

Interactions of Metal–Metal-Bonded Antitumor Active Complexes with DNA Fragments and DNA

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ABSTRACT

This Account summarizes the DNA binding properties of anticancer active dinuclear Rh, Re, and Ru compounds. The combined results of NMR spectroscopy, X-ray crystallography, and various biochemical tools provide incontrovertible evidence that dinuclear complexes are favorably poised to bind to purine nucleobases, DNA fragments, and double-stranded DNA. Moreover, direct DNA photocleavage *in vitro* is effected by dirhodium compounds in the presence of electron acceptors in solution or directly attached to the dirhodium core. This research has provided valuable insight in the interactions of dinuclear compounds with DNA, knowledge that is an excellent backdrop for rational design of promising dinuclear drugs.

Introduction

The medicinal properties of inorganic compounds are well-known, dating to ancient times, with the earliest recognition being traced back to the Egyptians who used copper to sterilize water in 3000 B.C. Presently, metal compounds that exhibit antiarthritic, antibacterial, anticancer, antidepressant, and anti-hypertensive properties are in routine clinical use. Interest in metal antitumor compounds stems from the extraordinary effectiveness of cisplatin (*cis*-[Pt(NH₃)₂Cl₂] or *cis*-DDP)^{1–3} and related complexes for the treatment of ovarian, testicular, head, neck, esophageal, and lung carcinomas, with a cure rate greater than 90% in the case of testicular cancer. Extensive investigations of this potent antitumor agent have established DNA as its primary intracellular target. The intras-

strand d(GpG) head-to-head (H-H) cross-links that are formed contribute to a cascade of events, including transcription inhibition and repair shielding of cisplatin–DNA lesions, which lead to cell death.^{1–3} The success of cisplatin notwithstanding, it is important to continue the search for new anticancer active metal compounds with different activities and resistances as well as lower toxicities.

Among the recognized non-platinum antitumor agents are dinuclear carboxylate species of rhodium (Rh),⁴ rhenium (Re),^{5,6} and ruthenium (Ru).⁷ The basic paddlewheel structure of these compounds, which consists of a metal–metal-bonded fragment with at least two bridging ligands (Chart 1), appears to defy most of the accepted guidelines for metal anticancer agents. The recognition of the anticancer activity of tetracarboxylate compounds spawned a number of investigations in the 1970s regarding plausible cellular targets,^{8,9} but research in this area steadily declined, in part, because the compounds did not surpass the anticancer activity of cisplatin. The revival of this research field in our laboratories over the past decade has been directed towards elucidating the biological activity of metal–metal-bonded systems *vis-à-vis* interactions with DNA, which is the primary target of platinum anticancer agents. Our findings provide valuable insight in the viable substitution pathways and binding modes of di-metal units with DNA and pave the way for rational design of promising anticancer and photochemotherapeutic candidates. Prior to highlighting our results, a brief overview of the biological activity of each class of compounds is presented.

Biological Activity of the Compounds

Dirhodium. Studies of the biological activity of dirhodium complexes conducted in the 1970s support the conclusion that tetracarboxylate compounds Rh₂(μ-O₂CR)₄ (R = Me, Et, Pr) exhibit significant *in vivo* antitumor activity against L1210 tumors,¹⁰ Ehrlich ascites,^{8,9,11,12} sarcoma 180, and P388 tumor lines.⁴ Although the precise antitumor mechanism of dirhodium carboxylate compounds has not been elucidated, it is known that they bind to DNA^{8,9,13–15} and inhibit DNA replication and protein synthesis^{16–18} in a manner akin to cisplatin.

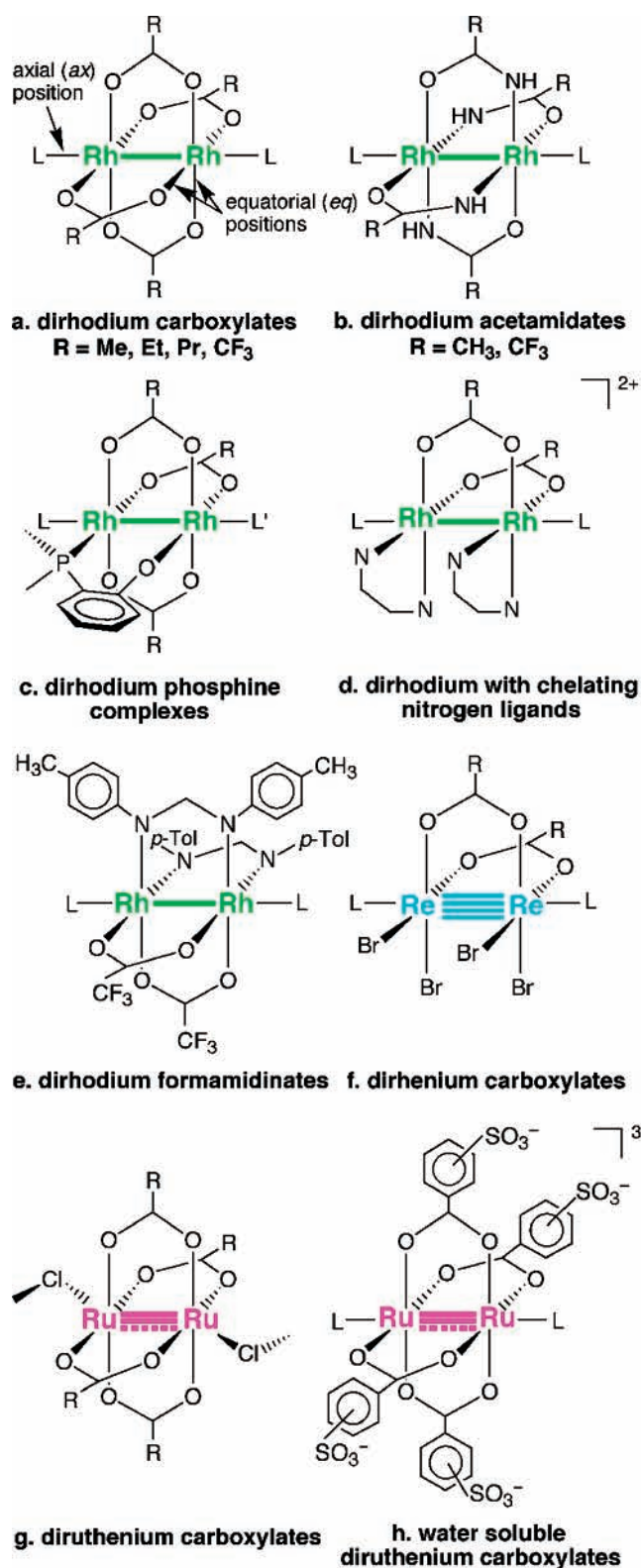
A systematic variation of the axial (*ax*) and equatorial (*eq*) ligands has shed light on the structure–activity relationships in this family of compounds. Of particular note is the fact that the antitumor activity increases in the series Rh₂(μ-O₂CR)₄ (R = Me, Et, Pr) (Chart 1, structure a) with the lipophilicity of the R group.^{8,10,11} The compounds Rh₂(μ-O₂CCF₃)₄ (Chart 1, structure a) and Rh₂(μ-HNCOCF₃)₄ (Chart 1, structure b) have been reported to significantly increase the survival rate of mice bearing Ehrlich ascites cells and have LD₅₀ values on the same order as that of cisplatin.^{19,20} The most active member of the methoxyphenylphosphine series is the

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Helen T. Chifotides was born in Providence, RI, and received her B.S. and Ph.D. degrees in Chemistry from the University of Athens, Greece. After a NATO Postdoctoral Fellowship at Michigan State University (1994–1995), she was appointed as a Lecturer in Chemistry at Oregon State University (1995–1997). She held a senior staff scientist position in the Biochemistry Laboratory of the Pulmonary Hospital ‘Sotiria’ in Athens, Greece, until 2001. Currently, she is a Senior Research Associate at Texas A&M University. Her research interests focus on the interactions of mononuclear and dinuclear metal complexes with DNA and other biologically active molecules.

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Chart 1



oxygen-metalated complex $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_3[\mu\text{-}(o\text{-OC}_6\text{H}_4)\text{P}(o\text{-OMeC}_6\text{H}_4)_2](\text{HOCCCH}_3)$ (Chart 1, structure c), which exhibits higher antitumor activity than cisplatin.²¹ Moreover, cationic compounds of general formulae $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{N-N})_2(\text{H}_2\text{O})_2]^{2+}$ (N-N = 2,2'-bipyridine (bpy) or 1,10-phenanthroline) (Chart 1, structure d) exhibit anticancer activity against human oral carcinoma KB cell

lines comparable to $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4$.²² The compound $\text{cis-Rh}_2(\text{DTolF})_2(\text{O}_2\text{CCF}_3)_2(\text{H}_2\text{O})_2$ (Chart 1, structure e; $\text{DTolF}^- = \text{N,N}'\text{-}p\text{-tolylformamidinate}$) with two robust formamidinate and two labile trifluoroacetate bridging groups represents a favorable compromise between antitumor activity and toxic side effects. The latter was evaluated for efficacy against Yoshida ascites and T8 sarcomas and was found to exhibit considerably reduced toxicity with comparable antitumor activity to cisplatin.²³ The homoleptic paddlewheel compound $\text{Rh}_2(\text{DTolF})_4$, however, exhibits no appreciable biological activity,²⁴ presumably due to steric factors that preclude access of biological targets to the *ax* and *eq* sites of the dirhodium core. Other strategies to improve dirhodium antitumor activity include the use of water-soluble ligands such as carbohydrate and cyclophosphamide derivatives,²⁵ adducts attached to carrier ligands such as isonicotinic acid,²⁶ and cyclodextrin encapsulated compounds; the latter allow for localized and controlled release of the drug with fewer side effects.²⁷

Dirhenium. Dirhenium compounds constitute a promising class of inorganic molecules for clinical development, given the noted lack of Re toxicity compared to other "heavy" metals (no LD₅₀ has been reported).⁵ The compounds $\text{Re}_2(\mu\text{-O}_2\text{CC}_2\text{H}_5)_2\text{Br}_4(\text{H}_2\text{O})_2$ ⁶ (Chart 1, structure f) and the water-soluble $[\text{Re}_2(\mu\text{-O}_2\text{CC}_2\text{H}_5)_4](\text{SO}_4)$,⁵ with quadruple Re-Re bonds, exhibit considerable antitumor activity against B-16 melanoma and sarcoma 180, respectively. Although no additional biological testings of dirhenium compounds have been reported since the studies by Eastland and co-workers,^{5,6} it is postulated that they inhibit DNA replication and protein synthesis similarly to dirhodium complexes.

Diruthenium. Diruthenium compounds have been much less investigated than their dirhodium counterparts vis-à-vis antitumor properties, but their biological mode of action is thought to be comparable. Mixed-valent carboxylato complexes of the type $\text{Ru}_2(\mu\text{-O}_2\text{CR})_4\text{Cl}$ (R = Me, Et) (Chart 1, structure g) were found to be moderately active against P388 lymphocytic leukemia cell lines.⁴ The in vitro antineoplastic activity of diruthenium compounds is greatly enhanced, however, for the highly water-soluble series $\text{M}_3[\text{Ru}_2(\mu\text{-O}_2\text{CR})_4(\text{H}_2\text{O})_2] \cdot 4\text{H}_2\text{O}$, R = *m*- or *p*-C₆H₄-SO₃⁻ and M = Na⁺ or K⁺ (Chart 1, structure h),⁷ a fact that reinforces the importance of solubility in increasing the biological activity of these potential drugs.

Diplatinum. The study of diplatinum complexes containing $\text{cis-}[\text{Pt}(\text{NH}_3)_2]^{2+}$ entities originated from the high activity of "platinum blues" against the ascites S-180 tumor system and their low toxicity, but there appears to be no consensus regarding the nature of their interactions with DNA.²⁸

Reactions of Dinuclear Rhodium Complexes with Nitrogen Donor Chelates

In an early effort to elucidate the possible binding modes of DNA to the dirhodium core, efforts in our laboratories were aimed at studying dirhodium interactions with bidentate nitrogen chelates, for example, bpy, which may

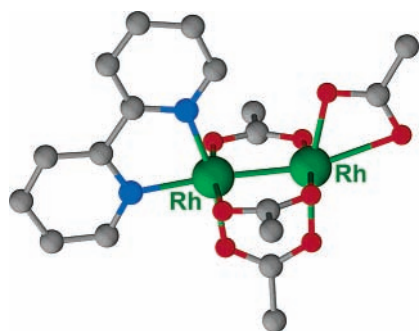


FIGURE 1. Molecular structure of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_3(\eta^2\text{-O}_2\text{CCH}_3)(\text{bpy})$.

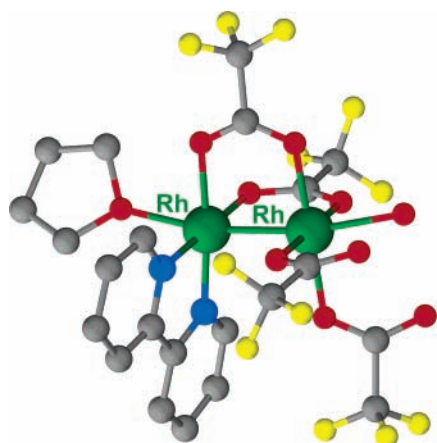
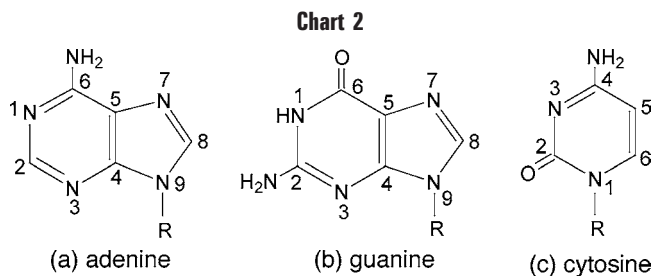
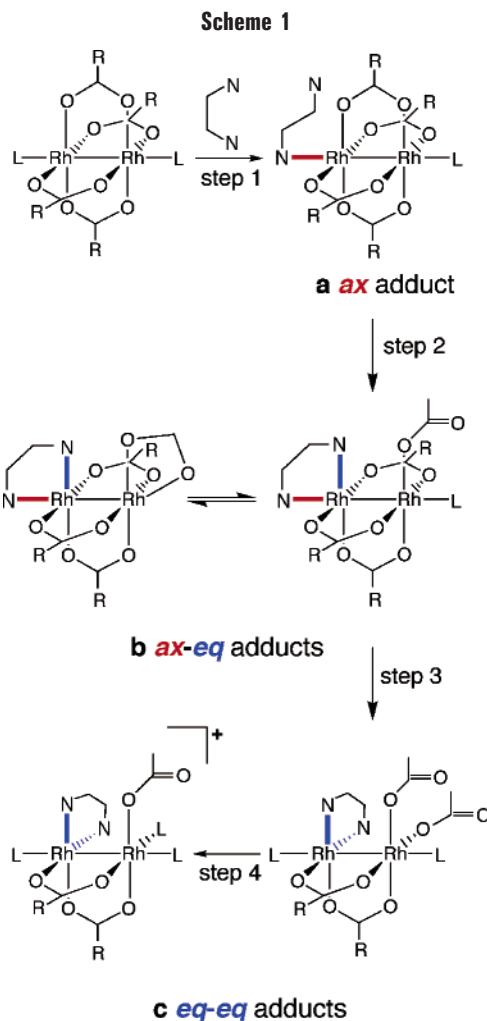


FIGURE 2. Molecular structure of $\text{Rh}_2(\mu\text{-O}_2\text{CCF}_3)_2(\eta^1\text{-O}_2\text{CCF}_3)_2(\text{bpy})(\text{THF})(\text{H}_2\text{O})$.

be considered mimics of adjacent DNA bases. Although the analogy was not fully appreciated until much later, we reasoned that if bpy could enter the coordination sphere of “lantern-type” structures, it should be possible for DNA bases to behave likewise. Indeed, the adduct $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_3(\eta^2\text{-O}_2\text{CCH}_3)(\text{bpy})$ ^{29,30} (Figure 1), as well as the related species $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\text{bpy})(\text{NCCH}_3)_4](\text{BF}_4)_2$ and $\text{Rh}_2(\mu\text{-O}_2\text{CCF}_3)_2(\eta^1\text{-O}_2\text{CCF}_3)_2(\text{bpy})(\text{THF})(\text{H}_2\text{O})$ (Figure 2),³⁰ exhibit structures with one chelating bpy molecule in *ax-eq* and *eq-eq* positions, respectively. By piecing together the data obtained from these collective studies, a mechanism for how nitrogen-containing chelates enter the coordination sphere of the dirhodium core was proposed (Scheme 1). The reaction involves an initial nucleophilic attack of the base at an *ax* site of the dimetal unit to afford the axially bound monodentate adduct **a**, followed by formation of a chelate ring by attack of a second donor atom at an *eq* site (**b**; *ax-eq* adducts) and conversion to the final *eq-eq* adducts **c**.³⁰ The rearrangement from *ax* to *eq* positions is a key feature of this chemistry and a major factor in dictating the outcome of purine reactions with dirhodium units.

Interactions with Nucleobases and Nucleos(t)-ides

Axial Nucleobases. A perusal of the literature reveals that, unlike cisplatin, dirhodium compounds bind strongly to polyadenylic acids and adenine nucleos(t)ides.^{8,9,31–33} These DNA binding preferences are considered to be



related to the fact that dirhodium compounds are well-known to react via trans substitution of *ax* ligands located at opposite ends of the dimer (Chart 1, structure a). Axial interactions between adenine (via position N7; Chart 2, structure a) and the dirhodium core are stabilized by formation of hydrogen bonds between the purine exocyclic NH_2 (6) amino group and the carboxylate oxygen atom of the dirhodium unit, as evidenced by the X-ray structural studies of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(1\text{-MeAdo})_2$ ³³ (Figure 3) and *trans*- $[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\mu\text{-HNCOCF}_3)_2(9\text{-MeAdeH}_2)_2](\text{NO}_3)_2$.³⁴ By comparison, the absence of guanine (Chart 2, structure b) *ax* adducts, when the dirhodium unit is supported solely by carboxylate groups, is attributed to electrostatic repulsions between the purine site O6 and the carboxylate oxygen atoms. When at least two of the carboxylate groups of the complex are replaced by lig-

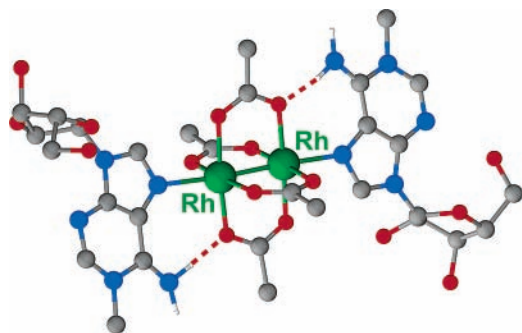


FIGURE 3. Molecular structure of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4(1\text{-MeAdo})_2$.

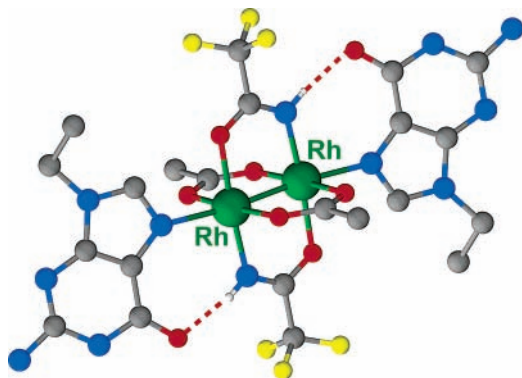


FIGURE 4. Molecular structure of $\text{trans-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\mu\text{-NHCOCF}_3)_2(9\text{-EtGuaH})_2]$.

ands with hydrogen-bonding donor moieties, however, *ax* binding of guanine via N7 as in $\text{trans-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(\mu\text{-NHCOCF}_3)_2(9\text{-EtGuaH})_2]$ (Figure 4) and $\text{Rh}_2(\mu\text{-NHCOCF}_3)_4(\text{dGuo})_2$, is favored.³⁴ The argument of unfavorable *ax* binding of guanine to dirhodium carboxylate units is supported by the fact that although the guanine analogue, theophylline, binds axially to $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_4$ via N9, in $[\text{Rh}_2(\text{HNCOCCH}_3)_4(\text{theophylline})_2]\text{-NO}_3$, the NH hydrogen-donor groups on the acetamide moieties favor binding via position N7.³⁵ Axial binding of cytosine via N3 (Chart 2, structure c) to $\text{Rh}_2(\text{HNCOCCH}_3)_4$ units is stabilized by formation of hydrogen bonds between the exocyclic $\text{NH}_2(4)$, O2 sites, and the tetraamidate cage.³⁶ Similarly, in diplatinum(III,III)–guanine *ax* adducts, the guanine O6 and the NH_3 groups of the dimetal unit engage in hydrogen bonding interactions (Figure 5).^{37–39} Axial binding of adenine and adenosine via N9/N3 and N7, respectively, has been proposed for their adducts with tetraacetatodiruthenium(II,III), but the compounds have not been structurally characterized.⁴⁰

Equatorial 9-Ethylguanine (N7/O6). Early claims in the literature that dirhodium compounds do not react with guanine³² and polyguanylic acids⁸ were largely based on the lack of perceptible color change upon reaction of guanine with the dirhodium core, in contrast to the immediate color change (from blue-green to pink-violet) upon *ax* binding of adenine and its derivatives to dirhodium carboxylate compounds. Findings in our laboratories have shown that adenine and guanine bases bind to the dirhodium core in an alternative manner that involves displacement of *eq* bridging groups. The crystal structure

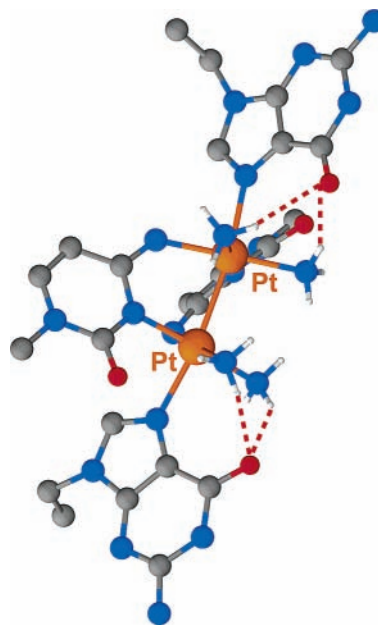


FIGURE 5. The cation in $\text{H-T cis-}[\text{Pt}_2(\text{NH}_3)_2(1\text{-MeCyt})_2(9\text{-EtGuaH})_2](\text{ClO}_4)_4$.

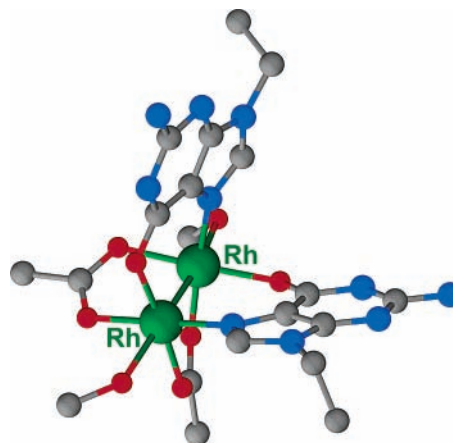


FIGURE 6. Molecular structure of $\text{H-T cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(9\text{-EtGua})_2(\text{MeOH})_2]$.

determinations of $\text{H-T cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(9\text{-EtGua})_2(\text{MeOH})_2]$ (Figure 6),⁴¹ $\text{H-T cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCF}_3)_2(9\text{-EtGuaH})_2(\text{Me}_2\text{CO})_2](\text{CF}_3\text{CO}_2)_2$ (Figure 7),⁴¹ and $\text{H-H cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(9\text{-EtGuaH})_2(\text{Me}_2\text{CO})(\text{H}_2\text{O})](\text{BF}_4)_2$ (Figure 8)⁴² revealed the presence of unprecedented bridging guanine groups spanning the dirhodium unit via N7/O6 in a *cis* disposition and H-H or head-to-tail (H-T)⁴³ orientations. These X-ray studies provided the first hard evidence for guanine O6 participation in binding to dimetal units. The 9-EtGuaH structures are intriguing in another respect, namely, that position N1 can be protonated or deprotonated, depending on the nature of the leaving group. In $\text{H-T cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2(9\text{-EtGua})_2(\text{MeOH})_2]$, the purine is deprotonated at N1, that is, the enolate form of guanine (9-EtGua^-) is stabilized⁴¹ (Scheme 2), whereas in $\text{H-T cis-}[\text{Rh}_2(\mu\text{-O}_2\text{CCF}_3)_2(9\text{-EtGuaH})_2(\text{Me}_2\text{CO})_2](\text{CF}_3\text{CO}_2)_2$, the purine N1 site is protonated,⁴¹ in accord with the lower basicity of CF_3CO_2^- ($\text{p}K_b \approx 13.5$) compared to CH_3CO_2^- ($\text{p}K_b \approx 9.2$). The substantial increase in the acidity of

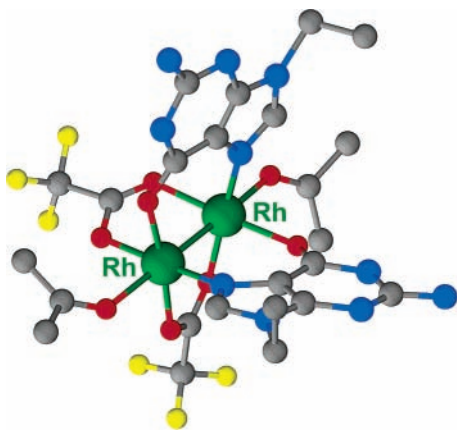


FIGURE 7. The cation in H-T cis -[Rh₂(μ-O₂CCF₃)₂(9-EtGuaH)₂(Me₂CO)₂](CF₃CO₂)₂.

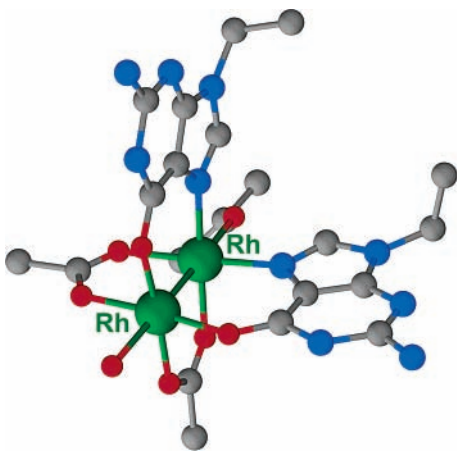
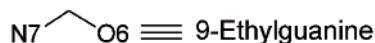
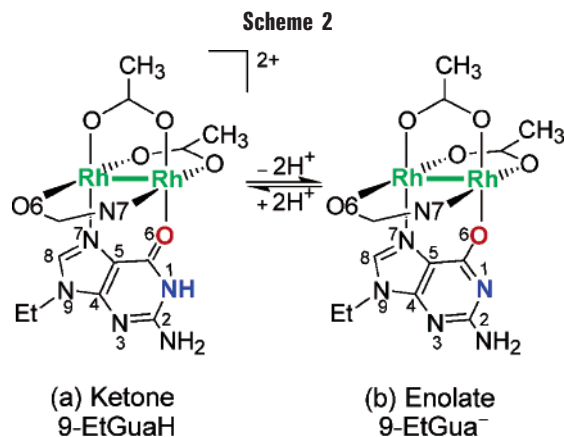


FIGURE 8. The cation in H-H cis -[Rh₂(μ-O₂CCH₃)₂(9-EtGuaH)₂(Me₂CO)(H₂O)](BF₄)₂.



N1–H for the aforementioned compounds (pH-dependent ¹H and ¹³C NMR titrations afford a value p*K*_a ≈ 5.7 compared to 8.5 for N7-bound only and 9.5 for the unbound purine) is attributed to the bidentate N7/O6 coordination of the base.⁴⁴ The reaction of Rh₂(μ-O₂CCH₃)₄ with guanosine-5'-monophosphate (GMP) follows similar trends, which consist in the formation of two isomers (H-H and H-T) with the guanine unit spanning the Rh–

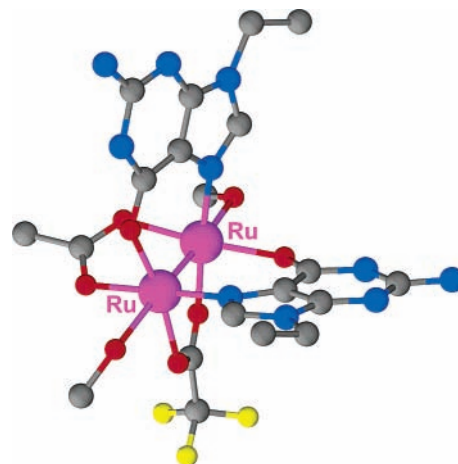


FIGURE 9. The cation in H-T cis -[Ru₂(μ-O₂CCH₃)_{2-x}(μ-O₂CCF₃)_x(9-EtGuaH)₂(MeOH)₂](O₂CCF₃)₂ ($x = 0.18$). The *eq* carboxylate group in yellow is occupied by CH₃CO₂⁻/CF₃CO₂⁻ with occupancies of 82%/18%, respectively.

Rh bond in a bridging fashion via N7/O6 and a substantially decreased p*K*_a value of the N1–H group (p*K*_a ≈ 5.6) due to N7/O6 coordination.⁴⁵ The pronounced electronic effects on the O6/N1 guanine sites, involved in base recognition and hydrogen bonding in duplex DNA and the shifting of the p*K*_a values to the physiological pH range, suggest that metal binding severely impairs normal Watson–Crick pairing, which bears directly on metal mutagenicity and cell death.⁴⁶

Bridging 9-EtGuaH groups coordinated via N7/O6 positions have also been observed in H-H cis -[Rh₂(DTolF)₂(9-EtGuaH)₂(NCCH₃)](BF₄)₂,⁴⁷ as well as in H-T cis -[Ru₂(μ-O₂CCH₃)_{2-x}(O₂CCF₃)_x(9-EtGuaH)₂(MeOH)₂](O₂CCF₃)₂⁴² (Figure 9) and H-H cis -[Mo₂(μ-O₂CCH₃)₂(9-EtGuaH)₂(NCCH₃)₂](BF₄)₂.⁴² Moreover, ¹H NMR studies performed on reaction solutions of 9-EtGuaH with Re₂(μ-O₂CR)₂Br₄ (R = Me, Et, Pr) support the loss of two *cis* carboxylate groups and coordination of the purine via positions N7/O6. The fact that dinuclear compounds with metal–metal bonds ranging from single to quadruple and M–M distances between 2.2 and 2.5 Å form stable bis-bridging 9-EtGuaH complexes argues strongly for the prevalence of this binding mode. The crystal structure determination of cis -[Rh₂(μ-O₂CCH₃)₂(bpy)(9-EtGuaH)(H₂O)₂](CH₃SO₄)](CH₃SO₄) (Figure 10),⁴⁸ however, revealed that 9-EtGuaH may also bind in a monodentate fashion via N7 to a single rhodium center at an *eq* position in the presence of a chelating agent, for example, bpy, which occupies *eq* sites of the other rhodium center.

Bridging 9-Ethyladenine. Our findings from adenine nucleobase reactions with dimetal units clearly demonstrate that the former are not restricted to binding solely to *ax* positions, as previously thought. In an effort to avoid the insoluble polymers that plague dirhodium/carboxylate reactions with adenine,^{31,49} two carboxylate ligands were replaced with DTolF⁻ groups. Reactions of [Rh₂(DTolF)₂(CH₃CN)₆](BF₄)₂ with 9-EtAdeH (Chart 2, structure a) afford H-T cis -[Rh₂(DTolF)₂(9-EtAdeH)₂(NCCH₃)](BF₄)₂ (Figure 11) with two 9-EtAdeH rings bridging at *eq* sites via N7/N6.^{47,50} This binding mode of adenine with dimetal

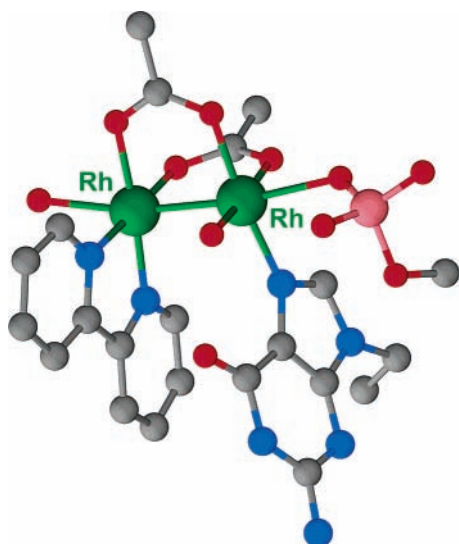


FIGURE 10. The cation in *cis*-[Rh₂(μ-O₂CCH₃)₂(bpy)(9-EtGuaH)(H₂O)₂(CH₃SO₄)](CH₃SO₄).

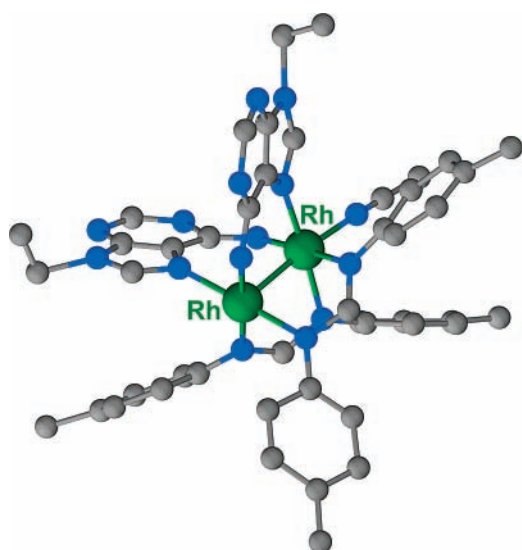


FIGURE 11. The cation in H-T *cis*-[Rh(DTolF)₂(9-EtAdeH)₂(NCCH₃)](BF₄)₂.

units was originally observed in the dimolybdenum analogue H-T *cis*-[Mo₂(μ-O₂CCHF₂)₂(9-EtAdeH)₂](BF₄)₂⁵¹ and complements the results observed for 9-EtGuaH (vide supra). As indicated by variable temperature ¹H NMR spectra,⁴⁷ 9-EtAdeH is present in the rare imino form, as a result of the metal-induced internal proton transfer from NH₂(6) to N1 (Scheme 3). The imino form of adenine results in alteration of the hydrogen bonding behavior of the base and an increase in the acidity of N1–H, the ultimate result of which is nucleobase mispairing and cell mutations.⁴⁶ In the case of H-H *cis*-[Re₂(μ-O₂CC₂H₅)₂(9-EtAde)₂]Cl₂ (Figure 12),⁵² the adenine bases are bridging via positions N1/N6 and NH₂(6) is deprotonated; metal binding to these sites, normally involved in Watson–Crick hydrogen bonding, may have important biological consequences.⁴⁶

Bridging Pyrimidine Rings. In the diplatinum(III,III) compound H-T *cis*-[Pt₂(NH₃)₂(1-MeCyt)₂(9-EtGuaH)₂](ClO₄)₄

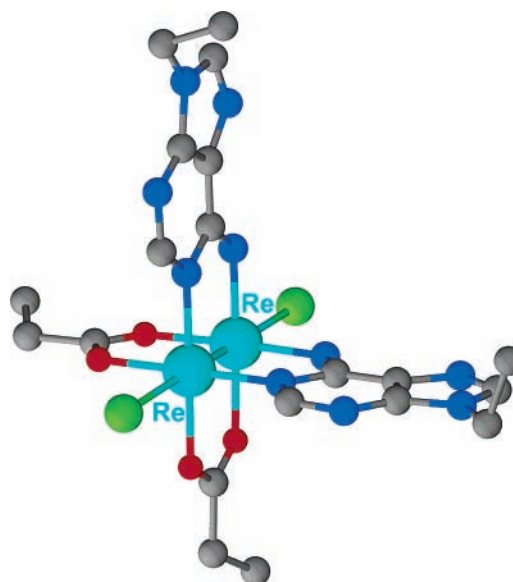
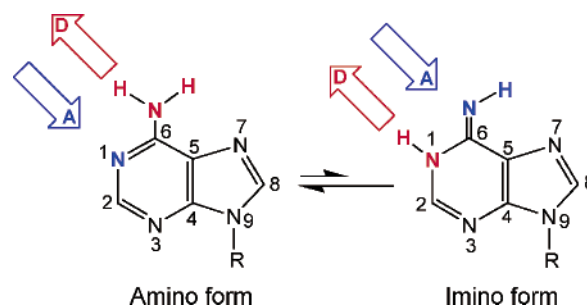


FIGURE 12. The cation in H-H *cis*-[Re₂(μ-O₂CC₂H₅)₂(9-EtAde)₂]Cl₂.

Scheme 3



(Figure 5) with H-T bridging cytosine moieties (Chart 2, structure c), binding takes place via N3/N4 with site NH₂(4) being deprotonated.^{37,38} Two cytosinato fragments acting as bridging ligands via N3/N4 are proposed in the paramagnetic compound Rh₂(DTolF)₂(Cyt)₂(O₂CCF₃), but no X-ray structure has been reported.²⁴

Dirhodium Adducts with Dinucleotides. Armed with the knowledge obtained from our studies of dirhodium unit interactions with the basic building blocks of DNA, we extended our work to the chemistry of small DNA fragments. It was reasoned that the 90° “bite” angle displayed by the d(GpG)–cisplatin “chelate” is well-suited to accommodate two *cis eq* positions of one metal atom in a dirhodium unit, despite the different geometries of the two metal atoms. The reactions of Rh₂(μ-O₂CCH₃)₄ with the dinucleotides d(GpG) and d(pGpG) were probed by NMR spectroscopy.^{44,45} One-dimensional ¹H and ¹³C NMR studies of Rh₂(μ-O₂CCH₃)₂{d(GpG)} (X = H) and Rh₂(μ-O₂CCH₃)₂{d(pGpG)} (X = HPO₃[−]) (Chart 3) revealed that both compounds contain dinucleotides bridging the dirhodium core via N7/O6 with considerable increase in the acidity of the purine sites N1–H (pK_a ≈ 5.7), as in the case of 9-EtGuaH. Intense H8/H8 ROE (rotating-frame nuclear Overhauser effect) cross-peaks in the 2D ROESY NMR spectra (Figure 13) indicate a H-H arrangement of the guanine bases for both adducts.^{44,45} The Rh₂(μ-O₂CCH₃)₂{d(GpG)} adduct exhibits two major right-

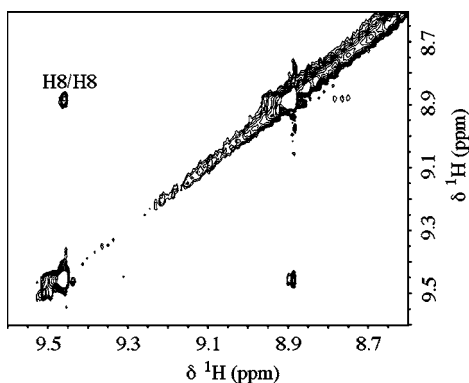
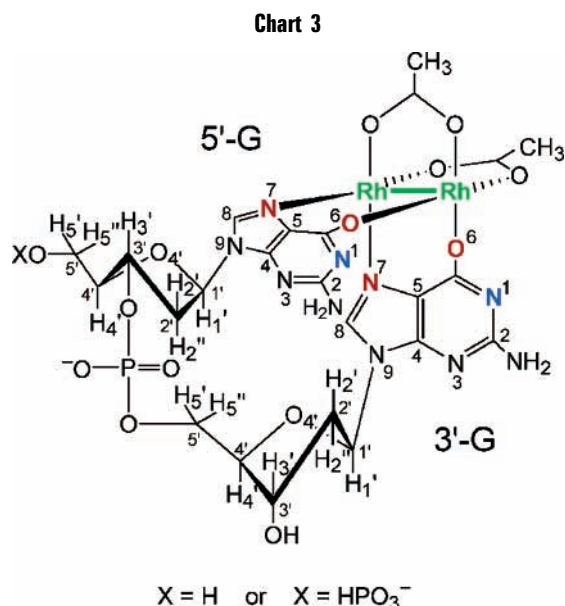


FIGURE 13. Aromatic region of the 2D ROESY NMR spectrum of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$ in D_2O displaying the H8/H8 ROE cross-peaks of the two guanine bases in a H-H arrangement.



handed conformers HH1R (~75%) and HH2R (~25%), which differ in the relative canting of the two bases,⁴⁴ whereas in $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$, the steric effect of the terminal 5'-phosphate group results in stabilization of only one left-handed HH1L conformer as in cisplatin–DNA adducts.⁴⁵ Detailed characterization of $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{GpG})\}$ and $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$ by 2D NMR spectroscopy reveals notable structural features that resemble those of *cis*-[Pt(NH₃)₂{d(pGpG)}]; the latter involve repuckering of the 5'-G sugar rings to type N, retention of type S conformation for the 3'-G sugar rings and *anti* orientation with respect to the glycosyl bonds.^{44,45} Superposition of the low-energy $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$ conformer, generated by simulated annealing calculations, and the crystal structure of *cis*-[Pt(NH₃)₂{d(pGpG)}] reveals remarkable similarities between the adducts (Figure 14); not only are the bases almost completely destacked (interbase dihedral angle 3'-G/5'-G $\approx 80^\circ$) upon coordination to the metal in both cases, but they are favorably poised to accommodate the bidentate N7/O6 binding to the dirhodium unit.⁴⁵ Contrary to conventional wisdom, the two octahedral rhodium atoms are capable of engaging in *cis* binding to GG intrastrand sites by establishing

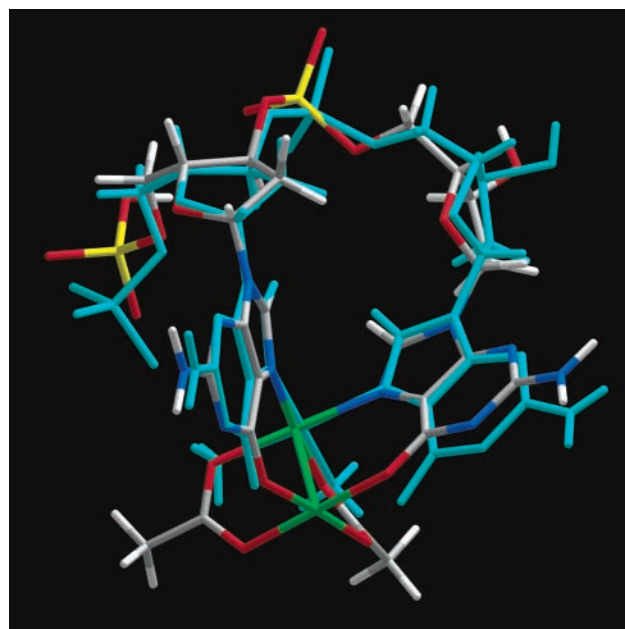
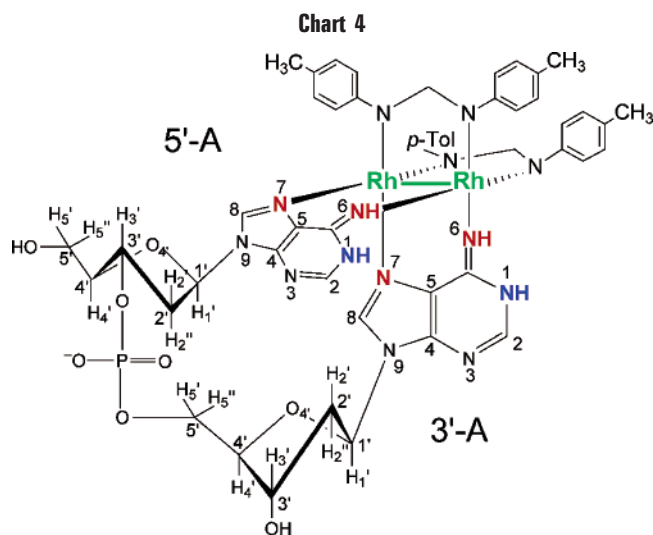


FIGURE 14. Superposition of the low-energy $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$ conformer, generated by simulated annealing calculations, and the crystallographically determined *cis*-[Pt(NH₃)₂{d(pGpG)}] (light blue).

N7/O6 bridges that span the Rh–Rh bond. The rigid steric demands of the tethered guanine bases bound to the square planar platinum atom in *cis*-[Pt(NH₃)₂{d(pGpG)}] are also satisfied in metal–metal-bonded dirhodium compounds. Our unprecedented findings that d(GpG) fragments establish *eq* bridging interactions with the dirhodium unit, via N7/O6, reveal new possibilities for metal–DNA interactions and lay a solid foundation for exploring similar structural motifs in related systems. Indeed, NMR spectroscopic data for $\text{Rh}_2(\text{DTolF})_2\{\text{d}(\text{GpG})\}$ reveal that binding also occurs via N7/O6 of the guanine bases in a H-H fashion and that both sugar rings are in an *anti* orientation with respect to the glycosyl bonds.⁵³ Contrary to *cis*-[Pt(NH₃)₂{d(pGpG)}] and $\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2\{\text{d}(\text{pGpG})\}$, in $\text{Rh}_2(\text{DTolF})_2\{\text{d}(\text{GpG})\}$ both sugar rings are of type N, a fact that implies possible conformational restriction in the adduct. The NMR studies of $\text{Rh}_2(\text{DTolF})_2\{\text{d}(\text{ApA})\}$ (Chart 4) indicate that metal binding occurs at *eq* sites via N7/N6 with a H-H arrangement of the adenine bases.⁵³ The latter are present in the rare imino form, as suggested by variable-temperature ¹H NMR spectra.⁵³ Metal N7/N6 binding leads to an increase in the acidity of the adenine N1–H site and a shift of its pK_a to values near physiological pH, with apparent biological implications.⁴⁶

Dirhodium Adducts with Single-Stranded (ss) DNA Oligonucleotides.^{13,14} In an attempt to address the issue of versatility and stability of dirhodium–DNA adducts, a comprehensive mass spectrometry study was undertaken. Dirhodium reactions with single-stranded oligonucleotides containing dipurine sites point to the following relative order of reactivity, which correlates with the lability of the leaving groups: *cis*-[Pt(NH₃)₂(H₂O)₂]²⁺ \approx $\text{Rh}_2(\mu\text{-O}_2\text{CCF}_3)_4 >$ *cis*-[Pt(NH₃)₂Cl₂] \gg *cis*-[$\text{Rh}_2(\mu\text{-O}_2\text{CCH}_3)_2$ –



(NCCH₃)₆(BF₄)₂ > Rh₂(μ-O₂CCH₃)₄. As indicated by the data, bis-acetate oligonucleotide dirhodium adducts dominate with tetramers, whereas longer oligonucleotides also form species with one or no acetate bridging groups.

At this point, it is pertinent to address the conclusions of an early study that brought into question the integrity of the dirhodium unit *in vivo*.⁵⁴ The study involved detecting the ¹⁴CO₂ produced during the respiration of mice that had been treated with a specific quantity of Rh₂(μ-O₂¹⁴CCH₃)₄. Although it was concluded, for a reason that is not clear to us, that the results point to total decomposition of the Rh₂ unit, actually the amount of excreted ¹⁴CO₂ accounts for ~50% of the administered Rh, which is more compatible with the conclusion that two acetate ligands remain bound to the dirhodium core *in vivo*. Our mass spectrometry results, as well as the dirhodium tetraacetate studies with nucleobases and dinucleotides (*vide supra*), clearly indicate that the reactions proceed with substitution of two acetate bridges with the other two remaining intact. Moreover, the noted kinetic stabilities of the solvated cations [Rh₂(NCCH₃)₁₀]⁴⁺ and [Rh₂(H₂O)₁₀]⁴⁺⁵⁵ are an excellent indication that the dirhodium core remains intact, even in the absence of carboxylate bridging groups.

The “gentle” nature of the electrospray ionization process permitted the observation of initial dirhodium–DNA adducts and reaction intermediates; the data imply that DNA purine sites bind to dirhodium compounds by establishing weak *ax* interactions followed by rearrangement to more stable *eq* positions. These findings corroborate the proposed mechanism for adduct formation of nitrogen donor chelating molecules (*vide supra*).

The adducts formed between dirhodium units and DNA oligonucleotides were subjected to enzymatic digestion studies by the DNA exonucleases phosphodiesterase I (3'→5') and phosphodiesterase II (5'→3'). The digestion products, which were detected by matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometries, indicate that the enzymes are inhibited *at* or *near* the dipurine sites. This observation supports the conclusion that purine rather than pyrimi-

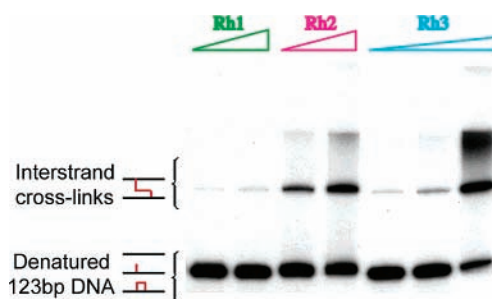


FIGURE 15. Denaturing PAGE (5%) of reactions between Rh₂(μ-O₂CCH₃)₄ (Rh1), *cis*-[Rh₂(μ-O₂CCH₃)₂(NCCH₃)₆](BF₄)₂ (Rh2), and Rh₂(μ-O₂CCF₃)₄ (Rh3) with radiolabeled 123 bp dsDNA at increasing metal concentrations.

dine bases preferentially interact with the dirhodium core.^{13,14} Detailed NMR spectroscopic studies of high-performance liquid chromatography (HPLC)-purified adducts are currently underway to determine the conformational changes and perturbations induced to the DNA structure upon metal binding.

Dirhodium Adducts of Double-Stranded (ds) DNA.⁵⁶

A number of studies have been performed that address the long-standing issue of whether and how dirhodium compounds bind to dsDNA.^{8,9,15} An investigation of the interactions of dsDNA with Rh₂(μ-O₂CCH₃)₄(H₂O)₂, [Rh₂(μ-O₂CCH₃)₂(CH₃CN)₆](BF₄)₂, and Rh₂(μ-O₂CCF₃)₄ supports the presence of covalently linked DNA adducts, including stable DNA interstrand cross-links (Figure 15). These findings refute earlier claims that no reaction between dirhodium compounds and dsDNA occurs.^{8,9} The reversal behavior of the dsDNA interstrand cross-links in 5 M urea at 95 °C implies the presence of a mixture of mono-functional or bifunctional adducts possibly bound at *ax-ax*, *ax-eq*, or *eq-eq* sites of the dirhodium core. The less stable adducts in the isolated band are most likely *ax*-DNA species, which are expected to exhibit enhanced exchange rates with heating as compared to *eq*-DNA adducts.⁵⁵ The reversal of additional dsDNA–dirhodium adducts in the isolated band by further heating in 40 mM thiourea indicates the presence of another subset of products that are stable to more harsh conditions (most likely *eq*-DNA adducts). The lability of the dirhodium leaving groups corresponds to the extent of interstrand cross-link formation on a 123 bp DNA fragment, as indicated by denaturing polyacrylamide gel electrophoresis (dPAGE) studies, and is in the order Rh₂(μ-O₂CCH₃)₄(H₂O)₂ << [Rh₂(μ-O₂CCH₃)₂(CH₃CN)₆](BF₄)₂ < Rh₂(μ-O₂CCF₃)₄. These results corroborate the conclusions of the mass spectrometry studies and provide insight into the possible mechanisms involved in biological activity of dinuclear metal compounds.

Photochemistry and DNA Photocleavage *In Vitro*

Dirhodium complexes are being investigated by the Turro group at The Ohio State University as potential antitumor agents in photochemotherapy, which involves triggering the toxicity of a compound by irradiation of the affected

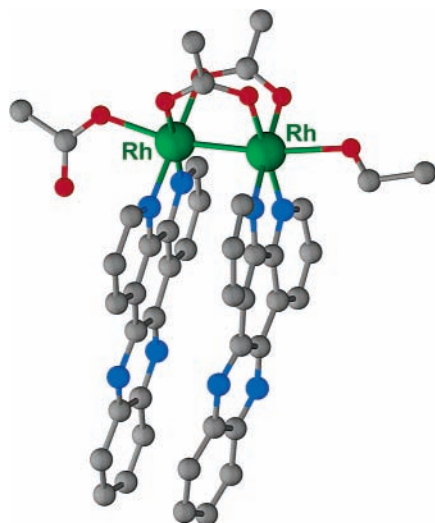


FIGURE 16. The cation in *cis*-[Rh₂(μ-O₂CCH₃)₂(dppz)₂(η¹-O₂CCH₃)-(EtOH)](BF₄).

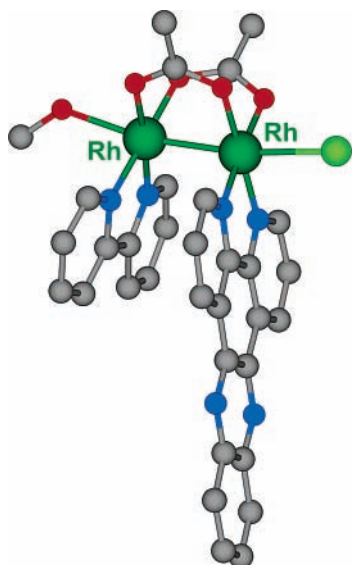


FIGURE 17. The cation in *cis*-[Rh₂(μ-O₂CCH₃)₂(bpy)(dppz)(MeOH)-Cl](BF₄).

area with low-energy light. It has been reported that Rh₂(μ-O₂CCH₃)₄ exhibits a long-lived excited state ($\tau = 3.5 \mu\text{s}$) that can be accessed with visible light ($\lambda_{\text{exc}} \approx 350\text{--}600 \text{ nm}$) and undergoes energy and electron transfer with a variety of acceptors.⁵⁷ Irradiation of Rh₂(μ-O₂CCH₃)₄ with visible light ($\lambda_{\text{irr}} = 400\text{--}610 \text{ nm}$), in the presence of electron acceptors, results in DNA photocleavage by the mixed-valent cation [Rh₂(μ-O₂CCH₃)₄]⁺.⁵⁸ Unlike Rh₂(μ-O₂CCH₃)₄, which requires an electron acceptor in solution,⁵⁸ Rh₂⁴⁺ complexes with dppz (dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine), *cis*-[Rh₂(μ-O₂CCH₃)₂(dppz)(η¹-O₂CCH₃)-(CH₃OH)]⁺⁵⁹ and *cis*-[Rh₂(μ-O₂CCH₃)₂(dppz)₂]²⁺ (Figure 16),⁶⁰ effect direct pUC18 plasmid photocleavage *in vitro* upon irradiation with visible light ($\lambda_{\text{irr}} \geq 395 \text{ nm}$) resulting in nicked circular DNA. An enhancement of photocleavage is observed for *cis*-[Rh₂(μ-O₂CCH₃)₂(dppz)(η¹-O₂CCH₃)-(CH₃OH)]⁺, which may be due to its ability to intercalate DNA bases.⁶⁰ The species *cis*-[Rh₂(μ-O₂CCH₃)₂(dppz)₂]²⁺ exhibits relatively low cytotoxicity toward human skin cells

in the dark, but its toxicity increases 3.4-fold upon irradiation of the cell cultures with visible light.⁶⁰ Likewise, the cytotoxicity of the heteroleptic species *cis*-[Rh₂(μ-O₂CCH₃)₂(bpy)(dppz)]²⁺ (Figure 17) increases 5-fold upon irradiation, with the advantage of 10-fold lower toxicity than hematoporphyrin (key component in Photofrin) in the dark.⁶¹ These results render the aforementioned compounds promising candidates for photochemotherapy.

Concluding Remarks and Future Prospects

The results presented in this Account constitute the current state of knowledge regarding DNA binding properties of dinuclear anticancer active agents. The combined results of NMR spectroscopy, X-ray crystallography, and various biochemical tools have contributed to an improved understanding of specific DNA binding motifs of dinuclear metal complexes. Among the important points that have emerged is the fact that, counter to conventional wisdom, two adjacent Lewis acid metal sites of a dinuclear complex are favorably poised to interact with two tethered purine bases in a manner akin to cisplatin. Interestingly, metal binding to the bases leads to generation of rare nucleobase tautomers and induces changes to their hydrogen-bonding properties that are known to lead to DNA mutations and cell death. By tailoring the bridging and other *eq* groups on the dimetal unit, one can effectively control the compound activity and toxicity, as well as the potential for the complexes to covalently bind to or intercalate DNA bases. Introduction of electron acceptor functionalities on the dirhodium core represents an important new direction in the design of future dinuclear photochemotherapeutic agents. Our recent findings unequivocally demonstrate that dirhodium compounds bind to dsDNA by forming interstrand cross-links and provide valuable insight into the possible underlying mechanism(s) of their antitumor behavior. The data obtained thus far constitute an excellent backdrop for further biochemical studies of metal–metal-bonded systems including rational design of promising anticancer candidates.

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