Synthesis of metal complexes of 2,9-bis(2-hydroxyphenyl)-1,10-phenanthroline and their DNA binding and cleaving activities

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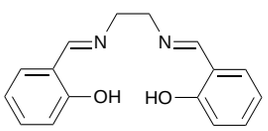
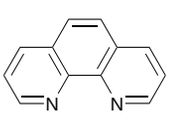
A series of metal complexes that combine the structure of phenanthroline and salen have been synthesized and characterized by electron paramagnetic resonance spectroscopy. The effects of the 2,9-bis(2-hydroxyphenyl)-1,10-phenanthroline compounds complexed with Cu^{II}, Ni^{II}, Co^{II} or Mn^{III} on the temperature-dependent helix-to-coil transition of DNA have been measured. The interaction with DNA is metal-dependent and the highest stabilization is observed with the Co complex. The DNA cleaving activities have been studied with plasmid DNA and/or with a ^{32}P-labelled duplex oligonucleotide depending on the redox properties of the complexes. The Cu complex is inactive whereas the Co chelate efficiently cleaves DNA in the presence of a reducing agent. Cleavage of DNA by the Mn complex can occur either in the presence of a reducing agent *via* the production of oxygen radicals (which are detected by EPR spectroscopy) or in the presence of an oxidant such as $KHSO_5$. In both cases, the cleavage of nucleic acids is very efficient whereas no cleavage is observed with the Ni complex. The complexes of bis(hydroxyphenyl)phenanthroline with Mn and Co complement the tool-box of reagents available for cleavage of DNA.

For a long time metallosalen catalysts have been used for selective olefin epoxidation and hydrocarbon oxidation.[1] Bis(salicylidene)ethylenediamine and other salen-type Schiff bases can form stable square-planar complexes with metal ions such as Cu^{II}, Ni^{II}, Co^{II} and Mn^{III}, forming four-membered chelate rings *via* coordination of two oxygen and two nitrogen atoms. In recent years, salen catalysis has been introduced in nucleic acid chemistry, as a probe for investigating DNA and RNA structure. We have previously shown that salen–copper complexes interact with double-stranded DNA and can induce non-selective cleavage in the presence of 2-mercaptopropionic acid.[2] A reducing agent is also needed to promote DNA cleavage by salen–cobalt complexes.[3] In contrast, due to differing redox properties, nickel- and manganese-bound salens produce DNA cleavage in the presence of an oxidant.[4–7]

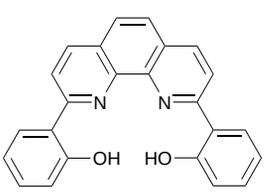
A wide range of metal complexes are available for the scission of nucleic acids. Peptides (*e.g.* GHK-Cu, VIHN-Ni), polypyridyl (*e.g.* 2,2′-bipyridyl–Fe) and ethylenediamine compounds can serve as chemical nucleases.[8] One of the most effective metal-based nucleases is the copper complex of 1,10-phenanthroline which has been extensively used for mapping protein and drug binding sites on DNA as well as for studying DNA structure.[8,9] It is also of considerable practical importance as a RNA cleaver. These considerations have prompted us to synthesize a new type of transition metal-based DNA cleavage agent that combines the features of both salen and phenanthroline ligand frameworks. The rationale behind this molecular architecture is that the introduction of the phenanthroline structure can reinforce the interaction with DNA and may stabilize the metal complex so as to promote the redox-dependent cleavage process. Here we report the synthesis and DNA-binding and cleaving properties of a 2,9-bis(2-hydroxyphenyl)-1,10-phenanthroline complexed with either Cu, Ni, Co or Mn.



salen



phenanthroline



1,10-bis(2-hydroxyphenyl)phenanthroline

Synthesis

The key intermediate for the synthesis of the different transition metal complexes was the symmetrical compound **3**. Initially, the protected ligand **2** was obtained by addition of 2-methoxyphenyllithium to 1,10-phenanthroline **1**, followed by an oxidation with MnO_2.[10–12] We used 40 equivs. of aryllithium to produce the disubstituted adduct **2**. Deprotection of the methoxy group by BBr_3 at low temperature[10–12] afforded ligand **3**. Finally, different complexations of **3** were achieved using copper,[2] nickel,[13] manganese[14] or cobalt[15] acetates to afford compounds **4**, **5**, **6** and **7**, respectively.

Results and discussion

The ability of the metal complexes to alter the thermal denatur-

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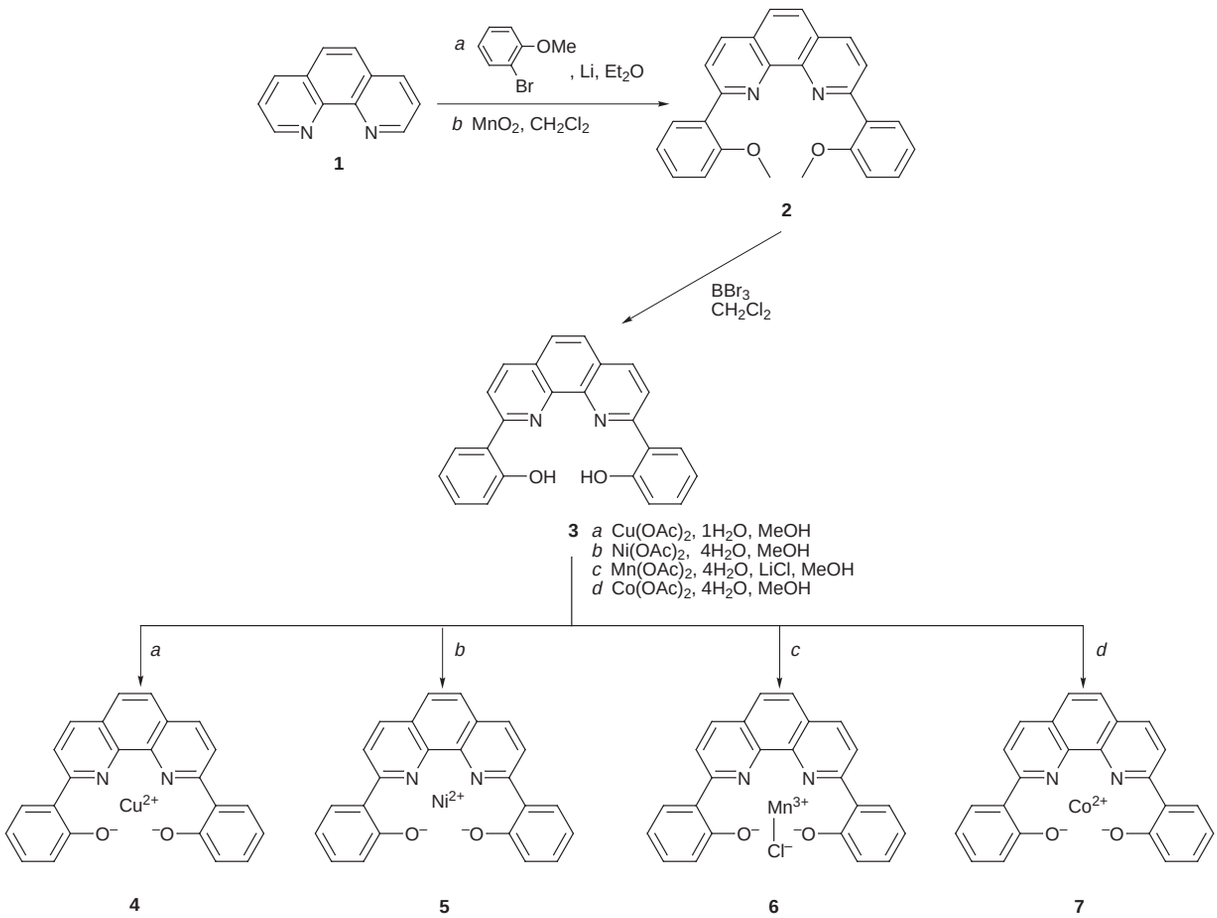

ation profile of DNA can be used as an indication of their propensity to bind to DNA. The complexes formed between DNA and compounds **4**–**7** displayed in each case simple, monophasic, melting curves (the transition remains sharp even though the T_m changes). The effects of increasing the concentration of compounds **4**–**7** on the T_m of the helix-to-coil transition of calf thymus DNA are shown in Fig. 1. The ΔT_m values correspond to the difference between the T_m measured in the presence of the ligand and that obtained with the free DNA. A large increase in the T_m of nucleic acids is observed for compound **7** and to a lower extent with compound **5** whereas the stabilisation of the duplex structure is weak with compound **6** and very little effect was observed with the copper complex **4**. The stabilization of the DNA double helix by binding of the Co complex increases smoothly with an increasing molar ratio of drug to DNA-phosphate (D/P). The stabilizing action of the Ni complex is substantial but always smaller than that of the Co analogue. The complexes rank in the order **7**–Co > **5**–Ni > **6**–Mn > **4**–Cu. The effect is manifestly metal-dependent.

Due to different redox properties, the metal complexes were studied separately depending on the requirement of a reducing or an oxidizing cofactor to initiate the cleavage reaction. In parallel to the DNA cutting studies, we performed a series of electron paramagnetic resonance (EPR) measurements either to identify the metal complex formation or to detect the production of oxygen-based radicals by means of spin-trapping.

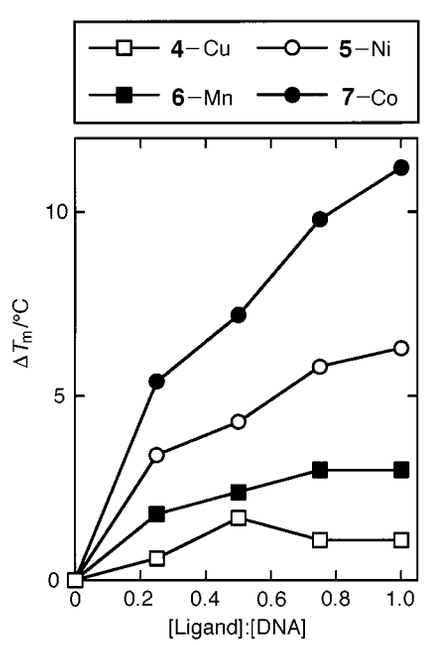

Fig. 1 Variation in difference melting temperature ($\Delta T_m = T_m^{\text{ligand-DNA}} - T_m^{\text{DNA}}$) measured with the four metal complexes at different ligand:DNA-phosphate molar ratios. T_m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1mM EDTA) using 20 μM calf thymus DNA (nucleotide conc) in 3 ml quartz cuvettes at 260 nm with a heating rate of 1 °C min^{-1}. Each drug concentration was tested in duplicate. The T_m of the DNA alone is 66 ± 1 °C.

Cu and Co complexes

The Co complex acts as a potent DNA cleaver in the presence of a reducing agent whereas, surprisingly, under identical conditions the Cu complex is totally inactive. No sharp EPR signal was observed with the copper complex attesting that the Cu atom is not properly complexed. Only a broad and unresolved spectrum was obtained from a frozen solution of **4**. The absence of a stable copper complex probably explains the lack of hydroxyl radical production and DNA cleavage. In contrast, the Co atom is perfectly complexed. Previous studies have shown that Co complexes give rise to an EPR spectrum typical

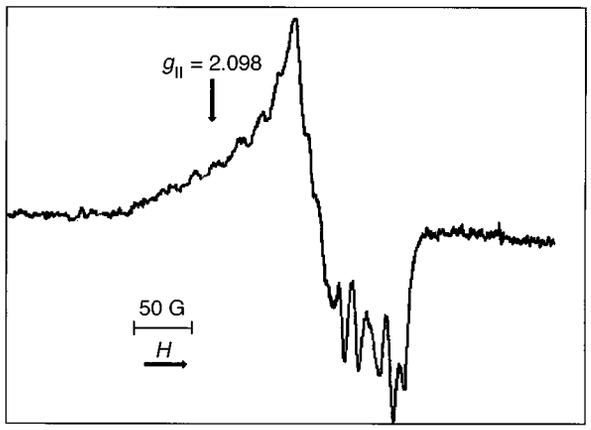

Fig. 2 EPR spectrum of Co^{II}–O_2 adduct complex at 77 K. Experimental settings: 20 mW power, 10 G modulation amplitude, gain 10^4 and scan rate 4 min.

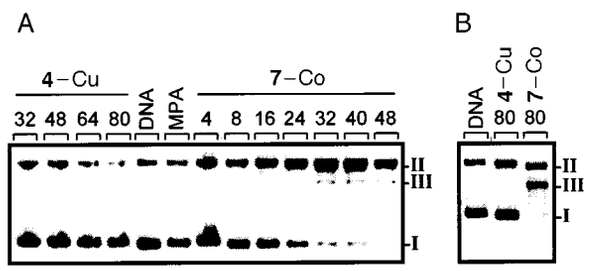

Fig. 3 Cleavage of closed circular pUC12 DNA (form I) by compounds **4**–Cu and **7**–Co in the presence of 2-mercaptopropionic acid (MPA) as reducing agent. Forms II and III refer to the nicked and linear DNA forms respectively. The drug concentration (μM) is indicated at the top of each gel lane. The lanes marked DNA and MPA refer to the plasmid DNA incubated without drug in the absence and presence of MPA, respectively. The DNA was incubated with the metal complex for (A) 1 h or (B) 6 h.

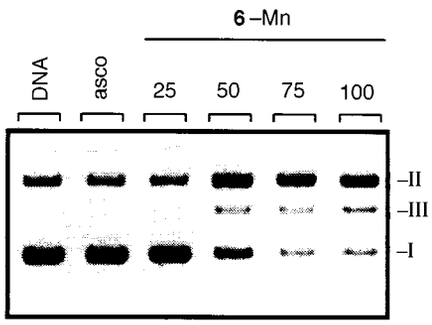

Fig. 4 Cleavage of closed circular pUC12 DNA (form I) by compound **6**–Mn in the presence of ascorbic acid as reducing agent. Forms II and III refer to the nicked and linear DNA forms respectively. The drug concentration (μM) is indicated at the top of each gel lane. The lanes marked DNA and asco refer to the plasmid DNA incubated without drug in the absence and presence of ascorbic acid, respectively.

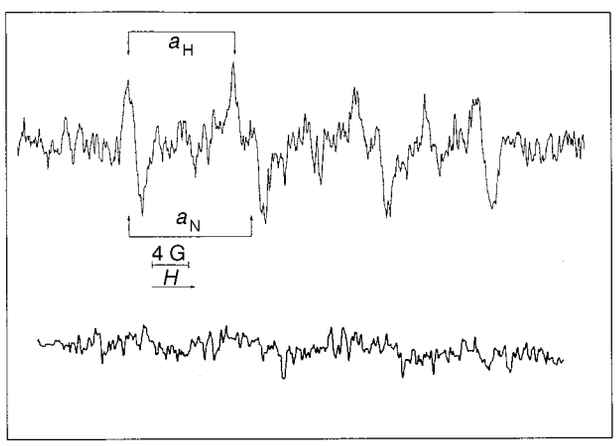

Fig. 5 EPR signal of DMPO–OOH adduct with complex **6** (upper trace) and DMPO control (lower trace) at room temperature. Experimental settings: 20 mW power, 1 G modulation amplitude, gain 3.2×10^4 and scan rate 4 min.

of a planar four coordinated Co^{II} type with nearly axial symmetry and an eight-line parallel hyperfine pattern due to interaction with the ^{59}Co ($I = 7/2$) nucleus.[16] Such a signal provides EPR parameters of $g_{\|} = 2.025$ and $g_{\perp} = 2.3$ and $A_{\|}^{Co} \approx 100$ G, characteristic of a low-spin Co^{II} form. Spin-trapping experiments failed to provide evidence for the formation of oxygen radicals. However, the Co complex reacts in the presence of oxygen. Under oxygenation the EPR parameters are drastically changed. As shown in Fig. 2, the spectrum of complex **7** exhibits the following EPR parameters: $g_{\|} = 2.098$, $g_{\perp} \approx 2.0$ and a hyperfine splitting constant A of 23 G due to ^{59}Co. Such EPR parameters are consistent with an oxygen adduct complex[17] and suggest that the unpaired spin density no longer resides on the cobalt metal center but instead on the dioxygen moiety.[18]

For both the Cu and Co complexes, DNA cleavage was analysed by monitoring the conversion of supercoiled plasmid DNA (form I) to the nicked circular molecules (form II) and linear DNA (form III). The tests were performed under aerobic conditions in the presence of 2-mercaptopropionic acid (MPA) as a reducing agent. No cleavage occurs in the absence of MPA or with uncomplexed Cu or Co (not shown). As shown in Fig. 3, the Co chelate efficiently cleaves DNA. Incubation of the plasmid at 37 °C for 1 h with 50 μM of compound **7** causes the complete conversion of form I to the nicked form II. We therefore conclude that the activation of the cobalt complex leads mainly to single strand cleavage of duplex DNA. As the ligand concentration increases the probability of double-strand scissions is enhanced once the DNA has undergone a single-strand break. This is manifested in the gel by the appearance of linearized DNA molecules (form III). The percentage of linear DNA molecules observed with the Co complex is much higher when the reaction is longer (6 h, Fig. 3, panel B) whereas the Cu complex remains inactive. The Co·bis(hydroxyphenyl)phenanthroline derivative **7** complements the tool-box of reagents which can be utilized to produce single-strand cleavage of DNA.

Mn complex

In this case the cleavage reaction can be activated by the addition of either ascorbic acid (reduction of Mn^{III} to Mn^{II}) or an oxidant such as $KHSO_5$ (oxidation of Mn^{III} to Mn^{V}). The two chemical pathways were tested.

A typical gel showing the cutting of DNA in the presence of the Mn complex is shown in Fig. 4. As with the Co complex, supercoiled DNA is easily converted into the nicked and linear forms. No cleavage was seen in the control lane in the absence of ascorbate which is required for the reduction of the manganese.

The complex **6** Mn^{III} redox state is an 'EPR silent' species due to its spin state $S = 2$. Therefore no relevant EPR signal of the metal could be detected. However, we employed EPR spectroscopy to monitor the production of oxygen radicals by spin-trapping using 5,5′-dimethylpyrroline *N*-oxide (DMPO). Fig. 5 shows the signal of the DMPO–OOH spin adduct with the characteristic hyperfine splitting constants $a_N = 14.0$ and $a_H = 12.1$ G which reflect the production of the superoxide anion radical $O_2^{\cdot -}$ by compound **6**.

The interaction of ascorbate (AH^-) with Mn^{III} complex resulted in two superimposed EPR signals (Fig. 6). The first one is characterised by a doublet centered around a g value of 2.005

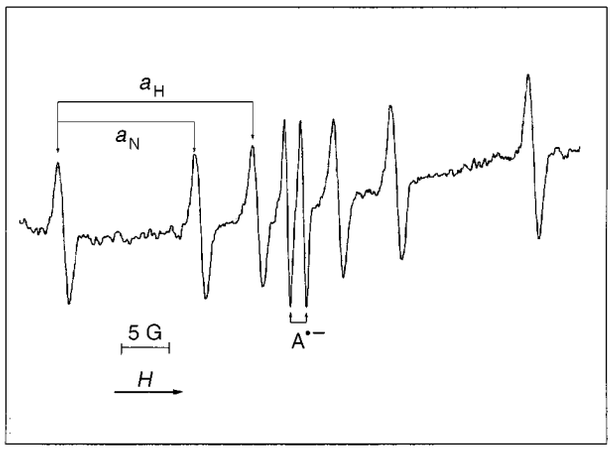

Fig. 6 EPR signal of DMPO–CH_3 adduct and ascorbyl radical ($A^{\bullet -}$) with complex **6** in the presence of ascorbic acid at room temperature. Experimental settings: 20 mW power, 1 G modulation amplitude, gain 3.2×10^3 and scan rate 4 min.

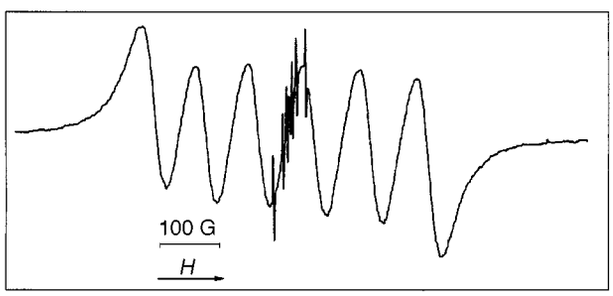

Fig. 7 EPR signal of Mn^{II} reduced form of complex **6** with signal of DMPO–CH_3 adduct and ascorbyl radical in the presence of ascorbic acid at room temperature. Experimental settings: 20 mW power, 5 G modulation amplitude, gain 2×10^3 and scan rate 4 min.

and a hyperfine splitting constant $a_H = 1.8$ G with a relative intensity of 1 : 1 for both peaks. The second one corresponds to the spin adduct DMPO–CH_3 with hyperfine splitting constants $a_N = 23.0$ and $a_H = 16.1$ G. For these spin-trapping experiments, the metal complexes, including compound **6**-Mn, were dissolved in pure DMSO at a concentration of 1 mM. The DMPO–CH_3 spin adduct indicates the production of hydroxyl radicals which then react with DMSO to give the methyl radicals. No DMPO–OH signal was detected. The use of pure DMSO explains the complete loss of the DMPO–OH spin adduct. It must be noted that the spectrum lineshape is modified indicating that another paramagnetic species is overlapped. This corresponds to the reduction of the Mn^{III} metal center upon interaction with ascorbate. The reduction of Mn^{III} yields the Kramer ion Mn^{II} ($S = 5/2$) detected by EPR spectroscopy. The spectrum in Fig. 7 shows six lines centered around a g value of 2.0 with hyperfine splitting constant $A_{\|} = 90$ G. The cleavage of DNA in the presence of compound **6** is therefore attributable to the hydroxyl radicals produced upon the ascorbate-induced reduction of the Mn ion. These hydroxyl radicals may arise *via* autooxidation of the low valent state with consequent formation of superoxide radicals and hence hydrogen peroxide.

Cleavage of DNA by salen ligands complexed with Ni and Mn can be achieved in the presence of oxygen donor compounds such as potassium monoperoxysulfate ($KHSO_5$) and magnesium monoperoxyphthalic acid (MMPP). Under these conditions, the plasmid assay employed with the other complexes is inapplicable because the DNA is partially cleaved in the presence of the activator alone. We therefore tested the cleavage activity of compounds **5** and **6** using a synthetic oligonucleotide duplex. The oligonucleotide 5′-CTCTTTTTGGTTATTCCC-3′ was ^{32}P-labelled at the 5′-end

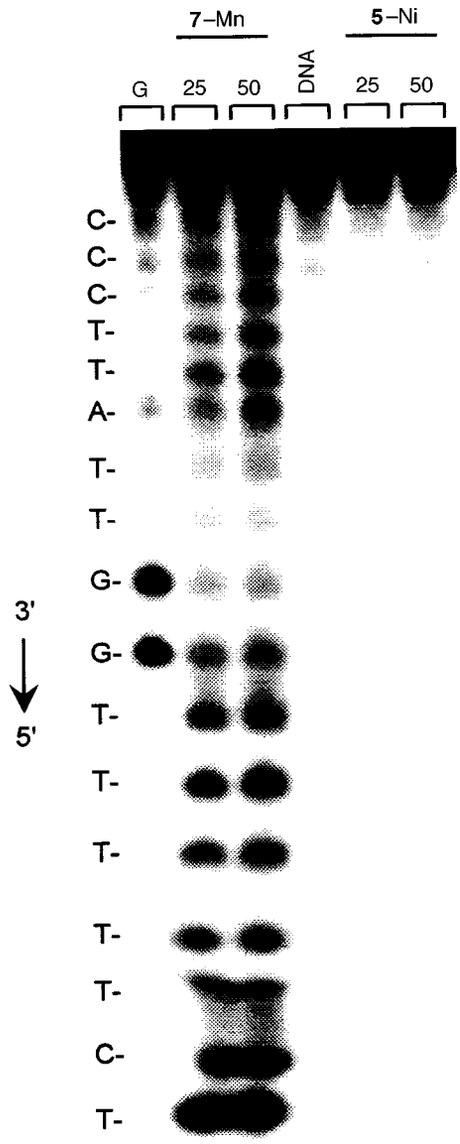

Fig. 8 Cleavage of the ^{32}P-labelled duplex oligonucleotides (2 μM) by compounds **5** and **7** at 25 and 50 μM in the presence of $KHSO_5$. The sequence of the labelled strand is indicated.

with polynucleotide kinase. After hybridisation with the complementary sequence and gel purification, the 20-mer DNA was subjected to cleavage by compounds **5** and **6** in the presence of $KHSO_5$. The cutting sites were visualized on the autoradiograms after boiling the DNA samples with piperidine. Practically no cleavage can be detected without alkaline treatment of the oxidized products. Also, there was absolutely no cleavage with the uncomplexed Ni and Mn metals or with $KHSO_5$ alone (not shown). As shown in Fig. 8, the Ni complex turned out to be inactive whereas the Mn complex cleaves DNA efficiently. The cleavage, which is probably mediated by a [salen–Mn^{V}–O]$^+$ intermediate, is essentially non-selective. Apart from the two T residues on the 3′ side of the GG doublet which are weakly cleaved, all the other bases are attacked by the Mn complex.

According to previous studies by Burrows and Rokita[4] and ourselves,[13] salen–Ni complexes can only cleave DNA at accessible guanine residues. The lack of cleavage of the 20-mer oligonucleotide by compound **5**–Ni may be attributed to the duplex structure that does not present unpaired guanines. However, we also tested the single-strand oligonucleotide with two adjacent unpaired guanine residues but no cleavage was observed with the Ni complex (data not shown).

In conclusion, we have designed a new type of chemical nuclease based on the 2,9-bis(2-hydroxyphenyl)-1,10-phen-

anthroline structure. There is a good agreement between the EPR data and the results of the DNA binding and cleaving experiments. The geometry of the designed ligand is apparently not well suited for complexation with Cu and Ni but is adequate for complex formation with Co and Mn. The ligand forms stable complexes with both Co and Mn. The Mn complex produces oxygen radicals that are responsible for the efficient cleavage of DNA under reducing conditions. A different mechanism must take place with the Co complex which does not generate detectable oxygen radicals but, however, behaves as a potent DNA cleaver. More elaborate analyses are required to define the nature of the intermediates and the exact mechanism of cleavage by the Co complex. The Mn complex can also function under oxidative conditions. With compound **6**, the objective of designing a versatile chemical nuclease has been fulfilled; this complex is now used as a footprinting probe for studying drug binding to nucleic acids.

Experimental

Materials

All chemicals were purchased from Aldrich Chemical Co. Solvents were distillated under argon prior to use. The purity of all compounds were assessed by TLC, ^{1}H and ^{13}C NMR spectroscopy, and by mass spectrometry. Kieselgel 60 (004–0063 mesh) was used for flash chromatography columns. TLC was carried out using silica gel 60F-254 (0.25 mm thick) precoated UV sensitive plate. Spots were visualized by inspection under visible light or UV at 254 nm. Melting points were determined using a hot plate microscope and are uncorrected. NMR spectra were recorded on a Bruker AC 300 NB. Chemical shifts were reported using trimethylsilane as an internal reference and are given in δ units and coupling constants (J) are given in Hz. MALDI (Mass Assisted Laser Desorption Ionisation) mass spectra were determined on a Finigan MAT vision 2000 (Bremen). The matrix used was dihydroxybenzoic acid–water.

2,9-Bis(2-methoxyphenyl)-1,10-phenanthroline 2

A solution of 2-bromoanisole (24 g, 128 mmol) in 12 cm^3 of dry diethyl ether, was added dropwise under argon to a suspension of lithium (1.97 g, 256 mmol) in 10 cm^3 of dry diethyl ether. After addition, the solvent was refluxed for 1 h to complete the reaction. The reaction mixture was cooled to room temperature and a solution of 1,10-phenanthroline (1.12 g, 6.4 mmol) in 30 cm^3 of toluene was added dropwise. The reaction mixture was then refluxed for 3 h and stirred for an additional 15 h at room temperature. The solution was cooled at 0 °C and quenched by addition of 64 cm^3 of dry methanol and 64 cm^3 of water. The reaction mixture was concentrated under reduced pressure and the aqueous solution extracted three times with 70 cm^3 of CH_2Cl_2. The combined organic phases were mixed with MnO_2 (50 g, 0.57 mol) for 3 h. After drying with $MgSO_4$ and filtration under Celite, the solvent was removed by distillation under reduced pressure. The product was purified by flash chromatography [light petroleum (bp 45–65 °C)–CH_2Cl_2 1:1] to afford compound **4** (1.04 g, 52%) as a pale yellow solid, mp 104–106 °C; ν_{max}(KBr)/cm^{-1} 2965, 1590, 1240; *m/z* (MALDI$^+$) 393.1 (M + 1)$^+$; R_f (Et_2O–CH_2Cl_2 90:10) 0.65; δ_H($CDCl_3$) 3.90 (s, 6H), 7.05 (d, *J* 8.3, 2H), 7.20 (td, *J* 7.5, 1, 2H), 7.40 (td, *J* 7.5, 1.7, 2H), 7.80 (s, 2H), 8.20 (d, *J* 1.7, 4H), 8.30 (dd, *J* 7.5, 1.7, 2H); δ_C($CDCl_3$) 55.79 (CH_3), 111.56 (CH), 120.56 (CH), 124.86 (CH), 125.84 (CH), 128.00 (Cq), 129.81 (Cq), 130.25 (CH), 132.46 (CH), 135.28 (CH), 146.24 (Cq), 156.26 (Cq), 157.57 (Cq). Anal. calc. for $C_{26}H_{20}N_2O_2$: C, 79.56; H, 5.14; N, 7.14. Found: C, 79.61; H, 5.09; N 7.18%.

2,9-Bis(2-hydroxyphenyl)-1,10-phenanthroline 3

In a 50 cm^3 flask flushed with argon, compound **4** (0.5 g, 1.3 mmol) was dissolved in 12 cm^3 of dry CH_2Cl_2 and cooled to −78 °C. A solution of BBr_3 (4.46 g, 18 mmol) in 5 cm^3 of CH_2Cl_2, was added dropwise over 1 h. After addition, the reaction mixture was stirred for 48 h. After hydrolysis was performed with 25 cm^3 of concentrated $NaHCO_3$, the precipitate was filtered and treated three times with 20 cm^3 of MeOH and *ca*. 30 mm^3 H_2SO_4. Solvents were distilled under reduced pressure. Crude material was purified by flash chromatography (CH_2Cl_2) to afford compound **3** as a white solid (46%, 0.220 g), mp 230–232 °C; ν_{max}(KBr)/cm^{-1} 3450, 2960, 1620; *m/z* (MALDI$^+$) 365.0 (M + 1)$^+$, 387.0 (M + Na)$^+$, 403.0 (M + K)$^+$; R_f (CH_2Cl_2) 0.4; δ_H([$^2H_6$]DMSO) 7.00 (td, *J* 8.3, 1.2, 2H), 7.10 (d, *J* 8.1, 2H), 7.40 (t, *J* 7.0, 2H), 8.00 (s, 2H), 8.20 (d, *J* 7.9, 2H), 8.55 (d, *J* 8.7, 2H), 8.65 (d, *J* 8.7, 2H), 10.15 (s, 2H); δ_C([$^2H_6$]DMSO) 117.82 (CH), 119.09 (CH), 120.28 (Cq), 121.18 (CH), 126.20 (CH), 127.30 (Cq), 128.53 (CH), 131.94 (CH), 138.20 (CH), 141.45 (Cq), 157.04 (Cq), 159.09 (Cq). Anal. calc. for $C_{24}H_{16}N_2O_2$: C, 79.09; H, 4.43; N, 7.69. Found: C, 79.15; H, 4.35; N, 7.74%.

2,9-Bis(2-hydroxyphenyl)-1,10-phenanthroline copper(II) complex 4

The compound **3** (0.1 g, 0.27 mmol) was dissolved in 2.2 cm^3 of dry ethanol. Then 2 equivs. of NaOH (0.02 g, 0.54 mmol) in 0.5 cm^3 of EtOH were added dropwise. After stirring for 15 min, a solution of $Cu(OAc)_2 \cdot 1H_2O$ (0.109 g, 0.54 mmol) in water (0.7 cm^3) was added. The reaction mixture was refluxed for 1.5 h, then concentrated under reduced pressure. After filtration, the residue was washed with ethanol. Compound **4** (60 mg, 52%) was obtained as a brown solid, mp >240 °C; ν_{max}(KBr)/cm^{-1} 2930, 2850, 1600, 1560, 1500; *m/z* (MALDI$^+$) 427.0 (M + 1)$^+$,448.1(M + Na)$^+$, 492.2 (M + K)$^+$, 852.9 (2M + 1)$^+$; R_f (CH_2Cl_2) 0.2. Anal calc. for $C_{24}H_{14}N_2O_2Cu$: C, 67.76; H, 3.32; N, 6.59. Found: C, 67.90; H, 3.29; N, 6.51%.

2,9-Bis(2-hydroxyphenyl)-1,10-phenanthroline nickel(II) complex 5

Compound **3** (0.27 mmol, 0.1 g) was dissolved in 2.2 cm^3 of dry ethanol. Then 2 equivs. of NaOH (0.02 g, 0.54 mmol) in 0.5 cm^3 of ethanol were added dropwise. After 15 min, $Ni(OAc)_2 \cdot 4H_2O$ (0.13 g, 0.54 mmol) was poured into the solution. The solvent was removed under reduced pressure and the crude product was washed with cold methanol. Compound **5** (40%, 44 mg) was obtained as a orange powder, mp >240 °C; ν_{max}(KBr)/cm^{-1} 3050, 2930, 2850, 1600, 1580, 1500; *m/z* (MALDI$^+$) 421.4 (M + 1)$^+$, 443.4 (M + Na)$^+$; R_f (CH_2Cl_2) 0.3; δ_H([$^2H_6$]DMSO) 7.03 (t, *J* 6.84, 2H), 7.09 (d, *J* 7.81, 2H), 7.41 (t, *J* 7.81, 2H), 8.09 (s, 2H), 8.28 (d, *J* 8.3, 2H), 8.61 (d, *J* 8.79, 2H), 8.71 (d, *J* 8.79, 2H); δ_C([$^2H_6$]DMSO) 117.83 (CH), 119.14 (CH), 120.63 (Cq), 121.30 (CH), 126.27 (CH), 127.36 (Cq), 128.59 (CH), 131.97 (CH), 138.27 (CH), 141.57 (Cq), 157.11 (Cq), 159.08 (Cq). Anal. calc. for $C_{24}H_{14}N_2O_2Ni$: C, 68.56; H, 3.36; N, 6.67. Found: C, 68.48; H, 3.30; N, 6.65%.

2,9-Bis(2-hydroxyphenyl)-1,10-phenanthroline manganese(III) chloride complex 6

Under argon, compound **3** (0.1 g, 0.27 mmol) was dissolved in 2.2 cm^3 of dry ethanol. Then 2 equivs. of NaOH (0.02 g, 0.54 mmol) in 0.5 cm^3 of freshly distilled ethanol were added dropwise. After 15 min, the solution was refluxed and the complex was obtained by addition of $Mn(OAc)_2 \cdot 4H_2O$ (0.134 g, 0.54 mmol) over 1.5 h. LiCl (0.104 g, 0.54 mmol) was then added to the reaction mixture which was placed in air and refluxed for an additional 30 min. The solvent was removed under vacuum. Water (25 cm^3) was added and the precipited was filtered and washed with cold ethanol. Compound **6** (58%, 72 mg) was obtained as a brown powder, mp >240 °C; ν_{max}(KBr)/cm^{-1} 3030, 2900, 2830, 1595, 1560, 1550, 1490; *m/z* (MALDI$^+$) 417.2 (M − Cl)$^+$, 833.8 (2M − 2Cl)$^{2+}$, *m/z* (MALDI$^-$) 34.9 (Cl$^-$), 36.9 (Cl$^-$); R_f (CH_2Cl_2) 0.0. Anal. calc. for $C_{24}H_{14}N_2O_2MnCl$: C, 63.72; H, 3.12; N, 6.20. Found: C, 63.68; H, 3.08; N, 6.15%.

2,9-Bis(2-hydroxyphenyl)-1,10-phenanthroline cobalt(II) complex 7

Under argon, compound **3** (0.05 g, 0.13 mmol) was dissolved in 25 cm^3 of deoxygenated methanol. The solution was stirred vigorously and $Co(OAc)_2 \cdot 4H_2O$ (0.035 g, 0.14 mmol) was then added. The solution was refluxed for 3 h. After cooling, the precipitate was filtered off and washed three times under argon with dry methanol. Complex **7** was obtained as a red powder (83%, 0.045 g), mp > 240 °C; ν_{max}(KBr)/cm^{-1} 3020, 2950, 1600, 1590, 1540, 1510, 1490; *m/z* (MALDI$^+$) 421.3 $(M)^{\cdot +}$, 422.5 $(M + 1)^+$, 444.5 $(M + Na)^+$, 460.5 $(M + K)^+$; R_f (CH_2Cl_2) 0.3. Anal. calc. for $C_{24}H_{14}N_2O_2Co$: C, 68.40; H, 3.35; N, 6.65. Found: C, 68.45; H, 3.29; N, 6.70%.

Melting temperature studies

Melting curves were measured using a Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. For each series of measurements, 12 samples were placed in a thermostatically controlled cell-holder (10 mm pathlength) and the quartz cuvettes were heated by circulating water. The measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette was monitored by using a thermocouple in contact with the solution. The absorbance at 260 nm was measured over the range 20–100 °C with a heating rate of 1 °C min^{-1}. The 'melting' temperature T_m was taken as the mid-point of the hyperchromic transition.

Product analysis by EPR experiments

The X-band EPR spectra of the metal complexes (1 mM in DMSO) were recorded at 77 K or at room temperature using a Varian E-9 spectrometer operating with a 100 kHz magnetic field modulation. The *g* values were determined taking the strong pitch ($g = 2.0028$) as a standard. For spin trapping experiments 5 mM 5,5′-dimethylpyrroline *N*-oxide (DMPO) were added to the metal complex solution at 1 mM.

DNA cleaving activity

Experiments with plasmid DNA on agarose gels. Each reaction mixture contained 4 μl of supercoiled pUC12 DNA (0.5 μg), 5 μl of the tested compound at the desired concentration and 2 μl of either MPA (25 mM) or ascorbic acid (1 mM) to initiate the reaction. After 1 h incubation at 37 °C, 1 μl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) was added to each tube and the solution was loaded on to a 1% agarose gel. The electrophoresis was carried out for about 2 h at 100 V in TBE buffer (89 mM tris-borate, pH 8.3, 1 mM EDTA). Gels were stained with ethidium bromide (1 μg ml^{-1}) then destained for 30 min in water prior to being photographed under UV light.

Experiments with ^{32}P-labelled DNA on sequencing gels. The synthetic oligonucleotide was labelled at the 5′-end with γ-[^{32}P]-ATP (6000 Ci $mmol^{-1}$) and T4 polynucleotide kinase and then mixed with the complementary oligonucleotide sequence. The 20-mer duplex formed after cooling was purified on a 15% non-denaturing polyacrylamide gel. DNA samples were treated with the metal complex in the presence of 50 μM $KHSO_5$ for 30 min at room temperature and then precipitated with cold ethanol. The DNA pellet was resuspended in 30 μl of 1 M piperidine, boiled for 12 min at 90 °C and then lyophilized. Cleavage products were resuspended in 5 μl of 80% formamide containing 10 mM EDTA and 0.1% tracking dyes. Samples were heated to 90 °C for 4 min and then chilled in an ice-bath just before being loaded on a sequencing gel (15% polyacrylamide, 7 M urea).

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