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Affinity and Nuclease Activity of Macrocyclic Polyamines and Their Cu^{II} Complexes

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Abstract: The stability constants of Cu^{II} complexes that consist of either an oxa-aza macrocycle with two triamine moieties linked by dioxa chains, or two macrocyclic ligands with a polyamine chain which are connecting the 2 and 9 positions of phenanthroline, have been determined by means of potentiometric measurements. The results are compared to those reported for other ligands with a similar molecular architecture. Of the complexes that contain phenanthroline in their macrocycle, the Cu^{II} ion of the complex with the smallest and most rigid macrocycle (**L3**) has an unsaturated coordination sphere, while in the complex with the largest macrocycle (**L5**) the Cu^{II} ion is coordinatively almost saturated. These results are corroborated by the crystal structure of the $[Cu\mathbf{L5}](ClO_4)_2$ complex. The affinity of the ligands and the complexes towards nucleic acids was studied by measuring the changes in the melting temperature, which showed that the affinity of the macrocyclic ligands towards double-stranded DNA or RNA is generally smaller than that of their linear analogues that bear a similar charge, with a strong preference for polyA–polyU, a model for RNA. However, the complexes of two of the changed macrocyclic ligands which contain a phenanthroline unit (**L4**, **L5**) showed a distinctly larger increase in their melting temperature ΔT_m with DNA (polydA–polydT), which is reversed again in favor of RNA upon metallation to the dinuclear copper complex with **L5**. Experiments with supercoiled plasmid DNA showed a particularly effective cleavage with a mononuclear Cu^{II} complex that contains a phenanthroline unit (**L6**). Related ligands showed less activity towards DNA, but not so towards the biocidic bis(*p*-nitrophenyl)phosphate (BNPP). In both cases (with DNA and BNPP) the activity seemed to increase with decrease of coordinative saturation of the Cu^{II} ion, with the exception of one particular ligand (**L6**). Experiments with radical scavengers in the DNA experiments showed some decrease in cleavage, which indicates the participation of redox processes.

Keywords: copper · DNA cleavage · macrocyclic polyamines · N ligands · nucleic acids

Introduction

Macrocyclic polyamines can effectively complex many transition metal ions which might then alter their binding properties to nucleic acids and thus be useful as a spectroscopic probe and could also act as chemical nucleases. The nuclease activity of the 1,10-phenanthroline(OP)-copper ion complex was discovered by Sigman et al. in 1979.[1] In this system, a tenfold excess of OP was used in order to obtain the 2:1 ligand/metal ion chelate which is necessary in the course of the DNA cleavage.[1, 2] The reaction proceeds in the presence of a thiol and H_2O_2 as a co-reactant. Generation of the copper-oxo species and binding to the minor groove of DNA initiated the cleavage, which occurs in an oxidative manner. The attack is initiated at the C1 hydrogen of a deoxyribose moiety.[3] A related ligand, in which two OP units are covalently attached by a carbon chain (to avoid the use of excess ligand) was probed for nuclease activity,[4] whereby mercaptopropionic acid was added as a reducing agent. In general, an excess of external reducing agent was used[5] in the study of various copper-mediated DNA cleavage reactions. Recently, the Tambjamine E–Cu^{II} complex was reported to cleave DNA also in the absence of any external reducing agent.[6]

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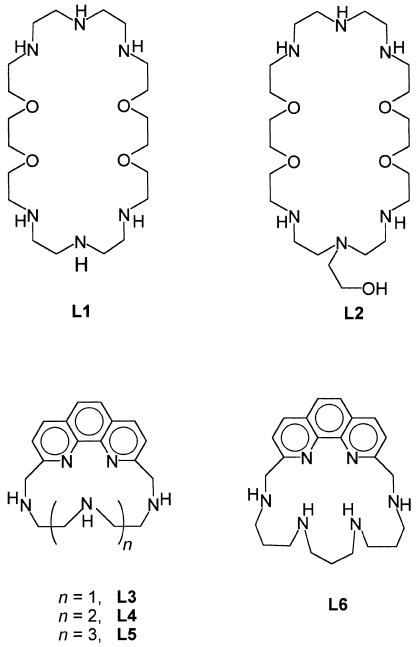
Earlier studies have shown that the hydrolytic activity of many metal complexes can also be significantly modified by the use of suitable ligands, particularly those that contain positively charged nitrogen centers and/or intercalators.[7] Several ligands used in the present study also allow the introduction of more than one metal ions, which can significantly enhance the activity of nucleases.[8] A particularly intriguing aspect for the use of macrocyclic polyamines is the possibility of investigating the change in the coordination sphere of the metal ion, which is also known to alter catalytic properties in enzymes. In particular, the activity of metallo-enzymes in catalytic processes is often related to unsaturated coordination environments of the metal cations, namely to the presence of free coordination sites for substrate binding and activation.[9]

Furthermore, macrocyclic polyamines themselves are of interest with respect to their interactions with nucleic acids, which may differ significantly from those of acyclic amines.[10] Some azoniacyclophanes show, for example, selective destabilization of RNA.[11] Similar effects might be expected with macrocyclic amines described previously[12] if their size is large enough to lead to binding modes other than those of the more flexible open-chain amines. Herein we report on the affinity of six macrocyclic ligands and their copper complexes towards double-stranded nucleic acids, and on the nuclease activity of new Cu^{II} complexes in the absence of H_2O_2 and of any reducing agent. The macrocyclic ligands **L1 – L6** (), the syntheses of which have been reported previously,[12] were of particular interest, as they allow either simultaneous intercalation (**L3 – L6**), or multisite binding of metal ions (**L1**, **L2**, and **L5**), and thus represent a step towards multinuclear chemical nucleases. The distinct geometry of these ligands could also have a significant influence on their binding properties to DNA and RNA-type double strands; these features were studied by thermal melting experiments (see Table 3).



Abstract in German: *Die Stabilitätskonstanten von Cu^{II}-Komplexen mit Liganden zum einen aus zwei mit Dioxaketten verknüpften Triamineinheiten, sowie mit Polyaminketten verbunden in der 2,9-Position eines Phenanthrolinrings wurden durch potentiometrische Messungen bestimmt und mit anderen ähnlicher Struktur verglichen. Bei den Phenanthrolinkomplexen zeigt der kleinste und besonders starre Markrocyclus **L3** das Cu-Atom in einer ungesättigten Koordinationssphere, im Gegensatz zum entsprechenden grössten Komplex mit **L5**. Dies wird durch eine Röntgenstrukturanalyse mit [Cu**L5**]$(ClO_4)_2$ erhärtet. Schmelzpunkt-Untersuchungen zeigen im Vergleich zu offenkettigen Aminen generell eine geringere Affinität der Makrocyclen zu doppelsträngigen Nukleinsäuren, mit einer ausgeprägten Präferenz für polyA – polyU als RNA Modell. Makrocyclen mit Phenanthrolineinheiten (**L4**, **L5**) führen jedoch zu einer bedeutend grösseren Schmelzpunkterhöhung ΔT_m bei DNA (polydA – polydT), welche sich bei **L5** durch Metallierung mit Cu^{II} in Richtung einer RNA-Stabilisierung umkehren lässt. Experimente mit superspiralisierter plasmidischen DNA zeigen eine besonders effektive Spaltung mit dem mononuklearen phenanthrolinhaltigen Komplex **L6** · $CuCl_2$. Ähnliche Komplexe besitzen eine geringere Aktivität gegen DNA, nicht aber gegen das biozide Bis(p-nitrophenyl)phosphat (BNPP). Sowohl mit DNA wie mit BNPP scheint die Spaltung mit einer abnehmenden koordinativen Sättigung der Cu^{II} ions anzusteigen, mit Ausnahme eines Liganden (**L6**). Der Zusatz von von Radikalfängern führt bei den DNA zu abnehmenden Spaltungensraten, was auf die Beteiligung von Redoxprozessen hinweist.*

Results and Discussion

Crystal structure of [CuL5](ClO$_4$)$_2$: The molecular structure consists of complexed cations [Cu**L5**]$^{2+}$ and perchlorate anions. The asymmetric unit contains two independent molecules. The ORTEP[13] drawings of the [Cu**L5**]$^{2+}$ cations in the two molecules (herein indicated **A** and **B**) are shown in Figure 1. Table 1 gives selected bond distances and angles for the coordination sphere of Cu^{II}. The Cu^{II} ions in **A** and **B** display slightly different coordination geometries. In both cases the coordination environments can best be described as distorted octahedral. In complex **A**, the metal is hexacoordinated by the phenanthroline nitrogens N1 and N2 and by the amine groups N3, N4, N5, and N6; N1, N4, N5, and N6 define the basal plane and N2 and N3 the apical positions of the distorted octahedron. The Cu – N2 and Cu – N3 bonds form dihedral angles of 16.1(2)° or 13.5(2)°, respectively, with the normal to the basal plane. The metal ion lies 0.845 Å above the basal plane, shifted toward N2. One of the benzylic amine groups (N7) does not bind to the metal.

In complex **B** the basal plane of the octahedron is defined by the N8, N11, N12, and N13 donors (max. deviation 0.234(8) Å for N12) while N9 and N10 occupy the apical positions. The Cu – N9 and Cu – N10 bonds form dihedral angles of 21.3° and 16.9° with the normal to the basal plane. The metal lies 0.164 Å out of this plane, shifted toward N9. As in molecule **A**, a benzylic nitrogen donor (N14) is not involved in coordination to the metal. In both molecules the

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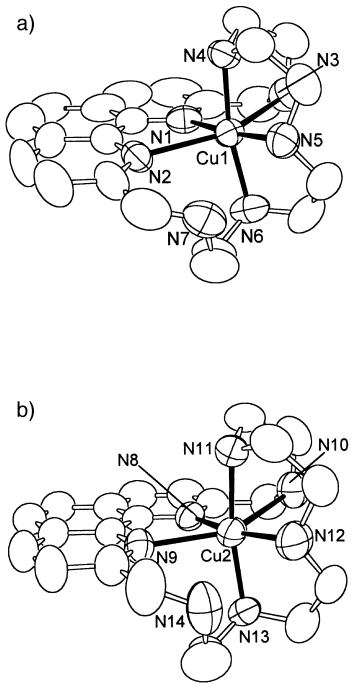

Figure 1. ORTEP drawings of the $[Cu\mathbf{L5}]^{2+}$ cations in the two independent molecules **A** a) and **B** b).

ligand assumes a screw conformation, "wrapping" around the metal ion so that it is embedded inside the macrocyclic cavity.

Coordination of Cu^{II} in aqueous solutions: A previous investigation[12a] on the coordination of Cu^{II} to ligand **L1** in aqueous solutions showed that this ligand behaves as a ditopic receptor for metal cations, with two almost independent binding sites for metals. In its binuclear Cu^{II} complexes, each metal is coordinated by a triamine unit, while the ether oxygens are less involved in metal coordination. We have now extended this study to **L2**, which shows a molecular architecture similar to **L1**, and displays two triamine moieties separated by two diether chains. Potentiometric measurements (0.1M, NMe_4Cl, 298.1 K) have shown that, in a similar manner to **L1**, **L2** can form both mono- and binuclear species in aqueous solution and that the stability constants of its Cu^{II} complexes[14] are similar to those found for the corresponding **L1** complexes ($\log K = 15.36$ and 16.1 for the equilibrium $Cu^{2+} + L \rightarrow [CuL]^{2+}$ for L = **L1** and **L2**, respectively; $\log K =$ 27.88 and 27.5 for the equilibrium $2\,Cu^{2+} + L \rightarrow [Cu_2L]^{4+}$ for L = **L1** and **L2**, respectively). Furthermore, the binuclear Cu^{II} complexes display similar UV/Vis spectral features in aqueous solutions (a broad band with $\lambda_{max} = 705$ nm ($\varepsilon =$ 139 $M^{-1}cm^{-1}$) and 698 nm ($\varepsilon = 148\ M^{-1}cm^{-1}$) for $[Cu_2\mathbf{L1}]^{4+}$ and $[Cu_2\mathbf{L2}]^{4+}$, respectively). These data suggest that, as previously found for **L1**,[12a] in both the mono- and binuclear complexes with **L2** each metal ion is located within a triamine moiety. Both mono- and binuclear complexes are very stable and, as a matter of fact, with a 1:2 ligand/metal molar ratio, all the Cu^{II} is complexed at slight acidic pH.

The stability constants of the Cu^{II} complexes of ligands **L3**–**L6** are given in Table 2. The equilibrium data in Table 2 clearly show that all ligands form stable 1:1 complexes with Cu^{II}. Actually, the Cu^{II} ion is completely complexed even in acidic aqueous solutions, as shown in Figure 2 for the **L3** and **L6** ligands. Interestingly, all $[CuL]^{2+}$ complexes show a marked tendency to protonate; protonated complexed species become predominant in neutral or slightly acidic solutions

Table 1. Selected bond lengths [Å] and angles [°] for $[Cu\mathbf{L5}](ClO_4)_2$.

molecule **A**	
Cu1–N1	2.035(6)
Cu1–N2	2.459(6)
Cu1–N3	2.432(6)
Cu1–N4	2.060(5)
Cu1–N5	2.003(6)
Cu1–N6	2.045(6)
N5-Cu1-N6	82.9(3)
N4-Cu1-N6	166.7(3)
N4-Cu1-N5	84.5(3)
N3-Cu1-N6	97.6(3)
N3-Cu1-N5	97.9(3)
N3-Cu1-N4	80.0(2)
N2-Cu1-N6	93.2(2)
N2-Cu1-N5	110.3(2)
N2-Cu1-N4	95.0(2)
N2-Cu1-N3	150.8(2)
N1-Cu1-N6	100.6(3)
N1-Cu1-N5	174.0(2)
N1-Cu1-N4	91.7(2)
N1-Cu1-N3	76.9(3)
molecule **B**	
Cu2–N8	2.123(5)
Cu2–N9	2.340(5)
Cu2–N10	2.578(7)
Cu2–N11	2.053(5)
Cu2–N12	1.970(7)
Cu2–N13	2.051(5)
N12-Cu2-N13	83.8(3)
N11-Cu2-N13	168.1(2)
N11-Cu2-N12	84.3(3)
N10-Cu2-N13	101.6(2)
N10-Cu2-N12	90.0(3)
N10-Cu2-N11	77.6(2)
N9-Cu2-N13	93.0(2)
N9-Cu2-N12	123.0(3)
N9-Cu2-N11	94.1(2)
N9-Cu2-N10	145.3(2)
N8-Cu2-N13	97.2(2)
N8-Cu2-N12	161.9(3)
N8-Cu2-N11	93.8(2)
N8-Cu2-N10	72.0(2)
N8-Cu2-N9	75.1(2)

Table 2. Stability constants for Cu^{II} complexation with ligands **L3**–**L6**.[a]

Reaction	log K			
	L3	**L4**[b]	**L5**[c]	**L6**
$Cu^{2+} + L \rightarrow [CuL]^{2+}$	16.17(3)	17.53	17.91	13.90(3)
$[CuL]^{2+} + H^+ \rightarrow [CuHL]^{3+}$	4.99(3)	5.94	6.63	9.03(3)
$[CuHL]^{3+} + H^+ \rightarrow [CuH_2L]^{4+}$			4.53	5.98(3)
$[CuH_2L]^{4+} + H^+ \rightarrow [CuH_3L]^{5+}$				5.46(3)
$[CuL]^{2+} + OH^- \rightarrow [CuL(OH)]^+$	4.00(3)	3.50	3.34	
$[CuL(OH)]^+ + OH^- \rightarrow [CuL(OH)_2]$	2.39(6)			
$[CuL]^{2+} + Cu^{2+} \rightarrow [Cu_2L]^{4+}$			7.37	
$[Cu_2L]^{4+} + OH^- \rightarrow [Cu_2L(OH)]^{3+}$			6.38	

[a] Reaction conditions: 0.1M, NMe_4Cl, 298.1 K. [b] From ref. [29]. [c] From ref. [12d].

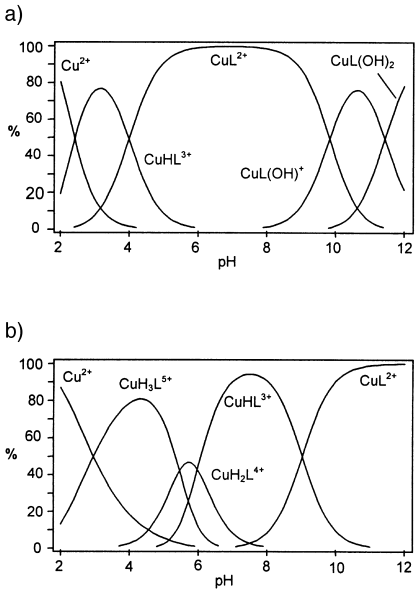

Figure 2. Distribution diagrams for the systems a) **L3**/Cu^{II} and b) **L6**/Cu^{II} ([**L3**] = [**L6**] = [Cu^{II}] = 1×10^{-4} M, NMe_4Cl = 0.1 M, 298.1 K).

(Figure 2). High values of protonation constants for metal complexes usually indicate that some nitrogen donors are not bound, or weakly bound, to the metal. On the other hand, the rigidity of the phenanthroline unit does not allow the simultaneous binding of metal to the aromatic nitrogens and to the adjacent benzylic amine groups. It can be suggested, therefore, that at least one of the benzylic nitrogens is not coordinated to the metal. This is in agreement with the crystal structure of the [Cu**L5**]$^{2+}$ complex (Figure 1): the phenanthroline donors (N1 and N2 in complex a) and one of the benzylic nitrogens (N3) are coordinated to the Cu^{II} ion, while the benzylic N7 donor is not involved in binding to the metal. The presence of an uncoordinated benzylic nitrogen seems to be a peculiar structural characteristic of the present [CuL]$^{2+}$ complexes.

A most interesting finding is the increase of the complex stability in the order **L6** $\ll$ **L3** < **L4** < **L5** (Table 2). The much lower stability of the [Cu**L6**]$^{2+}$ complex can be simply attributed to the replacement of the ethylenic chains that link the amine groups by propylenic chains. The increased N-Cu-N bond angle, as a result of the larger bite of the propylenic chain, reduces the stability of the complex. Actually, open-chain or macrocyclic tetraamines with propylenic chains which link the nitrogen donors form less stable Cu^{II} complexes than the corresponding ligands with ethylenic chains (e.g. $\log K = 20.32$ for the formation of the Cu^{II} complex with 1,4,7,10-tetraazadecane,[15] while $\log K = 16.36$ in the case of the Cu^{II} complex with 1,5,9,13-tetraazatridecane).[16] For the Cu^{II} complexes with the phenanthroline-containing macrocycles **L3**–**L5**, the observed increase in stability from [Cu**L3**]$^{2+}$ to [Cu**L5**]$^{2+}$ may be ascribed to the increased number of nitrogen donors available for metal coordination, that is, to the increase in the coordination number of the metal. This suggestion is supported by the UV/Vis spectra of these complexes, which show a blue shift of the d–d band from [Cu**L3**]$^{2+}$ to [Cu**L5**]$^{2+}$ ([Cu**L3**]$^{2+}$: λ_{max} = 687 nm, $\varepsilon = 84.1\ M^{-1}cm^{-1}$; [Cu**L4**]$^{2+}$: λ_{max} = 670 nm, ε = 52 $M^{-1}cm^{-1}$; [Cu**L5**]$^{2+}$: λ_{max} = 637 nm, $\varepsilon = 60\ M^{-1}cm^{-1}$).

A previous study on the binding features of **L3** towards Zn^{II}[17a] and Pb^{II}[12c] indicated that the rigidity of the macrocyclic framework, because of the short polyamine chain which connects the 2 and 9 positions of the rigid phenanthroline moiety, does not allow the ligand to change conformation upon coordination to a metal. In particular, in the Zn^{II} complex with **L3**, the metal is coordinated to the phenanthroline donors and to the central nitrogen of the aliphatic chain. The benzylic nitrogens are only weakly involved in coordination to the metal (at $\approx$2.5 Å). As a consequence, the metal ion shows a rather unsaturated coordination sphere and free binding sites are available at the Zn^{II} ion. A similar unsaturated coordination environment can also be proposed for the Cu^{II} complex with **L3**. Ligands **L4** and **L5** are more flexible and contain a larger number of donors. It is expected, therefore, that in [Cu**L4**]$^{2+}$ and [Cu**L5**]$^{2+}$ the metal presents a more coordinately saturated coordination sphere than in [Cu**L3**]$^{2+}$. This hypothesis is confirmed, once again, by the crystal structure of the [Cu**L5**]$^{2+}$ complex (Figure 1), which shows the six-fold coordination of the metal to the ligand donor.

Ligand **L5** gives also rise to binuclear complexes in aqueous solution and binuclear species are largely prevalent in solutions with 1:2 ligand to metal molar ratios. The seven nitrogen donors of the macrocycle cannot fill the coordination sphere of both metals, which therefore leads to low coordination numbers for each of the Cu^{II} ions in this complex. The remarkable red shift of the d–d band observed in the UV/Vis spectrum of [Cu_2**L5**]$^{4+}$ (λ_{max} = 729 nm, $\varepsilon = 121\ M^{-1}cm^{-1}$) with respect to the mononuclear complex [Cu**L5**]$^{2+}$, supports this hypothesis. Therefore, this complex is a promising receptor for substrate molecules through a bridging coordination to the two metal centers. Indeed, this complex shows a high value of the equilibrium constant for the binding of the OH^- anion, one of the simplest examples of anionic substrates ($\log K$ = 6.38, Table 2). Such a strong binding of the hydroxide ion is usually indicative of a bridging coordination of this group to two metal ions.[12a]

DNA affinity tests from melting temperature experiments: The interaction of the ligands and the Cu^{II} complexes with double stranded nucleic acids polyA–polyU (corresponding to RNA) and polydA–polydT (a model for DNA) are characterized by quite variable differences in the values of their melting temperatures (ΔT_m; Tables 3 and 4). All ligands as well as their copper complexes showed stabilization of the double-stranded nucleic acids as can been seen from the considerable increase of the melting temperature in each case. A large stabilization effect, which shows a preference for RNA, is observed for **L1** and **L2**, sometimes with a biphasic transition which is possibly caused by the presence of a different stoichiometry of the ligand/nucleic acid complex. We also observe that the macrocycles **L1** and **L2** are less efficient in increasing ΔT_m as compared to acyclic amines which bear approximately the same number of positive charges. Similar behavior has been observed with other macrocyclic polyamines that contain benzene rings in the macrocycle.[18] The

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Table 3. Ligand interaction with polyA–polyU and polydA–polydT.[a]

Ligand	r[b]	ΔT_m [°C] polyA–polyU	ΔT_m [°C] polydA–polydT
L1[c]	0.1	28.8	11.1
	0.2	16.6/42.1	22.0
	0.3	14.1/44.6	broad
L2	0.1	27.0	10.7
	0.2	16.6/39.0	16.6
	0.3	13.4/41.0	29.1
L3	0.1	5.6	6.5
	0.2	7.5	10.5
	0.3	9.0	12.1
L4	0.1	12.6	13.2
	0.2	13.1	18.3
	0.3	12.5	22.7
L5	0.1	12.4	14.6
	0.2	13.2	20.4
	0.3	13.9	27.1
L6	0.1	26.3	14.8
	0.2	32.1	26.6
	0.3	– 2.9/35.9	broad

[a] Reaction conditions: 0.01M MES buffer; pH 6.25; $I = 0.01$M; error in $\Delta T_m = \pm 0.5$ °C. [b] r = Molar ratio of ligand/nucleic acid phosphate. [c] Protonated species [calculated from the values of the protonation constants reported in refs. [12a, b] (for **L1** and **L2**) and refs. [17b, 12e] (for **L3–L6**)]: **L1**: $[H_4\mathbf{L1}]^{4+} = 90\%$, $[H_3\mathbf{L1}]^{3+} = 10\%$, **L2**: $[H_4\mathbf{L2}]^{4+} = 90\%$, $[H_3\mathbf{L2}]^{4+} = 10\%$, **L3**: $[H_2\mathbf{L3}]^{2+} > 95\%$, **L4**: $[H_2\mathbf{L4}]^{2+} = 60\%$, $[H_3\mathbf{L4}]^{3+} = 40\%$, **L5**: $[H_3\mathbf{L5}]^{3+} = 85\%$, $[H_2\mathbf{L5}]^{2+} = 15\%$, **L6**: $[H_3\mathbf{L6}]^{3+} = 60\%$, $[H_4\mathbf{L6}]^{4+} = 40\%$.

Table 4. Interaction of Cu^{II} complexes with polyA–polyU and polydA–polydT.[a]

Cu^{II} complex	r[b]	ΔT_m [°C] in polyA–polyU	ΔT_m [°C] in polydA–polydT
L1 · $2CuCl_2$[c]	0.1	25.2	12.0
	0.2	34.4	17.9/26.7
	0.3	9.0/34.5	37.2/42.4
L2 · $2CuCl_2$	0.1	20.5	11.9
	0.2	26.4	28.2
	0.3	5.3/34.0	34.7/42.2
L3 · $CuCl_2$	0.1	4.9	3.8
	0.2	6.8	7.0
	0.3	8.1	8.9
L4 · $CuCl_2$	0.1	6.7	7.0
	0.2	10.1	12.4
	0.3	14.6	16.7
L5 · $CuCl_2$	0.1	10.2	9.0
	0.2	12.6	17.3
	0.3	14.4	18.2
L5 · $2CuCl_2$	0.1	25.6	14.7
	0.2	38.6	21.0
	0.3	41.2	28.1
L6 · $CuCl_2$	0.1	19.6	16.2
	0.2	broad	25.5
	0.3	broad	broad

[a] and [b] See footnotes of Table 3. [c] Complexed species in solution at pH 6.25 (calculated from the values of the stability constants given in Table 2 for ligands **L3–L6** and from refs. [12b, 14] for **L1** and **L2**). **L1**: $[Cu_2\mathbf{L1}]^{4+} = 100\%$, **L2**: $[Cu_2\mathbf{L2}]^{4+} = 100\%$, **L3**: $[Cu\mathbf{L3}]^{2+} = 100\%$, **L4**: $[Cu\mathbf{L4}]^{2+} = 65\%$, $[CuH\mathbf{L4}]^{3+} = 35\%$; **L5**: $[Cu\mathbf{L5}]^{2+} = 30\%$, $[CuH\mathbf{L5}]^{3+} = 70\%$ (**L5**:Cu^{2+} in 1:1 molar ratio), $[Cu_2\mathbf{L5}]^{4+} > 90\%$, (**L5**:$Cu^{2+}$ in 1:2 molar ratio); **L6**: $[CuH\mathbf{L6}]^{3+} = 60\%$, $[CuH_2\mathbf{L6}]^{4+} = 35\%$ $[CuH_3\mathbf{L6}]^{4+} = 5\%$.

more rigid macrocycles will have less tendency than linear chains with more flexible amines to form a maximum number of contact ion pairs with the groove phosphates. With certain azoniacyclophanes we did indeed observe an unusually large ΔT_m value only in the case of close geometric matching between the cationic and anionic groups.[11]

The phenanthroline-containing ligand **L3** shows more affinity towards both DNA and RNA than the linear amine counterpart (diethylenetriamine),[18] which suggests that the phenanthroline moiety is indeed intercalating. Interestingly, **L3** shows discrimination behavior: it does not show a preference for RNA, which is in contrast to the difference observed with the other ligands. Similarly, both **L4** and **L5** have more affinity towards DNA; however the affinity for RNA is lower than the affinity of their linear amine counterparts[18] (triethylenetetramine and tetraethylenepentaamine, respectively). Both the ligands **L4** and **L5** also show discrimination behavior similar to that exhibited by **L3**. **L6** leads to a smaller affinity towards RNA than the linear amine analogue (tripropylenetetraamine),[18] while the binding to DNA is comparable to the linear amine; however, in contrast to **L3–L5**, it is not stronger than towards RNA. Thus, the phenanthroline-based macrocycles investigated in this work are promising ligands for double-stranded DNA.

The average number of positive charges carried by the free ligand and of the corresponding Cu^{II} complexes is similar (see footnotes of Tables 3 and 4), except in the case of the binuclear complex of **L5**. The ternary/quaternary complexes between of ligand, metal ion and double-stranded nucleic acids were studied to unveil a possible change of affinity in spite of their similar charges. The ΔT_m values of all Cu^{II} complexes (Table 4) show that the affinity of the free ligand and the metallated ligands are not dramatically different. However, the binuclear Cu^{II} complex of **L5** exhibits a markedly higher affinity towards RNA, unlike the mononuclear complex of the same ligand.

Nuclease activity: The ability of the Cu^{II} complexes of the ligands to cleave supercoiled plasmid DNA is summarized in Table 5 and the cleavage pattern is shown in Figure 3. As discussed above, all ligands form very stable complexes with the Cu^{II} ion and there is no free copper ion under the conditions employed. Very slow cleavage was observed with the Cu^{II} complex of **L1**. Unfortunately, for the Cu^{II} complexes with **L2**, no data could be obtained as the bands of DNA did not move in the gel even after treatment with an ion-exchange resin which has been successfully used with other polyamine

Table 5. Cleavage of pBR322 plasmid DNA.[a, b]

Cu^{II} complex	RFI [%]	RFII [%]	RFIII [%]
L1 · $2CuCl_2$	88	12	0
L2 · $2CuCl_2$	[c]		
L3 · $CuCl_2$	16	76	8
L4 · $CuCl_2$	25	75	0
L5 · $CuCl_2$	53	46	0
L5 · $2CuCl_2$	0	87	13
L6 · $CuCl_2$	smearing		

[a] Reaction conditions: [metal complex] = 1×10^{-3}M; [DNA] = 1.9×10^{-5}M (base pair); 0.01M EPPS buffer; pH 7.0; 37 °C; incubation time 2 h. [b] Corrected for the decreased stainability of RFI by a factor of 1.22; corrected for the impurity of RFII in starting material and background noise; double runs, the calculation error is ±2.5%. [c] No bands could be located.

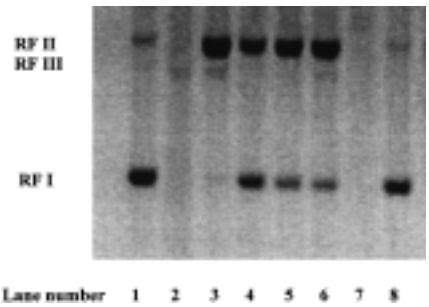

Figure 3. Gel electrophoresis of the cleavage of pBR322 plasmid DNA with the relevant RF bands (for conditions see footnote of Table 5). Lane 1: control DNA, lane 2: **L6**·$CuCl_2$, lane 3: **L5**·2$CuCl_2$, lane 4: **L5**·$CuCl_2$, lane 5: **L4**·$CuCl_2$, lane 6: **L3**·$CuCl_2$, lane 7: **L2**·2$CuCl_2$, lane 8: **L1**·2$CuCl_2$.

ligands.[7d] The results obtained with the Cu^{II} complexes of **L3**, **L4**, **L5**, and **L6** are more promising. These ligands contain one phenanthroline unit (OP) and a polyamine chain of different nature. The linear form, the RF III band, which is the result of more than one cleavage, was observed with **L3**·$CuCl_2$ along with the RF I and RF II bands. However, in the case of **L4**·$CuCl_2$ and **L5**·$CuCl_2$, only RF I and RF II were seen. Interestingly, for **L5**·2$CuCl_2$, the RF I form was completely cleaved and only RF II and RF III bands were observed. Smearing in the gels was observed in the case of **L6**·$CuCl_2$, which indicates multifragmented product.

Because of the high activity of the complexes, which contain the phenanthroline unit, these systems were investigated further in order to clarify the cleavage mechanism (Table 6).

Table 6. Cleavage of pBR322 plasmid DNA.[a, b]

Cu^{II} complex	Relative quantities [%] of RF I, RF II, and RF III, with addition of:			
	1M DMSO	1M *t*BuOH	0.1M NaN_3	0.1M EDTA
L3·$CuCl_2$	33, 67, 0	35, 65, 0	51, 49, 0	73, 27, 0
L4·$CuCl_2$	35, 65, 0	34, 66, 0	16, 84, 0	69, 31, 0
L5·$CuCl_2$	60, 40, 0	59, 41, 0	72, 18, 0	69, 31, 0
L5·2$CuCl_2$	0, 100, 0	0, 100, 0	41, 59, 0	68, 32, 0
L6·$CuCl_2$	smearing	smearing	0, 100, 0	51, 49, 0

[a] See footnotes of Table 5. [b] Complexed species in solution at pH 7 (calculated from the values of the stability constants given in Table 2 for ligands **L3**–**L6** and from refs. [12b, 14] for **L1** and **L2**): **L1**: $[Cu_2\mathbf{L1}]^{4+}$ > 95%, **L2**: $[Cu_2\mathbf{L2}]^{4+}$ > 95%, **L3**: $[Cu\mathbf{L3}]^{2+}$ = 100%, **L4**: $[Cu\mathbf{L4}]^{2+}$ = 90%, $[CuH\mathbf{L4}]^{3+}$ = 10%; **L5**: $[Cu\mathbf{L5}]^{2+}$ = 70%, $[CuH\mathbf{L5}]^{3+}$ = 30% (**L5**:Cu^{2+} in 1:1 molar ratio), $[Cu_2\mathbf{L5}]^{4+}$ = 75%, $[Cu_2\mathbf{L5}(OH)]^{3+}$ = 25% (**L5**:Cu^{2+} in 1:2 molar ratio); **L6**: $[CuH\mathbf{L6}]^{3+}$ = 90%, $[CuH_2\mathbf{L6}]^{4+}$ = 10%.

Metal-complexing agents, such as EDTA, inhibit the nuclease activity to a large extent, which indicates the essential role of the metal ion. Also, the smearing which was observed in **L6**·$CuCl_2$ was not observed if EDTA was added during incubation. Quenchers of hydroxyl radicals,[19] such as DMSO and *t*BuOH, were less effective as inhibitors compared with the singlet-oxygen quencher[20] NaN_3 (except in **L4**·$CuCl_2$). These findings suggest that there should be no or only a small contribution from hydroxyl radicals; however, participation singlet oxygen in redox processes cannot be ruled out.

Hydrolysis of BNPP (bis(*p*-nitrophenyl)phosphate): All hydrolysis reactions of BNPP with the Cu^{II} complexes showed pseudo-first order behavior; the rate constants are presented in Table 7. The binuclear complexes of **L1** and **L2**, namely **L1**·2$CuCl_2$ and **L2**·2$CuCl_2$ showed less efficiency and only

Table 7. Cleavage of BNPP.[a]

Cu^{II} complex	$k_{obs} \times 10^6\ s^{-1}$	k_{rel}
L1·2$CuCl_2$	2.3[b]	0.82×10^5
L2·2$CuCl_2$	4.0[b]	1.42×10^5
L3·$CuCl_2$	50	17.8×10^5
L4·$CuCl_2$	35[c]	12.5×10^5
L5·$CuCl_2$	6.2[d]	2.21×10^5
L5·2$CuCl_2$	140	50.0×10^5
L6·$CuCl_2$	61[e]	21.78×10^5

[a] Reaction conditions: [metal complex] = 1×10^{-3} M; [BNPP] = 3.76×10^{-5} M; 0.01M EPPS buffer; pH 7.0; 75 °C. $k_{rel} = k_{cor}/k_0$, where $k_{cor} = k_{obs}$/[metal complex] and $k_0 = 2.6 \times 10^{-8}\ s^{-1}$ (ref. [7e]). [b] Calculated for the first 10% release of *p*-nitrophenolate. [c] Calculated from 200 min onwards. [d] Calculated from 300 min onwards. [e] Calculated from 300 min onwards (biphasic curve).

≈10% of the *p*-nitrophenolate was released before saturation. In contrast, the Cu^{II} complexes of the phenanthroline-based macrocycles exhibited higher activity with complete hydrolysis of BNPP. The relative efficiencies of the mononuclear complexes were in the order **L6**·$CuCl_2$ > **L3**·$CuCl_2$ > **L4**·$CuCl_2$ > **L5**·$CuCl_2$, and the highest activity was shown by the binuclear complex **L5**·$CuCl_2$.[7e]

In order to explain the cleavage results, the following arguments may be plausible: When the mononuclear Cu^{II} complexes of **L3** to **L4** to **L5** are compared, the bound metal ion becomes more and more coordinatively saturated. The data of BNPP hydrolysis and DNA cleavage suggest that coordinative unsaturation around the complexed Cu^{II} ion favors its activity. Most interesting is the remarkable activity observed with the binuclear Cu^{II} complex of **L5**. The presence of a hydroxide anion bound to a metal (≈25% of the total complex at 298 K) even at neutral pH conditions could be responsible for the high activity. Alternatively, the remarkable efficiency of the Cu^{II} complex of **L6** can be explained by the fact that this system exists completely in its protonated form under neutral pH conditions. Such species could favor a salt bridge with the phosphate anion moiety and activate[21] the leaving group or stabilize the transition state. Also, the larger number of charges can lead to a higher concentration of the substrate–catalyst complex, which would lead to a correspondingly more favorable Michaelis–Menton K_M value. Intercalation of the phenanthroline units in **L3** to **L6** in double-stranded DNA or stacking with the phenyl groups in BNPP is also expected to enhance the activity by improved binding to the substrates.

Experimental Section

PolyA–polyU, polydA–polydT, BNPP, MES, and EPPS were obtained from Sigma while pBR322 plasmid DNA was acquired from Pharmacia. The synthesis of the ligands and their protonation behavior has already been reported;[12] the copper(II) complexes were obtained by the addition of

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the appropriate amount of a standardized solution of $CuCl_2$ to a solution of ligand and buffer. The pH was adjusted with a Knick digital pH-meter 646. Crystals of [Cu**L5**]$(ClO_4)_2$ suitable for X-ray analysis were obtained by slow evaporation of an aqueous solution which contained **L5** and $Cu(ClO_4)_2$ in an equimolecular ratio. UV/Vis spectra were recorded on a Shimadzu UV-2101PC spectrophotometer.

X-ray structure analysis: Analysis of a prismatic, blue, single crystal of [Cu**L5**]$(ClO_4)_2$ was carried out at 298 K with a Siemens P4 automatic four-circle X-ray diffractometer, equipped with a rotating anode and graphite-monochromated $Cu_{K\alpha}$ radiation ($\lambda = 1.5418$ Å). Cell parameters were determined by least-squares refinement of diffractometer setting angles for 25 carefully centered reflections (θ range 12–23°). The intensity of three standard reflections were monitored during data collection to check the stability of the diffractometer and of the crystal; no loss of intensity was observed. A summary of the crystallographic data is given in Table 8. A total of 6604 reflections ($\theta_{max} = 55°$) were collected. Intensity data were corrected for Lorentz and polarization effects and an absorption correction was applied, once the structure was solved, by the DIFABS[22] method (φ and μ corrections, max. = 1.240197, min. = 0.790898; θ correction: max. = 1.149838, min. = 0.474041). The structure was solved by the direct method of the SIR 97[23] program. The refinement was performed by means of the full-matrix least-square method of the SHELXL 93[24] program, which uses the analytical approximation for the atomic scattering factors and anomalous dispersion correction for all atoms from a reference.[25] All the non-hydrogen atoms were anisotropically refined while the hydrogen atoms were introduced in calculated position, and their coordinates refined in agreement with those of the atoms to which they are linked. The temperature factors of the hydrogen atoms were isotropically refined. Some degree of disorder was found for the perchlorate anions which gave rise to high thermal parameters for the oxygen atoms. A slight disorder also affected the carbon atoms of the phenanthroline unit in molecule **A**. The final agreement factors for 721 refined parameters were $R1 = 0.0869$ (for 6604 reflections with $I > 2\sigma(I)$) and $wR^2 = 0.2528$ (for all data).

Table 8. Crystal data and structure refinement for [Cu**L5**]$(ClO_4)_2$.

empirical formula	$C_{22}H_{26}Cl_2CuN_7O_8$
M_r	1301.88
T [K]	298
radiation	1.54178 Å
crystal system	monoclinic
space group	$P2_1/n$
a [Å]	14.668(2)
b [Å]	18.834(3)
c [Å]	19.975(4)
β [°]	100.490(1)
V [Å^3]	5426(2)
Z	4
ρ_{calcd} [g cm^{-3}]	1.594
absorption coefficient [mm^{-1}]	3.478
$F(000)$	2672
crystal size [mm]	0.3 × 0.25 × 0.1
θ [°]	3.25–55.00
index ranges	$-1 \le h \le 15$, $-1 \le k \le 20$, $-21 \le l \le 21$
reflections collected	8091
independent reflections	6604 [R(int) = 0.0493]
refinement method	full-matrix least-squares on F^2
data/restraints/parameters	6604/0/721
goodness-of-fit on F^2	1.021
final R indices [$I > 2\sigma(I)$]	$R1$[a] = 0.0869, wR^2[b] = 0.2463
R indices (all data)	$R1$[a] = 0.0921, wR^2[b] = 0.2528
largest diff. peak and hole [e Å^{-3}]	0.943 and −0.550

[a] $R = \Sigma \mid F_o \mid - \mid F_c \mid / \Sigma \mid F_o \mid$. [b] $wR^2 = [\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w F_o^4]^{1/2}$.

Crystallographic data (excluding structure factors) for the structure [Cu**L5**]$(ClO_4)_2$ reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-141 349. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). See also Supporting Information.

Potentiometric measurements: Equilibrium constants for Cu^{II} complexation with **L2**, **L3**, and **L6** were determined by pH-metric measurements at 298.1 K, with the equipment[26] and procedure[12b] which have already been described. The titrations were performed in the pH range 2.5–10.5 with CO_2-free aqueous solutions containing the ligand and/or the metal ion with concentrations of 1×10^{-3} to 2×10^{-3} mol dm^{-3}. At least three titration experiments (≈100 data points each) were performed for each system. The ionic strength was 0.1 mol dm^{-3} NMe_4Cl ($pK_w = 13.83$ at 298.1 K). The computer program HYPERQUAD[27] was used to calculate both protonation and stability constants from e.m.f. data.

Thermal melting curves: Thermal melting curves were obtained with a Cary 1 Bio UV/Vis spectrophotometer connected to a temperature controller and interfaced to a PC. The melting curves were recorded as described in the literature[28] at different compound-to-nucleic acid phosphate ratios (r) by following the absorption change at $\lambda = 260$ nm as a function of temperature with a heating rate of 0.5 °C min^{-1}. T_m values were determined from the maximum of the first derivative or tangentially from the graphs at the midpoint of the transition curves. ΔT_m values were calculated by subtracting T_m of the free nucleic acid from T_m of the complex.

DNA cleavage experiments: Samples were incubated at 37 °C for 2 h in 10 μL samples, as described previously.[7d] The reactions were quenched by the addition of 2 μL of a loading buffer that contained saccharose (40 wt.%), TRIS (0.89 M), boric acid (0.89 M), EDTA (1.0 M), and a little bromophenol blue. The macrocyclic polyamines inhibited the migration of DNA in the electrophoresis gel; this was overcome by the use of ion-exchange resin as previously reported.[7d] Electrophoresis was conducted on 0.9% agarose in a horizontal gel apparatus at 70 V for 2 h. The electrophoresis buffer contained TRIS (0.89 M), boric acid (0.89 M), EDTA (2 mM), and ethidiumbromide (0.5 μg mL^{-1}). Quantification after electrophoresis was performed with an "Eagle Eye II" densitometry system using the "Zero-Dscan" software from Scanlytics.

BNPP cleavage experiments: The rate of *p*-nitrophenolate release was monitored[7e] at $\lambda = 400$ nm ($\varepsilon = 6430$ M^{-1} cm^{-1}) with a Cary 1 Bio UV/Vis spectrophotometer at 75 °C. The required amount of BNPP solution was added to 1 mL of the reaction solution in a quartz semi-microcuvettes of 1 cm path length. The reaction was monitored for a period of 1000 min. First-order rate constants were calculated from treatment of the data that used a first-order rate law.

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