Targeting an artificial metal nuclease to DNA by a simple chemical modification and its drastic effect on catalysis

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Supplementary Material

EXPERIMENTAL SECTION

Synthesis of the complex

All starting materials were purchased from Aldrich, Acros, or Merck. The N^6-(aminoethyl)adenine (Aead) and 2-[(bis(pyridylmethyl)amino)methyl]-4-methyl-6-formylphenol (Hbpmamff) were prepared from methods already described in the literature[1, 2] IR spectra were recorded with a PerkinElmer FTIR Spectrum 100 spectrophotometer (KBr pellets). Elemental analysis was performed on a Carlo Erba E-1110 analyzer. Electronic absorption spectra in the 200-1200 nm range were recorded on a PerkinElmer Lambda 750 spectrophotometer. Electrospray ionization mass spectrometry (ESI-MS) of the complexes dissolved in an ultrapure CH_3CN solution (500 nM) was performed using an AmaZon X Ion Trap MS instrument (Bruker Daltonics) with an ionspray source using electrospray ionization in positive-ion mode. The ion-source voltage was 4500 V. Nitrogen was used as the nebulizing (20 psi) and curtain (10 psi) gases. The samples were directly infused into the mass spectrometer at a flow rate of 180 μL/h. The scan range was from *m/z* 100 to 1500. The simulated spectra were calculated using the Mmass software[3].

Synthesys of HLPu

In a 125 mL round-bottom flask, 0.62 g (3.50 mmol) of N6-(aminoethyl) adenine was dissolved in 40 mL of metanol. In the next step, 30 mL of a methanol solution containing 1.22 g of Hbpmamff (3.50 mmol) was added slowly. The mixture was stirred at room temperature for 4 h. Subsequently, the mixture was cooled to 0 °C in an ice bath, and small portions of NaBH4 were added directly (0.13 g, 3.50 mmol) under constant stirring. The solution was stirred for another 3 h, and it was possible to observe the decrease in the intensity of the yellow color. A 1.0 M HCI solution was then added in small portions to adjust the pH to 6, and the solvent was removed by rotary evaporation. To the resultant oil was added 80 mL of DCM, and the solution was washed with a saturated aqueous solution of NaHCO3 (3 × 20 mL). The organic layer was dried with solid anhydrous Na2SO4, and the solvent was removed under reduced pressure. As a result, 1.21 g of a yellow sólid was obtained (yield: 68%). IR (KBr pellets, cm-1): ν(O-H) 3430, ν(N-H) 3206, ν(C-HAr) 3043, ν(C-HAliph) 2922, 2810, ν(C=C) 1593, ν(C=N) 1535, δ(O-H) 1387, δ(C-HAr) 798, δ(C-HAr) 761. 1H NMR (CDCl3): δ - 2.18 (3 H, s), 2.61 (2 H, t), 3.30 (2 H, t), 3.66-3.82 (4 H, s), 4.10 (4 H, s), 5.39 (1 H, s), 6.92 (2 H, s), 7.21-7.37 (4 H, m), 7.69 (2 H, t), 8.15 (1 H, s), 8.52 (2 H, s), 11.29 (1 H, s).

Synthesis of [Cu^{II}(LPur)] (1)

The complex was obtained by mixing solutions in acetonitrile of the HLPu ligand (0.52 g, 1.0 mmol, in 25 mL) and $CuCl_2.2H_2O$ (0.17 g, 1.0 mmol, in 15 mL), while stirring, at 25 °C for 15 min. In the next step, drops of hydrochloric acid 2.0 M were added until a change from green to light blue color and precipitation of a blue solid, which was filtered off and washed with cold isopropyl alcohol and diethyl ether. Yield: 0.31 g, 52%. Anal. Calcd. for $CuC_{28}H_{35}N_9O_3Cl$: C, 52.17; H, 5.47; N, 19.56. Found: C, 51.79; H, 5.32; N, 19.21).

Solution Studies

In order to establish the most likely structure of the complex in solution, ESI-MS, electronic spectroscopy studies were carried out in ACN or ACN/water solutions. ESI-MS analysis of complex [CuII(LPur)] was performed in a pure ACN solution (0.5 μM) (Figure S1, see also S12). A group of peaks with a maximum (100%) at m/z 286.1 with a 2+ charge was observed. This signal can be assigned to the system [CuII(LPur)]. A second group of peaks with a maximum (100%) at m/z 607.1 with a 1+ charge was observed, and was assigned to the system [Cu^{II}(LPur)] + $2H_2O$. This adducts formed with some ions and solvent molecules commonly occur in mass spectrometry experiments[4]. The UV-vis absorption spectra of complex [Cu^{II}(LPur)] were investigated in the range of 300-900 nm, using ACN and ACN/water as the solvents (Figure S2). In pure ACN solutions, the complex shows a broad band at λ_{max}, 678 nm (ε, 95 $M^{-1}cm^{-1}$), assigned to an inner d-d type bands of the metal, for the Cu^{II} ion in a d^9 system. With successive additions of an aqueous

solution of NaOH it was possible to observe the appearance of a second band at λ_{max}, 476 nm (ε, 198 $M^{-1}cm^{-1}$) assigned to a ligand-to-metal charge-transfer (LMCT) transition from the pπ orbitals of the phenolate to the dπ* orbitals of the Cu^{II} ion.

DNA cleavage

For DNA cleavage assays, plasmid pBSK-II (Stratagene, USA) was used. The reactions consisted of 330ng (~30μM base pair) of plasmid DNA with increasing concentrations of buffered complex **1** in specific solutions – as indicated in the figure legends - for 16 hours at controlled temperature and protected from light. The reactions with complex **1** were buffered with 10mM CHES (pH 9.0) at 37°C. The influence of the pH in the cleavage reactions was assayed at different pH values: 10mM MES (pH 5.5 and 6.0), 10mM HEPES (pH 7.0 and 8.0), and 10mM CHES (pH 9.0). Finally, reactions were stopped with the addition of 5 μL of 6X concentrated running buffer (0.25M EDTA, 50% glycerol and bromophenol blue 0.01% - pH 8.0), and subsequently separated by agarose gel electrophoresis.

Mechanism of DNA cleavage and interaction

The effect of ionic strength on DNA cleavage was established by adding increasing concentrations of sodium chloride to DNA prior to the treatment with complex **1**. These reactions were conducted in standard conditions (100μM complex, 16h incubation time, 37°C, 10mM CHES pH 9.0) at concentrations of 100, 200, 625, and 1250mM NaCl. To elucidate the mechanism of DNA cleavage (hydrolytic or oxidative) the reactions described above were prepared with different reactive oxygen species scavengers: 40mM DMSO, hydroxyl radical scavenger ($^{\bullet}OH$); 0.5mM KI peroxide scavenger; 20U SOD enzyme, scavenger for superoxide anion ($O_2^{\bullet-}$); 0.5mM NaN_3, singlet oxygen scavenger. Small molecules may interact with DNA through one or both grooves (minor and major). To determine the preference of complex **1** for each DNA groove, the plasmid was previously treated with groove binding molecules and then added to the cleavage reactions. Netropsin was used as a minor groove binder[5] and methyl green as a major groove preference binder[6].

Kinetic Parameters

In order to estimate the kinetic parameters, reactions were performed as described previously: 330ng DNA in 10mM CHES (pH 9.0) and different concentrations of complex 1 (50, 75, 100, 200, 300, 500μM) for up to 4h at 37°C and protected from light.

The k_{obs} value was obtained directly from the slope of the linear regression originated from the plot of the natural logarithm of the amount of the intact form of DNA as a function of reaction time. The plot of k_{obs} values versus the concentration of the complex follows saturation kinetics (pseudo-Michaelis-Menten kinetics[7]).

DNA cleavage under argon atmosphere

Using the methodology previously described[8], DNA cleavage reactions were carried out under argon atmosphere in order to evaluate the influence of molecular oxygen in the reactions of the metal complex studied.

Circular Dichroism (CD)

CD of calf thymus DNA (CT-DNA) was used to assess the interaction of complex **1** with DNA and the potential of structural alterations in the nucleic acid upon binding[9]. CD was performed on a Jasco J-815 spectropolarimeter using 200 μM CT-DNA, at 37°C, assays were done in duplicate, with increasing concentrations of complex **1**.

High resolution Urea-Polyacrylamide gel electrophoresis

In order to detect specificity associated to DNA cleavage, a hairpin oligonucleotide (see Fig. S6, S7, S8) was treated with increasing concentrations of complex **1** (250, 500, and 750μM) for 16h at 37°C, following the methodology previously described[10], or with the modifications described in the figure legends.

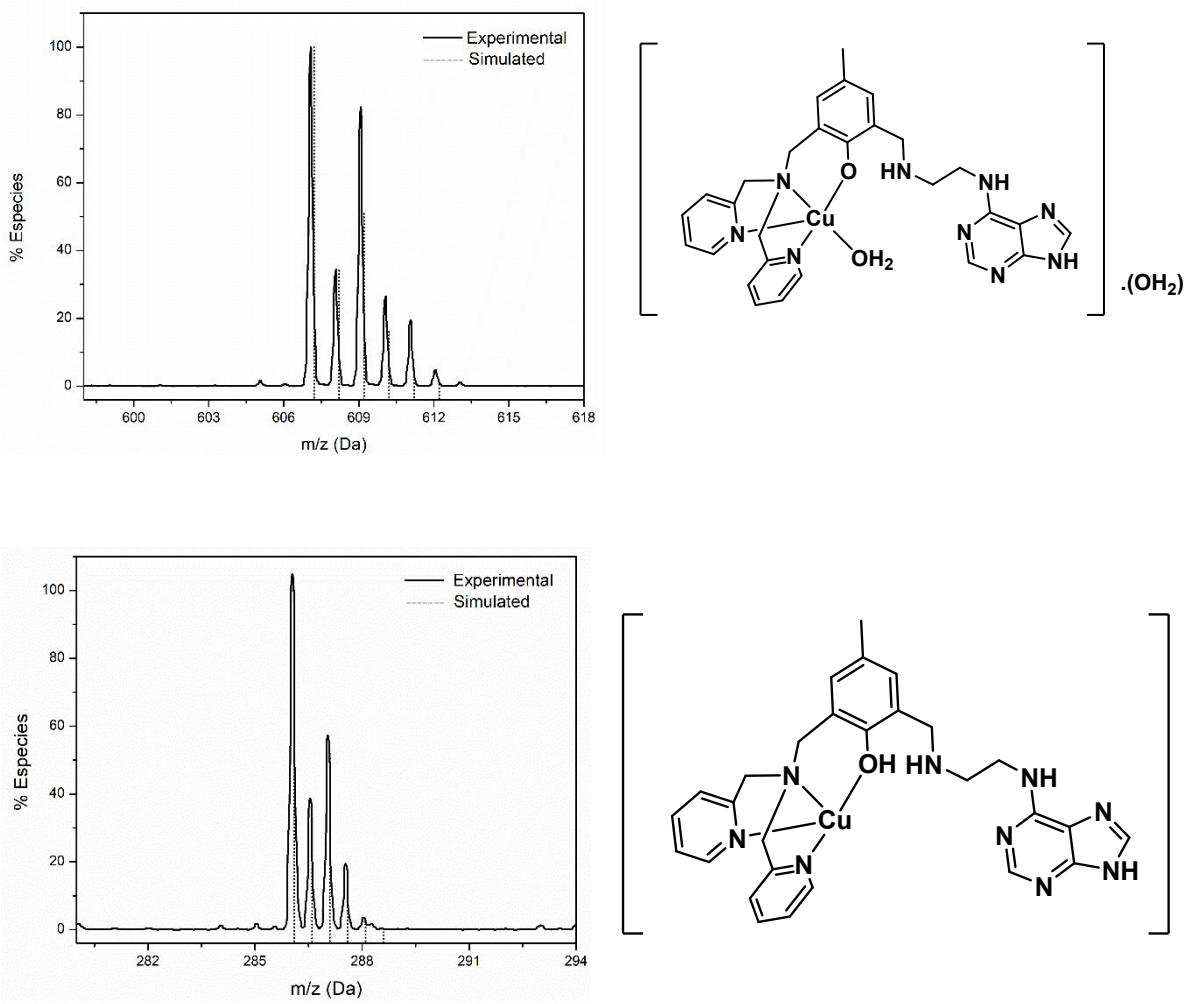

Figure S1. ESI-MS Positive ion spectra in pure acetonitrile for the complex **1** (up) peak (100%) in 607.1 (1+) for $CuC_{28}H_{34}N_9O_3$ and (down) peak (100%) in 286.1 (2+) for $CuC_{28}H_{31}N_9O$.

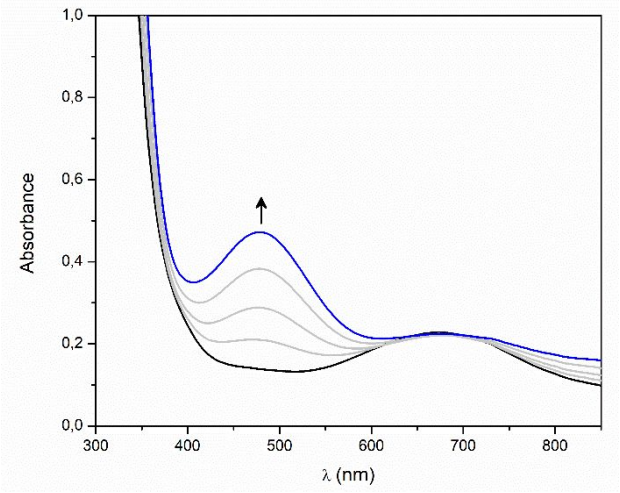

Figure S2. Electronic spectrum of the complex **1** in pure acetonitrile with NaOH additions (0.25-1 Equivalent). [CuLPu] = 2.5 Mm.

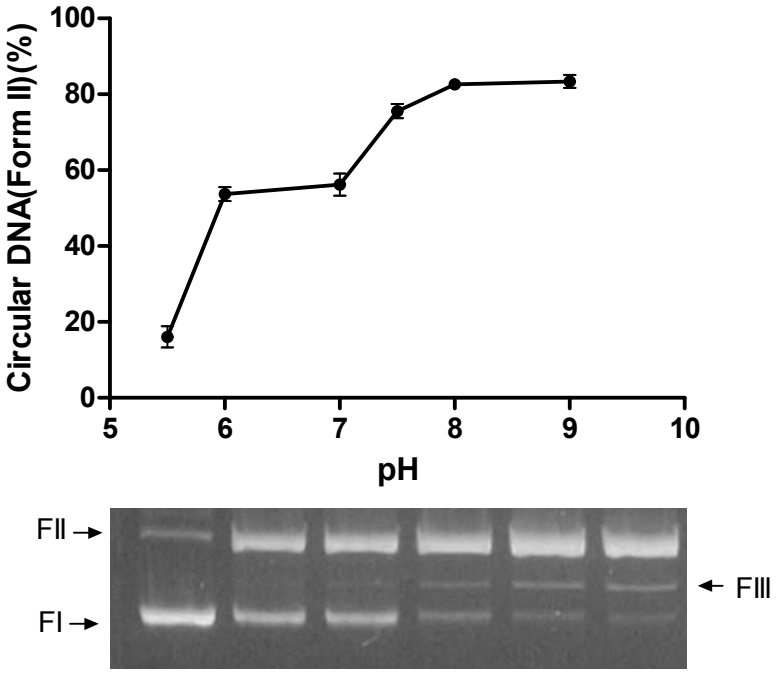

Figure S3: Influence of the reaction pH for DNA cleavage ([Buffer] = 10 mM; MES pH 5.5 and 6.0; HEPES pH 7.0, 7.7 and 8.0, and CHES pH 9.0), (**1**), 100μM at 37 ° C for 16h. Representative data from two different experiments expressed as mean ± SD.

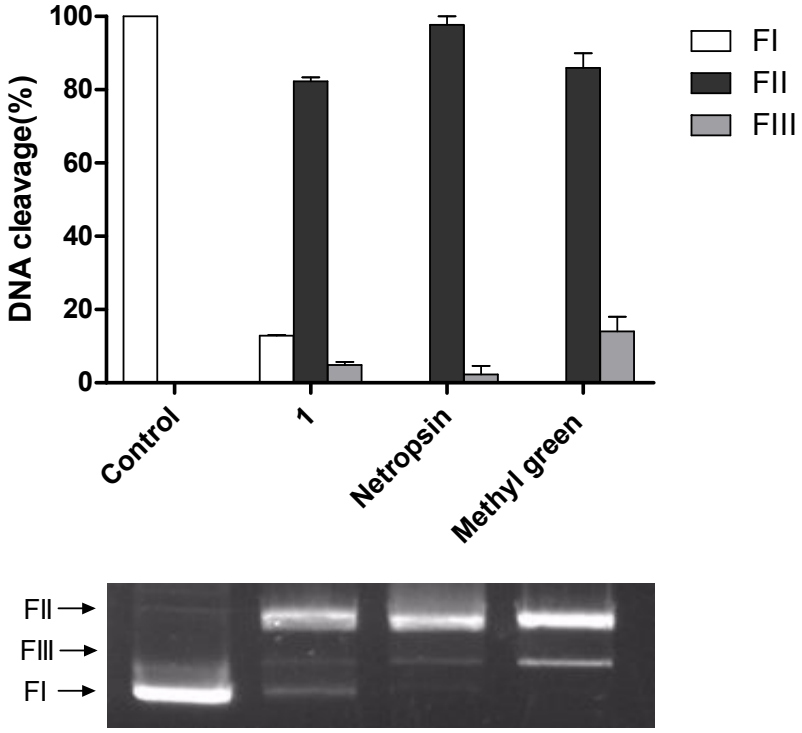

Figure S4: Effect of DNA groove binders netropsin (50 μM) and methyl green (50 μM) on cleavage of supercoiled DNA by **1** at 100 μM for 16h at 37ºC in 10 mM CHES (pH9.0).

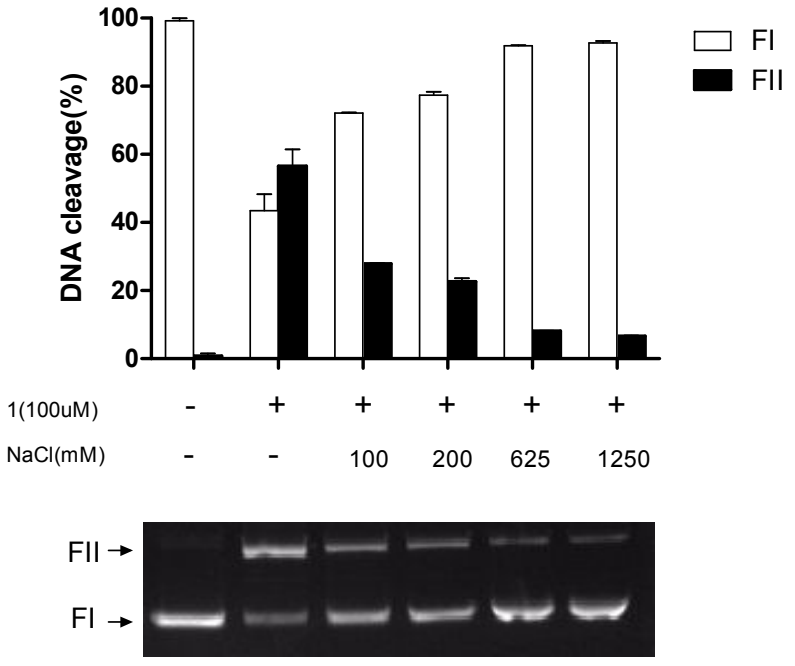

Figure S5: Effect of ionic strength (NaCl) on cleavage of supercoiled DNA by **1** at 100μM for 16 h at 37ºC in CHES buffer (10 mM, pH 9.0). Before the addition of the complexes, different concentrations of NaCl (100 to 1250mM) were added to the reaction media.

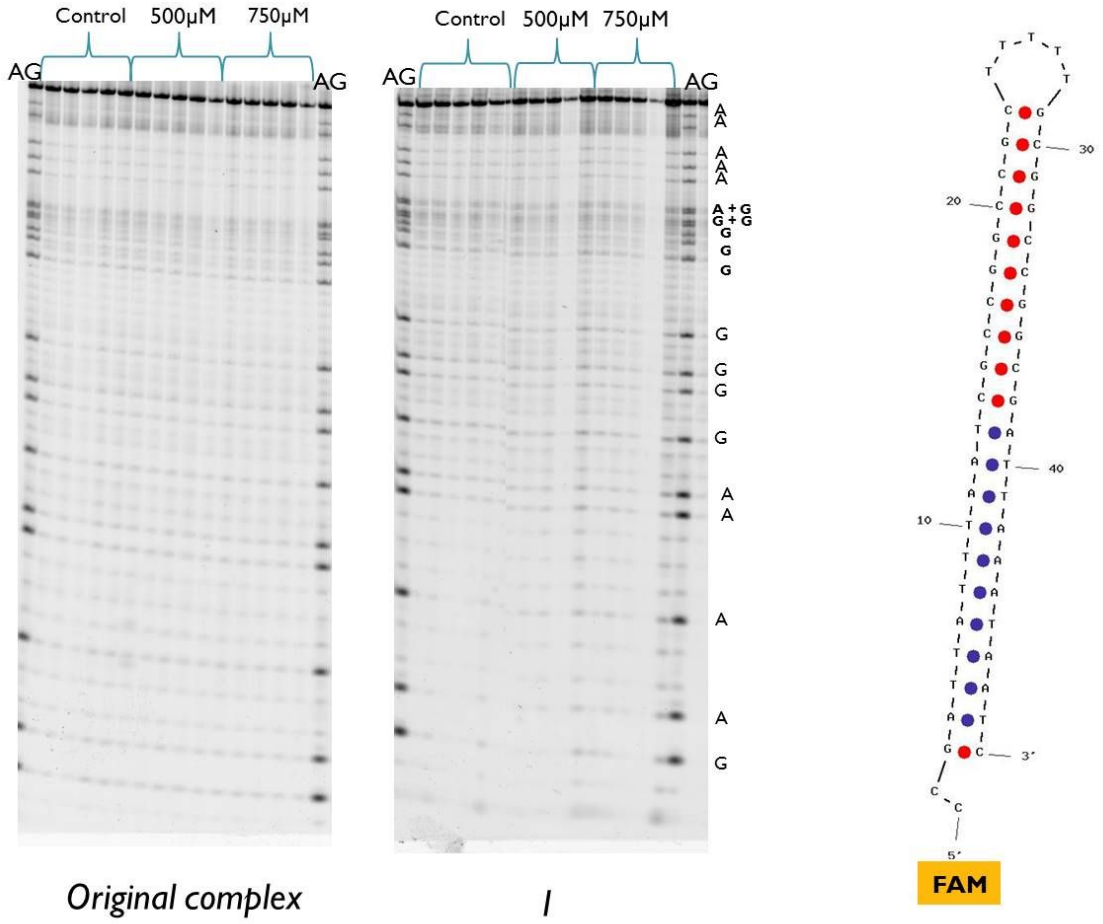

Figure S6: High resolution gel electrophoresis of the cleavage products of the DNA probe depicted at the right. Complexes ("original complex") (Cu-*bpmamff*) and **1**, at concentrations of 500 and 750μM were incubated for 0 to 24 h at 37ºC. Cleavage occurs specifically at purine nitrogen bases (A and G), as shown in the figure. "Control" at the left in each panel indicates the reaction in the absence of metal complex.

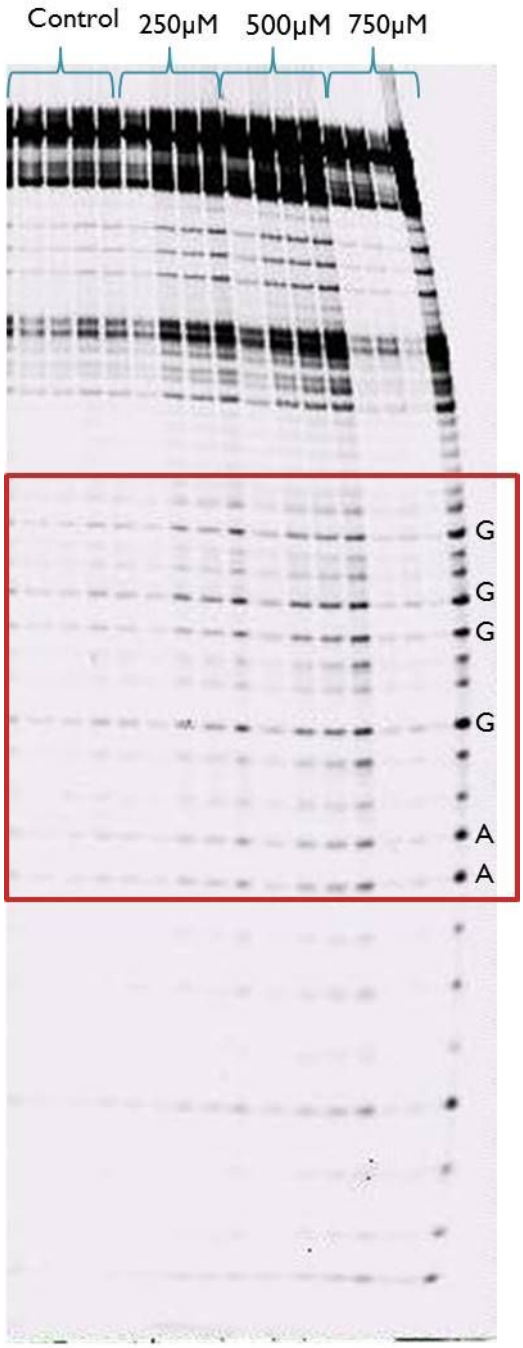

Figure S7: Same as S6, but with the addition of ascorbate (50μM) in the reaction medium – a huge increase in activity is seen. Channels: 1 to 4 – Control – Complex **1** (without ascorbate) – 5 to 8: Complex **1** (250μM) + Ascorbate (50μM); 9 to 12 - Complex **1**(500μM) + Ascorbate (50μM); 13 to 16 - Complex **1** (750μM) + Ascorbate (50μM) at different incubation times 0, 0.5, 1 and 2h, and 90 seconds with ascorbate.

Figure S8. High resolution polyacrilamide electrophoresis of DNA cleavage products obtained from control, CuII complex without (Cu) or with purine moiety (Cu-Pur) at 500 μM. Incubation in the presence of ascorbate for 0 (channel 1), 30 (channel 2) or 90 min (channel 3). A/G, Maxam Gilbert sequence ladder. Control, no complex; Cu, copper complex without purine moiety; Cu-Pur, copper complex with purine moiety.

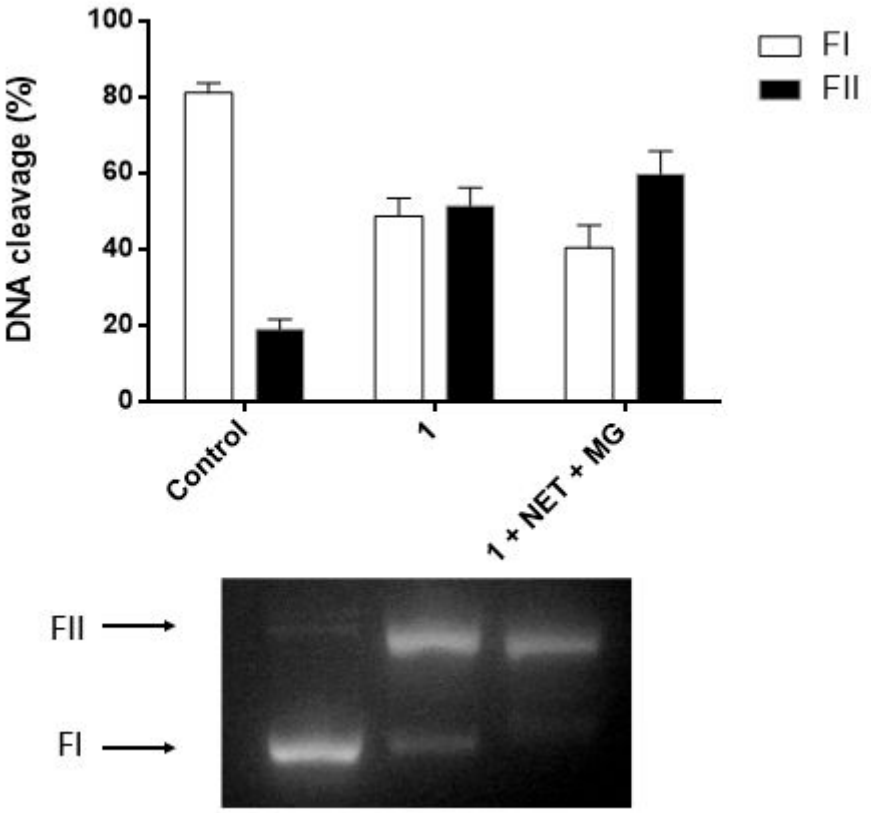

Figure S9. Effect of both DNA groove binders netropsin (50 μM) and methyl green (50 μM) on cleavage of supercoiled DNA by **1** at 100 μM for 16h at 37ºC in 10 mM CHES (pH9.0).

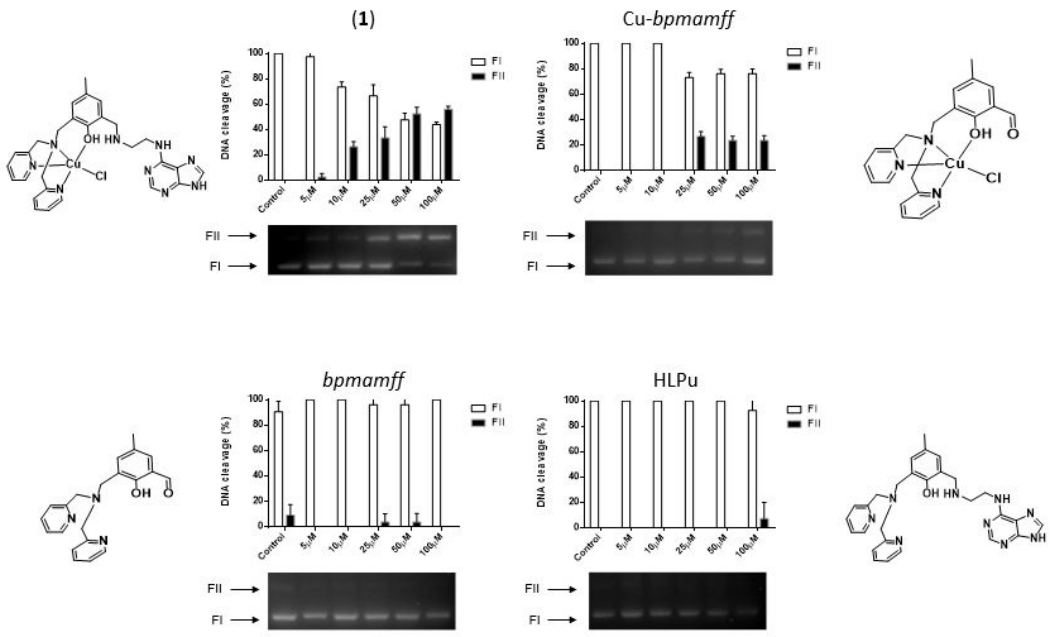

Figure S10 Cleavage of supercoiled DNA by **1** and controls (Cu-*bpmaamff, bpmaamff*, HLPu) for 16 hours at 37°C in CHES buffer (10 mM, pH 9.0) and different concentrations of the complex (5 to 100 μM). Representative data from three different assays expressed as mean ± SD.

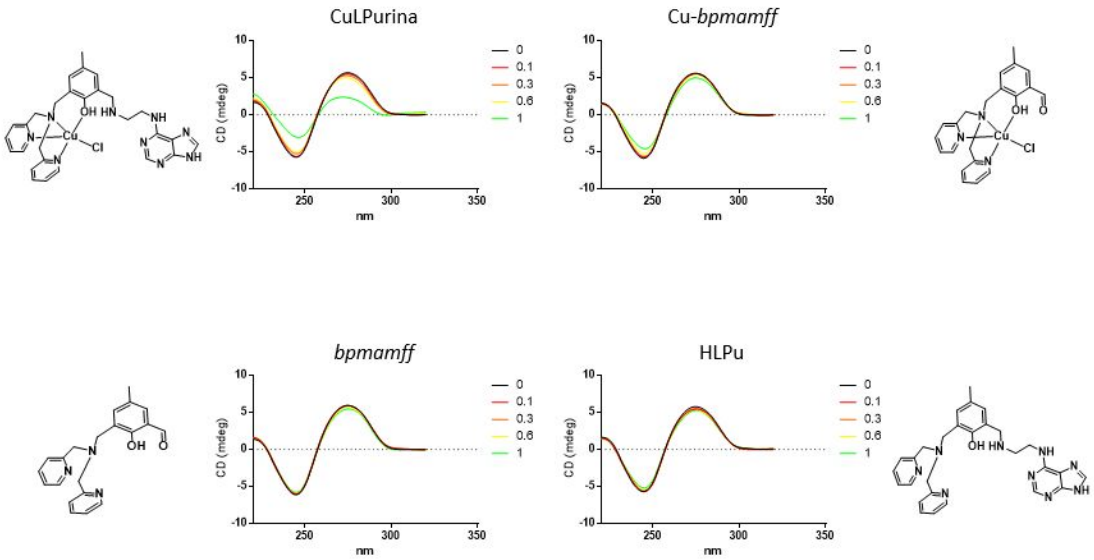

Figure S11 Circular dichroism spectra of CT-DNA (200µM) in the absence or presence of varying concentrations of complex **1** and controls (Cu-*bpmaamff, bpmaamff*, HLPu) (r = 0.10, r = 0.30, r = 0.60, r = 1.0) where r =[complex]/[CT-DNA].

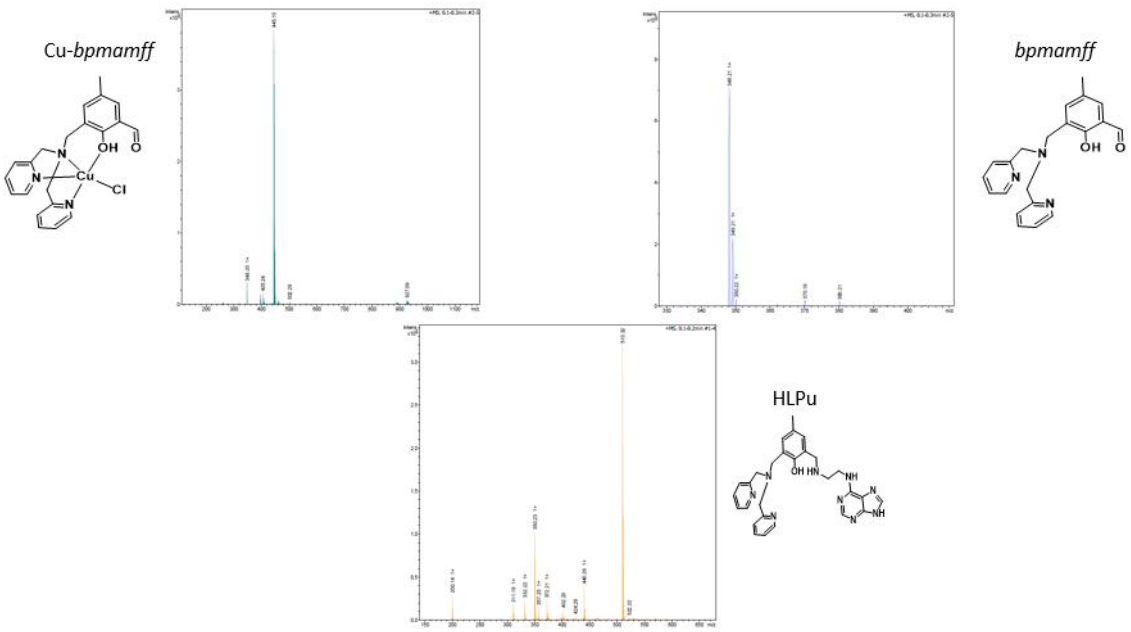

Figure S12. ESI-MS spectra of each ligand and complex described, in addition to those described in Figure S1, under the same conditions.

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