Effect of Axial Coordination on the Electronic Structure and Biological Activity of Dirhodium(II,II) Complexes

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The reactivities toward biomolecules of a series of three dirhodium(II,II) complexes that possess an increasing number of accessible axial coordination sites are compared. In cis-[Rh₂(OAc)₂(np)]^2+ (1; np = 1,8-naphtyridine) both axial sites are available for coordination, whereas for cis-[Rh₂(OAc)₂(np)(pynp)]^2+ (2; pynp = 2-(2-pyridyl)1,8-naphtyridine) and cis-[Rh₂(OAc)₂(pynp)]^2+ (3) the bridging pynp ligand blocks one and two of the axial coordination sites in the complexes, respectively. The electronic absorption spectra of the complexes are consistent with strong metal-to-ligand charge transfer transitions at low energy and ligand-centered peaks localized on the np and/or pynp ligands in the UV and near-UV regions. Time-dependent density functional theory calculations were used to aid in the assignments. The three complexes exhibit metal-centered oxidations and reductions, localized on the aromatic ligands. The ability of the complexes to stabilize duplex DNA and to inhibit transcription in vitro is greatly affected by the availability of an open axial coordination site. The present work shows that open axial coordination sites on the dirhodium complexes are necessary for biological activity.

Introduction

The principal mode of action of many antitumor drugs and antiviral agents takes place through the disruption of transcription and related processes.1−4 These include anticancer agents such as actinomycin D,5−7 anthracycline antibiotics,8,9 daunorubicin,10,11 and cisplatin,12,13 among others.14−21 In general, the inhibition of transcription by these drugs takes place through their interaction with, modification of, or damage to template DNA,5−13 but in some cases the mechanism involves the binding of the drug to the active

(18) Aune, G. J.; Furuta, T.; Pommier, Y. Anti-Cancer Drugs 2002, 13, 545.
site of RNA polymerase, blocking of the DNA/RNA channel, or by targeting transcription factors. The developed cellular resistance and the effectiveness of each agent against certain cancers but not others are limitations of the current drugs, which have prompted the search for new compounds.

Dirhodium(II) tetracarboxylate complexes, R₂H₅(O₂CR)₄ (R = Me, Et, Br, Bu), and related compounds exhibit carcinostatic activity against Ehrlich ascites and leukemia L1210 tumors, and they have been shown to be antibacterial and antitumor agents. In some cases, the antitumor activity in vitro was reported to be of the same order of magnitude as that of the anticancer drug cisplatin. Cisplatin covalently binds to double-stranded DNA (ds-DNA) by forming primarily 1,2-(dGpG) intrastrand cross-links, which disrupt the normal function of proteins and the transcription process. Although the mechanism of action of the dirhodium complexes remains largely unknown, it has been established that the complexes inhibit DNA replication and transcription in vitro. Transcription inhibition by Rh₂(II) complexes is believed to involve binding of the compound to either template DNA or RNA polymerase and to depend, among other factors, on the ligation sphere around the dirhodium core.

Dirhodium complexes have been shown to bind to nucleobases, dinucleotides, and DNA dodecamer single strands through both axial and equatorial ligand substitution. It is believed, however, that for complexes where equatorial substitution takes place, the initial interaction is axial followed by shifting of the new axial ligand to an equatorial position. Therefore, it is expected that the presence of strong, nonlabile ligands in the axial position of a dirhodium(II) complex would decrease the observed bioactivity through decreased interactions or binding of the compounds to biomolecules. In the present work, complexes that possess one or two accessible axial coordination sites are compared to a compound in which the axial positions are blocked. In the case of cis-[Rh₂(OAc)₂(pyp)₂]²⁺ (1: pyp = 1,8-naphthyridine), both axial sites are available for coordination whereas for cis-[Rh₂(OAc)₂(pyp(pypn))₂]²⁺ (2: pypn = 2-(2-pyridyl)1,8-naphthyridine) and cis-[Rh₂(OAc)₂(pypn(pypn))₂]²⁺ (3), the bridged Axen, R.; Rainen, L.; Chang, I. M.; Howard, R.; Serio, G.; Howard, R. A.; Spring, T. G.; Bear, J. L. Proc. Soc. Exp. Biol. Med. 1979, 157, 3904. (e) Catalan, K. V.; Mindiola, D. J.; Ward, D. A.; Chirotos, H. T.; Fu, P. K.-L.; Dunbar, K. R.; Turo, C. Inorg. Chem. 2004, 43, 1175. (f) Aoki, K.; Salam, M. A. Inorg. Chim. Acta. 2001, 359, 297. (g) Aslam, M. A.; Chirotos, H. T.; Fu, P. K.-L.; Dunbar, K. R.; Turo, C. Inorg. Chem. 2004, 43, 1175. (h) Aoki, K.; Salam, M. A. Inorg. Chim. Acta. 2001, 359, 297.
Experimental Section

Materials. Biotech grade acetonitrile was purchased from Sigma-Aldrich, while tetra-n-butylammonium hexafluorophosphate (TBAPF$_6$) was purchased from Fluka. Agarose, ethidium bromide, EDTA, Tris/HCl, MgCl$_2$, and RNA loading solution were purchased from Invitrogen and used as received. RhCl$_3$-$\text{H}_2\text{O}$ was purchased from TCI (Tokyo, Japan), while pynp was prepared following literature procedures. The bridging ligand np was purchased from Pressure Chemicals, and the starting materials were collected and dried (0.074 g, 74%).$^1$H NMR in CH$_3$CN-$\text{d}_6$, $\delta$/ppm (splitting): 9.05 (q, 4H), 8.37 (d, 4H), 7.61 (m, 4H), 2.40 (s, 6H).

$cis$-$[\text{Rh}_2(\mu-O\text{CCH}_3)_2(np)],[\text{BF}_4]_2$. A procedure similar to that for the synthesis of $[\text{Rh}_2(\mu-O\text{CCH}_3)_2(np)]$ was used, with the addition of 2 equiv (0.056 g, 0.27 mmol) of pynp ligand to cis-$[\text{Rh}_2(\mu-O\text{CCH}_3)_2(CH_3\text{CN})_6]$ (0.1 g, 0.13 mmol) in acetonitrile (20 mL) to obtain a dark-red solid (0.084 g, 84%).$^1$H NMR in CH$_3$CN-$\text{d}_6$, $\delta$/ppm (splitting): 9.70 (d, 2H), 8.87 (m, 4H), 8.70 (d, 2H), 8.67 (m, 4H), 8.50 (dd, 2H), 8.37 (d, 2H), 7.48 (q, 2H), 2.25 (s, 6H). Anal. Calcd for Rh$_2$C$_{24}$H$_{21}$N$_4$O$_6$B$_2$F$_8$: C, 31.70; H, 2.39; N, 7.39. Found: C, 31.65; H, 2.53; N, 7.43.

$cis$-$[\text{Rh}_2(\mu-O\text{CCH}_3)_2(pynp)],[\text{BF}_4]_2$. A solution of Rh$_2(\mu$-$O\text{CCH}_3)_3$ (0.2 g, 0.39 mmol) was stirred overnight in acetone (20 mL) in the presence of 1 equiv of pynp (0.08 g, 0.39 mmol). The product precipitated in the reaction mixture and was collected by filtration. The solid was suspended in methanol (40 mL) and stirred at room temperature until it went into solution. To this solution was added 4 equiv of NaBF$_4$ (0.16 g, 1.44 mmol) and 1 equiv of np (0.05 g, 0.38 mmol). The mixture was stirred overnight, the volume of the solution was decreased to $\sim$6 mL, and filtered using a fine frit. The red-orange solution was treated with toluene to afford a red-orange powder (0.06 g, 30%).$^1$H NMR in CH$_3$CN-$\text{d}_6$, $\delta$/ppm (splitting): 10.04 (d, 1H), 9.81 (d, 1H), 9.71 (d, 1H), 9.02 (d, 1H), 8.71 (m, 2H), 8.71 (d, 1H), 8.64 (m, 2H), 8.52 (m, 2H), 8.33 (dd, 1H), 8.15 (dd, 1H), 7.87 (dd, 1H), 7.56 (dd, 1H), 2.21 (s, 6H), 1.82 (s, 6H). MS $m/z$: 330.5, [Rh$_2(\mu-O\text{CCH}_3)_3$]($\text{pynp}+[\text{np}]$).$^{35}$ Anal. Calcd for Rh$_2$C$_{25}$H$_{21}$N$_5$O$_4$B$_2$F$_8$: C, 35.97; H, 2.54; N, 8.39. Found: C, 35.93; H, 2.39; N, 8.41.

Instrumentation. X-ray diffraction data were collected on a Bruker SMART 1000 CCD diffractometer with graphite monochromated Mo K$_\alpha$ radiation ($\lambda = 0.71073$ Å). The frames were integrated with the Bruker software, and a semimeipirical absorption correction using multiple-measured reflections was applied using SADABS.$^{34}$ The structures were solved and refined using X-SEED.$^{35}$ A graphical interface to SHELX97.$^{36}$ Cyclic voltammetric measurements were performed on a HCH electrochemical analyzer, model 620A. Absorption measurements were performed on a Shimadzu UV 1601PC spectrophotometer, a HP 8453 diode array spectrometer, or a Perkin Elmer Lambda 900 spectrometer. Mass spectra were acquired on a PE SCIEX QSTAR Pulsar electrospray ionization mass spectrometer. The ethidium bromide stained agarose gels (1%) were imaged on an Alphalmager 2000 transilluminator (Alpha Innotech Corporation).

Methods. For the $X$-ray crystallographic analysis, a red prismatic crystal of I (approximate dimensions: $0.20 \times 0.16 \times 0.10 \text{mm}^3$) was selected. The crystal was coated with Paratone oil, transferred to a nylon loop, and placed in a cold N$_2$ stream at 110(2) K. An indexing of the preliminary diffraction patterns indicated that the crystal was monoclinic, and selected refinement parameters are listed in Table 1. A total of 24 689 reflections were collected in the range $2.36 \leq \theta \leq 26.37^\circ$. The data collection covered approximately a hemisphere of reciprocal space by a combination of three or four sets of exposures; each set had a different $\phi$ angle for the crystal, and each exposure covered 0.3$^\circ$ in $\Omega$. The crystal decay, which was monitored by analyzing duplicate reflections, was found to be less than 1%; therefore, no decay correction was applied. During the final cycles of refinement, all atoms with the exception

$^{54}$ SADABS, Version 2.03; Bruker AXS Inc.: Madison, WI, 2002.
$^{56}$ Sheldevic, G. M. SHELX Programs for Solving and Refining Crystal Structures; University of Göttingen: Germany, 1997.
Table 1. Crystal Data and Structure Refinement for Compound I–(Cl)₂

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
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<tbody>
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<td>empirical formula</td>
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</tr>
<tr>
<td>fw</td>
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</tr>
<tr>
<td>crystal syst</td>
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<td>a</td>
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</tr>
<tr>
<td>b</td>
<td>17.293(2) Å</td>
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<tr>
<td>c</td>
<td>17.336(2) Å</td>
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<td>independent refls</td>
<td>5224</td>
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<tr>
<td>GOF on F²</td>
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<td>R indices (I &gt; 2σ(I))ᵃ</td>
<td>R₁ = 0.0508, wR₂ = 0.1193</td>
</tr>
<tr>
<td>R indices (all data)ᵃ</td>
<td>R₁ = 0.0604, wR₂ = 0.1266</td>
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</table>

The binding constant, K₉, was determined by optically titrating 5 μM metal complexes in a 5 mM Tris/HCl, pH 7.2 buffer at room temperature with ct-DNA up to a final concentration of 200 μM. The dilution of the compound concentration at the end of each titration was negligible. The K₉ values were calculated from fits of the absorption changes as a function of [DNA] as previously described in detail. The relative changes in viscosity were measured on a Cannon-Manning semimicroviscometer. The viscometer was immersed in a constant-temperature water bath (25 °C) controlled by a Neslab (model RTE-100) circulator.

The transcription assay has been previously reported.

In the in vitro transcription experiment, the pHGE linear DNA template (120 μM bases) was used with T7–RNAP, resulting in two transcripts of length 1065 and 2346 bases, and each trial was conducted three times. The transcription reaction was conducted for 45 min at 37 °C (40 mM Tris/HCl, pH = 8.0) in nuclease-free water in the presence of 1.25 units of T7–RNAP, 25 mM NaCl, and 1.0 mM of each ATP, CTP, GTP, and UTP. The inhibition of mRNA production by the dirhodium complexes was detected in vitro by the measurement of the mRNA generated upon addition of increasing amounts of metal complex to the assay. The concentration of each complex at which 50% of the mRNA is transcribed, C₅₀, was calculated by interpolation of the integrated areas of the mRNAs signal of each lane of the gel conducted with various concentrations of a given complex. Modifications of these methods were utilized in the various control assays, including those designed to determine the role of binding of the complexes used instead of acetates in the computationally modeled complexes.

This procedure has been found to be acceptable in other reported computational studies. All geometries were fully optimized under the conditions of the respective programs. Orbital analysis was completed with Molekel 4.3.6 Vertical electronic transitions were calculated using TDDFT methods implemented within G03.

The concentration of DNA stock solutions was determined from its absorbance at 260 nm (ε = 10 000 M⁻¹ cm⁻¹). The methods used to determine the DNA binding constants, K₉, of the compounds and the DNA melting temperature have been previously reported, and the errors in the value of K₉ typically range from 3 to 7% from the fits and multiple measurements. The melting temperature experiments were carried out by monitoring the absorption change at 260 nm while varying the temperature from 25 to 95 °C of a mixture containing 20 μM complex and 100 μM calf-thymus DNA (ct-DNA) in 1 mM phosphate buffer, 2 mM NaCl, pH 7.2. The binding constant, K₉, was determined by optically titrating 5 μM metal complexes in a 5 mM Tris/HCl, pH 7.2 buffer at room temperature with ct-DNA up to a final concentration of 200 μM. The dilution of the compound concentration at the end of each titration was negligible. The K₉ values were calculated from fits of the absorption changes as a function of [DNA] as previously described in detail. The relative changes in viscosity were measured on a Cannon-Manning semimicroviscometer. The viscometer was immersed in a constant-temperature water bath (25 °C) controlled by a Neslab (model RTE-100) circulator.

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The transcription assay has been previously reported.
that of \( \text{Rh}_2(\text{CCH}_3)_2(\text{np})_2 \) complex

\[ \text{O}_2 \text{CCH}_3 \] 2 (phen) 2 Cl 2 and somewhat shorter than that found

longer than that reported for \( \text{Rh}_2(\text{CCH}_3)_2(\text{np})(\text{pynp})^2 \) 2

atom. The structure of the dichloride adduct of \( \text{Rh}_2(\text{CCH}_3)_2(\text{np})(\text{pynp})^2 \) 2

Figure 2. Thermal ellipsoid plot at the 50% probability level of 1–Cl2.

to T7–RNAP. All stock solutions of the metal complexes were prepared in pure H2O.

Results and Discussion

Synthesis and Characterization. The bisheteroleptic complexes were prepared by substitution of the four equatorial CH3CN ligands in cis-[Rh2(μ-O2CCH3)2(CH3CN)] 2+ with the bidentate ligands, np (1,8-naphthyridine) and pynp (2-(2-pyridyl)1,8-naphthyridine), to generate cis-[Rh2(μ-O2CCH3)2(np)] 2+ (1) and cis-[Rh2(μ-O2CCH3)2(pynp)] 2+ (3), respectively. The synthesis of the new tri-heteroleptic complex cis-[Rh2(μ-O2CCH3)2(np)(pynp)] 2+ (2) was carried out by stepwise addition of np followed by pynp to an acetone solution of Rh2(μ-O2CCH3)4 at room temperature. The crystal structure of the dichloride adduct of 1, which has not been previously reported, is shown in Figure 2.

The molecular structure of 1, determined by X-ray crystallographic methods, consists of a dinuclear Rh 2 (II,II) core with a pair of bridging np ligands coordinated to each Rh atom and two bridging acetate groups occupying the remaining equatorial sites. Two chloride ions complete a distorted octahedral coordination sphere around each Rh atom. The structure of 1 is shown in Figure 2 and is similar to those reported for related dirhodium complexes. A selection of bond distances and angles is listed in Table 2. The Rh–Rh distance in 1, 2.4251(5) Å, is well within the expected range for a Rh–Rh single bond. The distance is longer than that reported for [Rh2(μ-O2CCH3)2(pynp)] 2+ , 2.206(9) Å, and shorter than those reported for Rh2(μ-O2CCH3)2(phen)2Cl2 (phen = 1,10-phenanthroline) and [Rh2(np)(μ-O2CCH3)] 2+ , 2.606(11) Å, and 2.448(1) Å, respectively. The average Rh–N bond length in 1, 2.027(4) Å, is similar to that of [Rh2(μ-O2CCH3)2(pynp)] 2+ , 2.018(4) Å, and longer than the reported Rh–Nav distance of 2.010(5) Å in Rh2(μ-O2CCH3)2(phen)2Cl2 and somewhat shorter than that found

Table 2. Selected Bond Lengths (Å) and Angles (deg) for Compound 1–Cl2

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<th>bond</th>
<th>length (Å)</th>
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<td>Rh1–Rh2</td>
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<td>Rh1–N1</td>
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<td>Rh2–Cl1</td>
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<td>Rh2–Cl2</td>
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Table 3. Electronic Absorption and Electrochemical Properties of 1–3

<table>
<thead>
<tr>
<th>complex</th>
<th>( \lambda_{\text{abs}}/\text{nm} ) (ε \text{M}^{-1} \text{cm}^{-1})</th>
<th>( E_{\text{1/2}}/\text{V} )</th>
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<tbody>
<tr>
<td>1</td>
<td>268 (6 260), 307 (5980), 315 (5830), 393 (4880)</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>266 (21 320), 320 (19 490), 356 (5410), 398 (3380)</td>
<td>0.63, 1.35</td>
</tr>
<tr>
<td>3</td>
<td>316 (31 390), 329 (31 400), 356 (12 790), 465 (5680)</td>
<td>0.64, 1.03</td>
</tr>
</tbody>
</table>

* In CH3OH/H2O (50:50 v/v). ** In acetonitrile with 0.1 M Bu4NPF6 vs SCE.

for [Rh2(np)(μ-O2CCH3)] 2+ , 2.054(3) Å. The axial chloride ligands are at an average distance of 2.563(1) Å in 1 whereas the reported Rh–Cl distance for Rh2(μ-O2CCH3)2(phen)2Cl2 is 2.532(2) Å. This increase of 0.03 Å, as compared to the bis-phenanthroline analogue, can be attributed primarily to the presence of the hydrogen atoms on the np ligands pointing toward the chloride ions.

Electronic Absorption and Electrochemistry. The absorption maxima and molar extinction coefficients of complexes 1–3 are listed in Table 3. Since the free np ligand exhibits ππ* transitions at 258, 300, and 309 nm in CH3OH/H2O (50:50 v/v), it is likely that the peaks observed in 1 at 268 and 307 nm are centered on the np ligand. Similarly, the free pynp ligand exhibits absorption maxima at 271, 322, and 333 (sh) nm in CH3OH/H2O (50:50 v/v), which leads to the assignment of the peaks at 316 and 329 nm in 3 as ligand centered. In the mixed-ligand complex 2, the peaks observed at 262 and 356 nm are likely due to a superposition of ππ* transitions of the np and pynp ligands. Additional absorption peaks in the UV and near-UV are observed with maxima at 315 nm (ε = 5830 M⁻¹ cm⁻¹) in 1 and at 356 nm in both 2 (ε = 5410 M⁻¹ cm⁻¹) and 3 (ε = 12 790 M⁻¹ cm⁻¹), which may be attributed to metal-to-ligand charge transfer (MLCT) transitions. The latter may correspond to Rh2 → pynp, which is expected at a lower energy than the corresponding MLCT transition to the np ligand in 1. Each complex also exhibits an absorption peak in the 393–465 nm region with intensity that ranges from 3380 to 4880 M⁻¹ cm⁻¹. In the related complex Rh2(μ-O2CCH3)4, the transitions observed in water in the visible region are weak (441 nm, ε = 106 M⁻¹ cm⁻¹ and 585 nm, ε = 241 M⁻¹ cm⁻¹) and have been previously assigned to Rh–O(σ) → Rh2(σ*) and Rh2(π*) → Rh2(σ*), respectively.

Owing to the intensity of the transitions observed in 1–3 in the visible region, the transitions can be assigned as arising from MLCT from Rh2 to the np and/or pynp ligand depending on the complex. The position and intensity of these transitions are comparable to those observed in cis-


Dirrhodium(II,II) Complexes

\[ \text{[Rh}_2(\mu-O_2CCH_3)_2(bpy)_2]^{2+} \] (bpy = 2,2’-bipyridine, \( \lambda_{\text{abs}} = 408 \text{ nm, } \epsilon = 2920 \text{ M}^{-1} \text{ cm}^{-1} \) in CH_3CN) and cis-[\text{Rh}_2(\mu-O_2CCH_3)_2(phen)_2]^{2+} (phen = 1,10-phenanthroline, \( \lambda_{\text{abs}} = 408 \text{ nm, } \epsilon = 3050 \text{ M}^{-1} \text{ cm}^{-1} \) in CH_3CN), which also possess aromatic ligands coordinated to the dirhodium core and have been previously assigned as MLCT from the Rh_2 to the bpy and phen ligand, respectively.\(^{(77)}\)

Additional evidence for the assignment of the lowest energy transition in complexes 1–3 as MLCT can be obtained by examining the spectrum of 1 with axially coordinated pyridine (py). The presence of strong \( \sigma \)-donor axial ligands has been shown to have a profound effect on the electronic structure of dirhodium complexes. For example, in \text{Rh}_2(\mu-O_2CCH_3)_4 addition of ligands that bind to the axial positions results in a blue shift of the lowest energy, \text{Rh}_2(\sigma^*) \rightarrow \text{Rh}_2(\sigma^*) metal-centered (MC) transition.\(^{(78–80)}\) This shift is due to the interaction of the antisymmetric linear combination of the filled orbitals on the axial ligands with the \text{Rh}_2(\sigma^*) molecular orbital, thus raising the energy of the latter.\(^{(81–83)}\) Addition of excess py (547 \mu M) to 1 (22 \mu M) in H_2O does not result in spectral shifts in the near-UV–vis regions. This result is consistent with the assignment of these low-energy transitions as \text{Rh}_2 \rightarrow np MLCT, which are not expected to be affected greatly by the presence of axial ligands.

The reduction potentials of 1–3 are listed in Table 3. The first reduction is dependent on the identity of the aromatic ligand. Complexes 2 and 3, with one and two coordinated pynp ligands, respectively, exhibit \( E_{1/2} \sim -0.64 \text{ V vs SCE} \). In contrast, complex 1 is more difficult to reduce by \( \sim 0.17 \text{ V} \), with the first reduction observed at \( -0.82 \text{ V vs SCE} \). The free pynp ligand is easier to reduce than the np ligand, with \( E_{1/2} \) values of \(-1.68 \text{ and } -1.88 \text{ V vs SCE (CH}_3\text{CN, 0.1 M Bu_4NPF_6)} \), respectively. The difference in the reduction potentials of the free ligands, 0.20 V, is comparable to that between 1 and complexes 2 and 3. The values of the first two reduction waves of complexes 1–3 are similar to those reported for other dirhodium complexes possessing aromatic ligands, such as cis-[\text{Rh}_2(\mu-O_2CCH_3)_2(bpy)_2]^{2+} (\( E_{1/2} = -0.93 \text{ V vs SCE in CH}_3\text{CN)} and cis-[\text{Rh}_2(\mu-O_2CCH_3)_2(phen)_2]^{2+} (\( E_{1/2} = -0.87 \text{ V vs SCE in CH}_3\text{CN).} \(^{(77)}\) The oxidation potentials, \( E_{1/2} ([\text{Rh}_2(\mu-O_2CCH_3)_4]^{2+} = +1.17 \text{ V vs SCE in CH}_3\text{CN,} \(^{(84)}\) These results point at the oxidation of 1–3 as being centered on the dirhodium core.

**Electronic Structure Calculations.** Electronic structure calculations were conducted in order to aid in the assignments of the absorption spectra and electrochemistry of complexes 1–3, and comparisons were made regarding the structural and electronic changes that take place upon axial coordination of solvent and as the bridging ligands are varied. As shown in Figure 3, the electronic structures of the model complexes cis-[\text{Rh}_2(\mu-O_2CCH_3)(np)]^{2+} (1a) and cis-[\text{Rh}_2(\mu-O_2CCH_3)(pynp)-](np)]^{2+} (2a) are sensitive to the nature of the coordinating solvent in the axial position as previously reported for other dirhodium complexes.\(^{(65)}\)

In aqueous solution, water molecules coordinate to the open axial sites of 1a and 2a generating 1a–(H_2O)_2 and 2a–(H_2O), respectively (Figure 3). Interaction of the symmetric and antisymmetric linear combinations of the axial water molecules with metal-centered MOs in 1a results in the destabilization of the \text{Rh}_2(\sigma^*) and \text{Rh}_2(\sigma^*) orbitals in 1a–(H_2O)_2 (Figure 3). As shown in Figure 3, these axial interactions change the identity of the LUMO from \text{Rh}_2(\sigma^*) in 1a to np(\( \pi^* \)) in 1a–(H_2O)_2. The electronic structure calculations with NCH axial ligands as models for acetonitrile molecules also result in np(\( \pi^* \)) LUMO in 1a–(NCH)_2. These results are in agreement with the observation of ligand-centered reduction of 1 in CH_3CN. DFT calculations on 2a, 2a–(H_2O), and cis-[\text{Rh}_2(\mu-O_2CCH_3)(pynp)]^{2+} (3a) also result in ligand-centered LUMO in agreement with the electrochemical reduction discussed above (Figure 3). It should be noted that the calculations also predict that the reduction potentials of 2 and 3 should be similar in a coordinating solvent whereas 1 should be more difficult to reduce (Figure 3). Selected molecular orbitals of 1a–(H_2O)_2, 2a–(H_2O), and 3a are shown in the Supporting Information.

The changes to the orbital energies of 1a–(H_2O)_2 upon substitution of one and two np ligand(s) for pynp to generate 2a–H_2O and 3a, respectively, are also shown in Figure 3. In order to make a semiquantitative comparison among the complexes, the energy of the \text{Rh}_2(\alpha^*) MO of 1a was set to 0.0 eV. In addition, the lowest energy np(\( \pi^* \)) orbitals of 1a,
1a−(H₂O), 2a, and 2a−(H₂O) were matched in energy in Figure 3, since this orbital is not expected to be affected by axial substitution. Similarly, the lowest energy pynp(π*) in 2a−(H₂O) and 3a were set to the same energy. As expected, substitution of H₂O molecules in the axial position for the stronger pyridine portion of the pyn ligand results in an increase in the energy of both the Rh₂(σ) and Rh₂(σ*) MOs (Figure 3).

Time-dependent DFT (TDDFT) calculations can be used to predict observed transition energies and can be used to elucidate their parentage. As discussed above, the peaks observed in the UV region can be ascribed to ππ* transitions centered on the aromatic ligands, while the two lowest energy transitions are assigned as Rh₂ np/pyn MLCT. In general, the MLCT peaks red-shift across the series 1 to 3 (Table 3). TDDFT calculations on 1a−(H₂O)₂ predict a strong nπ-centered transition at 259 nm and four weaker ones in the 261−275 nm range (Supporting Information). Two low-energy vertical Rh₂(π*) → np(π*) MLCT transitions are calculated at 402 and 410 nm. The latter has the largest oscillator strength (Supporting Information) and may be correlated to the peak observed at 393 nm in the complex (Table 3). In 3a, MLCT transitions from Rh₂(δ*) and Rh₂(σ*) to pynp(π*) are calculated at 466 and 489 nm, respectively (Supporting Information), which may be correlated with the absorption peak with maximum at 465 nm in 3. Additional MLCT transitions of 3a are predicted at 315, 320, and 331 nm; however, a very strong pynp-centered transition of significantly greater intensity is calculated at 326 nm (Supporting Information). The latter may be associated with the peak observed at 356 nm in 3. A transition at 340 nm, calculated to exhibit both MLCT and LC character in 3a, may also contribute to the peak observed at 356 nm in 3. Owing to the presence of both np and pynp ligands in the tri-heteroleptic complex, there is a greater number of calculated transitions for 2a−(H₂O) in the same energy range. Two low-lying MLCT transitions of similar intensity from Rh₂(π*) to pynp(π*) and to np(π*) are calculated at 471 and 423 nm, respectively (Supporting Information). These are at significantly lower energy than the observed MLCT peak in 2 with maximum at 398 nm (Table 3). A fairly strong pyn ππ* transition is calculated at 342 nm for 2a−(H₂O) with several additional peaks with MLCT character between 318 and 335 nm. As a result of a large number of calculated peaks, a correlation with the experiment in solution for this complex is not possible. However, the calculated transitions lie in the region where the peaks are observed. As previously assigned, the calculations show that the lowest energy transition in each of these complexes is MLCT in character.

As discussed above, the metal-centered Rh₂(π*) → Rh₂(σ*) transition of the parent complex Rh₂O₃(C₂H₄)₂ is known to shift to higher energy as a function of the axial ligands.85,86 Although this weak transition is not observed experimentally in complexes 2 and 3 owing to the overlapping high-intensity MLCT peaks, its position can be calculated. As expected, the calculated energy of Rh₂(π*) → Rh₂(σ*) transition shifts as the number of pyn ligands with strong axial coordination is increased in the series 1a−(H₂O)₂, 2a−(H₂O), and 3a with predicted maxima at 521 nm (f = 0.0011), 474 nm (f = 0.0022), and 455 nm (f = 0.0008), respectively. The calculated maximum of the metal-centered transition for 1a−(H₂O)₂ agrees well with the observed shoulder for 1 in water at ~519 nm.

**DNA Binding.** The binding constants of 1 to 3 to DNA, Kₖ, determined from fits of the changes in the absorption of each complex as a function of nucleic acid concentration are listed in Table 4. The values of Kₖ vary with the availability of the axial position. For complex 1, with both axial positions available for coordination with Lewis bases, Kₖ = 5.6 × 10⁵ M⁻¹ (s = 1.1) was measured, while Kₖ = 3.4 × 10⁶ M⁻¹ (s = 0.9) was calculated for 2. The value of Kₖ measured for 3 (3.3 × 10⁷ M⁻¹ (s = 2.0)), which has the two axial positions blocked by the pyn ligand, is approximately 2 orders of magnitude smaller than the DNA binding constants of 1 and 2. The magnitude of Kₖ obtained for 3 is consistent with electrostatic interactions between the complex and DNA, where similar values have been reported in the literature for [Ru(tpy)(bpy)(OH)₂]²⁺ (tpy = 2,2',6,2″-terpyridine, Kₖ = 1.3 × 10⁴ M⁻¹), [Ru(tpy)(bpy)(OH)₂]²⁺ (Kₖ = 6.6 × 10³ M⁻¹), and [Ru(bpy)₃]²⁺ (Kₖ = 6.8 × 10² M⁻¹), which bind DNA through electrostatic interactions.87 For comparison, the DNA binding constant for the partial intercalator [Ru(phen)]²⁺ was reported87b to be 4.8 × 10⁵ M⁻¹ while a Kₖ value of 1.7 × 10³ M⁻¹ has been reported for the intercalator ethidium bromide.88 Since complexes 1 to 3 have the same overall charge, the differences in measured Kₖ values are not due to electrostatic interactions. Previous reports have shown that dirhodium compounds can interact with DNA forming a variety of interstrand cross-link adducts.89 Therefore, it is possible that there is a covalent interaction between the open axial position of the compound and the DNA such that the decrease in the value of the binding constant from 1 to 2 is related to the difference in the number of open axial positions.

The shift in the melting temperature of 100 μM DNA, ΔT_m, in the presence of each complex (20 μM) was measured relative to that of DNA alone, for which T_m = 59(1) °C (1 mM phosphate buffer, 2 mM NaCl, pH 7.2). The largest shift, ΔT_m = +10(2) °C, was observed for compound 1 followed by 2 and 3 in that order.

![Table 4. DNA Binding Constants, ΔT_m Values, and C_m of 1−3](image)

<table>
<thead>
<tr>
<th>complex</th>
<th>Kₖ (M⁻¹)</th>
<th>ΔT_m °C</th>
<th>C_m (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6 × 10⁵</td>
<td>10.3</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>3.4 × 10⁵</td>
<td>7.0</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>3.3 × 10³</td>
<td>4.2</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>

* T_m = 61(1) °C was measured for DNA alone. The value measured for cisplatin under the same experimental conditions was 4.1 μM.87

by 2 with $\Delta T_m = +7(2) ^\circ C$. Only a modest shift in the DNA melting temperature was measured for 3, $\Delta T_m = +4(2) ^\circ C$. Intercalating complexes possessing a phi (phi = 9,10-phenanthrenquinone diimine) ligand in their coordination sphere, such as [Rh(phen)$_2$phi]$^{3+}$ and [Ru(phen)$_2$phi]$^{2+}$, were previously shown to raise the melting temperature of the duplex by 7 and 11 $^\circ C$, respectively. $^{72}$ Similar stabilization of the DNA double helix has been reported for other intercalators, such as [Ru(bpy)$_2$(DAP)]$^{2+}$ (DAP = 1,12-diazaperylene) and ethidium bromide. $^{72}$ Therefore, it is possible that the shift in $T_m$ observed for 1 is due to DNA intercalation by the complex. Intercalation of molecules between DNA bases is known to increase the viscosity of the solution owing to unwinding and elongation of the double helix. $^{90}$ Experiments using 1 mM herring sperm DNA in the presence of up to 300 $\mu$M of 1 did not result in an increase of the relative viscosity of the solution (Supporting Information). Since relative viscosity measurements are the most reliable means to determine intercalation, $^{90}$ these results indicate that the greater DNA binding constant and $\Delta T_m$ value measured for 1 is not due to intercalation. A possible explanation for these observations is the presence of one and two axial sites available for coordination in 1 and 2, respectively; whereas the two rhodium centers in 3 are coordinatively saturated, since the pypn ligands block both axial sites in the complex. It is well known that cations, such as Na$^+$ and Mg$^{2+}$, stabilize the duplex DNA structure by charge screening through interaction with the anionic phosphate groups in the phosphodiester backbone. $^{91-93}$ It has been previously shown that divalent alkaline-earth ions are able to stabilize the duplex structure to a significantly greater extent than monovalent cations. $^{91-93}$ For example, the melting temperature of a duplex composed of the sequence d(GC-CAGTTTAA) and its complementary strand was reported to increase from 32.0 $^\circ C$ in the presence of 100 mM NaCl (10 mM Na$_2$HPO$_4$, 1 mM Na$_2$EDTA, pH = 7.0) to 39.0 $^\circ C$ in 100 mM MgCl$_2$ (10 mM sodium cacodylate, pH = 7.0). $^{94}$ A similar shift in $T_m$ was measured for the same duplex in the presence of 10 mM MgCl$_2$ ($T_m = 36.0 ^\circ C$) and in mixtures of 100 mM NaCl with 10 mM MgCl$_2$ ($T_m = 35.1 ^\circ C$) and with 100 mM MgCl$_2$ ($T_m = 38.5 ^\circ C$). $^{94}$ These results show the greater activity of divalent ions in duplex stabilization compared to monovalent cations. In contrast to the increase in the DNA melting temperature typically observed for divalent alkaline earth ions, transition metal cations, such as Co$^{2+}$, Ni$^{2+}$, and Cd$^{2+}$, result in destabilization of the duplex. $^{95}$ It is believed that cations that interact with the phosphate backbone stabilize the duplex DNA structure, and those that coordinate to the nucleobases have a destabilizing effect. $^{95}$


In contrast, no decrease in the mRNA transcribed is observed upon addition of up to 300 μM of 3 (Figure S3). The concentration of each complex required to inhibit 50% of the transcription, $C_{\text{inh50}}$, was determined from interpolation of plots of percent inhibition as a function of increasing complex concentration. The values of $C_{\text{inh50}}$ for 1 and 2 are 3.4 and 54 μM, respectively, while that for 3 could not be measured but can be estimated to be $>600$ μM (Table 3).

A strong correlation between the DNA melting temperature of mononuclear Ru(II) and Rh(III) complexes with transcription inhibition has been previously reported. It is believed that the stabilization of the DNA duplex structure suppresses bubble formation thus reducing the amount of transcribed RNA. In the present work, this trend is also observed with an increase in transcription inhibition with greater duplex stabilization. Additional studies are currently underway to gain further understanding of whether the interactions of the complex with DNA or with the enzyme result in the reduced production of RNA.

**Conclusions**

Dirhodium(II,II) complexes that possess one or two accessible axial coordination sites, cis-[Rh₂(OAc)₂(np)]²⁺ (1) and cis-[Rh₂(OAc)₂(np)(pynp)]²⁺ (2), respectively, were compared to a compound in which the axial sites are blocked, cis-[Rh₂(OAc)₂(pynp)]²⁺ (3). In the latter, the bridging pynp ligand blocks both the axial coordination sites in the complex. The electronic and electrochemical properties of the complexes were investigated, and TDDFT calculations were used to aid in the assignments. The ability of the complexes to stabilize duplex DNA and to inhibit transcription *in vitro* shows a profound effect on the availability of an axial coordination site on reactivity toward biomolecules.

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**Supporting Information Available:** Selected molecular orbitals, TDDFT energies and assignments, relative viscosity experiments, additional transcription inhibition gels in the presence of 3, DNA binding titrations, and crystallographic parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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