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Cu (II) induced twisting of biphenyl core: Exploring the effect of structure and coordination environment of biphenyl based chiral Copper(II) complexes on interaction with calf thymus DNA

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Experimental Methods

A. Determination of affinity constants

Binding affinities of the complexes to ct-DNA have been determined from absorbance and fluorescence titrations. From the titration data the concentrations of bound and the free ligand (C_b and C_f) have been determined from the absorbance or fluorescence change at a fixed wavelength, which usually corresponds to the wavelength of maximum changes. If A_F(or I_F), A_B(or I_B), and A(I) represent the absorbance (or fluorescence) of the initially, finally, and partially titrated ligands, respectively, then the fraction of the bound ligand molecules α_b is given by

$$\alpha_b = [A_F(\text{or } I_F) - A(I)]/[A_F(I_F) - A_B(I_B)] \text{ ……(1)}$$

The molar concentration of free (C_f) and bound (C_b) ligands molecules and r could be evaluated from the following equations, where D and P represent the total input ligand and DNA phosphate concentrations, respectively

$$C_f = (1 - \alpha_b)D\text{…….. (2)}$$

$$Cb = \alpha_b D \text{ ……(3)}$$

$$r = C_b/P = \alpha_b D/P \text{ ……(4)}$$

Binding data obtained from spectrophotometric titration were cast into the form of Scatchard plot of r/C_f versus r, where r is the number of ligand molecules bound per mole of nucleotide. Non-linear binding isotherms were fitted to a theoretical curve drawn according to the excluded site model [1] for a non-linear non-cooperative ligand binding system using the following equation,

$$r/ C_f = K^{/}(1-nr)[(1-nr)/\{1-(n-1)r\}]^{n-1} \text{ ……..(5)}$$

where $K^{/}$ is the intrinsic binding constant to an isolated binding site, and n is the number of nucleotides excluded by the binding of a single ligand molecule. The binding data were analyzed using the programme Origin 7.0.

B. Circular dichroic study

All circular dichroism (CD) measurements were performed with a JASCO: J-815 spectropolarimeter. To measure the circular dichoric spectra 0.1M stock solution of the complexes was prepared in water DMSO mixture. At first a blank CD spectrum was drawn with

citrate phosphate buffer at pH7.4. Then CD spectra of all the three complexes were recorded in the buffer.

C. X-Ray crystallography

Intensity diffraction data of H_2L^1, H_2L^2, and of copper(II) complexes **1**, **2**, and **3** were collected at room temperature on a Bruker APEX-II CCD diffractometer with Mo-Kα monochromatic radiation (0.71073 Å). Cell refinement, indexing and scaling of the data set were done by using programs Bruker Smart Apex and Bruker Saint packages.[1] All the structures were solved by direct methods and subsequent Fourier analyses[2] and refined by the full-matrix least-squares method based on F^2 with all observed reflections.[2] Hydrogen atoms were included at calculated positions except some of the NH groups which were located on the Fourier map.

Diffraction data of H_2L^1 were treated with SQUEEZE tool of Platon package[3] to take into account a small residual difficult to model. In H_2L^2 the –O-Ethyl group of ethylacetate molecule was found disordered over two positions with refined occupancies 0.751(9)/ 0.249(9). Data of complex **1** are at low accuracy, and taking into account a perchlorate anion at 0.5 occupancy, the proton at the NH groups was considered disordered over the two arms being the complex located on a two-fold axis. The ClO_4^- anion in **3** was found disordered over two positions with refined occupancies 0.565(10)/0.435(10). All calculations were performed using programs implemented in the WinGX System, Ver 2018.3.[4]

CCDC 1990271 (**H_2L^1**), 1856436 (**H_2L^2**), 2019991 (**1**), 1856433 (**2**), and 1856434 (**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures

1 Bruker, (2000). *SMART, SAINT. Software Reference Manual Bruker AXS Inc.* Madison, Wisconsin, USA

2 (a) G. M. Sheldrick, Acta Cryst. (2015) A71, 3-8. (b) O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Cryst.* (2009). 42, 339-341.

3 A. L. Spek, *Acta Crystallogr.,* (1990) *A46*, C-34.

4 L.J. Farrugia, *J. Appl. Crystallogr*. 32 (1999) 837-838.

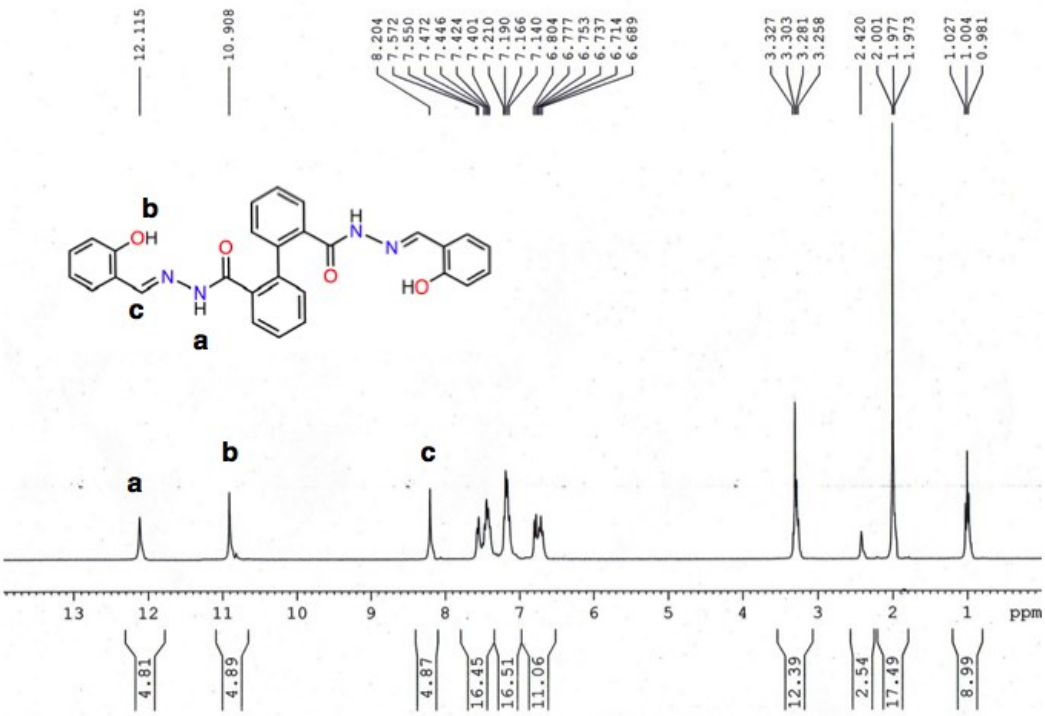

Figure S1. ^{1}H NMR (300 MHz) spectrum of $\mathbf{H_2L^1}$ in DMSO-d_6 at 20 ºC.

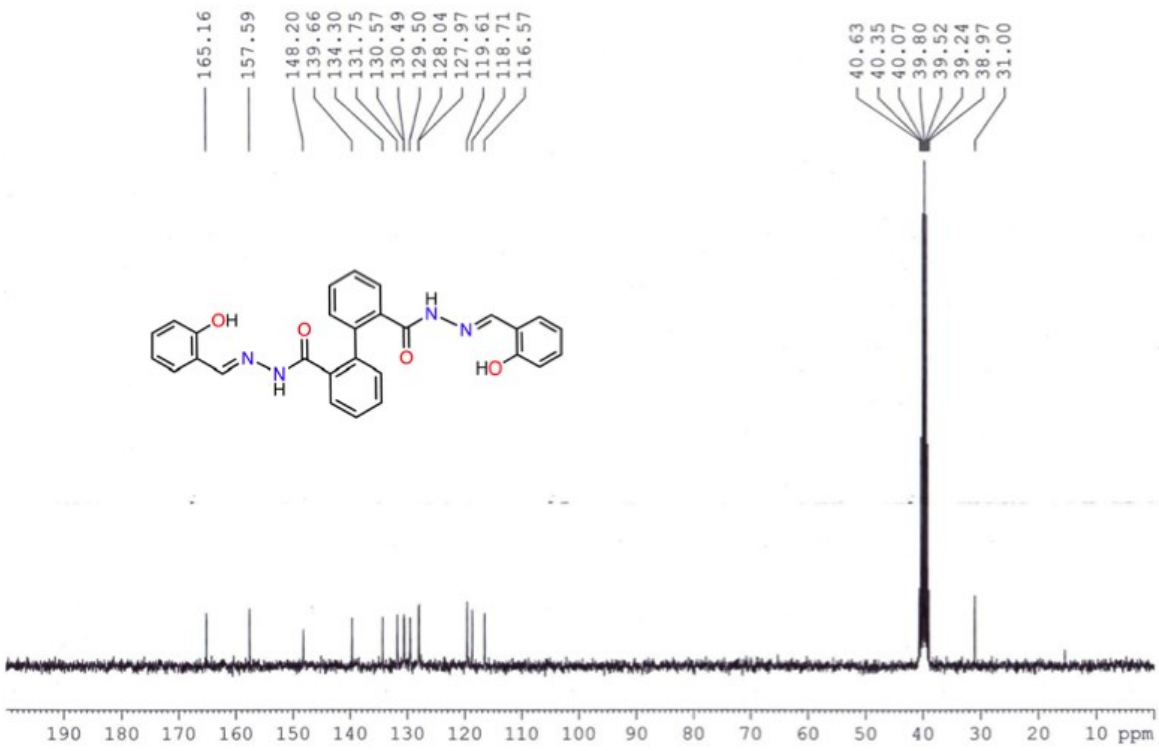

Figure S2. ^{13}C NMR (75.5 MHz) spectrum of $\mathbf{H_2L^1}$ in DMSO-d_6 at 20 ºC.

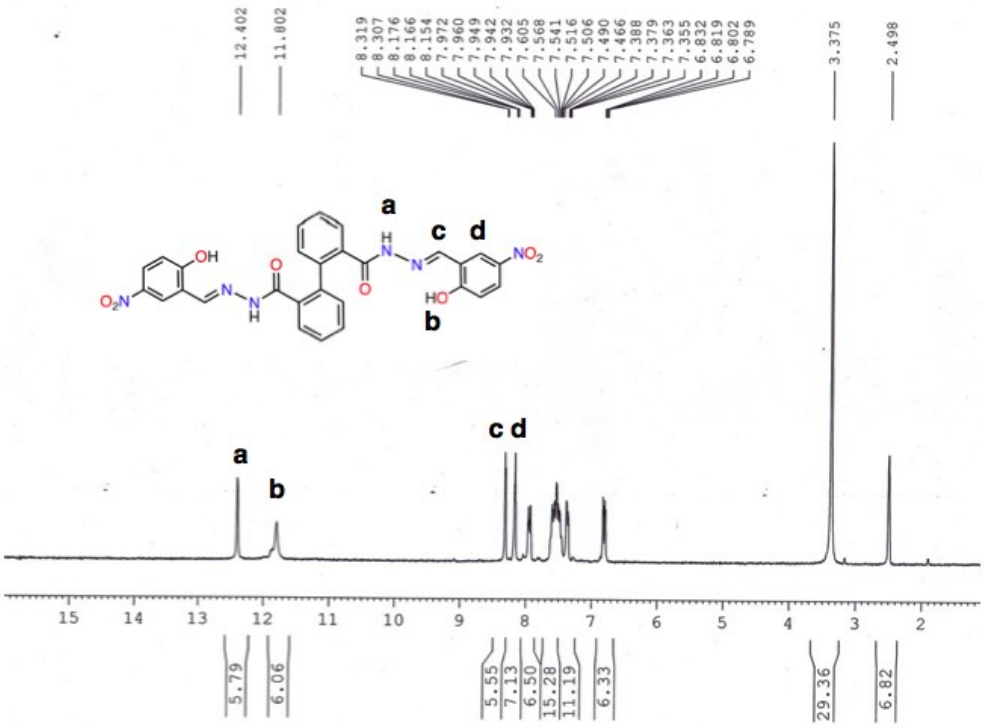

Figure S3. ^{1}H NMR (300 MHz) spectrum of $\mathbf{H_2L^2}$ in DMSO-d_6 at 20 ºC.

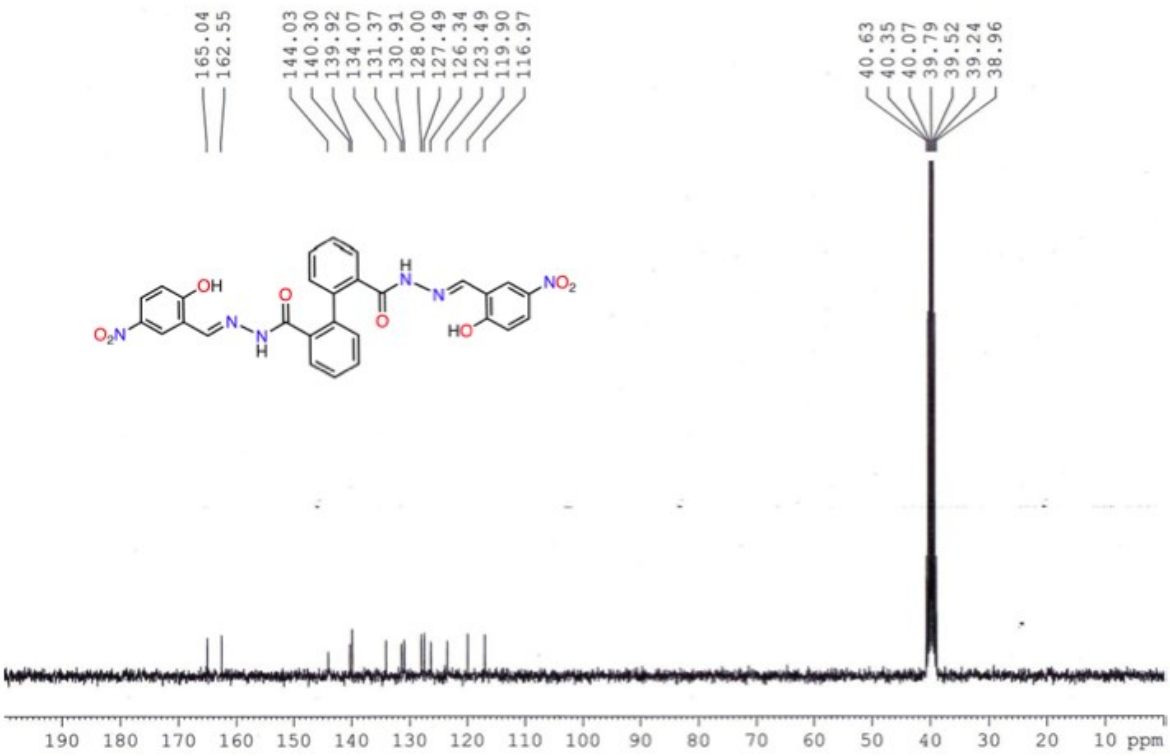

Figure S4. ^{13}C NMR (75.5 MHz) spectrum of $\mathbf{H_2L^2}$ in DMSO-d_6 at 20 ºC.

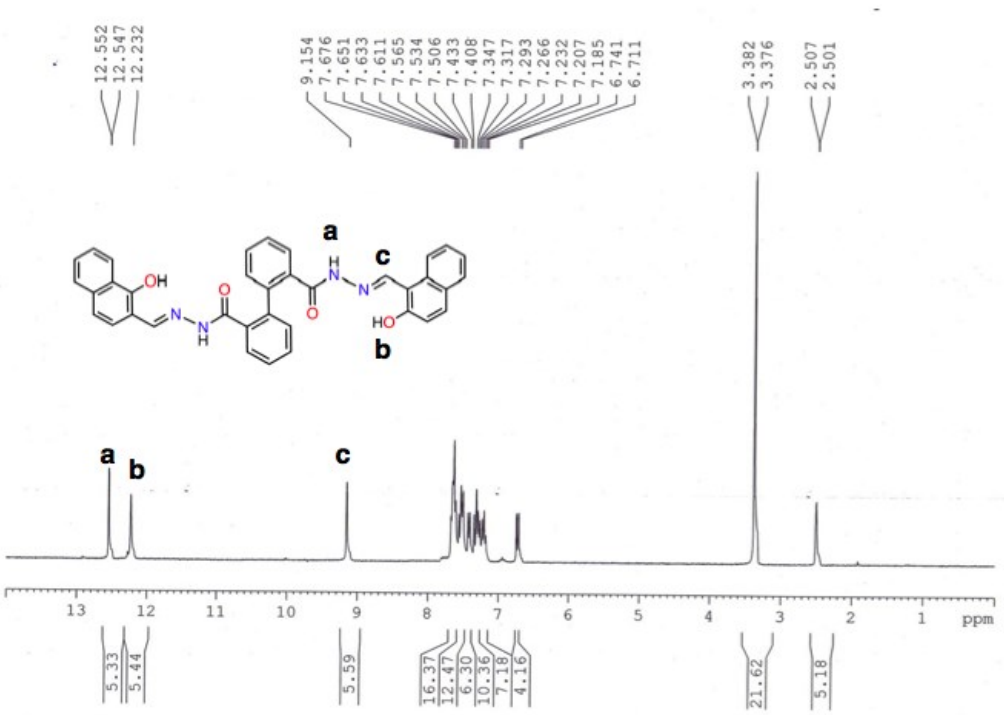

Figure S5. ^{1}H NMR (300 MHz) spectrum of $\mathbf{H_2L^3}$ in DMSO-d_6 at 20 ºC.

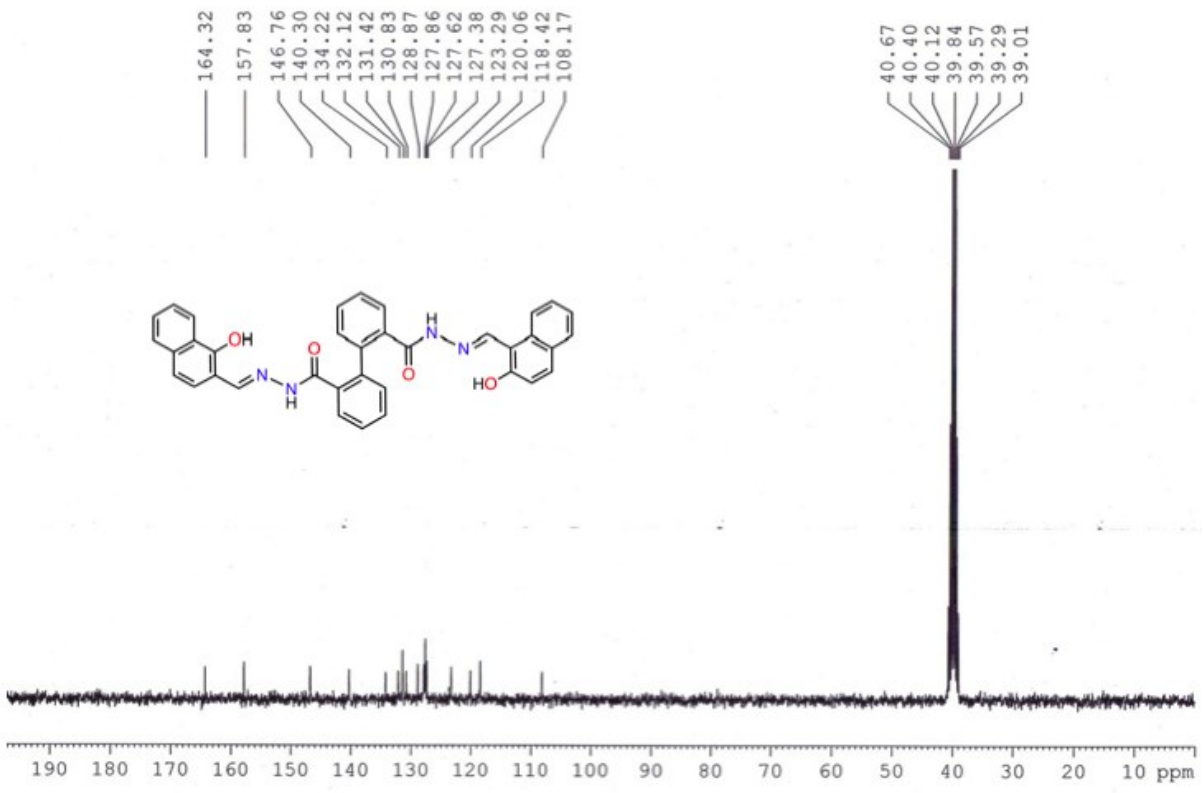

Figure S6. ^{13}C NMR (75.5 MHz) spectrum of **H_2L^3** in DMSO-d_6 at 20 ºC.

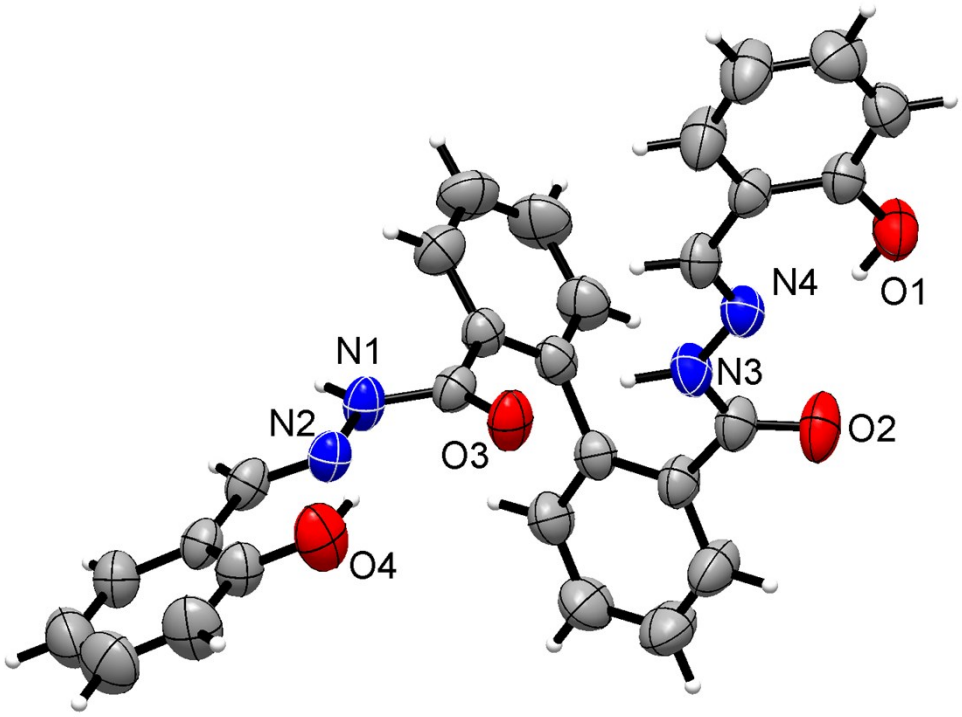

Figure S7. Ortep diagram of $\mathbf{H_2L^1}$ (thermal ellipsoid probability at 50%)

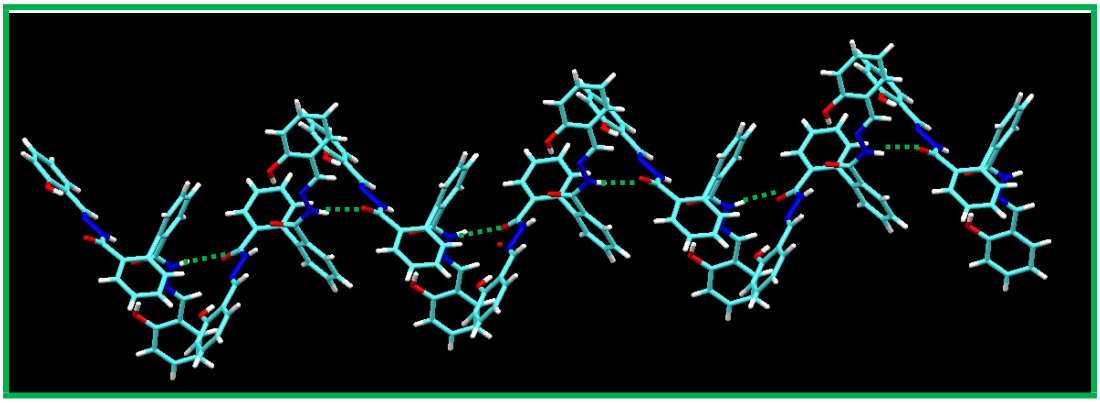

Figure S8. Intermolecular H-bonding among $\mathbf{H_2L^1}$ molecules (Atom colors: O red, N blue, H white).

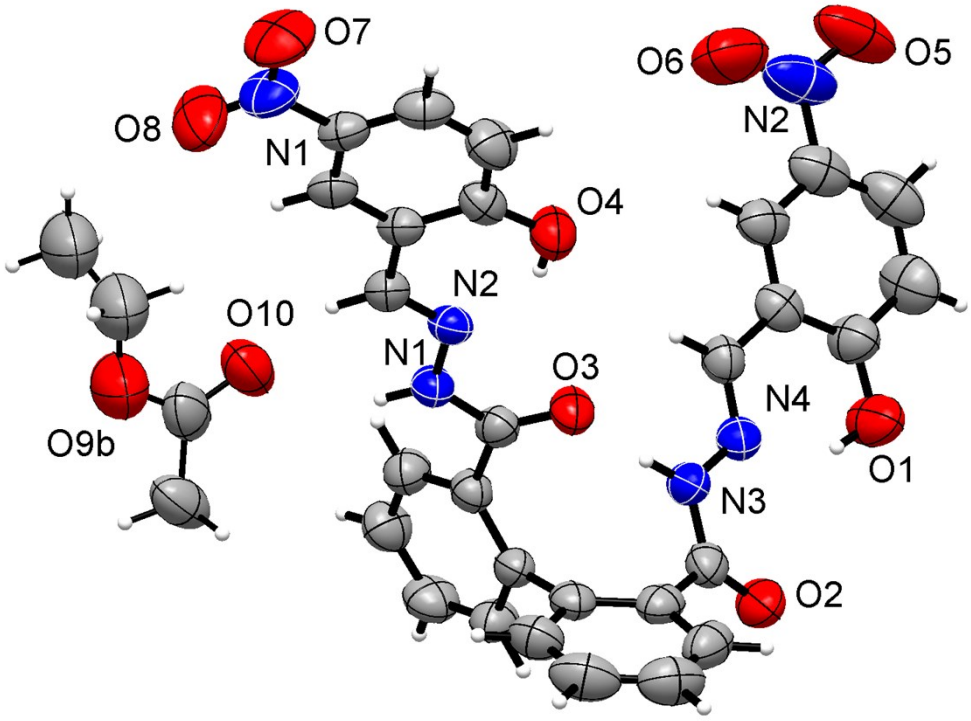

Figure S9. Ortep diagram of $\mathbf{H_2L^2}$ (thermal ellipsoid probability at 50%). Of the disordered ethylacetate molecule, only one orientation is shown.

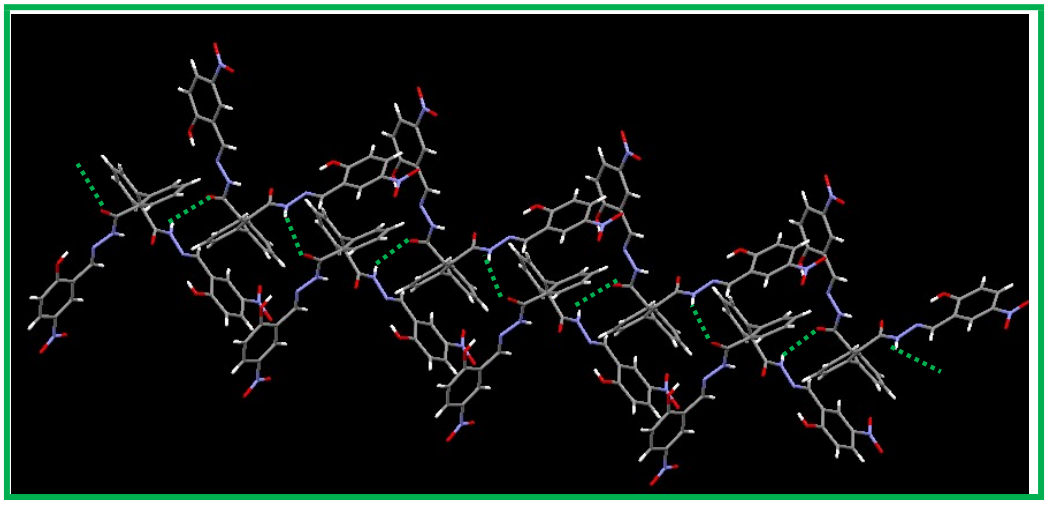

Figure S10. Intermolecular H-bonding among $\mathbf{H_2L^2}$ molecules (Atom colors: O red, N blue, H white).

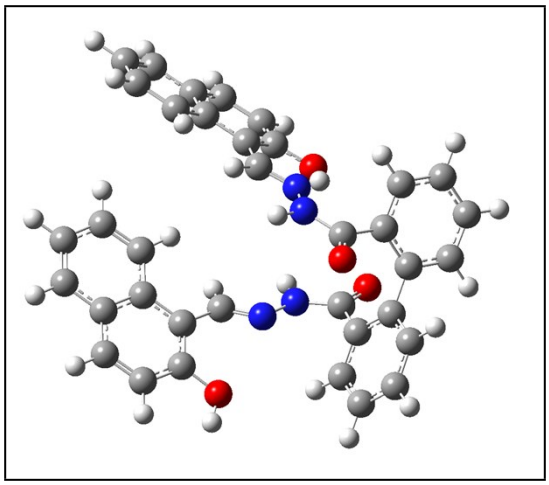

Figure S11. Optimized structure of $\mathbf{H_2L^3}$ molecule at theoretical DFT level calculation (B3LYP/6-31G(*d,p*))

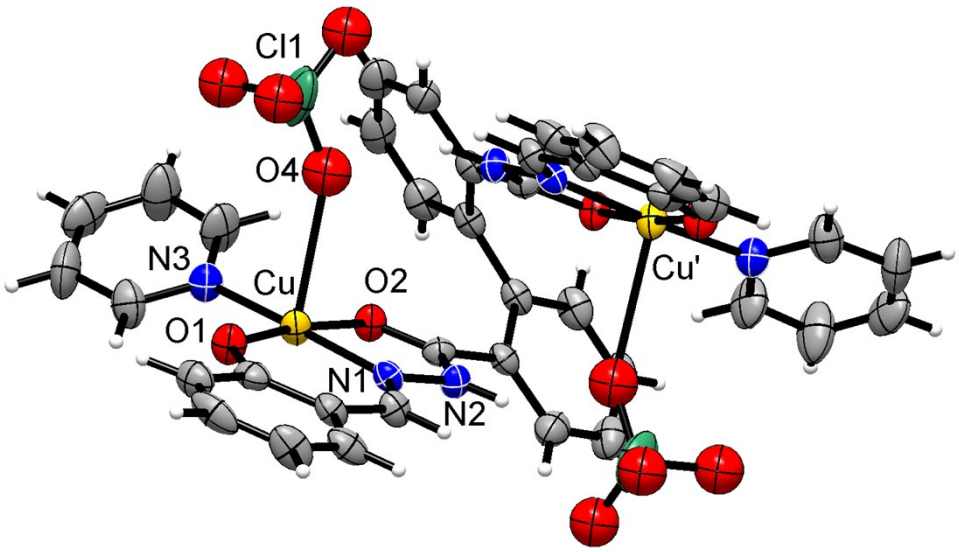

Figure S12. Ortep diagram of complex **1** (thermal ellipsoid probability at 30%) located on a crystallographic two-fold axis. Due to the high thermal factors of the perchlorate oxygen atoms, these are depicted as sphere of fixed radius.

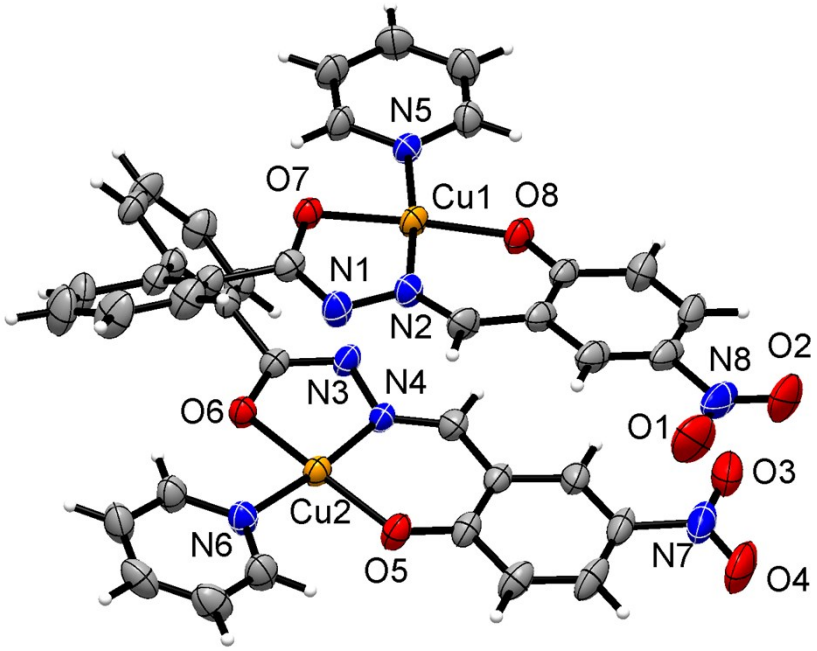

Figure S13. Ortep diagram of complex **2** (thermal ellipsoid probability at 40%). The lattice pyridine molecule not shown for clarity.

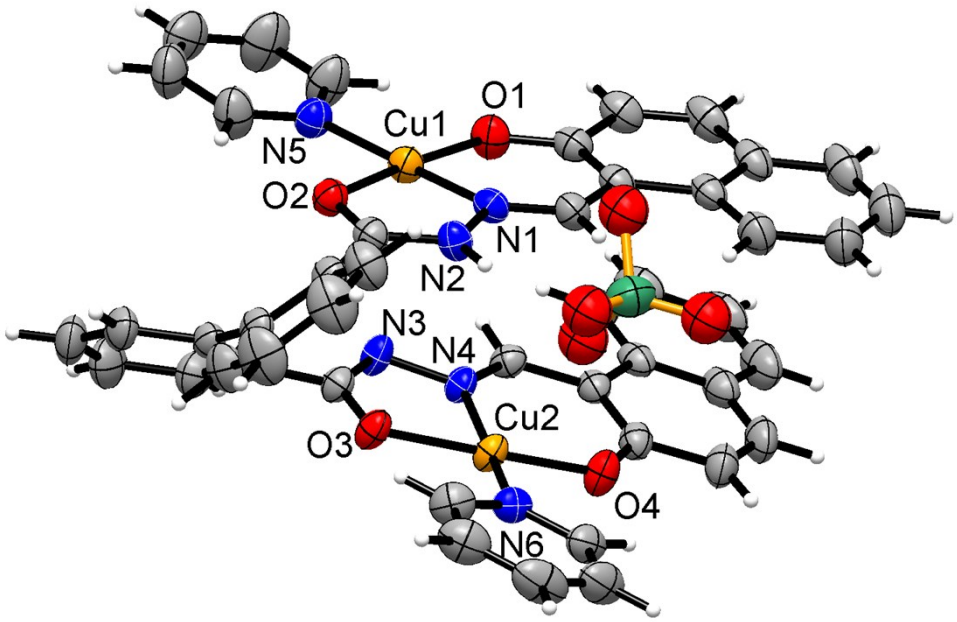

Figure S14. Ortep diagram of complex **3** (thermal ellipsoid probability at 40%). Due to the high thermal factors of the disordered perchlorate oxygen atoms, these are depicted as sphere of fixed radius.

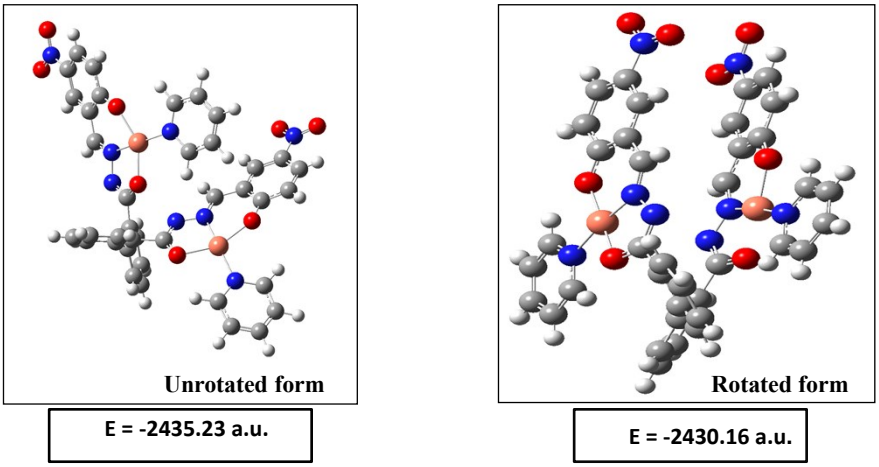

Figure S15. Theoretically optimized structure of complex **2** (left) without twisting and (right) after twisting.

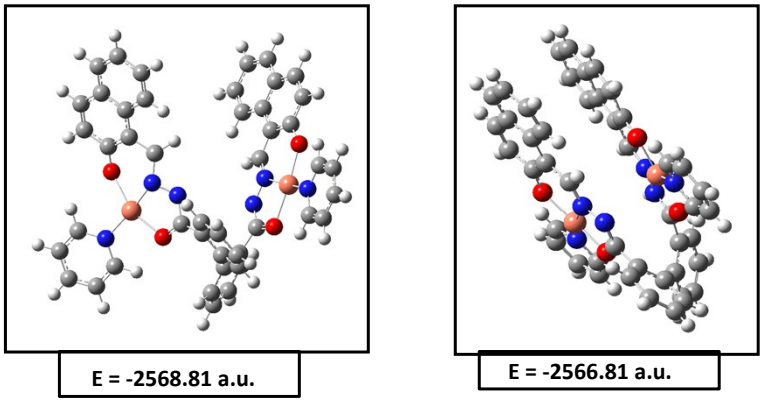

Figure S16. Theoretically optimized structure of complex **3** (left) without twisting and (right) after twisting.

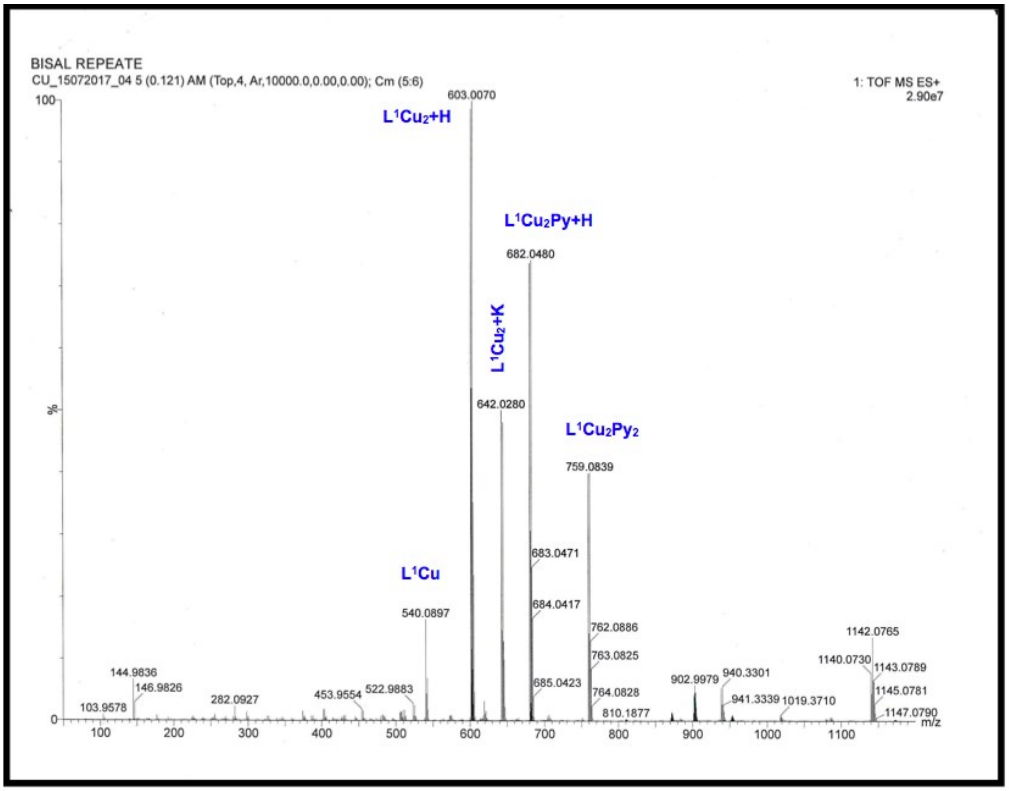

Figure S17. Mass spectrum of complex **1**

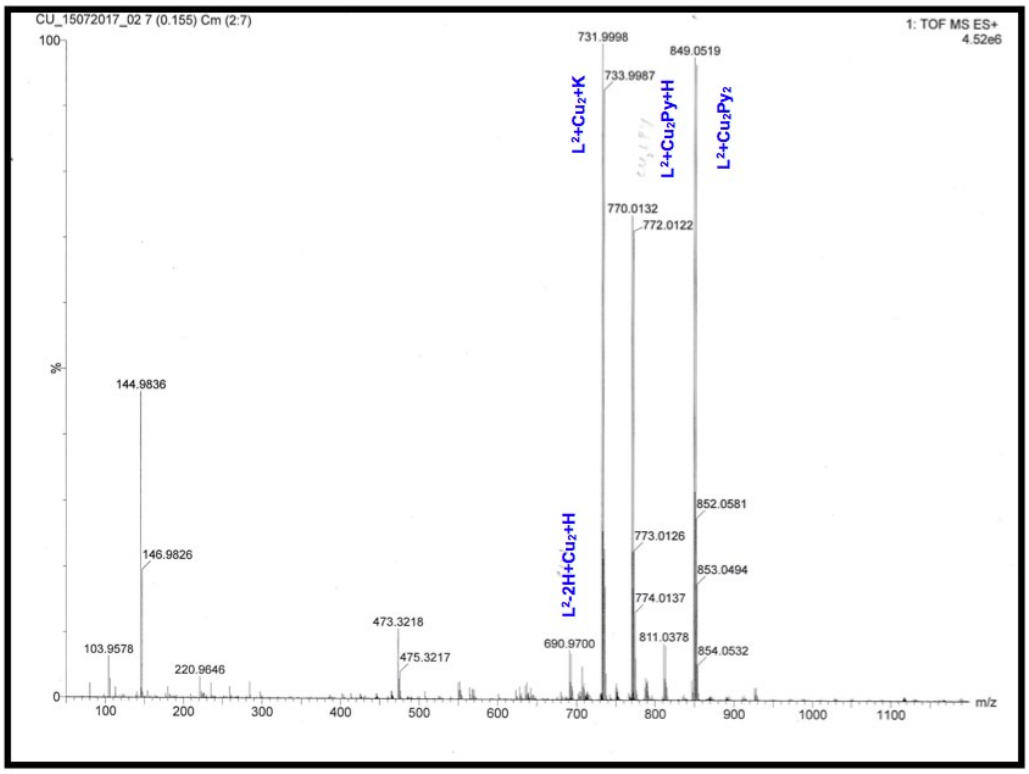

Figure S18. Mass spectrum of complex **2**

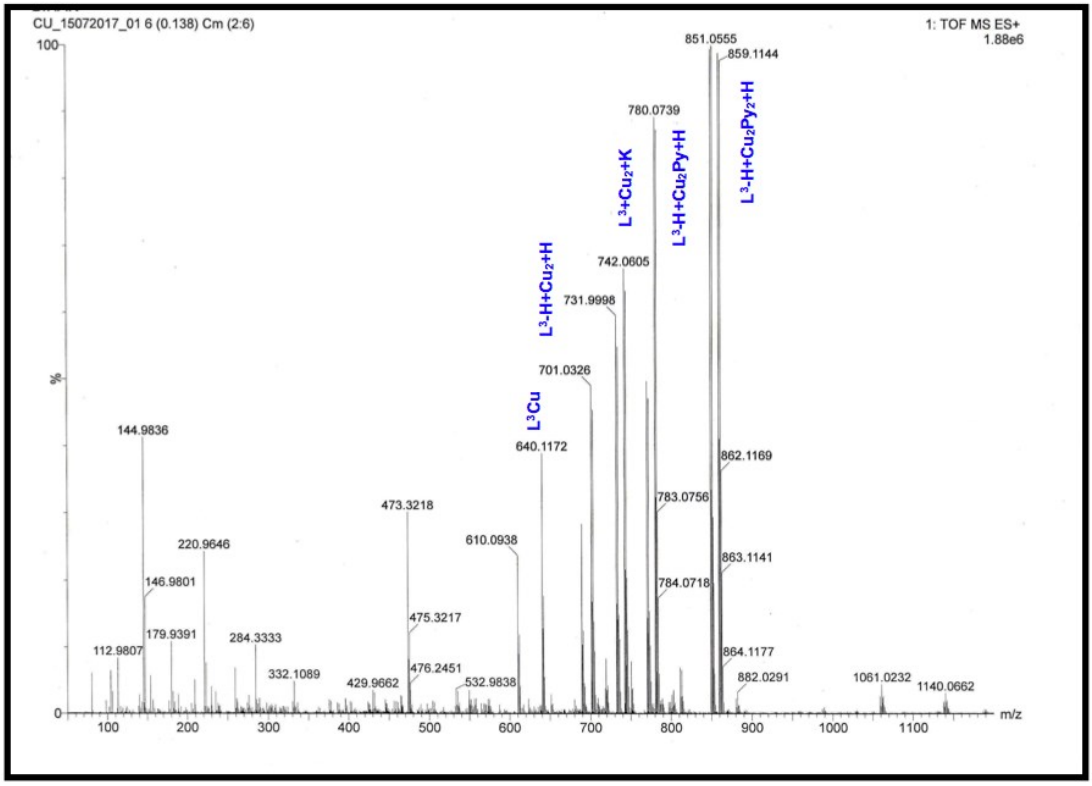

Figure S19. Mass spectrum of complex **3**

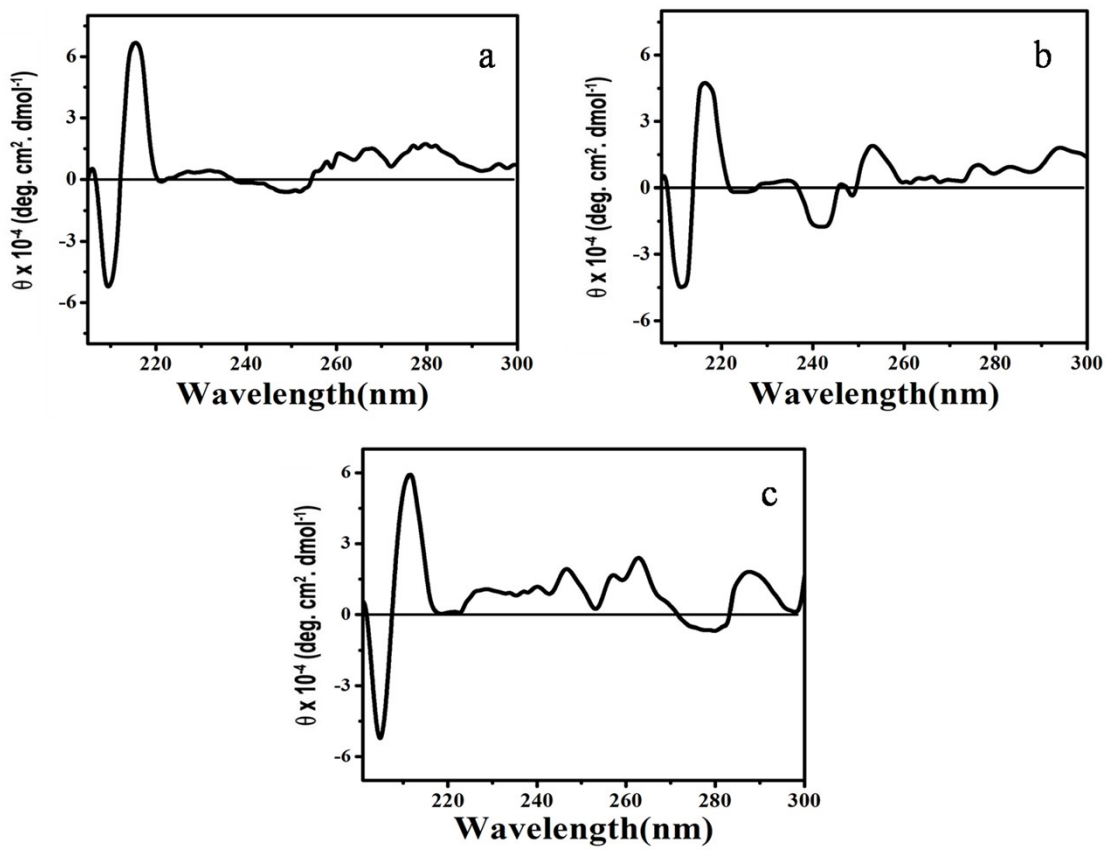

Figure S20. CD spectra of (a) complex **1**, (b) complex **2**, and (c) complex **3** in methanol

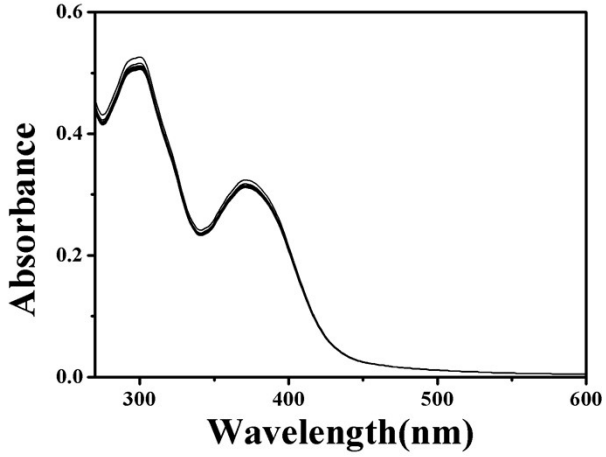

Figure S21. UV-Vis titration of complex **1** (1 x 10^{-6} M) with increasing concentration of ct-DNA (1860 μM)

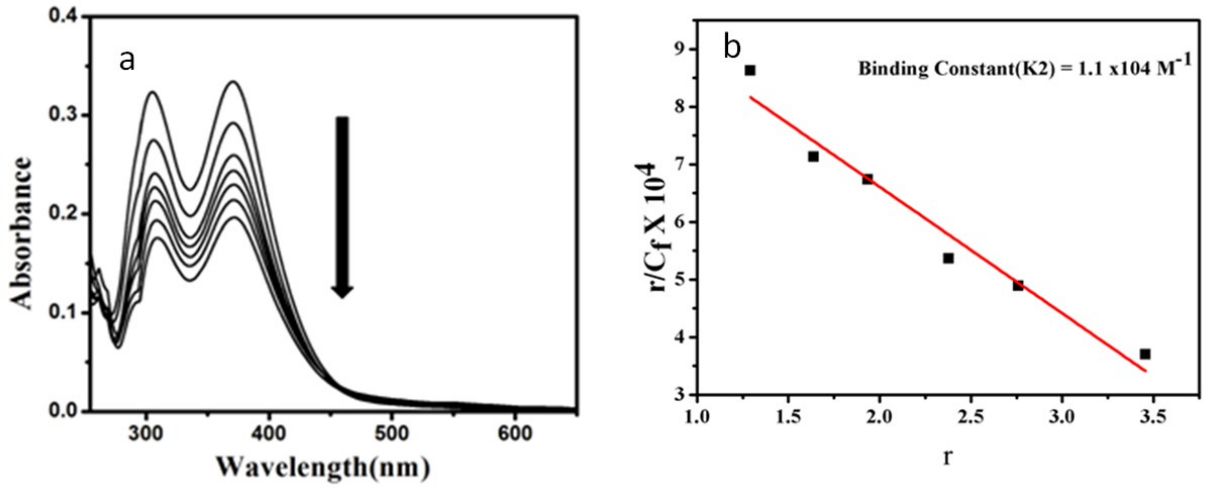

Figure S22. (a) UV-Vis titration of complex **2** (1 x 10^{-6} M) with increasing concentration of ct-DNA (1860 μM); (b) Scatchard plot obtained from absorbance study

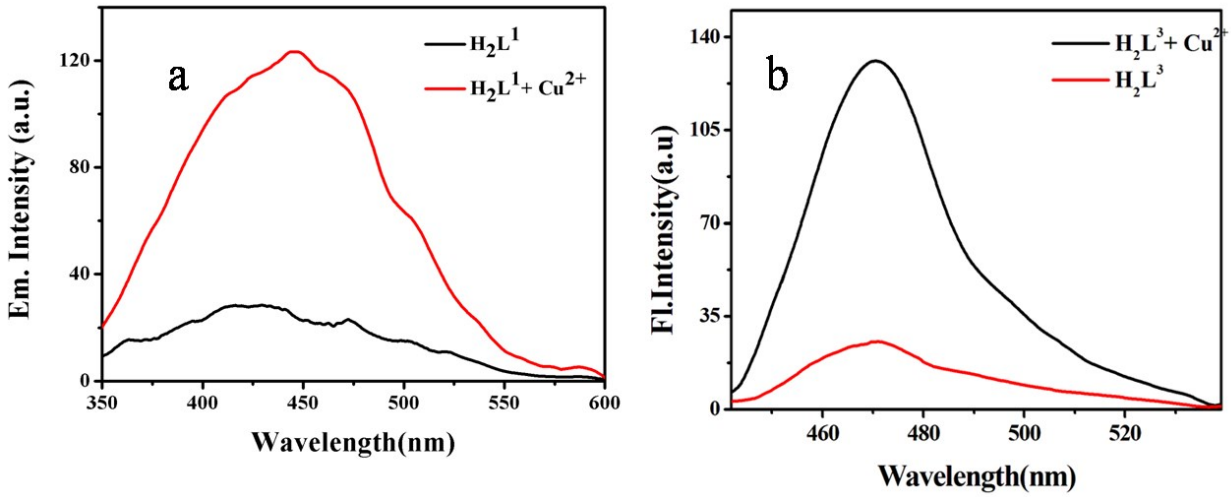

Figure S23. Fluorescence spectra of (a) only $\mathbf{H_2L^1}$ (1 x 10^{-6} M**)** and after addition of Cu^{2+} (3 equiv.) (b) only $\mathbf{H_2L^2}$ and after addition of Cu^{2+} (3 equiv.).

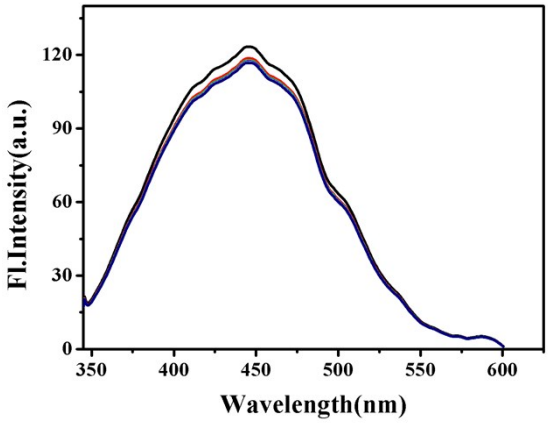

Figure S24. Fluorescence titration of complex **1** (1 x 10^{-6} M) with increasing concentration of ct-DNA (1860 μM).

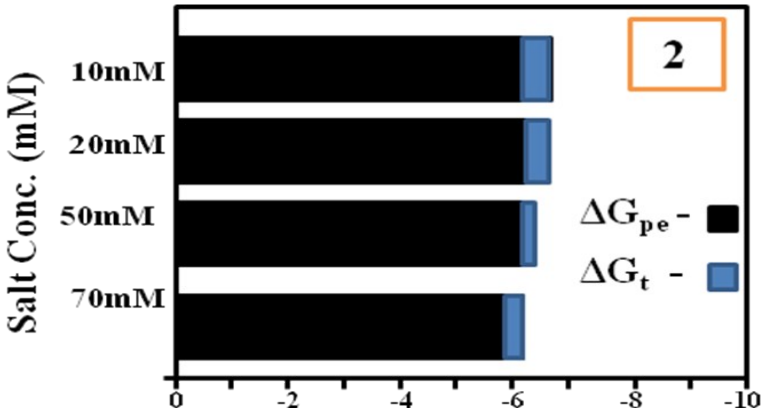

Figure S25. Plot of salt concentration vs free energy change for complex **2** (the blue part indicate the nonpolyelectrolytic (ΔG_t) and the black part the polyelectrolytic (ΔG_{pe}) contribution, respectively, to the ΔG binding).

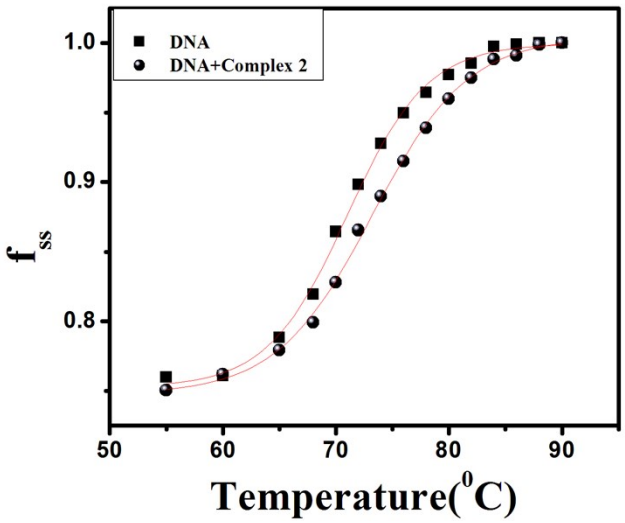

Figure S26. Plot indicating fraction of denaturation vs temperature for free DNA and **2**-DNA

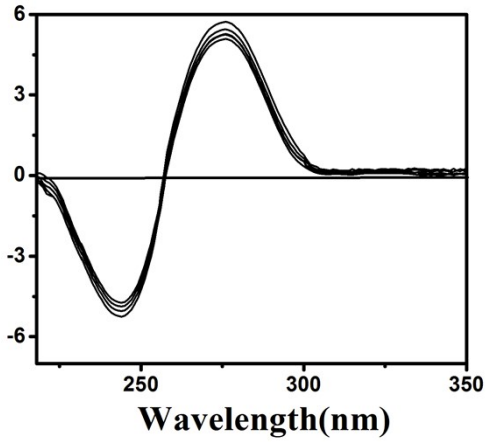

Figure S27.Intrinsic CD spectra of ct-DNA (50 μM) in presence of 0, 10, 15, 20 and 25 μM of complex **2**

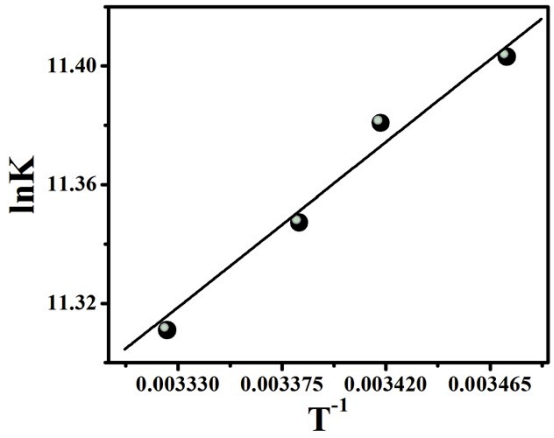

Figure 28 . Van't Hoff plot for the binding of **2** with CT-DNA.

Table S1. H-bonding parameters of H_2L^1, H_2L^2 and complex 3.

Compound	D-H…A	D-H (Å)	H…A (Å)	D…A (Å)	Symmetry code of A
H_2L^1	N1-H1n...O2	0.89(2)	1.96(2)	2.826(2)	1/2-x,1/2+y,1/2-
	O1-H1o...N4	0.91(3)	1.81(3)	2.603(2)	z
	N3-H3n...O3	0.95(2)	1.83(2)	2.771(2)	-
	O4-H4o...N2	0.98(3)	1.86(3)	2.675(2)	-
H_2L^2	N1-H1n…O10a	0.86	2.23	3.022(5)	1+x,y,z
	N1-H1n...O2	0.86	2.55	3.014(5)	3/2-x,1/2+y,1/2-
	O10-H10…N4	0.82	1.89	2.596(5)	z
	N3-H3n...O3	0.86	2.01	2.840(5)	-
	O4-H4o...N2	0.82	1.89	2.609(5)	-
Complex 3	N2-H2..O6a	0.86	2.08	2.881(9)	x,1/2-y,1/2+z
	N2-H2..O8a	0.86	2.14	2.902(14)	x,1/2-y,1/2+z

Table S2. C=O bond distances (Å) in the ligands and in Cu complexes

compound		C-O		C-O
H_2L^1	C8-O2	1.222(2)	C21-O3	1.233(2)
H_2L^2	C8-O2	1.211(5)	C21-O3	1.234(5)
1	C13-O2	1.259(10)	-	-
2	C16-O5	1.299(4)	C25-O7	1.285(4)
3	C12-O2	1.251(3)	C25-O3	1.286(2)

Table S3. CH…O interaction parameters (Å/°) in Cu complexes

Complex	D-H…A	D-H	H…A	D…A	<D-H…A	Symmetry code of A
1	C8-H8...O2	0.93	2.49	2.965(19)	112	-
	C12-H12...O1	0.93	2.54	2.982(16)	110	-
	C17-H17...O5	0.93	2.33	3.182(18)	152	-x,1+y,1/2-z
2	C6-H6…O1	0.93	2.37	2.692(5)	100	-
	C8-H8…O8	0.93	2.41	2.951(4)	117	-
	C11-H11…O1	0.93	2.49	3.370(5)	158	x,1+y,z
	C12-H12…O7	0.93	2.32	2.896(4)	120	-
	C20-H20…O6	0.93	2.36	2.932(5)	120	-
	C24-H24...O5	0..93	2.35	2.905(5)	118	-
3	C11-H11...O6	0.85(3)	2.60(3)	3.334(10)	145(2)	x,1/2-y,1/2+z
	C16-H16…O5a	0.93	2.58	3.303(9)	135	-
	C18-H18…O8a	0.93	2.48	3.224(14)	137	x,1/2-y,1/2+z
	C32-H32...O7a	0.93	2.46	3.149(15)	131	-x,-y,1-z

Table S4. The C-N single bond distances (Å) in the ligands and copper complexes

Compound	bond	C-N	bond	C-N
H_2L^1	N3-C8	1.339(3)	N1-C21	1.334(2)
H_2L^2	N3-C8	1.347(5)	N1-C21	1.356(5)
1	N2-C13	1.324(11)	-	-
2	N1-C25	1.319(4)	N3-C38	1.320(4)
3	N2-C12	1.330(3)	N3-C25	1.315(3)