ELECTRONIC SUPPORTING INFORMATION (ESI) FOR:

A dinucleating ligand which promotes DNA cleavage with one and without a transition metal ion

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Experimental

Materials and Methods.

Materials. All reagents and chemicals were obtained from commercial sources and used without further purification. Metal nitrate salts, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), dimethylarsonic acid (Cacodylate), 4-morpholineethanesulfonic acid (MES), sodium azide, (*S*)-2-amino-3-(4-imidazolyl)propionic acid (L-Histidine), sodium formiate and2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride hydrate (Hoechst 33258) were purchased from Sigma Aldrich. Sodium chloride and potassium iodide were obtained from Panreac. Methyl green was supplied by Alfa Aesar. Plasmid pUC18 DNA (0.5 μg/μL) in 10 mMTris-HCl (pH 7.6) and 1 mM EDTA was purchased from Fermentas. The ligands 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-ol (L) and bis(pyridin-2-ylmethyl)amino-propan-2-ol (L') were prepared as previously described.[1]

Methods. DNA cleavage. The DNA cleavage ability of the complexes was examined following the conversion of pUC18 supercoiled DNA (Form I) to nicked circular (Form II) and linear DNA (Form III) using agarose gel electrophoresis to separate the cleavage products.

Solutions of the metal salt were freshly prepared prior to each experiment. In a typical experiment, reaction mixtures were made by addition of the appropriate metal salt concentration (dissolved in water) and of the desired ligand concentration (dissolved in methanol). After 10 minutes, 0.5 μL of pUC18 DNA (0.5 μg/μL, 1500 μM nucleotides) and appropriate amounts of buffer solution were added to complete 20 μL total volume. Then, the samples were incubated at 37 ºC during 2 h in a Thermocycler. The final percentage of MeOH was below 5 % in all cases and the final DNA concentration was 37.5 μM in nucleotides.

To stop the reaction a quench buffer solution (4 μL) consisting of bromophenole blue (0.25%), xylenecyanole (0.25%), and glycerol (30%), and EDTA (250 mM) was added. The solution was then subjected to electrophoresis on 0.8% agarose gel in 0.5×TBE buffer (0.045 M Tris, 0.045 M boric acid, and 1 mM EDTA) containing 2 μL/100 mL of a solution of ethidium bromide (10 mg/mL) at 120 V for 2 h. The bands were photographed on an UVIdoc HD2 capturing system (UVItec Cambridge).

The relative amounts of different plasmid structures were quantified with the aid of ImageJ 1.34s (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). A correction factor of 1.31 was used to compensate for the reduced ethidium bromide uptake capacity of supercoiled DNA.[2] The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane.

To test for the presence of reactive oxygen species (ROS) generated during strand scission and to probe the possible metal complex-DNA interaction sites, various and groove binders were added to the reaction mixtures. The scavengers used were: sodium azide (0.5 mM), sodium formiate (0.5 mM), L-Histidine (0.5 mM) and potassium iodide (0.5 mM). To probe the groove binding preferences of the complexes, the major groove binder methyl green (5 μM) and the minor groove binder Hoechst 33258 (20 μM) were assayed. Samples were treated as described above.

The kinetics of DNA cleavage with Co-L complex (40 μM) was investigated as follows.Samples were treated as described above but quenched by placing on ice and adding loading dye at different times. Time-dependent supercoiled, and nicked DNA concentration data were fit to a first-order consecutive model defined by eq. 1, where *S*corresponds to the respective concentrations of supercoiled at time t, S_0 corresponds to the initial concentration, and k_{obs}correspond to the observed first-order rate constants of DNA nicking. Observed rate constants were expressed as min^{-1}.

$$S = S_0 \exp(-k_{obs}\, t) \qquad (1)$$

In order to investigate the contribution of electrostatic interactions in the plasmid DNA cleavage promoted by complexes Co-L and Fe-L, assays were conducted as described above but increasing the ionic strength of the reaction media by the addition of NaCl (from 0 to 100 mM).

Anaerobic experiments were conducted in a glove box under an argon atmosphere. All solutions were subject to five freeze-pump-thaw cycles under Ar prior to introduction to the glove box. Reaction mixtures were prepared immediately by addition of the appropriate amounts of stock solutions to reaction tubes, which were subsequently sealed and incubated at 37 ºC. All other conditions were the same as for the cleavage experiments performed under aerobic condition.

All of the results are the average of experiments performed at least in triplicate.

Fluorescence competitive binding studies.[3] The competitive ethidium displacement assays were performed by the measurement of the emission intensity of ethidium bromide (EB) on a JASCO FP-6200 spectrofluorometer at room temperature. The experiments were carried out by adding serial aliquots of the different complexes into a solution containing EB (3.78 μM) and CT-DNA (3 μM CT-DNA (ε_{260}= 13200 $M(bp)^{-1}cm^{-1}$) in HEPES buffer (50 mM, pH 7.0) until 50% of the initial fluorescence is lost. The different complexes were prepared previously by mixing of L (or L') and $M(NO_3)_2$ (M= Co(II), Fe(III) or Zn(II)) at a 2:1 (or 1:1) molar ratio in Milli-Q water during 30 minutes at room temperature. Excitation and emission wavelengths were set to 500 and 595 nm, respectively. The apparent binding constants were calculated using $K_{app}= K_{EB}\times (3.78/C_{50})$ where $K_{EB} = 9.5\times 10^6 M\ (bp)^{-1}$.[4] The fluorescence was normalized to 100% relative fluorescence.

High resolutions ESI-MS. High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed on a Waters LCT Premier XE (Waters, Milford, MA, USA) in W-optics positive ionization scan mode. Mass spectra were obtained by direct infusion with parameters optimized to achieve the best signal-to-noise ratio: capillary voltage 1 kV, sample cone voltage 15 V, desolvation gas flow 600 Lh-1, cone gas flow 50 Lh^{-1}, desolvation temperature 220 ^{0}C, source temperature 80 ^{0}C and flow rate 20 $\mu Lmin^{-1}$. 30 scans are summed to obtain representative spectra. The instrument was calibrated over the range m/z 100-1000 before each measurement using a standard NaI solution (1 μM). All the calibrations were carried out using the reference probe in order to avoid a contamination of the sample. Data analysis was performed with Masslynx software version 4.1 (Waters, Milford, MA, USA). Theoretical isotopes distributions were determined by the Compass IsotopePattern software (Bruker, Bremen, Germany).

Electromotive force measurements. The potentiometric titrations were carried out at 298.1±0.1 K using NEt_4ClO_4 0.15 M as supporting electrolyte. The experimental procedure (burette, potentiometer, cell, stirrer, microcomputer, etc.) has been fully described elsewhere.[5] The acquisition of the electromotive force data was performed with the computer program PASAT.[6] The reference electrode was an Ag/AgCl electrode in saturated KCl solution. The glass electrode was calibrated as an hydrogen-ion concentration probe by titration of previously standardized amounts of HCl with

CO_2-free NaOH solutions and determining the equivalent point by the Gran's method,[7] which gives the standard potential, E^o', and the ionic product of water (pKw = 13.73(1)).

Prior to the determination of the Co(II) stability constants, a titration was performed to calculate the values of the protonation constants of L. The values obtained were in agreement with those reported.[8] To obtain the stability constants solutions containing $CoCl_2 \cdot 6H_2O$ (10^{-3} M) and L in molar ratio 1:1 and 2:1 acidified with HCl were titrated with a NaOH solution. The computer program HYPERQUAD[9] was used to calculate the protonation and stability constants. The different datasets were merged together and treated simultaneously to give the final stability constants.

References:

1. (a) J. T. Groves and I. O. Kady, *Inorg. Chem*., 1993, **32**, 3868; (b) G. Feng, J. C. Mareque-Rivas and N. H. Williams, *Chem. Commun*., 2006, 1845.

2. T. A. van den Berg, B. L. Feringa and G. Roelfes, *Chem. Commun*., 2007, 180.

3. (a) W. Wilson, F. Tanious, M. Fernandez-Saiz, C. Rigl, In Drug−DNA Interaction Protocols; Fox, K. R., Ed.; Methods in Molecular Biology, Vol. 90; Humana Press: Totowa, NJ, 1997; Chapter 15, pp 219−240; (b) W. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski, D. Boykin, Biochemistry 1993, **32**, 4098.

4. (a) B. C. Baguley, W. A. Denny, G. J. Atwell, B. F. Cain, *J. Med. Chem.*, 1981, **24**, 170; (b) B. C. Baguley, *Mol. Cell Biochem.*1982, **43**, 167.

5. E. García-España, M. J. Ballester, F. Lloret, J. M. Moratal, J. Faus and A. Bianchi, *J. Chem. Soc. Dalton Trans.* 1988, 101.

6 M. Fontanelli and M. Micheloni, Proceedings of the I Spanish-Italian Congress on Thermodynamics of Metal Complexes, Diputación de Castellón, Castellón, Spain, 1990.

7 (a) G. Gran, *Analyst (London)* **1952**, *77*, 661-671. (b) F. J. Rossotti and H. Rossoti, *J. Chem. Educ.* 1965, **42**, 375-378.

8 L. Ciavatta, J. C. Mareque, D. Natale, and F. Salvatore, *Ann. Chim.*, 2006, **96**, 317.

9 P. Gans, A. Sabatini and A. Vacca, *Talanta* **1996**, *43*, 1739.

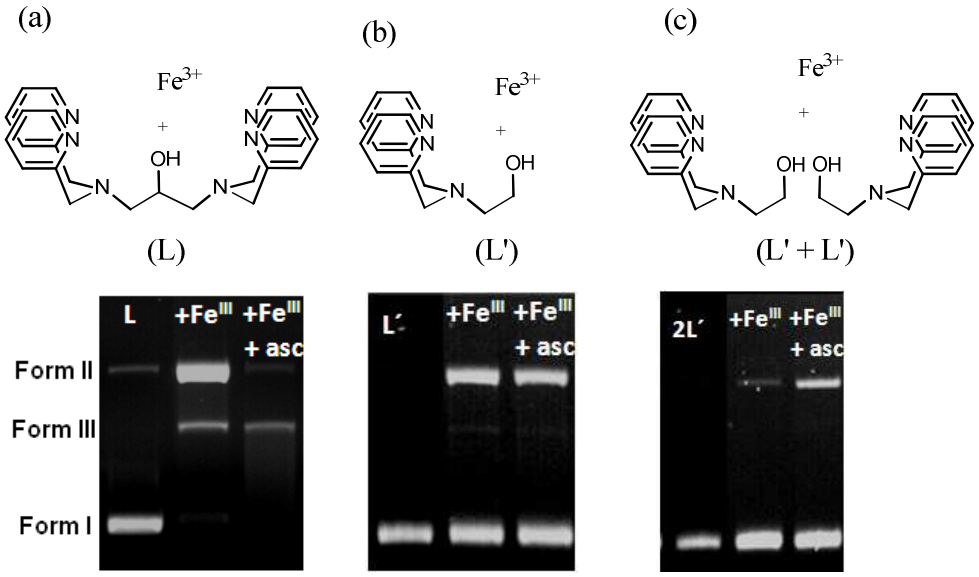


Fig. S1. Representative plasmid cleavage assays of pUC18 DNA (37.5 μM nucleotides) promoted by L (40 μM) and L´ (40 μM and 80 μM) in the presence of Fe(III) ions (40 μM) incubated in HEPES buffer (50 mM, pH = 7.0) for 2 h at 37 ºC; asc = ascorbate (40 μM). The quantitation of the different bands with errors is provided in Table S1.

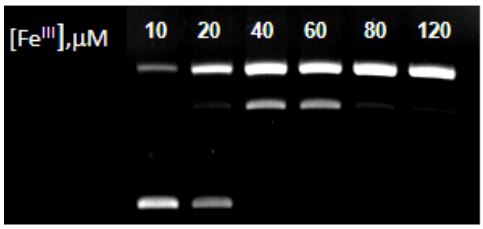


Fig. S2. Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated with L (40 μM) and various concentrations of Fe(III) ions in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC. The quantitation of the different bands is provided in Table S2.

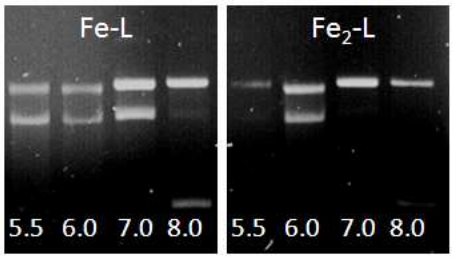


Fig. S3. Plasmid cleavage assays of pUC18 (37.5 μM nucleotides) promoted by Fe-L and Fe_2-L (40 μM) at different pHs after an incubation period of 2 h at 37 ºC. Buffers used: pH 5.5 (50 mM MES), pH 6 (50 mM cacodylate), pH 7 and 8 (50 mM HEPES). The quantitation of the different bands with errors is provided in Table S3.

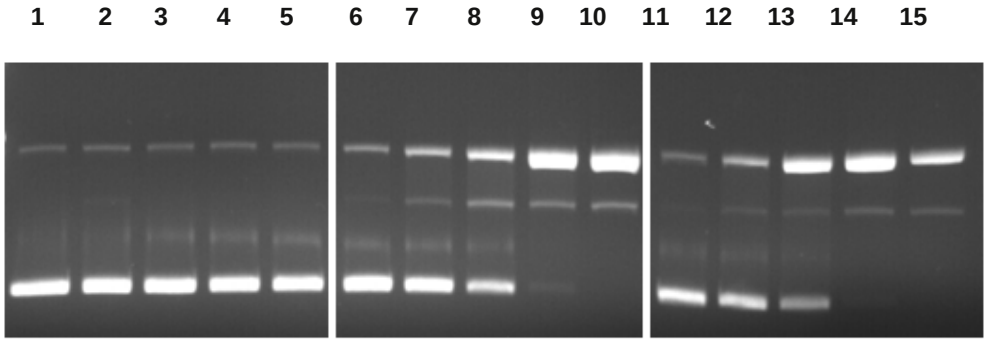


Fig. S4. Comparison of the DNA cleavage activity of1:1 and 2:1 M:L species (M = Co(II)) at different L concentrations. Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated in HEPES buffer (50 mM, pH = 7.0) for 2 h at 37 ºC. Lane 1: DNA control. Lane 2: 120 μM $Co(NO_3)_2$. Lane 3: 20 μM L. Lane 4: 40 μM L. Lane 5: 80 μM L. Lane 6: 5 μM Co-L. Lane 7: 10 μM Co-L.Lane 8: 20 μM Co-L.Lane 9: 40 μM Co-L.Lane 10: 80 μM Co-L.Lane 11: 5 μM Co_2-L.Lane 12: 10 μM Co_2-L.Lane 13: 20 μM Co_2-L.Lane 14: 40 μM Co_2-L.Lane 15: 80 μM Co_2-L.

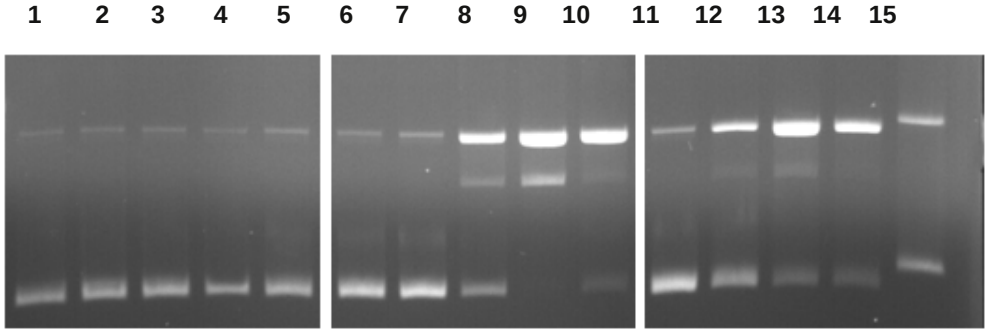


Fig. S5. Comparison of the DNA cleavage activity of 1:1 and 2:1 M:L species (M = Fe(III)) at different L concentrations. Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated in HEPES buffer (50 mM, pH = 7.0) for 2 h at 37 ºC. Lane 1: DNA control. Lane 2: 120 μM $Fe(NO_3)_3$. Lane 3: 20 μM L. Lane 4: 40 μM L. Lane 5: 80 μM L. Lane 6: 5 μM Fe-L. Lane 7: 10 μM Fe-L.Lane 8: 20 μM Fe-L.Lane 9: 40 μM Fe-L.Lane 10: 80 μM Fe-L.Lane 11: 5 μM Fe_2-L.Lane 12: 10 μM Fe_2-L.Lane 13: 20 μM Fe_2-L.Lane 14: 40 μM Fe_2-L.Lane 15: 80 μM Fe_2-L.

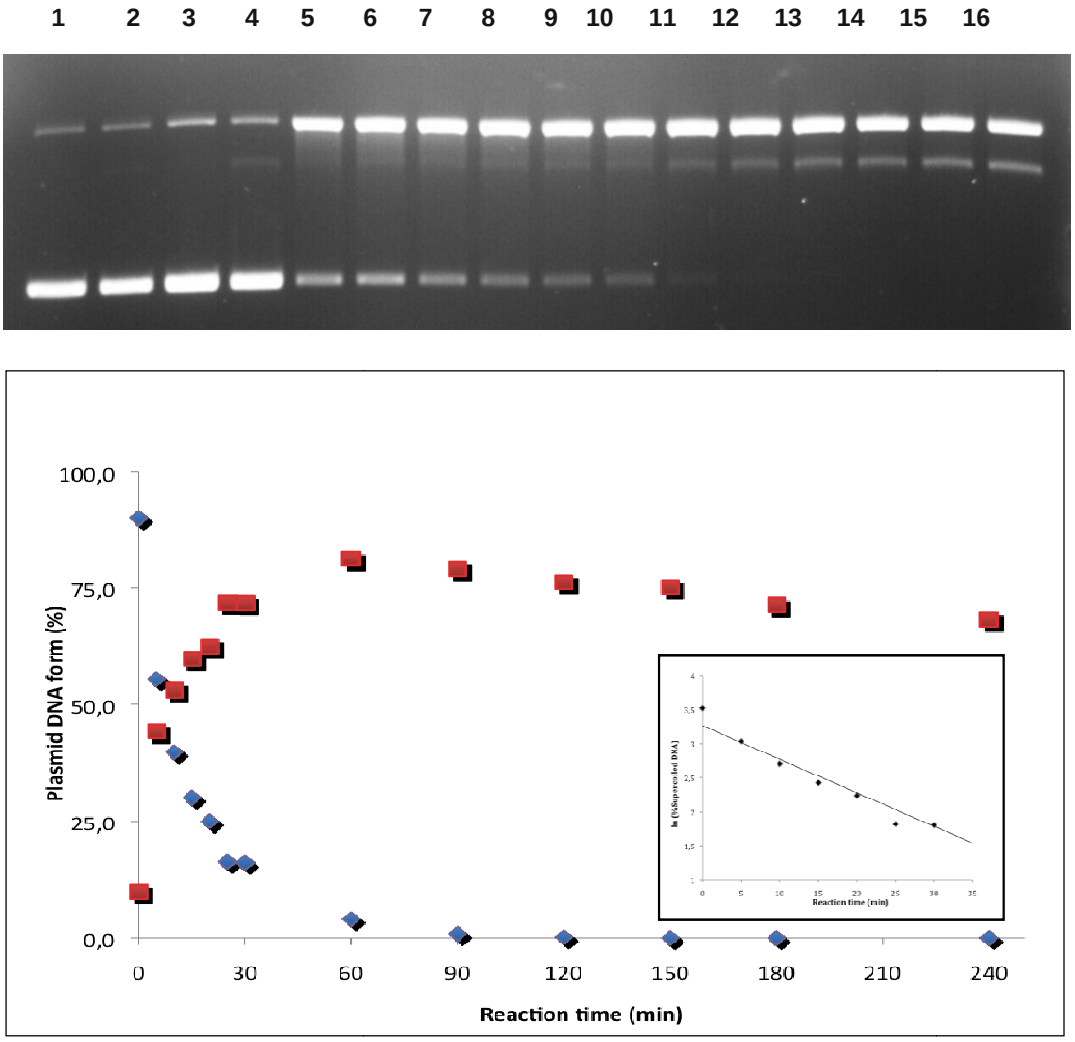


Fig. S6. Kinetics of supercoiled pUC18 DNA cleavage promoted by Co-L complex. Representative agarose gel images (*top*) at different incubation times: 0 min (lane 4), 5 min (lane 5), 10 min (lane 6), 15 min (lane 7), 20 min (lane 8), 25 min (lane 9), 30 min (lane 10), 40 min (lane 11), 50 min (lane 12), 60 min (lane 13), 75 min (lane 14), 180 min (lane 15) and 240 min (lane 16). Controls at t = 240 min: SC DNA (lane 1), DNA with 40 μM $Co(NO_3)_2$ (lane 2) and DNA with 40 μM L (lane 3). Variation of the relative concentrations of supercoiled (■) and nicked (◆) form(*bottom*). Inset: pseudo-first-order kinetics of DNA cleavage. [DNA]= 37.5 μM nucleotides, [Co-L] = 40 μM, HEPES buffer (pH= 7.0) at T = 37 ºC.

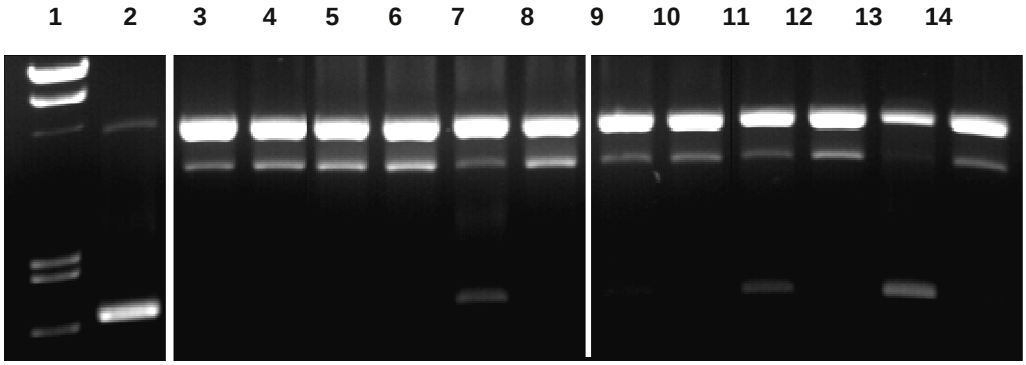


Fig. S7. Agarose gel showing the cleavage of supercoiled (SC) pUC18 DNA (37.5 μM nucleotides) promoted by Co-L and Fe-L (40 μM) in the presence of radical scavengers and groove binders incubated in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC. Lane 1: Marker. Lane 2: DNA control. Lane 3: 40 μM Co-L. Lane 4: 40 μM Co-L and 0.5 mM NaN_3. Lane 5: 40 μM Co-L and 0.5 mM Sodium formiate. Lane 6: 40 μM Co-L and 0.5 mM L-Histidine. Lane 7: 40 μM Co-L and 20 μM Hoechst 33258. Lane 8: 40 μM Co-L and 5 μM methyl green. Lane 9: 40 μM Fe-L. Lane 10: 40 μM Fe-L and 0.5 mM NaN_3. Lane 11: 40 μM Fe-L and 0.5 mM Sodium formiate. Lane 12: 40 μM Fe-L and 0.5 mM L-Histidine. Lane 13: 40 μM Fe-L and 20 μM Hoechst 33258. Lane 14: 40 μM Fe-L and 5 μM methyl green.

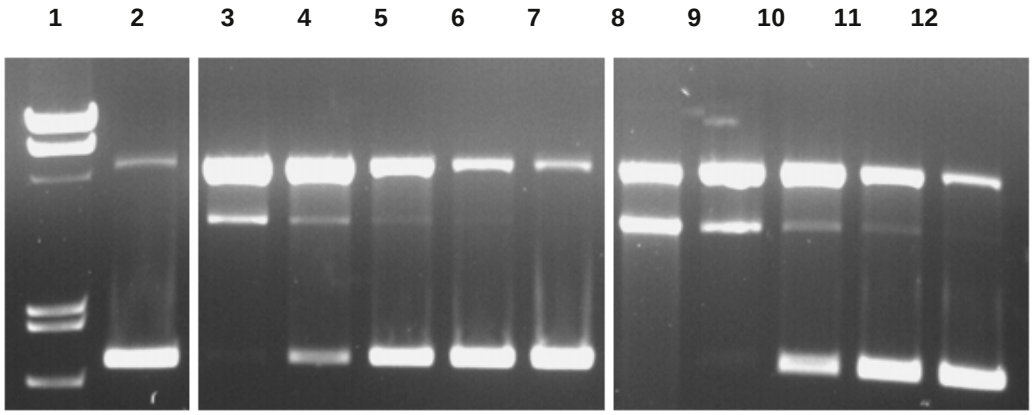


Fig. S8. Ionic strength dependence of supercoiled (SC) DNA cleavage promoted by Co-L and Fe-L (40 μM) incubated in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC. Lane 1: Marker. Lane 2: DNA control. Lane 3: 40 μM Co-L. Lane 4: 40 μM Co-L and 12.5 mM NaCl. Lane 5: 40 μM Co-L and 25 mM NaCl. Lane 6: 40 μM Co-L and 50 mM NaCl. Lane 7: 40 μM Co-L and 100 mM NaCl. Lane 8: 40 μM Fe-L. Lane 9: 40 μM Fe-L and 12.5 mM NaCl. Lane 10: 40 μM Fe-L and 25 mM NaCl. Lane 11: 40 μM Fe-L and 50 mM NaCl. Lane 12: 40 μM Fe-L and 100 mM NaCl.

Plasmid cleavage assays of pUC18 DNA (37.5 μM nucleotides) promoted by L (40 μM) and L´ (40 μM and 80 μM) in the presence of added metal ion (40 μM) incubated in HEPES buffer (50 mM, pH = 7.0) for 2 h at 37 ºC; asc = ascorbate (40 μM).

Table S1. Quantitation of bands (representive example shown in Fig. 1 and S1) based on at least three independent experiments.

(a)	DNA % form		
	Supercoiled (Form I)	Nicked (Form II)	Linear (Form III)
L	95 ± 3	5 ± 1	0 ± 0
CoL	5 ± 6	71 ± 8	24 ± 9
CoL + asc	4 ± 5	68 ± 10	27 ± 9
FeL	11 ± 6	62 ± 6	28 ± 9
FeL + asc	smearing		
NiL	92 ± 3	8 ± 3	0 ± 0
NiL + asc	82±10	16± 8	2 ± 4
CuL	90± 8	10±6	0 ± 0
CuL + asc	49 ± 11	51 ±12	0 ± 0
ZnL	89 ± 3	10 ± 2	1 ± 1
ZnL + asc	80±15	20± 10	0 ± 0

(b)	DNA % form		
	Supercoiled (Form I)	Nicked (Form II)	Linear (Form III)
L’	94 ±2	6 ±3	0 ± 0
CoL’	88 ±2	12 ±2	0 ± 0
CoL’ + asc	69 ±2	31 ±3	0 ± 0
FeL’	66 ± 5	34 ± 6	0 ± 0
FeL’ + asc	63 ± 8	37 ± 8	0 ± 0
NiL’	93 ±2	7 ±2	0 ± 0
NiL’ + asc	92 ± 2	8 ± 2	0 ± 0
CuL’	87 ±3	13 ±3	0 ± 0
CuL’ + asc	25 ±2	74 ±3	0 ± 0
ZnL’	91 ± 3	9 ± 3	0 ± 0
ZnL’ + asc	65 ±10	35 ±11	0 ± 0

(c)	DNA % form		
	Supercoiled (Form I)	Nicked (Form II)	Linear (Form III)
L'	97 ± 2	3 ± 2	0 ± 0
CoL'	90 ± 3	10 ± 3	0 ± 0
CoL' + asc	68 ± 16	32 ± 16	0 ± 0
FeL'	94 ± 4	6 ± 4	0 ± 0
FeL' + asc	70 ± 10	30 ± 10	0 ± 0
NiL'	96 ±3	4 ± 1	0 ± 0
NiL' + asc	96 ±3	4 ±2	0 ± 0
CuL'	90 ±4	10 ±2	0 ± 0
CuL' + asc	27 ± 6	71 ±5	2 ± 1
ZnL'	95 ±3	5 ± 1	0 ± 0
ZnL' + asc	75 ± 13	25 ± 15	0 ± 0

Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated with L (40 μM) and various concentrations of Co(II) and Fe(III) ions in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC.

Table S2. Quantitation of bands (representative example shown in Fig. 2 and Fig. S2) based on at least three independent experiments.

	DNA % form			
	Supercoiled (Form I)	Nicked (Form II)	Linear (Form III)	nicked + linear
[Co]				
10	50 ± 30	38 ± 7	12 ±7	50 ± 11
20	16 ± 11	61 ± 6	14 ±5	75±10
40	5 ± 2	71± 4	24 ± 4	95±4
60	5 ± 1	82±4	13 ± 6	95±5
80	4 ± 1	83 ± 3	13± 2	96±3
120	7 ± 1	85 ± 5	8 ± 5	93 ±5
[Fe]				
10	86 ± 1	14 ±5	0 ± 0	14 ±5
20	53 ± 4	28 ± 9	19 ± 5	47 ±7
40	18 ± 4	44±8	46 ± 8	90±6
60	10± 6	45± 2	44± 3	89±6
80	16 ± 10	54 ±4	30 ±6	84 ±5
120	19 ± 4	56 ± 4	25 ± 5	81 ± 5

Fig. S6 (refer to Fig. 2 and S2 and Table S2). Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated with L (40 μM) and various concentrations of Co(II) and Fe(III) ions in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC, represented as % Cleaved DNA **(% nicked + % linear)** vs metal ion concentration.

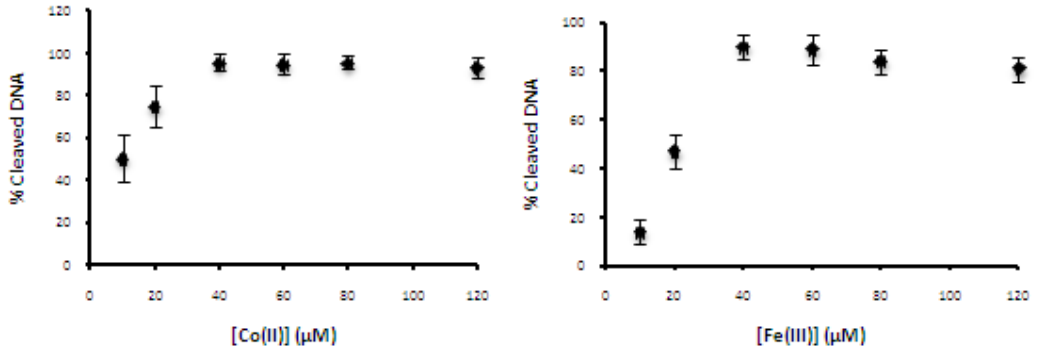
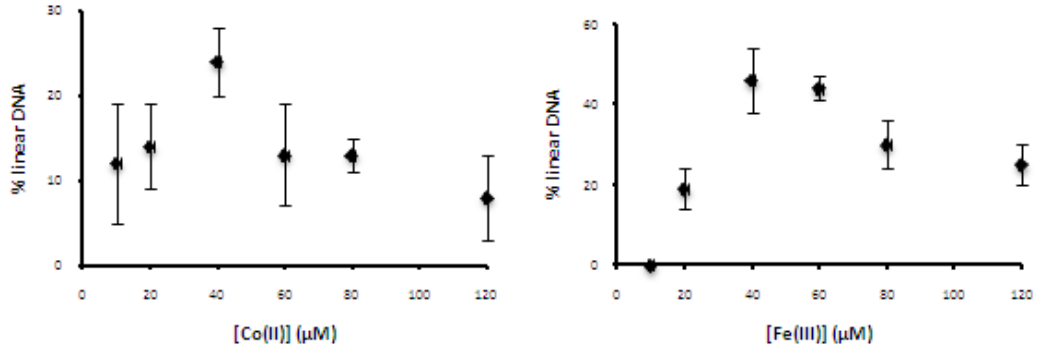


Fig. S7 (refer to Fig. 2 and S2 and Table S2). Cleavage of pUC18 DNA (37.5 μM nucleotides) incubated with L (40 μM) and various concentrations of Co(II) and Fe(III) ions in HEPES buffer (50 mM, pH 7.0) for 2 h at 37 ºC, represented as % Cleaved DNA **(% linear DNA)** vs metal ion concentration.



Plasmid cleavage assays of pUC18 (37.5 μM nucletotides) promoted by L, Co-L, Co_2-L, Fe-L and Fe_2-L (40 μM) at different pHs after an incubation period of 2 h at 37 ºC. Buffers used: pH 5.5 (50 mM MES), pH 6 (50 mM cacodylate), pH 7 and 8 (50 mM HEPES).

Table S3 (refer to Fig. 3 and S3). Quantitation of bands (representative example shown in Fig. 3) based on at least three independent experiments.

		DNA % form		
		Supercoiled (Form I)	Nicked (Form II)	Linear (Form III)
L				
	pH = 5,5	40 ± 4	50 ± 7	10 ± 4
	pH = 6,0	63 ± 4	35 ± 5	1 ± 1
	pH = 7,0	90 ± 1	10 ± 1	0 ± 0
	pH = 8,0	91 ± 1	9 ± 1	0 ± 0
Co-L				
	pH = 5,5	12 ± 4	74 ± 14	14 ± 10
	pH = 6,0	33 ± 11	60 ± 14	8 ± 3
	pH = 7,0	14 ± 3	68 ± 10	18 ± 6
	pH = 8,0	69 ± 2	29 ± 1	2 ± 1
Co_2-L				
	pH = 5,5	2 ± 1	80 ± 3	18 ± 4
	pH = 6,0	2 ± 1	97 ± 1	2 ± 1
	pH = 7,0	1 ± 0	86 ± 5	13 ± 4
	pH = 8,0	48 ± 8	50 ± 8	2 ± 1
Fe-L				
	pH = 5,5	0 ± 0	57 ± 11	43 ± 11
	pH = 6,0	0 ± 0	46 ± 16	54 ± 16
	pH = 7,0	0 ± 0	46 ± 4	54 ± 5
	pH = 8,0	60 ± 10	38 ± 9	3 ± 1
Fe_2-L				
	pH = 5,5	0 ± 0	71 ± 16	29 ± 16
	pH = 6,0	0 ± 0	50 ± 3	50 ± 3
	pH = 7,0	4 ± 5	73 ± 14	23 ± 19
	pH = 8,0	49 ± 4	51 ± 4	0 ± 0

Fluorescence competitive binding studies (ethidium bromide displacement assays).

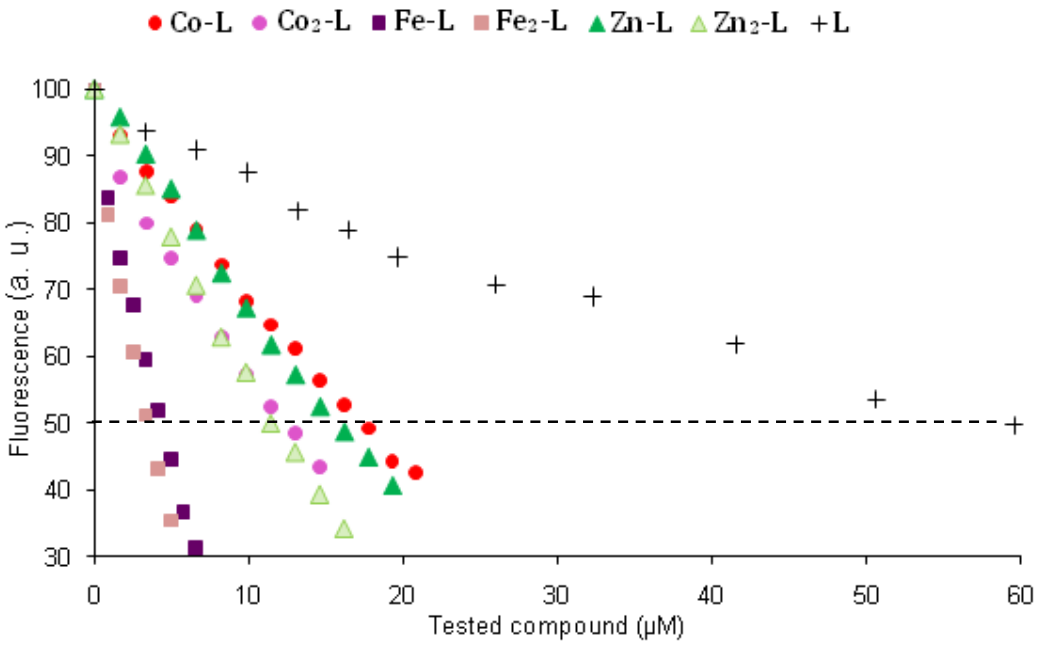


Fig. S7. Competitive ethidium bromide displacement for L, M-L and M_2-L complexes with CT-DNA.

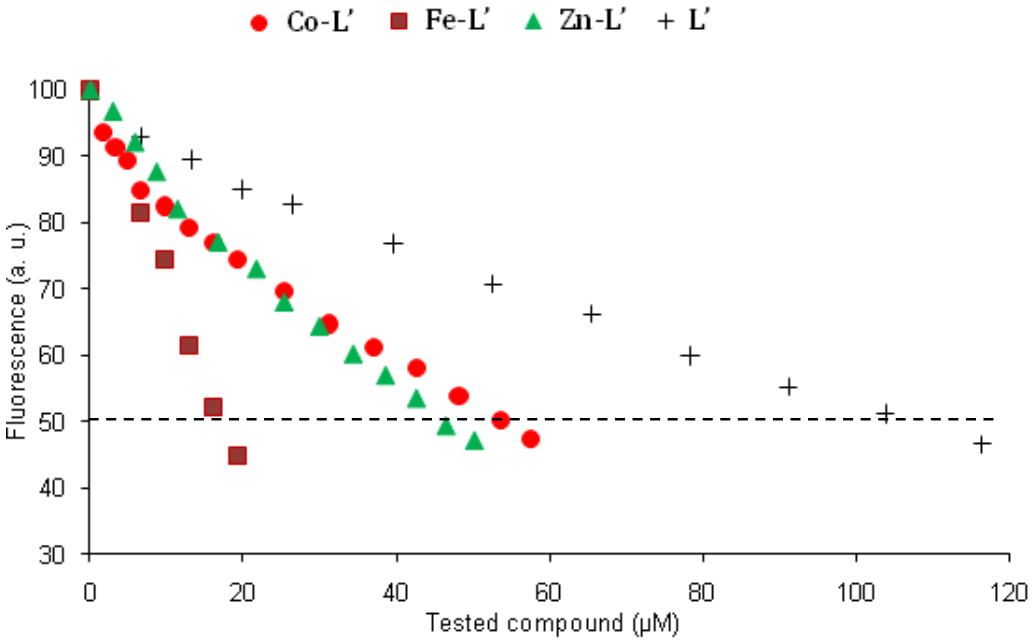


Fig. S8. Competitive ethidium bromide displacement for L' and M-L'complexes with CT-DNA.

Table S4 (refer to Fig. S7 and S8). Apparent DNA binding constants (K_{app}) for ligands and metal complexes (pH=7.0).

Compound	C_{50} [a] (μM)	K_{app} [b] (M^{-1})
Fe_2-L	3.5	1.0×10^7
Fe-L	4.0	9.0×10^6
Fe-L'	17.0	2.1×10^6
Zn-L	15.5	2.3×10^6
Zn-L'	46.0	7.8×10^5
Co_2-L	12.0	3.0×10^6
Co-L	17	2.1×10^6
Co-L'	53	6.8×10^5
Zn_2-L	11.5	3.1×10^6
Zn-L	15.5	2.3×10^6
Zn-L'	46.0	7.8×10^5
L	37.0	9.7×10^5
L'	104	$3{,}5 \times 10^5$

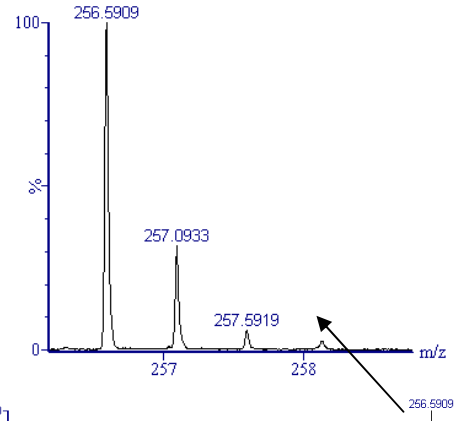
[a] C_{50} = concentration required to reduce fluorescence by 50%.

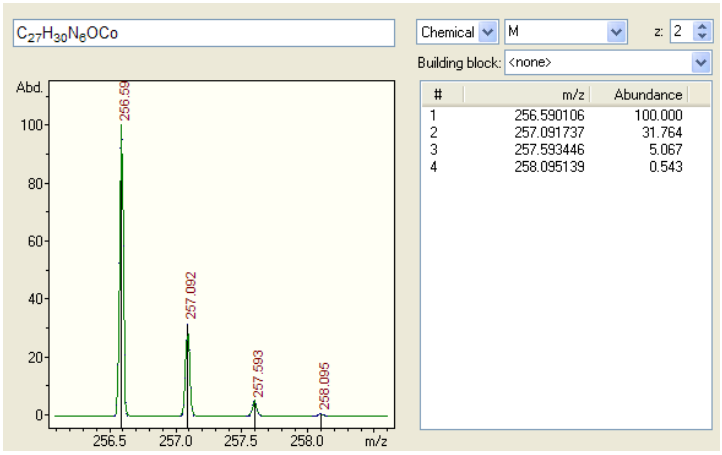
[b] $K_{app} = K_{EB} \times 3.78 / C_{50}$ where $K_{EB} = 9.5 \times 10^6$ $M(bp)^{-1}$

High resolution ESI-MS spectra of the complexes (M^{II}:L = 1:1) generated under the conditions used for the DNA cleavage reactions.

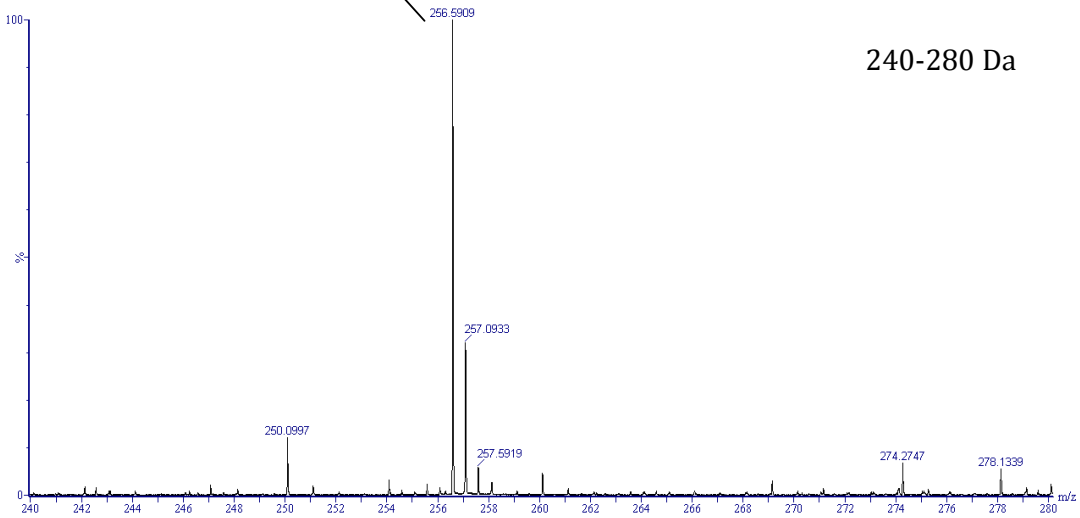
$[C_{27}H_{29}N_6OCo]^{2+}$

Accurate mass (experimental) = 259.5909 Exact mass (theoretical) = 259.5901 / Accuracy = 3.12 ppm

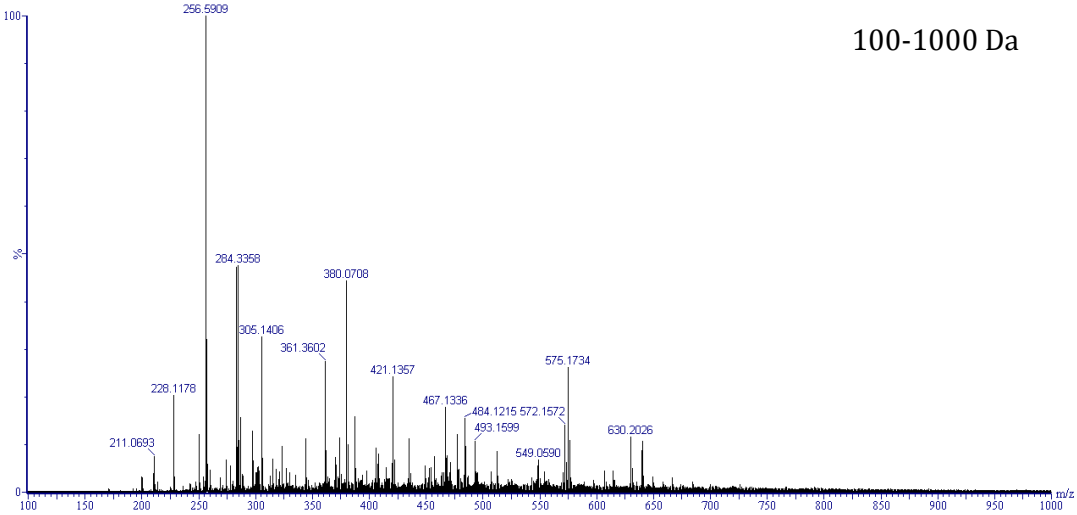




240-280 Da

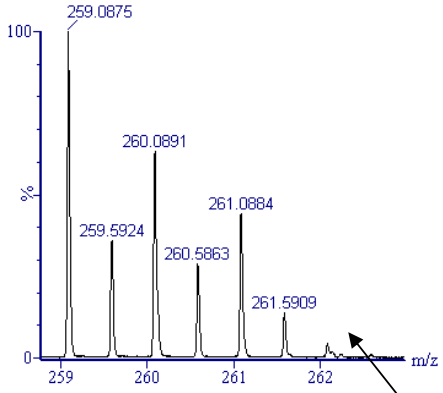


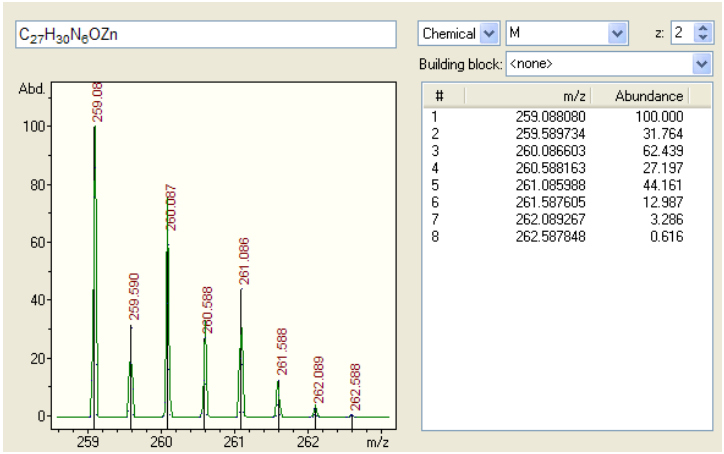
100-1000 Da



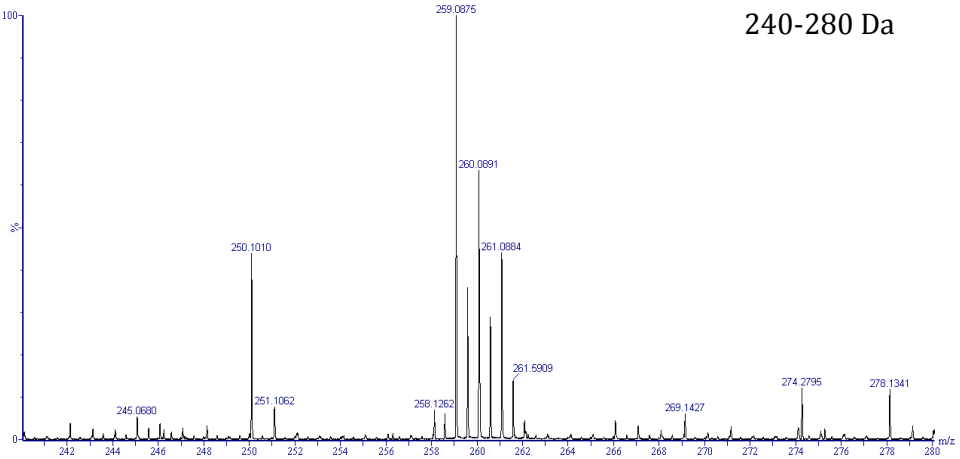
$[C_{27}H_{29}N_6OZn]^{2+}$

Accurate mass (experimental) = 259.0875 / Exact mass (theoretical) = 259.0881 / Accuracy = 2.32 ppm

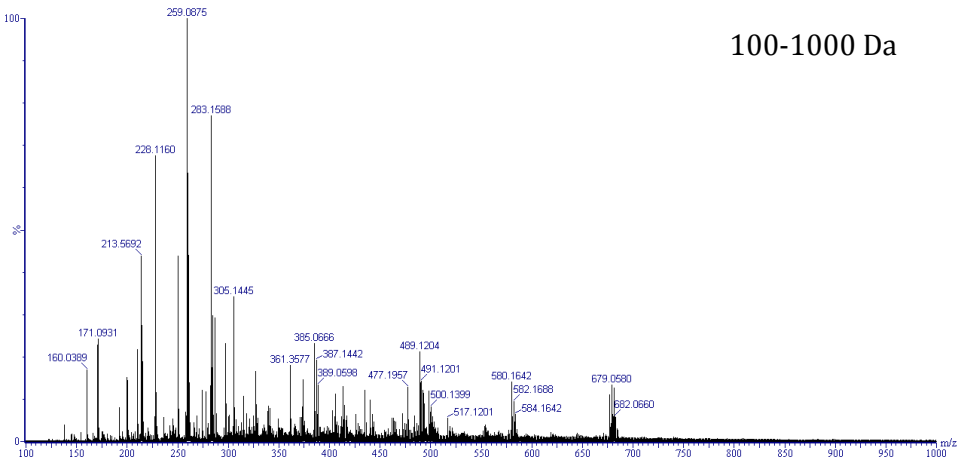




240-280 Da

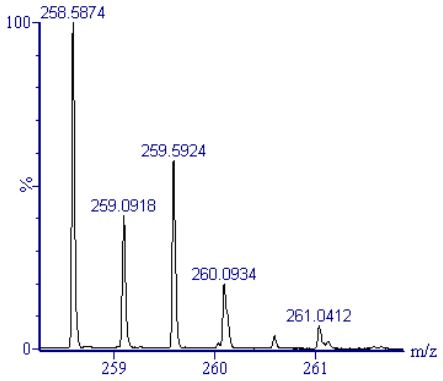


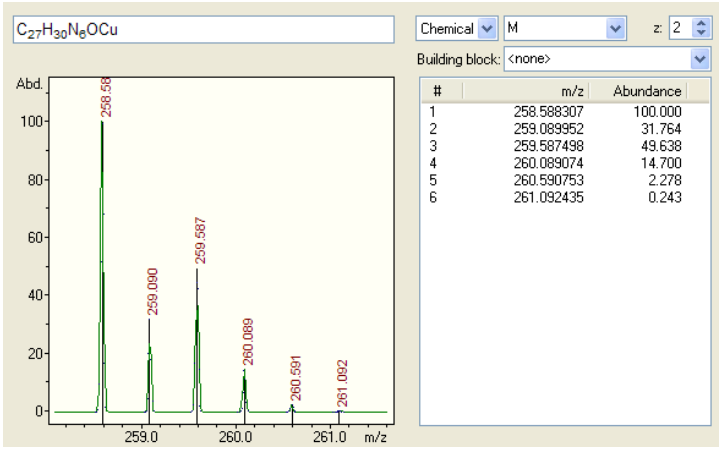
100-1000 Da



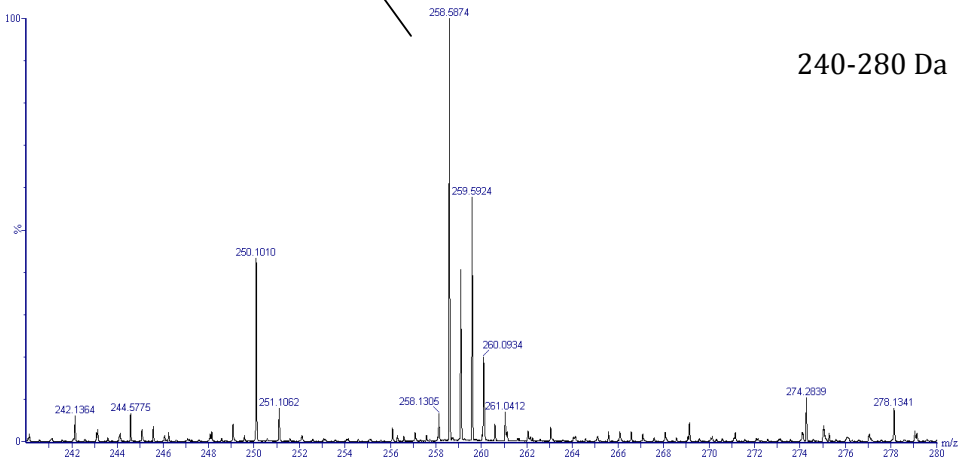
$[C_{27}H_{29}N_6OCu]^{2+}$

Accurate mass (experimental) = 258.5874 / Exact mass (theoretical) = 258.5883 / Accuracy = 3.15 ppm

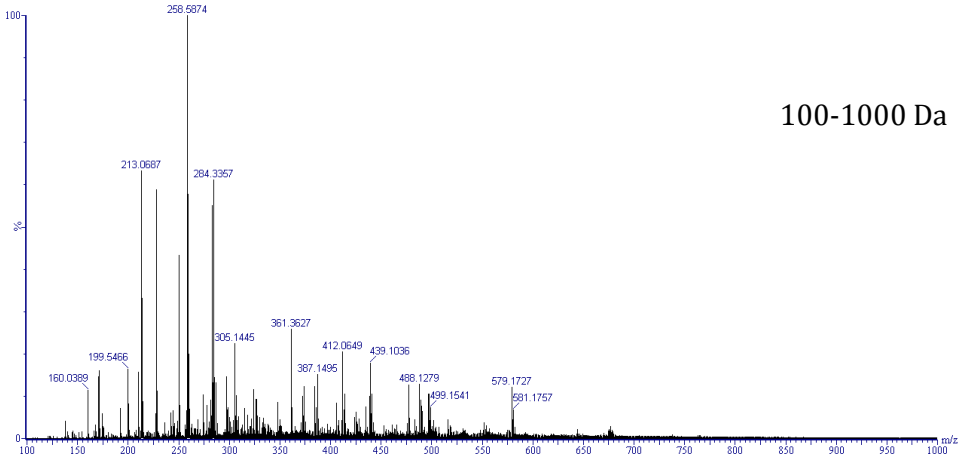




240-280 Da

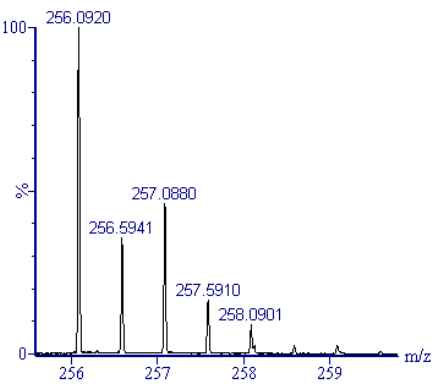


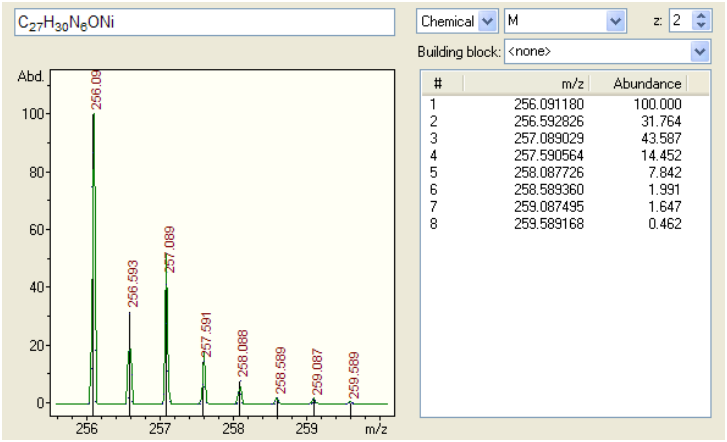
100-1000 Da



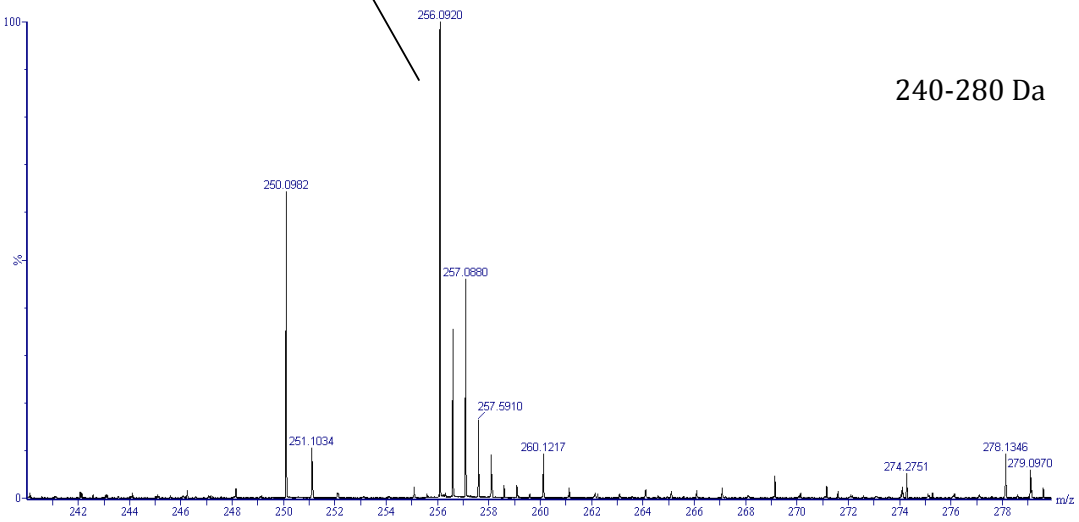
$[C_{27}H_{29}N_6ONi]^{2+}$

Accurate mass (experimental) = 256.0920 / Exact mass (theoretical) = 256.0912 / Accuracy = 3.13 ppm





240-280 Da



100-1000 Da

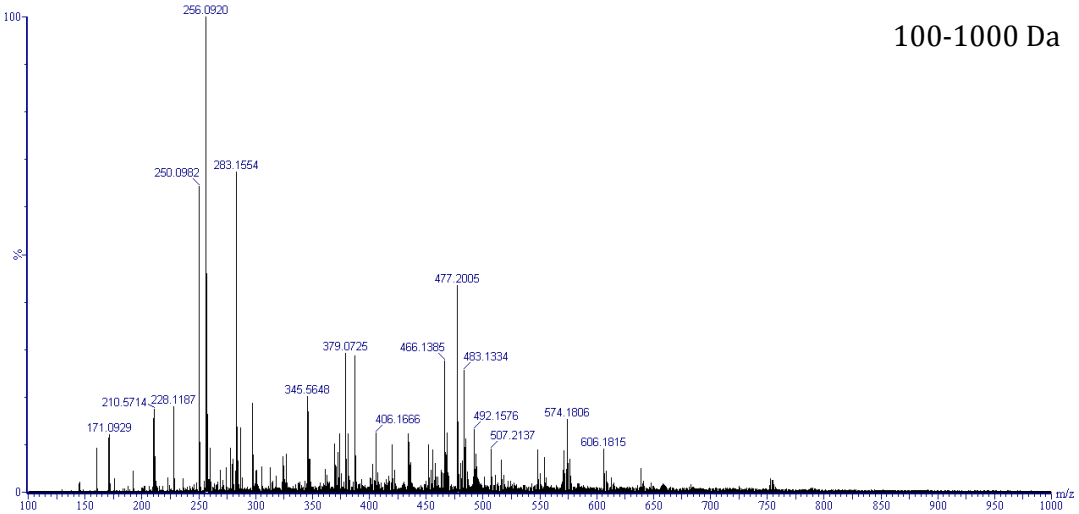


Table S5. Stability constants (as log *K*) for the Co-L system determined in 0.15 M NEt_4ClO_4 at 298.1 K.

Reaction[a]	Co(II)
$M + L + 2H = MH_2L$	18.16(9)
$M + L + H = MHL$	14.90(8)
$M + L = ML$	10.37(9)
$M + L = MH_{-1}L + H$	1.9(1)
$2M + L = M_2H_{-1}L + H$	10.0(1)
$2M + L = M_2H_{-2}L + 2H$	1.2(1)

a) Charges omitted.