Guanine and Plasmid DNA binding of Mono- and Trinuclear *fac*-$[Re(CO)_3]^+$ Complexes with Amino Acid Ligands

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We have synthesized and fully characterized four new complexes comprising the fac-*$[Re(CO)_3]^+$ moiety and the ligands NH_3, L-proline (Pro), or* N,N*-dimethylglycine (dmGly). The reaction of $[Re(H_2O)_3(CO)_3]^+$ with the two amino acids gives trinuclear complexes of general formula $[Re(L)(CO)_3]_3$ (where L = amino acid). We have studied the in vitro behavior of these compounds with guanine and DNA in order to understand whether the cytotoxicity exhibited by certain rhenium complexes based on the* fac-*$[Re(CO)_3]^+$ core is due to the formation of nucleobase complexes and inter- or intrastrand links between DNA bases. We have performed model studies with guanine and studied the structural effects induced by different rhenium(I) tricarbonyl complexes on ΦX174 plasmid DNA by electrophoretic methods. Our results show that rhenium complexes with two available coordination sites interact with plasmid DNA to form a stable adduct that is likely to involve two bases.*

Introduction

In the last few decades metal-based antitumor drugs have been playing an important role as therapeutic agents in antiblastic chemotherapy. Cisplatin and other Pt^{II}-based complexes remain the most effective inorganic drugs used in clinics but other transition-metal ions have received much attention as well. Among the more recent metal complexes that have been studied for their therapeutic potential, the octahedral geometry is often encountered. Ruthenium(II) and (III) compounds, for example, have raised great interest, and complexes of the type $[trans\text{-}RuCl_4(X)(X')]^+$ (X = imidazole, indazole; X′ = Me_2SO, indazole) have already entered phase I clinical trials.[1] Although the mode of action of these complexes is not yet well understood, there is evidence for DNA as a likely target, in a manner similar to the well-established platinum drugs.[2–19] Dinuclear rhodium acetate $[Rh_2(\mu\text{-}(O_2CCH_3)_4)(H_2O)_2]$ and related complexes also showed good antitumor activity,[20] and structural studies suggest an analogous activity to that of cisplatin by binding two adjacent purines on DNA.[21–24]

In the context of developing metal-based chemo- and radiopharmaceuticals, our attention was attracted to recent studies that reported the cytotoxicity of a number of different compounds, all based on the *fac*-$[Re(CO)_3]^+$ core. Studies on L1210 lymphoid leukemia and other cell lines indicated that rhenium(I) alkoxo/hydroxo carbonyl complexes were effective in suppressing DNA synthesis through the inhibition of dihydrofolate reductase and other enzymes in the purine and pyrimidine pathways. Interaction with DNA, however, was not ruled out and it was suggested that the compounds may bind to the purine bases after displacement of the alkoxide or hydroxide ligands.[25] Similarly, the cytotoxicity of rhenium(I) carbonyl 2-(dimethylamino)ethoxide complexes may involve binding to DNA bases or side chains of amino acid residues in peptides,[26] while phosphine-derivatized amine complexes seem unlikely to act as alkylating agents.[27]

We have recently shown that the $[M(CO)_3]^+$ moiety (M = Re, ^{99}Tc) can bind two guanine bases in a *cis* fashion,[28] and X-ray crystallography confirmed that the two bases assume both a head-to-head (HH) and a head-to-tail (HT) conformation around the Re core.[29] The two bases can freely rotate about the Re–N(7) bond, and neither intramolecular hydrogen bonding nor steric hindrance imposed by the carbonyl oxygen atom of the coordinated guanines are driving forces for the preference of one or the other conformation in the octahedral complex.[29]

In order to understand whether the cytotoxicity exhibited by some *fac*-$[Re(CO)_3]^+$ based complexes is due to an alkylating event resulting in the formation of inter- or intrastrand links between DNA bases, we have studied the interaction of a series of rhenium tricarbonyl complexes (Scheme 1) with ΦX174 plasmid DNA. For these studies we have prepared and fully characterized new complexes, three of which are based on L-proline (Pro) and *N*,*N*-dimethylglycine (dmGly). The complexes $[Re(Pro)(CO)_3]_3$ (**1**), $[Re(dmGly)(CO)_3]_3$ (**2**), and $[Re(Pro)(9\text{-}MeG)(CO)_3]$ (**3**; 9-MeG = 9-methylguanine) are rare examples of structurally characterized complexes with an amino acid bound to an organometallic Re moiety.

As $[Re(H_2O)_3(CO)_3]^+$ (**5**) interacts unspecifically with potential coordination sites of proteins in human serum, this drug is not available in relevant concentrations for therapeutic use unless the coordination sites are protected. Once the prodrug is inside a cell, the protecting ligands should be released (for example, due to a decreased pH value), thereby setting free the active form of the metal drug. Amino acids seem to be good candidates for this purpose, mainly for two reasons: they afford robust complexes and are not foreign to biological systems. Once displaced from the metal, they will not exhibit

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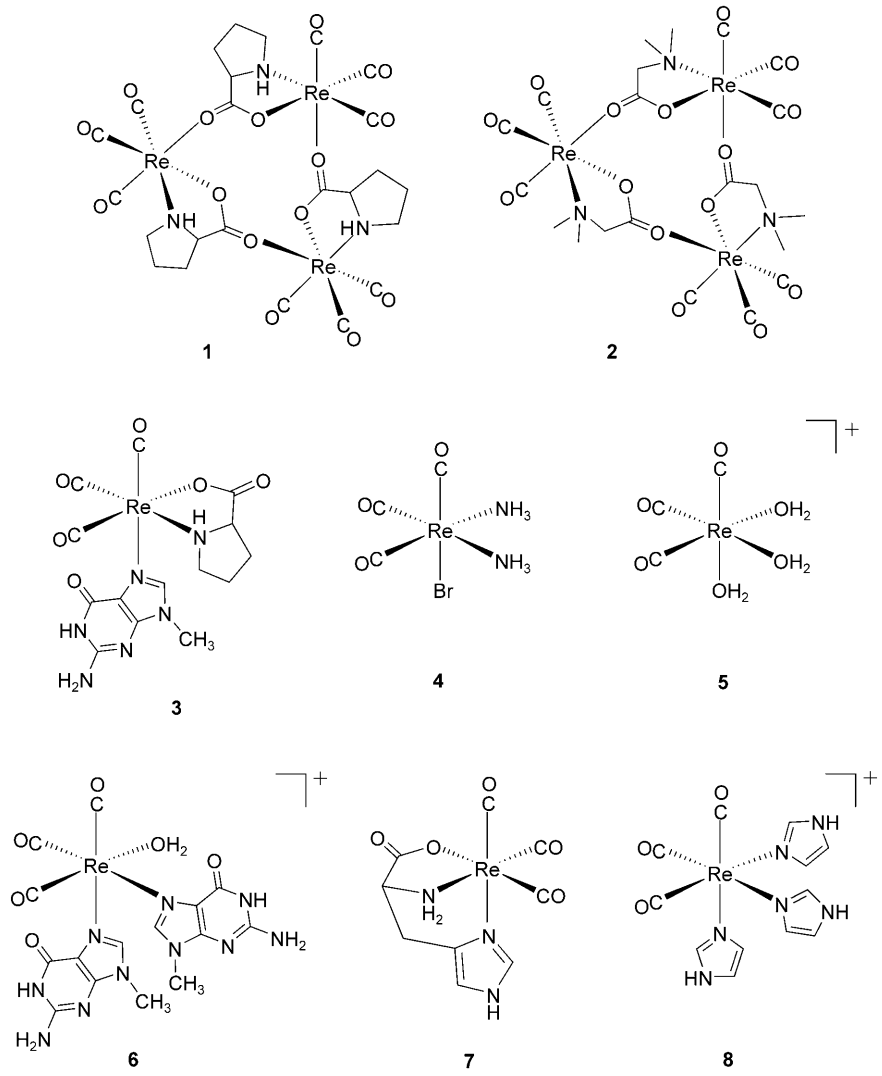

Scheme 1. Rhenium complexes investigated in this study.

toxic side effects but will simply be recycled in the biochemical pathways.

We show in this study that the reaction of **5** with the amino acids Pro and dmGly gives complexes **1** and **2** with an unusual trimeric structure in the solid state. Depending on the stability of the amino acid complex, the tertiary structure of ΦX174 plasmid DNA is influenced, as confirmed by gel electrophoresis. Complexes **1** and **2** can therefore be considered as prodrugs. The induced changes involve covalent binding to two bases, rather than a simple electrostatic interaction, a fact indicating that rhenium complexes might have potential as novel metal-based DNA-binding drugs.

Results and Discussion

Synthetic aspects

As previously mentioned, **5** is readily trapped in human serum by the serum proteins and is only released very slowly. Protection of the $[Re(CO)_3]^+$ moiety by ligands is required to prevent its interaction with serum proteins but the ligands must be labile enough to be displaced by the targeted molecules, for example, the bases in DNA.

Originally, the two NH_3 groups in $[ReBr(NH_3)_2(CO)_3]$ (**4**) seemed attractive as *cis*-labile exchangeable ligands, comparable to the chloride ligands in cisplatin and other transition-metal complexes with antitumor activity. In contrast to the latter, the NH_3 ligand was chosen to confer an overall neutral charge to the complex. Behrens and Pässler first prepared $[ReCl(NH_3)_2(CO)_3]$ by reaction of $[ReCl(CO)_5]$ in a benzene/liquid ammonia mixture under controlled pressure.[30] We found that the bromide can be more conveniently obtained by treating $[ReBr(CO)_5]$ with a NH_3-saturated benzene solution at 60 °C and normal pressure. The crystal structure has been elucidated and is discussed later. Complex **4** is soluble in polar organic solvents but hydrolyzes in a water/methanol mixture (1:1) to give **5** within a few minutes. The NH_3 ligand is, thus, too labile to serve as a protecting group for the $[Re(CO)_3]^+$ core, and we instead focused our attention on amino acids without coordinating side chains. The interaction of organometallic complexes with amino acids and peptides pioneered bioorganometallic chemistry[31–33] and has received much attention again in recent years.[34]

The reaction of **5** with Pro and dmGly gave the trinuclear complexes **1** and **2**, respectively. (ORTEP presentations are shown in Figures 3 and 4 and are discussed later.) The reaction goes to completion within a few hours in methanol/water (9:1) and in the presence of a slight excess of the amino acid, required to neutralize the protons released during reaction. Proline was chosen first because secondary amines are more weakly coordinating and are easier to replace.

Prior to the studies with DNA, the interaction of **1** with 9-MeG was studied as a model system. Complex **1** reacts with 9-MeG in a methanol/water mixture to give $[Re(Pro)(9\text{-MeG})(CO)_3]$ (**3**) in good yield as the only product even if excess nucleobase is applied. (The X-ray crystal structure could be elucidated and an ORTEP presentation is given in Figure 5). Evidently, proline, forming a stable five-membered ring upon co-

ordination, is still a strong bidentate ligand for the $[Re(CO)_3]^+$ core and cannot easily be displaced by free guanine or incorporated into DNA. Since **1** has a single available coordination site, the complex is unlikely to be able to form *cis*-bis intra- or interstrand adducts with the nitrogen atoms in purine or pyrimidine bases. Under the same conditions, DNA bases other than guanine showed little or no reaction with **1**.

The stability of amine coordination can be decreased for steric and electronic reasons by going from secondary to tertiary amines. Consequently, the reaction of **5** with dmGly was investigated. The reaction of **5** with dmGly yields the trinuclear complex **2**. It is noteworthy to mention at this point that **1** and **2** are obtained as trinuclear species in the solid state. In aqueous solution however, ^{1}H NMR spectroscopy and ESI-MS analysis suggests that the corresponding monomeric species $[Re(L)(H_2O)(CO)_3]$ (L = amino acid) are present. The related equilibria have not been investigated in detail as it is beyond the scope of this study, and we refer always to the trinuclear rather than the mononuclear species.

The behavior of **2** with 9-MeG differs greatly from that of **1**. In a water/methanol mixture at 50 °C, **2** reacts with an excess of 9-MeG in a stepwise manner. Complex **2** displays a retention time (R_t) of 15.5 min (Figure 1). After 1 h a second peak can be observed with an R_t value of 18.5 min. HPLC–MS analysis indicates that this species is $[Re(dmGly)(9\text{-MeG})(CO)_3]$ (**9**). Concomitantly, a third peak appears with an R_t value of 17.5 min; this peak was identified as $[Re(9\text{-MeG})_2(H_2O)(CO)_3]^+$ (**6**) by coinjection with a pure sample of this complex. The reaction proceeds further until a fourth peak appears with an R_t value of 16.5 min and an equilibrium is finally reached. This last species was identified by HPLC–MS analysis as $[Re(9\text{-MeG})(H_2O)_2(CO)_3]^+$ (**10**). The conversion of **2** into complexes **9** and **10** implies that dmGly can be displaced from the Re coordination sphere in favor of 9-MeG. As described in more detail later, the Re–N(1) and Re–O(1) bond lengths in **2** are 0.06 and 0.04 Å, respectively, longer than the equivalent bonds in **1**, and the N(1)–Re–O(1) bite angle is about 0.7° more acute in **2** (see the X-ray crystallography subsection below). These differences support weakening of the Re–N/O bonds in **2** and, consequently, an increased reactivity of **2**. Complex **2** might therefore be a good candidate for a model Re prodrug. While dmGly protects the $[Re(CO)_3]^+$ core from reaction with coordinating sites in proteins, the steric hindrance imposed on one (or both) of the coordinated atoms renders the ligand weak enough to be displaced by stronger (even monodentate) ligands such as guanine. We observed little or no transmetallation of **2** to serum proteins in human serum over 12 h at 37 °C.

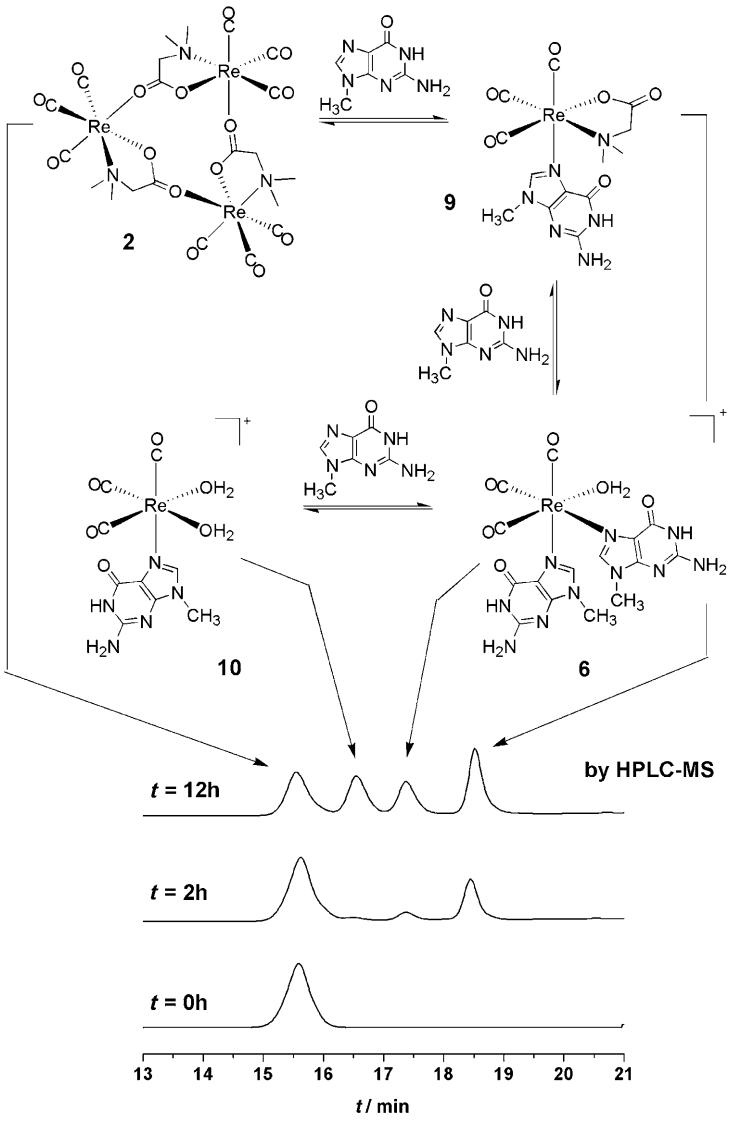

Figure 1. HPLC chromatogram of the reaction of **2** with excess 9-MeG and the proposed equilibrium scheme.

X-ray crystallography

Crystal data and experimental details are listed in Table 1. The crystal structure of compound **4** is shown in Figure 2. The structure consists of discrete molecules with short contacts occurring exclusively between Br, the carbonyl oxygen atoms, and the protons of NH_3 (on average NH–Br = 2.940(5) Å, NH–OC = 2.605(7) Å). The molecule crystallizes in the orthorhombic space group *Pnma* with Br, Re, and C(2)O lying on a mirror plane at $x,{}^3/_4,z$. The geometry around rhenium is octahedral with an Re–N(1) distance of 2.240(6) Å, which is comparable to the average Re–NH_2R distance (2.238 Å) in the bidentate ethylenediamine (en) of the related $[Re(en\text{–}N,N')(en\text{–}N)(CO)_3]^+$ complex and significantly longer than the average distance in the monodentate en.[35]

Figures 3 and 4 depict the molecular structure in the crystal of complexes **1** and **2**, respectively. Both molecules are macrocyclic trimers in which the rhenium atoms are bridged by the

Table 1. Summary of X-ray crystallography data.

	1	2	3	4
formula	$[C_{12}H_{16}NO_6Re]_3$	$C_{25}H_{34}N_3O_{16}Re_3$	$C_{17.5}H_{25}N_6O_7Re$	$C_3H_6BrN_2O_3Re$
M_w	1369.38	1191.15	617.64	384.21
crystal system	trigonal	monoclinic	monoclinic	orthorhombic
crystal size [mm^3]	0.25×0.07×0.06	0.26×0.04×0.02	0.57×0.17×0.08	0.15×0.12×0.08
space group	*R*3	$P2_1/c$	$P2_1$	*Pnma*
a [Å]	21.6864(11)	22.0917(10)	15.1130(5)	10.8300(6)
b [Å]	21.6864(11)	7.8752(4)	15.4589(7)	9.8501(7)
c [Å]	8.1989(3)	20.2707(12)	15.4093(5)	7.2163(8)
β [°]		92.768(6)	90.629(4)	
V [Å^3]	3339.3(3)	3522.5(3)	3599.9(2)	769.81(11)
Z	3	4	6	4
T [K]	183(2)	183(2)	183(2)	183(2)
goodness of fit on F^2	0.874	0.966	0.996	1.000
$R^{[a,b]}$	0.0302	0.0708	0.0328	0.0407
$wR^{2[a,c]}$	0.0596	0.1259	0.0821	0.0975
$\Delta\rho$ (max, min) [e Å^{-3}]	1.417, −0.587	2.492, −1.744	1.857, −0.550	1.687, −2.438

[a] Observation criterion: $I > 2\sigma(I)$. [b] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [c] $wR^2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.

carbonyl oxygen atom of the amino acid ligand, with the metal atoms being 5.43 Å apart from each other. In **1** the three Re atoms are related to each other by a threefold rotation axis, while in **2** there is no symmetry element that relates the three metal atoms. In both structures the rhenium is bonded in a distorted octahedral geometry with N–Re–O bite angles of 75–79°. All bond lengths are in good agreement with the structure of $[Re(His)(CO)_3]$ (**7**, see Scheme 1). Complexes **1** and **2** are rare examples of structurally characterized molecules in which an amino acid is bound to the $[Re(CO)_3]^+$ core and, to our knowledge, they represent the first example of a macrocyclic trimer of the $[Re(CO)_3]^+$ core that does not involve a μ-type bridge.

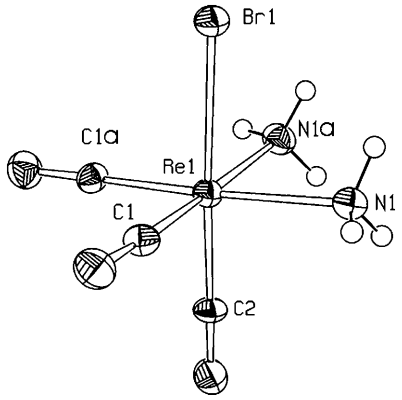

Figure 2. ORTEP view of $[ReBr(NH_3)_2(CO)_3]$ (**4**) with 50% probability for thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Re(1)–N(1) 2.240(6), Re(1)–Br(1) 2.6604(14); N(1)–Re(1)–Br(1) 84.65(17), N(1)–Re(1)–N(1a) 81.4(3).

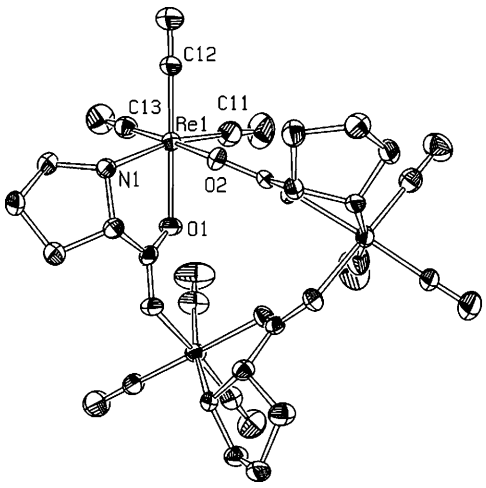

Figure 3. ORTEP view of $[Re(Pro)(CO)_3]_3$ (**1**) with 50% probability for thermal ellipsoids. Selected bond lenths [Å] and angles [°]: Re(1)–N(1) 2.205(5), Re(1)–O(1) 2.138(4), Re(1)–O(2) 2.195(4), Re(1)–C(11) 1.944(8), Re(1)–C(12) 1.900(6), Re(1)–C(13) 1.899(7); N(1)–Re(1)–O(1) 75.69(17), N(1)–Re(1)–O(2) 76.93(17), O(1)–Re(1)–O(2) 83.29(17).

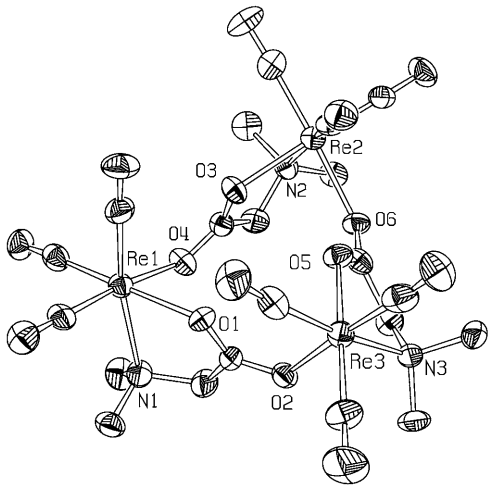

Figure 4. ORTEP view of $[Re(dmGly)(CO)_3]_3$ (**2**) with 50% probability for thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Re(1)–O(1) 2.172(8), Re(1)–O(4) 2.180(9), Re(1)–N(1) 2.260(10), Re(2)–O(3) 2.167(8), Re(2)–O(6) 2.200(8), Re(2)–N(2) 2.261(10), Re(3)–O(5) 2.146(8), Re(3)–O(2) 2.189(8), Re(3)–N(3) 2.263(10); O(1)–Re(1)–O(4) 83.5(3), O(1)–Re(1)–N(1) 75.0(4), O(4)–Re(1)–N(1) 78.6(4), O(3)–Re(2)–O(6) 84.2(3), O(3)–Re(2)–N(2) 75.7(3), O(6)–Re(2)–N(2) 79.0(4), O(5)–Re(3)–O(2) 83.2(3), O(5)–Re(3)–N(3) 75.6(3), O(2)–Re(3)–N(3) 77.4(3).

These unusual Re trinuclear complexes are closely related to a family of chiral-at-metal trimers of general formula $[\{(\eta^n\text{-ring})M(L)\}_3](BF_4)_3$ (where M = Ru, Os, Rh, Ir; L = amino acid) which result from the reaction of $[(\eta^n\text{-ring})MCl(L)]$ with $AgBF_4$.[34] In these cationic complexes, the aminocarboxylate ligand also acts as a tridentate bridging group where, as in **1** and **2**, the nitrogen atom and one the carboxylic oxygen atoms are bonded to a metal center, thereby forming a five-

membered metallacycle, and the remaining oxygen atom coordinates to a second neighboring metal center. The structures of **1** and **2** also resemble the self-assembled ruthenium macrocyclic ionophores with high affinity and selectivity for Li^+ and Na^+ that were recently described by Severin and co-workers.[36,37] The average O–O distance in **1** and **2** (where O= bridging carboxyl oxygen atom of the amino acid) is about 1 Å longer than the average O–O distance in the ionophores of Severin and co-workers. Compounds **1** and **2** might therefore be selective for larger cations like K^+ and/or Rb^+, although a high thermodynamic stability is not expected.

The molecular structure of **3** is shown in Figure 5. The complex crystallizes in the monoclinic space group $P2_1$ with three independent molecules in the unit cell. In the crystal, the molecules show an extensive network of intermolecular hydrogen-bonding interactions between the guanines and the amino acids. Two types of such interactions are shown in Figure 6. The proton bound to N(1) and one of the protons of the extracyclic $N(2)H_2$ group always form hydrogen bonds with the oxygen atoms O(1) and O(2), respectively, of the carboxylate group of the coordinated proline. The average mean lengths of these interactions are N(1)H–O(1) = 2.068(6) Å and N(2)H–O(2) = 1.992(3) Å. The second proton of the $N(2)H_2$ group forms one of three unique hydrogen bonds, either with the carboxylic oxygen atom O(2) of an adjacent complex (Figure 6A, on average N(2)H′–O(2) = 2.064(4) Å), or with N(3) of an adjacent complex (Figure 6B, on average N(2)H′–N(3) = 2.116(3) Å), or finally with the oxygen atom of a solvent methanol molecule (on average N(2)H′–$OHCH_3$ = 2.170(7) Å). The geometry is octahedral with all bond lengths and angles falling within expected values. Finally, the position of the guanine in the crystal state is stabilized by an intramolecular hydrogen bond (on average 2.041(6) Å) between the proline N(4)H proton and the carbonyl oxygen atom O(6) of the base.

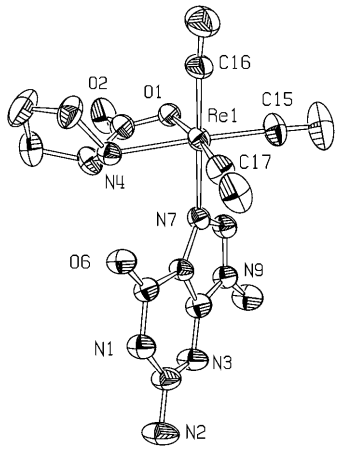

Figure 5. ORTEP view of [Re(Pro)(9-MeG)$(CO)_3$] (**3**) with 50% probability for thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Re(1)–O(1) 2.163(4), Re(1)–N(7) 2.208(4), Re(1)–N(4) 2.216(4); O(1)–Re(1)–N(7) 81.58(15), O(1)–Re(1)–N(4) 75.95(15), N(7)–Re(1)–N(4) 83.29(15).

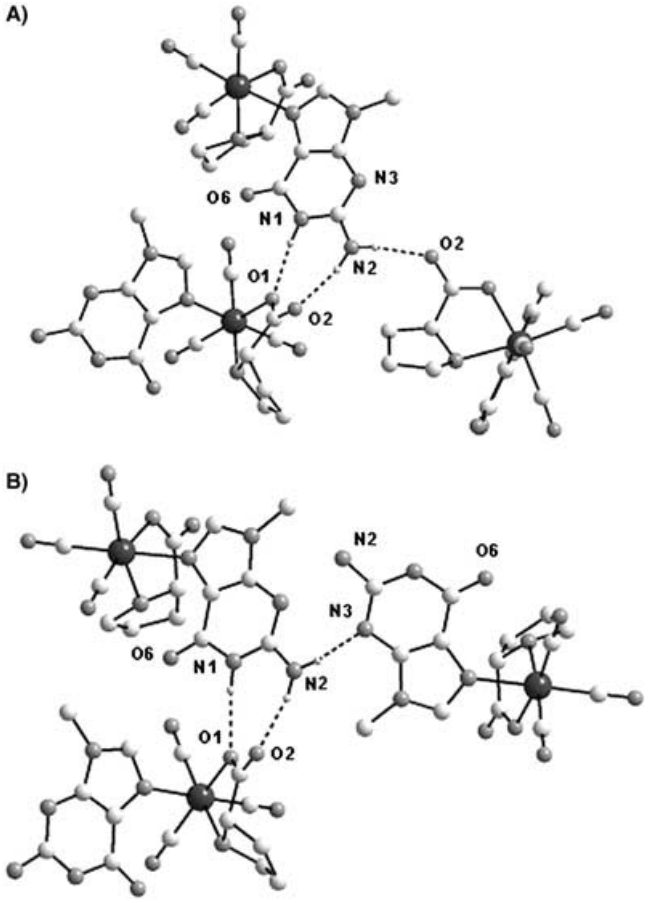

Figure 6. Two types of intermolecular hydrogen-bonding interactions in the crystal of **3**. The numbering scheme matches that in Figure 5. Solvent methanol molecules are not shown for clarity.

Conformational changes in ΦX174 DNA induced by Re^I complex binding

Gel mobility shift assays demonstrate conformational changes in macromolecules. The study of metal–DNA binding and of the changes induced by metal complexes on the tertiary structure of DNA by this technique is well established. Different Pt and Ru complexes, for example, cause unwinding of supercoiled (sc) or winding of open circular (oc) plasmid DNA (pDNA).[38–51] These structural changes can be observed on the gel matrix. Unwinding of sc pDNA, for example, causes relaxation of the DNA molecule, and the frictional force between DNA and the gel matrix during electrophoresis consequently increases. Thus, the band corresponding to the DNA–metal adduct moves relatively slower than the one of native sc DNA. The opposite is true if a metal complex causes winding of oc pDNA. In this case, winding of oc DNA renders the molecule more compact, the frictional force is reduced, and the band of the corresponding DNA–metal adduct moves relatively faster.

The analysis of the binding and of the influence of compounds **1**–**8** on the tertiary structure of DNA was determined by their ability to alter the electrophoretic mobility of the open circular and supercoiled forms of ΦX174 plasmid DNA. Figure 7A shows the mobility of native ΦX174 plasmid DNA

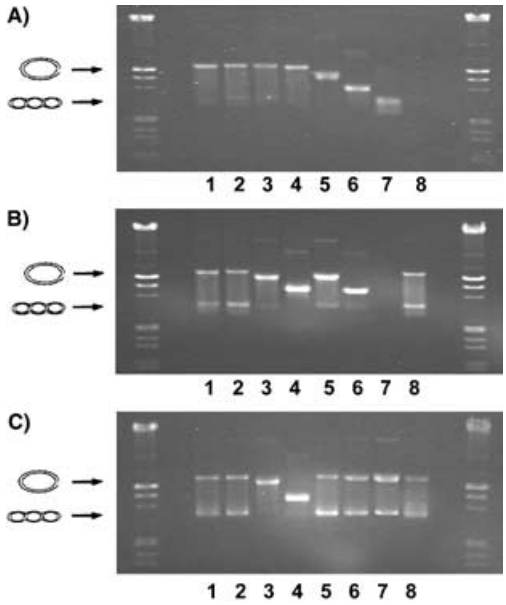

Figure 7. A) Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 1) incubated with various concentrations of **5** (lanes 2–8). Lane 1: reference, ΦX174 DNA only. r_b levels for lanes 2–8: (2) 0.00018, (3) 0.0018, (4) 0.018, (5) 0.18, (6) 1.8, (7) 18, and (8) 180. B) Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 2) incubated with various concentrations of **5** (lanes 2–4) or cisplatin (lanes 5–7). Lanes 1 and 8: reference, ΦX174 DNA only. r_b levels for lanes 2–7: (2, 5) 0.018, (3, 6) 0.18, and (4, 7) 1.8. C) Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 2) incubated with various concentrations of **5** (lanes 2–4) or **8** (lanes 5–7). Lanes 1 and 8: reference, ΦX174 DNA only. r_b levels for lanes 2–7: (2, 5) 0.018, (3, 6) 0.18, and (4, 7) 1.8.

and of pDNA incubated with compound **5**. Clearly, increasing the metal/base pair ratio (r_b) for the incubation of DNA with **5** induces a gradual increase in mobility of the oc form. Increase in mobility starts at $r_b = 0.18$ (lane 5) and progresses until $r_b = 18$ (lane 7) when a high degree of DNA mobility is reached. At a value of $r_b = 180$ (lane 8), the band disappears from the gel.

The same effect is observed when ΦX174 pDNA is incubated with cisplatin. Figure 7B shows the mobility of native ΦX174 plasmid DNA and of pDNA incubated with compound **5** and cisplatin under the same conditions. The influence on the tertiary structure and mobility of DNA produced by the two compounds is similar, although an analogous effect to that induced by **5** can already be obtained with a concentration of cisplatin that is ten times lower. At $r_b = 0.018$ (lane 2), in fact, **5** causes no change in the mobility of DNA, as indicated by the presence of both the oc and the sc forms of the plasmid, exactly as in the reference lanes 1 and 8. At $r_b = 0.18$ (lane 3), however, **5** induces a shift similar to the one caused by cisplatin at $r_b = 0.018$ (lane 5). The same trend appears when lanes 4 and 6, where the r_b values are 1.8 and 0.18 for **5** and cisplatin, respectively, are compared. At higher cisplatin concentrations ($r_b = 1.8$, lane 7), the band disappears from the gel, as previously noted.[40,51]

It is well established that Pt binding to pDNA causes winding of the oc form and unwinding of the sc form.[45–55] The same effect is known to occur with other metal ions like Ru when covalent binding to the N7 atom in guanine and noncovalent, hydrophobic interactions take place.[49–52] It is therefore likely that the shift in the mobility of ΦX174 plasmid DNA caused by Re is due to similar molecular events to those that take place with Pt and Ru. Since both the oc and sc forms are present at the same time in almost all of our experiments, binding of Re to DNA results in a concerted unwinding of the sc form together with winding of the oc form. These events result in an equilibrium tertiary DNA structure, which is revealed by a single band on the gel.

In order to evaluate whether these changes are due to an electrostatic rather than a covalent interaction, ΦX174 pDNA was incubated with **5** and $[Re(Im)_3(CO)_3]^+$ (**8**; Im = imidazole) under the same conditions (Figure 7C). The high kinetic stability of complex **8**[55] rules out possible ligand exchange and inner-sphere coordination to DNA. Any eventually observed change of the tertiary structure of the plasmid can therefore not be attributed to coordination to one of the nucleobases. However, while **5** induced the shift in mobility (lanes 2–4), complex **8** had no effect at all (lanes 5–7), a result indicating that metal binding to DNA is responsible for the observed changes, rather than electrostatic interactions.

To answer the question of whether the structural changes induced by **5** result from binding to one or two DNA bases, complexes **1**, **5**, **6**, and **7** were subjected to the same study and incubated with pDNA. Compounds **5** and **6** have two coordination sites, thereby enabling the formation of *cis*-bis adducts of guanine. Compound **1** has only one such site, as demonstrated with the formation of compound **3**, while compound **7** is inert towards ligand substitution.[54] Figure 8 shows the

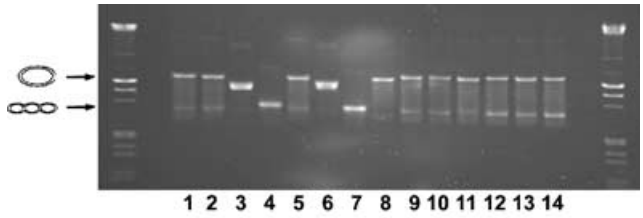

Figure 8. Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 2) incubated with various concentrations of **5** (lanes 2–4), **6** (lanes 5–7), **1** (lanes 8–10), or **7** (lanes 11–13). Lanes 1 and 14: reference, ΦX174 DNA only. r_b levels for lanes 2–13: (2, 5, 8, 11) 0.018, (3, 6, 9, 12) 0.18, and (4, 7, 10, 13) 1.8.

mobility of ΦX174 plasmid DNA after incubation with **1**, **5**, **6**, and **7** under the same conditions. Complex **5** (lanes 2–4) and complex **6** (lanes 5–7) induce the increase in mobility of pDNA, while complexes **1** (lanes 8–10) and **7** (lanes 11–13) do not. These results imply that (at least) two available coordination sites are required to induce a DNA mobility shift. Accordingly, the $[Re(CO)_3]^+$ core most likely binds to two DNA bases.

Furthermore, compound **2** revealed a similar action on the tertiary structure of ΦX174 plasmid DNA to that found with **5** or **6** (Figure 9). As discussed before, the reaction of **2** with 9-MeG establishes an equilibrium between **6** and **10**, a fact indicating that dmGly is displaced from rhenium by the base. The mobility shift induced by **2** is visible at $r_b = 0.13$ (lane 7), 0.36 (lane 9), 3.6 (lane 10), 0.54 (lane 12), and 5.4 (lane 13). These

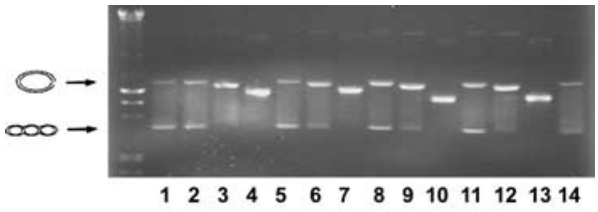

Figure 9. Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 2) incubated with various concentrations of **5** (lanes 2–4) or **2** (lanes 5–13). Lanes 1 and 14: reference, ΦX174 DNA only. r_b levels for lanes 2–13: (2, 5) 0.013, (3, 6) 0.13, (4, 7) 1.3, (8) 0.036, (9) 0.36, (10) 3.6, (11) 0.054, (12) 0.54, and (13) 5.4.

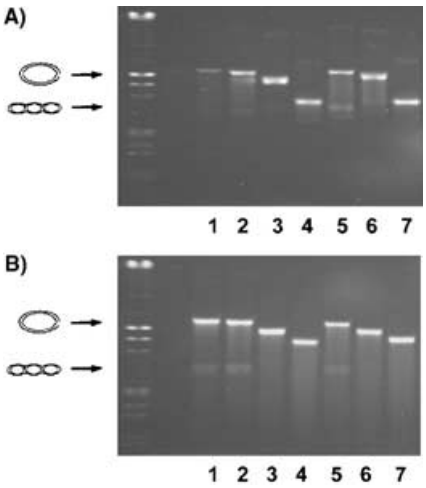

Figure 10. A) Electrophoresis in 0.75% agarose gel of ΦX174 DNA (5 nM, batch 1) incubated with various concentrations of **5** (lanes 2–4) or **4** (lanes 5–7). B) Electrophoresis in 0.75% agarose gel of the same solutions as in (A) after challenge with histidine for 22 h. Lane 1: reference, ΦX174 DNA only. r_b levels for lanes 2–7: (2, 5) 0.018, (3, 6) 0.18, and (4, 7) 1.8.

results further suggest that the shift in mobility of ΦX174 plasmid DNA is due to binding of rhenium to two DNA bases following, in this case, displacement of dmGly.

The interaction of Re with DNA may also involve the phosphate backbone. However, binding of the $[Re(CO)_3]^+$ core to phosphate in model ligands has not been observed in our studies; thus, binding to the bases is more likely to be the molecular event responsible for the structural changes in DNA. Since two available coordination sites on the metal are the prerequisite to induce changes in the tertiary structure of pDNA, bidentate binding to the phosphate backbone would give rise to a rather strained, thermodynamically unfavored, four-membered ring, while interaction with the bases can easily be rationalized with our previous results.

The stability of the Re–DNA adduct is an important factor for the use of such Re complexes as DNA-binding agents in cancer therapy. One principal hypothesis, originally advanced by Pil and Lippard to explain the activity of cisplatin, suggests that the head-to-head form in the intrastrand lesion is recognized by a damage-localizing protein whose binding prevents DNA repair. The cisplatin adduct then persists long enough to activate apoptosis.[52] If this hypothesis is correct, a relatively stable Re–DNA adduct is required to activate the same cellular response.

Figure 10A shows the mobility of native ΦX174 plasmid DNA incubated with compounds **4** and **5**. Figure 10B shows the same samples after a further 22 h incubation period in the presence of 6 equiv of histidine. When the adducts are challenged with histidine no appreciable changes in lanes 2 and 3 ($r_b = 0.018$ and 0.18, compound **5**) or 5 and 6 ($r_b = 0.018$ and 0.18, compound **4**) are observed. Lanes 4 and 7 ($r_b = 1.8$, compound **5** and **4** respectively), however, show a remarkable decrease in mobility when compared to the same lanes in Figure 10A. This effect is most likely due to trapping of unspecifically bound Re (for example, weakly bound to adenine). The Re–DNA adduct formed, however, is clearly stable.

Conclusion

The search for complexes with cytostatic or cytotoxic properties is a major field in current inorganic medicinal chemistry. We have shown that the $[Re(CO)_3]^+$ moiety exhibits a principally similar reactivity pattern with plasmid DNA to that of, for example, cisplatin. It binds selectively to two free guanines, a result implying a possible interaction with adjacent guanines in DNA as well. To protect the Re center in plasma from undesired coordination (trapping) to serum proteins, we used coligands such as proline or *N*,*N*-dimethylglycine to obtain the Re complexes in the form of prodrugs. *N*,*N*-dimethylglycine in particular can be replaced by coordination to the N7 atom of guanine. The complexes with proline are too stable in this respect but both complexes do not cross react with human serum. If the coligands are labile enough, the corresponding complexes influence the tertiary structure of ΦX174 plasmid DNA by altering the electrophoretic mobility of the open circular and the supercoiled forms. The induced changes most likely involve covalent binding to two bases, as is found with cisplatin. Although less potent than cisplatin, the model complexes depict the in vitro reactivity pattern that is required for therapeutic agents and might serve as lead structures for future inorganic medicinal drugs. Cell uptake, targeting, and cytotoxicity studies are currently under investigation.

Experimental Section

Materials and methods: All reagents and solvents were purchased from Fluka and were used as received. The complexes $[Et_4N]_2[ReBr_3(CO)_3]$ (in water $[Re(H_2O)_3(CO)_3]^+$ (**5**)), $[Re(H_2O)(9\text{-MeG})_2(CO)_3](ClO_4)$ (**6**), $[Re(His)(CO)_3]$ (**7**), and $[Re(Im)_3(CO)_3]Br$ (**8**) were prepared according to the reported procedures.[29,53–55] ΦX174 plasmid DNA was purchased by Promega and used without further purification. Two different batches were used: batch 1 consisted mostly of the open circular plasmid form and batch 2 consisted of a mixture of open circular and supercoiled forms. Cisplatin was purchased from Aldrich. HPLC chromatograms were measured on a Merck Hitachi LaChrom D-7000 instrument. HPLC system: RP-18 column (A = 0.1% CF_3COOH in H_2O, B = MeOH): 0–3 min, 100% A; 3–9 min, 75% A; 9.1 min, 66% A; 9.1–20 min, 66→0% A; 20–25 min, 0% A; 25.1–30 min, 100% A. Infrared spectra were recorded on a Perkin–Elmer BX II spectrometer from KBr pellets. All NMR spectroscopy

samples were prepared by dissolving crystals of the desired complex (typically 2 mM) in the required solvent and were immediately transferred to the probe. All ^{1}H NMR 1D spectra were recorded on a Bruker 500 MHz spectrometer. The residual solvent peak was used as the reference. Mass spectra were recorded on a Merck Hitachi M-8000 mass spectrometer.

Synthesis of complexes:

*[Re(Pro)(CO)$_3$]$_3$ (**1**)*: [Et$_4$N]$_2$[ReBr$_3$(CO)$_3$] (100 mg, 0.13 mmol) was dissolved in a methanol/water mixture (9:1, 5 mL). L-Proline (Pro) (52 mg, 0.46 mmol) was added and the mixture was stirred for 5 h at 50 °C under a slight N_2 pressure. The reaction was monitored by HPLC and stopped when no further change could be observed. The solution was allowed to equilibrate to room temperature, concentrated, and purified on a short C18 filter. A white crystalline solid (40.1 mg, 60%) was obtained: IR (KBr): $\tilde{\nu}$ = 2027 (s, C=O), 1908 (b, C=O), 1888 cm^{-1} (b, C=O); MS (30 V, ESI negative mode): *m/z*: 1266.9 [*M*+Pro]$^-$; elemental analysis: calcd (%) for $C_{24}H_{24}N_3O_{15}Re_3$ (1153.08): C 25.00, H 2.10, N 3.64; found: C 25.39, H 2.42, N 4.00. Crystals suitable for X-ray diffraction were obtained by slow diffusion of tetrahydrofuran (THF) into a methanolic solution of the complex.

*[Re(dmGly)(CO)$_3$]$_3$ (**2**)*: [Et$_4$N]$_2$[ReBr$_3$(CO)$_3$] (100 mg, 0.13 mmol) was dissolved in a methanol/water mixture (4:1, 10 mL). *N*,*N*-dimethylglycine (dmGly; 70 mg, 0.7 mmol) was added and the mixture was stirred for 12 h at 50 °C under a slight N_2 pressure. The solution was allowed to equilibrate to room temperature, concentrated, and purified on a short C18 filter. A white crystalline solid (20 mg, 40%) was obtained: ^{1}H NMR ([d$_6$]-DMSO, 25 °C, D_2O): δ = 4.18 (q, ^4J(H,H) = 5 Hz, 2H; CH_2), 3.46 (s, 3H; CH_3), 3.15 ppm (s, 3H; CH_3); IR (KBr): $\tilde{\nu}$ = 2022 (s, C=O), 1911 (b, C=O), 1890 (s, C=O), 1866 (s, C=O); MS (30 V, ESI positive mode): *m/z*: 1117.0 [*M*]$^+$; elemental analysis: calcd (%) for $C_{21}H_{24}N_3O_{15}Re_3$ (1117.05): C 22.58, H 2.17, N 3.76; found: C 23.19, H 2.78, N 3.84. Crystals suitable for X-ray diffraction were obtained by slow diffusion of diethyl ether into a CH_3CN solution of the complex.

*[Re(Pro)(9-MeG)(CO)$_3$] (**3**)*: [Et$_4$N]$_2$[ReBr$_3$(CO)$_3$] (18 mg, 0.02 mmol) was dissolved in methanol (3 mL). L-Proline (8 mg, 0.06 mmol) was added; the solution was stirred and heated to 50 °C under a slight N_2 pressure until all of the compound **5** had reacted (as revealed by HPLC, 3 h). 9-MeG (5 mg, 0.03 mmol) was added and allowed to react until no further change could be observed in the HPLC (4 h). The solution mixture was cooled to room temperature. Pentane was then allowed to diffuse into the mixture, thereby depositing crystals (10 mg, 78%) suitable for X-ray analysis: ^{1}H NMR ([D_2O, 25 °C, D_2O): δ = 8.01 (s, 1H; CH), 5.92 (s, 1H; NH), 3.39 (m, 1H; CH), 3.20 (m, 1H; CH), 2.66 (s, 3H; CH_3), 2.19 (m, 1H; CH), 1.98 (m, 2H; CH_2), 1.81 (m, 1H; CH), 1.70 ppm (m, 1H; CH); IR (KBr): $\tilde{\nu}$ = 2021 (s, C=O), 1894 cm^{-1} (b, C=O); MS (30 V, ESI negative mode): *m/z*: 548.5 [*M*−1]$^-$; elemental analysis: calcd (%) for $C_{14}H_{15}N_6O_6Re$ (549.51): C 30.60, H 2.75, N 15.29; found: C 30.96, H 2.47, N 15.70.

*[ReBr(NH$_3$)$_2$(CO)$_3$] (**4**)*: [ReBr(CO)$_5$] (75 mg, 0.19 mmol) was dissolved in a NH_3-saturated benzene solution (15 mL) and the mixture was heated to 60 °C under N_2 pressure. After 2.5 h, a white precipitate appeared; this was filtered, washed with benzene, and dried in vacuo to yield product (35 mg, 49%): IR (KBr): $\tilde{\nu}$ = 2016.9 (s, C=O), 1883.5 cm^{-1} (b, C=O); elemental analysis: calcd (%) for $C_3H_6BrN_2O_3Re$ (384.20): C 9.83, H 1.57, N 7.29; found: C 10.01, H 1.79, N 7.67. Crystals suitable for X-ray diffraction were obtained by slow diffusion of hexane into a THF solution of the complex.

X-ray crystallography: Suitable crystals were covered with Paratone N oil, mounted on top of a glass fiber, and immediately transferred to a Stoe IPDS diffractometer. Data were collected at 183(2) K by using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). A total of 8000 reflections distributed over the whole limiting sphere were selected by the program SELECT and used for unit-cell parameter refinement with the program CELL.[56] Data were corrected for Lorenz and polarization effects as well as for absorption (numerical). Structures were solved with direct method by using the SHELXS-97[57] or SIR-97[58] programs and were refined by full-matrix least-squares methods on F^2 with the SHELXL-97 program.[59]

CCDC 239971–239974 (**1**–**4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Gel mobility shift assay: ΦX174 RF plasmid DNA (0.1 μg) was mixed with the rhenium complexes in H_2O at [complex]/[bp] ratios of 0.018–1.8:1. The mixtures were incubated in water at 37 °C for 22 h in the dark before being analyzed by gel electrophoresis. The pH value of the mixtures remained constant at ≈7 in all cases. Experiments performed in 1 mM or 10 mM $NaClO_4$ showed no significant difference in the binding of **5** to ΦX174 RF plasmid DNA. DNA binding was examined by gel electrophoretic mobility shift assays through 9 cm 0.75% agarose slab gels with Tris–acetate–EDTA (TAE) running buffer. The gels were run at RT, with voltages of 50–75 V. The running time depended upon the voltage and was usually 1.5–2 h. The resultant gels were stained with ethidium bromide in the buffer at a concentration of ≈0.3 μg mL^{-1}. Bands were visualized by software UV transillumination equipped with a digital camera.

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Keywords: carbonyl complexes · chemotherapy · DNA binding · radiotherapy · rhenium

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