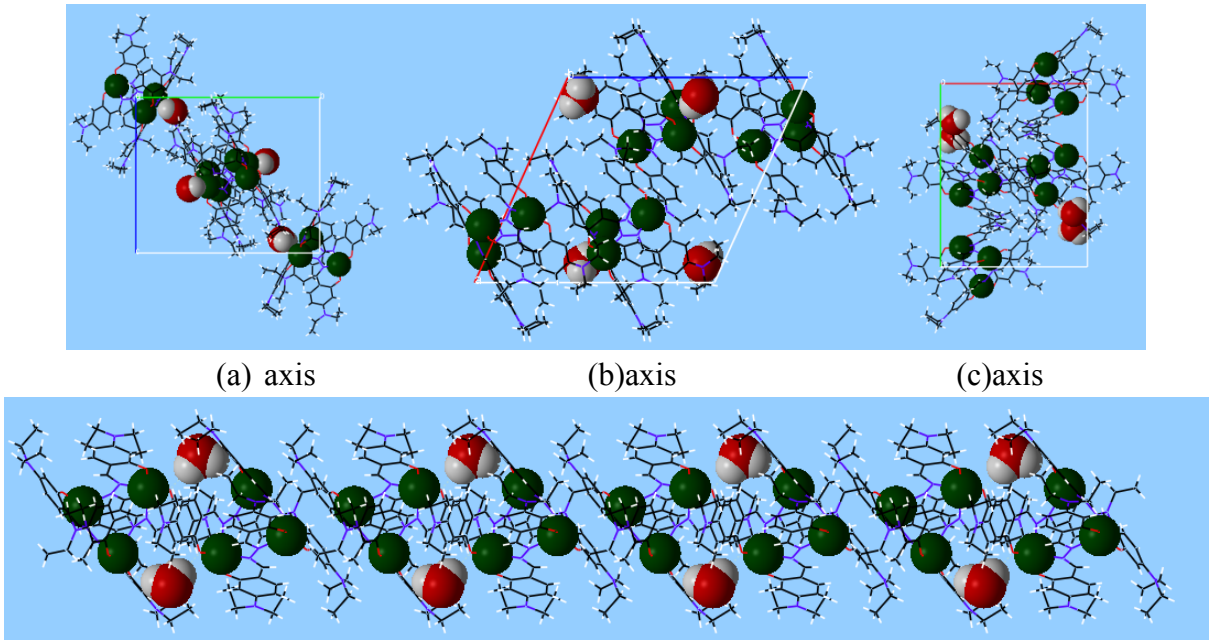


Figure S14: Crystal packing diagram of the complex through different a, b and c axis and the chain extension (below) through C----C interactions. The two water molecules is shown to lie between two molecules of complex.

S14. UV-VIS EXPERIMENT:

The potential binding ability of complex towards CT-DNA was studied through UV-vis spectroscopy. The absorption spectra were recorded on a UV-vis pharmaspec spectrophotometer using cuvettes of 1 cm path length. Calf Thymus DNA for binding studies was purchased from SRL chemical Pvt. Ltd. and was used as such. The titration experiments were performed in 5 mM Tris-HCl, 50 mM NaCl buffer at pH 7.4 and by maintaining a constant metal complex concentration (4 μM). The nucleotide (CT-DNA) concentration was varied between 0-40 μM while keeping the total volume constant (2.0 mL). After each addition of DNA to the metal complex the resulting solution was allowed to equilibrate at 25°C for 2 min followed by recording of absorption patterns.

The data thus obtained were then fitted to eq. 1 to obtain intrinsic binding constant K_b.[1]

$$[DNA]/(\mathcal{E}_a - \mathcal{E}_f) = [DNA]/(\mathcal{E}_b - \mathcal{E}_f) + 1/K_b(\mathcal{E}_b - \mathcal{E}_f) \text{ ----------(1)}$$

Where, [DNA] is the concentration of DNA in terms of base-pairs, $\mathcal{E}_a$ is the apparent extinction coefficient obtained by calculating A_{obs}/[complex], $\mathcal{E}_f$ corresponds to the extinction coefficient of the complex in its free form and $\mathcal{E}_b$ refers to the extinction coefficient of the complex in the bound form. Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(\mathcal{E}_b - \mathcal{E}_f)$ and a y-intercept of $1/K_b(\mathcal{E}_b - \mathcal{E}_f)$ and K_b was determined from the ratio of the slope to intercept.

S15. FLUORESCENCE EXPERIMENT:

Emission spectra were recorded on Varian Cary Eclipse Fluorescence spectrophotometer using cuvette of 1 cm path length. The titration experiments were performed in 5 mM Tris-HCl, 50 mM NaCl buffer at pH 7.4 and by maintaining a constant metal complex concentration (1μM). The nucleotide (CT-DNA) concentration was varied between 0-40 μM while keeping the total volume constant (2.0 mL). After each addition of DNA to the metal complex the resulting solution was allowed to equilibrate at 25°C for 2 min followed by recording of spectrum.

The quenching plots (figure 4b) followed the Stern-Volmer relationship of the form:

$$I_0/I=1+Ksv.r \text{ ---------- (2)}$$

Where I_0 and I are the fluorescence intensities of the excited DNA-EB in the absence and presence of the complexes, K is the Sterne-Volmer quenching constant and r is the ratio of the concentration of the metal complexes to DNA.

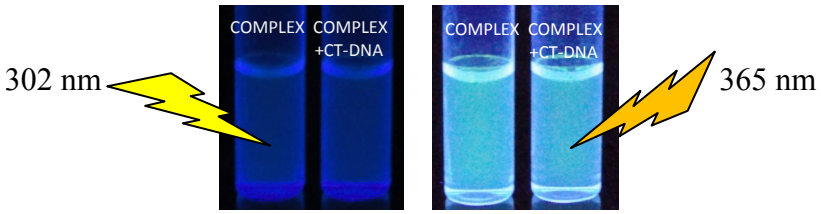


Figure S15: Showing florescence properties of complex (2μM) in absence and presence of CT-DNA (20μM) in different UV light (a) 302 nm (b) 365 nm

S16. CIRCULAR DICHROIC STUDIES:

CD measurements were done on a Jasco J 500A spectropolarimeter equipped with a constant temperature cell holder. The instrument was calibrated using ammonium (+)-10-camphorsulfonate. Temperature of the cell holder was controlled using Julabo F 25 water bath. Conformational changes in the DNA were monitored in the region between 300 and 220 nm with a DNA concentration of 500 μM in a cuvette of 1mm pathlength. After subtracting appropriate blanks, mean residue ellipticities were calculated, using the formula

$$[\theta]MRW = \frac{\theta_{obs}MRW}{10\,cl} \quad \text{---------- (3)}$$

Where θ_{obs} is the observed ellipticity in degrees, MRW is mean residue weight, c the concentration of DNA (gm/cm^3) and l is the path length in centimeter. A mean residue molecular weight 110 was used.

S17. AGAROSE GEL ELECTROPHORESIS EXPERIMENT:

S17.1. Plasmid DNA:

The plasmid pBR322 (4361 bp) was isolated and purified from the overnight grown *E. coli* culture using the HiPura Plasmid DNA Miniprep purification spin kit (HiMedia, India) as per the manufacturer's instructions. The plasmid DNA thus purified was analyzed further on 1% agarose (Bangalore GeNei Ltd., India) in order to check the integrity and quality of the purified DNA. This DNA was used as a template for all cleavage setup reactions.

S17.2. DNA cleavage Experiments:

The gel electrophoresis was setup by mixing 1µg of plasmid DNA with varying amount of complex ranging from 25 to 100µg dissolved in dichloromethane and the required amount of 50mM Tris-Cl buffer (pH 7.4) so as to make the final reaction volume to 20 µl. The reaction mixture was incubated at 37°C for various time intervals (6, 12, 24 and 36 hours) followed by the addition of 2µl loading buffer containing 0.25% bromophenol blue, 50% glycerol, 10mM Tris-HCl (pH 7.4), and 60mM EDTA at the end of incubation period. The reaction mixture was finally loaded on 1% agarose gel containing 1.0 µg/ml ethidium bromide. The electrophoresis was carried out for 1 hour at 50 volts in 1X TAE buffer containing 40mM tris, 20mM glacial acetic acid and 2mM EDTA (pH 8.0).

Bands were visualized under UV light and photograph was taken and analyzed by Alpha Digi Doc RT Gel documentation system (Alpha Innotech, CA, USA). The cleavage was confirmed by comparing the banding pattern of untreated and treated (with complex) plasmid DNA.

S18. DNA CLEAVAGE EXPERIMENT FOR LIGAND:

Similar experiment was also carried out for the ligand also under identical condition which did not showed cleavage property.

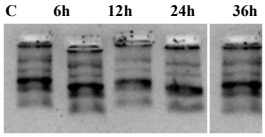


Figure S18. Showing the photographs of ethidium bromide stained gel at various time points, starting from 6 hours to 36 hours. No cleavage was observed even up to 36 hours incubation of ligand (100µg) with the circular plasmid DNA pBR322 (1µg). The C indicates control pBR322 plasmid DNA.

References:

1. A. M. Pyle, J. P. Rehmann, R. Meshoyer, C. V. Kumar, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1989, **111**, 3051.