Dirhodium(II,II) DNA Head-to-Head Cross-Links

$[\text{Rh}_2(\text{DTolF})_2\{\text{d(GpG)}\}]$

ROESY NMR

$[\text{Rh}_2(\text{O}_2\text{CCH}_3)_2\{\text{d(GpG)}\}]$
Head-to-Head Cross-Linked Adduct between the Antitumor Unit Bis(\(\mu\)-\(N,N\'-di-p\)-tolyiformamidinato)dirhodium(II,II) and the DNA Fragment d(GpG)

Helen T. Chifotides* and Kim R. Dunbar*\([a]\)

Abstract: Reactions of the compound \(\text{cis-[Rh}_2(D\text{TolF})_2(CH_3CN)_6](BF_4)_2\), a formamidinate derivative of the class of antitumor compounds \([\text{Rh}_2(O_2\text{CR})_4]\) (\(R = \text{Me}, \text{Et}, \text{Pr}\)) with 9-ethylguanine (9-EtGuaH) or the dinucleotide (R(GpG)) proceed by substitution of the acetoni trile groups, with the guanine bases spanning the Rh–Rh bond, in a bridging fashion, through sites N7/O6. In the case of 9-EtGuaH, both head-to-head (HH) and head-to-tail (HT) isomers are formed, whereas with the tethered bases in d(GpG), only one right-handed conformer HH1R \([\text{Rh}_2(D\text{TolF})_2[d(GpG)]]\) is present in solution. For both \(\text{cis-[Rh}_2(D\text{TolF})_2(9\text{-EtGuaH})_3](BF_4)_2\) and \([\text{Rh}_2(D\text{TolF})_2[d-(GpG)]]\), the absence of N7 protonation at low pH and the substantial decrease of the pK\(_a\) values for N1-H deprotonation, support N7/O6 binding of the bases to the dirhodium core. The N7/O6 binding of the bases is further corroborated by the downfield shift by \(\Delta \delta \sim 4.0\) ppm of the \(^{13}\)C NMR resonances for the C6 nuclei as compared to the corresponding resonances of the free ligands. The HH arrangement of the guanine bases in \([\text{Rh}_2(D\text{TolF})_2[d(GpG)]]\) is indicated by the intense H8/H8 ROE cross-peaks in the 2D ROESY NMR spectrum. Complete characterization of the \([\text{Rh}_2(D\text{TolF})_2[d-(GpG)]]\) conformer by 2D NMR spectroscopy supports anti-orientation and N (C3'-endo) conformation for both deoxyribose residues. The N-pucker for the 5'-G base is universal in such cross-links, but it is very unusual for platinum and unprecedented for dirhodium HH cross-linked adducts to have both deoxyribose residues in the N-type conformation. The bulk, the nonlabile character, and the electron-donating ability of the formamidinate bridging groups spanning the dirhodium core affect the nature of the preferred dirhodium DNA adducts. Molecular modeling studies performed on \([\text{Rh}_2(D\text{TolF})_2[d(GpG)]]\) corroborate the structural features obtained by NMR spectroscopy.

Keywords: nucleic acids · antitumor agents · bioinorganic chemistry · formamidinates · N ligands · rhodium

Introduction

Metal–metal-bonded dirhodium compounds\([1]\) with a lantern type structure have attracted scientific interest due to their appreciable carcinostatic activity against various tumor cell lines. Pioneering studies that emanated in the 1970s revealed that dirhodium tetracarboxylate compounds of the type \([\text{Rh}_2(O_2\text{CR})_4]\) (\(R = \text{Me}, \text{Et}, \text{Pr}; \text{Scheme 1a}\)) exhibit significant in vivo antitumor activity against L1210 tumors\([2,3]\) Ehrlich ascites\([4-7]\) as well as sarcoma 180 and P388 tumor lines\([8]\). Although the exact mechanism of their antitumor activity has not yet been elucidated, previous studies support the conclusions that dirhodium compounds bind to DNA\([4,5,9-12]\) and inhibit DNA replication, protein synthesis, and in vitro transcription\([13-16]\). Another closely related class of dirhodium compounds to the tetracarboxylate series has emerged by substituting the carboxylate for the more robust amidinate groups\([1]\). Despite the lack of any appreciable biological activity of the homoleptic paddlewheel compound \([\text{Rh}_2(D\text{TolF})_4]\) (DToF = anion of \(N,N\'-di-p\)-tolyiformamidinate) due to steric factors\([13]\) the compound \(\text{cis-[Rh}_2(D\text{TolF})_2(O_2\text{CCF}_3)_2(H_2\text{O})_2]\) (Scheme 1b) exhibits antitumor activity comparable to that of the dirhodium carboxylates and cisplatin (when supplied in the same quantity) against Yoshida ascites and T8 sarcomas with considerably reduced toxicity\([10]\). The two labile trifluoroacetate bridging groups impart an appreciable reactivity to the complex, yet its toxic side effects are minimal. It is
notable that it was not possible to establish the highest non-toxic dose for cis-[Rh₂(DTolF)₃(O₂CCF₃)₂(H₂O)]₂, since it would need to be dissolved in a volume of solvent (dimethyl sulfoxide) that would itself become toxic before the compound would be cytotoxic.[18]

In light of DNA being the primary target of most metal-based anticancer agents, the reactions of dirhodium compounds with purine nucleobases, nucleos(t)ides, single- and double-stranded DNA[11] have received considerable attention.[12] Early claims in the literature that dirhodium carbonylate compounds do not react with guanine (Scheme 2a) and polyguanylic acids[9] were unequivocally settled by findings in our laboratories that guanine bases bind to the dirhodium core in a manner involving unprecedented equatorial (eq) bridging interactions. In particular, the crystal structural determinations of HT cis-[Rh₂(O₂CCF₃)₂(9-EtGuaH)(MeOH)][19] HH cis-[Rh₂(O₂CCF₃)₂(9-EtGuaH)₂(Me₂CO)·(H₂O)](BF₄)₂[20] and HT cis-[Rh₂(O₂CCF₃)₂(9-EtGuaH)(Me₂CO)₃](CF₃CO₂)₂[19] revealed that bridging 9-EtGuaH groups (Scheme 2a) span the dirhodium unit through the N7/O6 groups (Scheme 2a) span the dirhodium unit through the N7/O6.

Related studies involving the reactions of cis-[Rh₂(DTolF)₂(CH₃CN)]₂(BF₄)₂ with N–N chelates, which mimic the binding of two adjacent DNA bases (2,2'-bipyridine and 1,10-phenanthroline), demonstrated that the latter bind to the dirhodium core in a chelating fashion by substituting acetonitrile molecules in equatorial positions.[24]

Armed with the knowledge obtained from our studies of the dirhodium unit interactions with the basic building blocks of DNA, we extended our work to the chemistry of small DNA fragments. Reactions of [Rh₂(O₂CCH₃)₃] with the dinucleotides d(GpG) (Scheme 2b) and d(pGpG) afford [Rh₂(O₂CCH₃)₂[d(GpG)]] and [Rh₂(O₂CCH₃)₂[d(pGpG)]] respectively, with bidentate N7/O6 bridging bases spanning the Rh–Rh bond. The bidentate N7/O6 coordination of the bases is corroborated by the notable increase in the acidity of N1-H and the substantial downfield shifts of the ¹³C NMR resonances of the base C6 carbon atoms.[22–25] For both dinucleotide complexes, intense H8/H8 ROE (Rotating frame nuclear Overhauser effect) crosspeaks in the 2D ROESY NMR spectra indicate a HH arrangement of the tethered guanine bases.[22–25] The [Rh₂(O₂CCH₃)₂[d(GpG)]] complex exhibits two major right-handed conformers HH1R (≈75%) and HH2R (≈25%).[22] The terms HH1R and HH2R, initially proposed for platinum compounds by Kozelka et al.[26–27] and refined by Marzilli et al.[28–31] refer to the relative base canting and the direction of propagation of the phosphodiester backbone with respect to the 5’ base (Scheme 3).[32] HH1L platinum ad-

![Scheme 1. Structures of metal–metal-bonded dirhodium compounds.](Image 47x366)

![Scheme 2. Structure and atom numbering of a) nucleobase 9-ethylguanine (9-EtGuaH) and b) dinucleotide d(GpG).](Image 110x541)
Bis(formamidinato)dirhodium–d(GpG) Adduct

Results

1D 1H NMR spectroscopy

cis-[Rh2(DTolF)2(9-EtGuaH)2(BF4)2]: The 1H NMR spectrum of [Rh2(DTolF)2(9-EtGuaH)2(BF4)2] in CD3CN displays two H8 resonances in the aromatic region at δ = 8.05 and 8.08 ppm, downfield from the H8 resonance of free 9-EtGuaH at δ = 7.49 ppm (in CD3CN). The two H8 resonances are attributed to the two isomers (HH and HT) of cis-[Rh2(DTolF)2(9-EtGuaH)2(BF4)2] produced from the reaction of cis-[Rh2(DTolF)2(NCCH3)2(BF4)2] with 9-EtGuaH. In the same region, there are two triplets at δ = 7.41 and 7.54 ppm, which are ascribed to the sets of N–CH–N groups of the bridging formamidinate groups for each isomer (the triplets are attributed to 1H coupling to the two equivalent rhodium nuclei, 3J[H8–Rh1] = 3.8 Hz). [Rh2(DTolF)2(NCCH3)2(BF4)2] iso-

moters differ by about 0.05 ppm, thus the titration curve for the second isomer has been omitted from the plot.

Detailed characterization of the HH1R and HH2R [Rh2-

(O2CCH3)2(dGpG)] conformers by 2D NMR spectroscopy,[22] revealed notable structural features that resemble those of cis-[Pt(NH3)2(dGpG)]]; the latter involve repuck-

ering of the 5'-G sugar rings to the C3'-endo (N-type) con-

formation, retention of the C2'-endo (S-type) conformation for the 3'-G sugar rings and anti-orientation of the bases with respect to the glycosyl bonds.[22,23] Herein, we report the structural characterization of the biologically relevant adduct [Rh2(DTolF)2(dGpG)] by one- (1D) and two-di-

mensional (2D) NMR spectroscopy along with molecular modeling studies. The conformational and structural characteristics of [Rh2(DTolF)2(dGpG)] are discussed in light of the conferred effects of substituting the acetate for the form-

amidinate bridging groups on the dirhodium core.

Figure 1. pH dependence of the H8 1H NMR resonance for [Rh2-

(DTolF)2(9-EtGuaH)2(BF4)2] in CD3CN at 20°C. At each pH value, the H8 NMR chemical shifts of the two [Rh2(DTolF)2(9-EtGuaH)2(BF4)2] iso-

moters differ by about 0.05 ppm, thus the titration curve for the second isomer has been omitted from the plot.

behavior of the H8 1H NMR resonance at low pH corroborates N7 binding to the rhodium center (the bound metal prevents protonation of this site). For free 9-EtGuaH in D2O, protonation of N7 and (de)protonation of NI take place at pKα ~2.5 and pKα ~9.5, respectively.[22] For [Rh2-

(DTolF)2(9-EtGuaH)2(BF4)2], the (de)protonation of NI takes place at pKα ~7.3 in CD3CN (Figure 1).

Owing to the very low solubility of 9-EtGuaH in CH3CN, it was not possible to perform a pH-dependence titration of its H8 proton in CD3CN. It has been demonstrated, however, that the pKα values of neutral acids (and their conjugated bases) increase in organic solvents as compared to water (the decrease in the dielectric constant of the medium disfa-

vors dissociation of neutral acids because it produces charged species, it thus increases the pKα values);[24] for example, the pKα of acetic acid is 4.76 and 22.3 in H2O and CH3CN, respectively.[18] It is thus inferred that the pKα of 9-EtGuaH

products with d(GpG) appear to dominate in solution, except for three reported cases.[26,30,32] The HH2 variants of d(GpG) adducts, however, have been elusive due to the degree of rotational freedom in cisplatin; they were first identified by Marzilli et al. by invoking retro models with carrier ligands (e.g., 2,2'-bipiperidine) on the platinum center that decrease the fluxional motion above and below the conformational plane.[28–31,33] In the case of the [Rh2(O2CCH3)2(dGpG)] adducts, the unusual HHR conformers were most likely observed because of the combined effects of restricted rotation of the guanine about the Rh–N7 bond due to the bidentate N7/O6 binding, and the presence of the acetate bridging groups.[22]

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in CD$_3$CN should be considerably higher than 9.5 (i.e., the pK$_a$ of benzylamine is 9.33 and 16.8 in H$_2$O and CH$_3$CN, respectively). Therefore, the pK$_a$ value (~7.3) for [(Rh$_2$-(DTolF)$_2$(9-EtGuaH)$_2$](BF$_4$)$_2$ in CD$_3$CN has decreased considerably compared to free 9-EtGuaH. The substantial increase in the acidity of N1-H is attributed to the bidentate N7/O6 binding to the dirhodium unit, a fact confirmed by the X-ray crystal structure of HH cis-[Rh$_2$((DTolF)$_2$(9-EtGuaH)$_2$](NCCH$_3$)](BF$_4$)$_2$ ([32] the N1-H sites are not deprotonated in the crystal structure as in the acetate adduct HT cis-[Rh$_2$(O$_2$CC$_2$H$_5$)(9-EtGuaH)$_2$](MeOH)$_2$, [19] presumably because CH$_3$CN does not need to be protonated to become a good leaving group).

[Rh$_2$(DTolF)$_2$(d(GpG))]: In the aromatic region of the $^1$H NMR spectrum of the dirhodium adduct in CD$_3$CN/D$_2$O 80/20% at 20°C, the two inequivalent nonexchangeable H8 protons of d(GpG) give rise to two resonances at $\delta$ = 8.73 and 7.84 ppm (Figure 2; Table 1). These downfield and upfield resonances are assigned to the 5'-G and 3'-G H8 protons, respectively, by analysis of 2D NMR spectroscopic data (vide infra).

Despite the different solvents used to collect the NMR data for [Rh$_2$(DTolF)$_2$(d(GpG))] and free d(GpG), [(Rh$_2$-(DTolF)$_2$(d(GpG))] is not soluble in D$_2$O only, the resonance of 5'-G H8 for the adduct is considerably downfield-shifted (Δ$\delta$ ~1 ppm) as compared to free d(GpG). As will be inferred from the 2D NMR spectroscopic data (vide infra), the guanine bases in [Rh$_2$(DTolF)$_2$(d(GpG))] have a HH orientation (Scheme 4), and it has been established from platinum-d(GpG) adducts that HH dinucleotide adducts give rise to one or both H8 signals that are ~1 ppm downfield from free d(GpG). [29,33] The pH-dependence $^1$H NMR titration for [Rh$_2$(DTolF)$_2$(d(GpG))] performed in CD$_3$CN (see Figure S1 in the Supporting Information), indicates the absence of protonation of the N7 sites at low pH values, which corroborates binding of the metal to the N7 sites. Moreover, in the pH-dependence $^1$H NMR titration curves, inflection point(s) are observed at pK$_a$ ~ 7.5 (see Figure S1 in the Supporting Information), which correspond to the (de)protonation(s) of the N1 sites of the two bases. By applying the same argumentation for the pK$_a$ values of the dinucleotide as for the 9-EtGuaH adduct (vide supra), it is inferred that the pK$_a$ values of the N1-H sites in [Rh$_2$-(DTolF)$_2$(d(GpG))] have decreased considerably compared to free d(GpG) (pK$_a$ ~10.0 in D$_2$O); [22] the pK$_a$ should be higher in CD$_3$CN [34]. The notable increase in the acidity of N1-H is attributed to the bidentate N7/O6 binding of the guanine bases to the dirhodium unit.

In the aromatic region, there also is a triplet at $\delta$ = 7.47 ppm (with twice the intensity of each H8 resonance), which is ascribed to the two N-C3H-N proton nuclei of the bridging formamidinate groups (Figure 2; the triplet is attributed to $^1$H coupling to the two equivalent rhodium nuclei, $^3$$J$[103Rh,$^1$H] = 3.8 Hz).

$^{13}$C NMR spectroscopy

For the aforementioned compounds, guanine binding to the dirhodium core through N7/O6 was corroborated by means of $^{13}$C NMR spectroscopy. In Table 2, a compendium of the

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**Table 1.** $^1$H and $^31$P NMR chemical shifts (δ, ppm) for [Rh$_2$(DTolF)$_2$(d(GpG))].

<table>
<thead>
<tr>
<th>d(GpG) species</th>
<th>G</th>
<th>H8</th>
<th>H1'</th>
<th>$^3$$J$(H1'::H2')</th>
<th>H2'</th>
<th>H2''</th>
<th>H3</th>
<th>H4</th>
<th>H5/H5''</th>
<th>Base sugar</th>
<th>$^3$P NMR[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Rh$_2$(DTolF)$_2$(d(GpG))][b]</td>
<td>5'</td>
<td>6.84</td>
<td>6.12</td>
<td>0/6(d)</td>
<td>2.57</td>
<td>2.18</td>
<td>4.68</td>
<td>3.95</td>
<td>3.81/3.85(c)</td>
<td>anti</td>
<td>-2.38</td>
</tr>
<tr>
<td></td>
<td>3'</td>
<td>7.82</td>
<td>6.19</td>
<td>0/14(d)</td>
<td>2.40[d]</td>
<td>2.40[d]</td>
<td>4.45</td>
<td>4.00</td>
<td>3.76/3.90(b)</td>
<td>anti</td>
<td>-4.00</td>
</tr>
<tr>
<td>d(GpG)[a]</td>
<td>5'</td>
<td>7.71</td>
<td>5.96</td>
<td>2.36</td>
<td>2.16</td>
<td>4.74</td>
<td>4.17</td>
<td>3.68[d]</td>
<td>anti</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3'</td>
<td>8.00</td>
<td>6.08</td>
<td>2.77</td>
<td>2.47</td>
<td>4.77</td>
<td>4.18</td>
<td>4.09[d]</td>
<td>anti</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[a] 2D NMR spectra collected in CD$_3$CN/D$_2$O: 80/20% at 10°C. [b] 2D NMR spectra collected in D$_2$O at 5°C. [c] In Hertz. [d] Overlapped resonances. [e] Not stereospecifically assigned. [f] Referenced to TMP at 0 ppm.

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**Figure 2.** Aromatic region of the 1D $^1$H NMR spectrum of [Rh$_2$-(DTolF)$_2$(d(GpG))] in CD$_3$CN/D$_2$O 80/20% at 20°C.
their bis-formamidinated dirhodium adducts is reported.

On the other hand, the quaternary carbon atoms due to carbon atoms with an even number of attached protons (including quaternary carbon atoms) face upwards. To this effect, for the [Rh₂(DTolF)₂(NCC₃H₄)₂(BF₄)] complex, the ¹³C NMR resonances (Table 2) attributed to the formamidinate group N=C=N (δ = 169.88 ppm; this group gives rise to the most downfield ¹³C NMR resonance in the spectrum) as well as the ortho- and meta-carbon atoms (1*) of the tolyl rings (δ = 127.29, 129.68 ppm) face downwards. [38, 39] On the other hand, the quaternary carbon atoms (4*) of the tolyl rings (δ = 135.19, 148.62 ppm) face upwards.

Although the ¹³C NMR data for 9-EtGuaH and free d(GpG) were collected in a different solvent from their dirhodium bis-formamidinate complexes, a comparison of the chemical shifts between the free ligands and the complexes is still viable. For both [Rh₂(DTolF)₂(9-EtGuaH)₂(BF₄)] and [Rh₂(DTolF)₂(d(GpG))], the ¹³C NMR resonances of the C6 nuclei have shifted downfield by Δδ ~ 4.0 ppm compared to the corresponding resonances of the unbound ligands (Table 2). Since the ¹³C NMR spectra of both [Rh₂(DTolF)₂(9-EtGuaH)₂(BF₄)] and [Rh₂(DTolF)₂(d(GpG))] were acquired at pH values below the inflection points for N1 (de)protonation (pKᵣ values 7.3 and 7.5, respectively; vide supra), the N1 positions of the bases are protonated and thus the downfield shifts of the C6 ¹³C NMR resonances, due to O6 binding of the bases to the rhodium centers, are not as pronounced as they would have been if the N1 sites were deprotonated. The Δδ ~ 4.0 ppm downfield shifts observed for the ¹³C NMR resonances of the C6 nuclei for [Rh₂(DTolF)₂(9-EtGuaH)₂(BF₄)] and [Rh₂(DTolF)₂(d(GpG))] are comparable to the downfield shifts of the corresponding resonances for [Rh₂(O₂CCH₃)₂(9-EtGuaH)₂] [32] and [Rh₂(O₂CCH₃)₂(S-GMP)] [33] and [Rh₂(O₂CCH₃)₂(d(pGpG))] [25] at pH 4, wherein the N1 sites of the guanine bases are protonated and the bases are binding to the dirhodium units through N7/O6. In the bis-formamidinate complexes, the ¹³C NMR resonances for C2 have not been essentially affected, because C2 is sensitive to deprotonation only, whereas C6 is sensitive to O6 complexation as well as N1 deprotonation. [38-40] The C5 nuclei for the dirhodium compounds being studied experienced only small downfield shifts upon complexation as reported in the literature for metal O6 binding. [40] The downfield impact on C5 upon O6 binding may be partially counterbalanced by the expected upfield shift of about 3 ppm (observed in cisplatin adducts) due to N7 binding of the metal. [39, 42] The ¹³C NMR resonance of the C8 carbon usually experiences a downfield shift of about 3 ppm upon N7 metal coordination. [41, 43] Although this trend is followed by the 9-EtGuaH adducts with dirhodium bis-formamidinate (Δδ ~ 4 ppm; Table 2), for the tethered d(GpG) adduct, the expected downfield shift of C8 was not observed as in a few other reported cases. [22, 25, 44, 45] In the case of the single-stranded [d(TG*G*T)-N7/N7]-Pt(en) complex, it is attributed to the “nonideal” overlap of the N7 lone pairs of both guanine bases with the metal center due to metal-induced distortion of the DNA structure, as well as to heavy-atom anisotropic effects of platinum on the ¹³C NMR chemical shifts. [44]

2D ¹H NMR spectroscopy

2D ROESY, DQF-COSY and [¹H–³¹P] HETCOR NMR spectra were collected to assess the structural features and assign the nonexchangeable proton resonances of the dirhodium bis-formamidinate (dGpG) species (Table 1). In the aromatic region of the 2D ROESY NMR spectrum of [Rh₂(DTolF)₂(d(GpG))], the two H8 resonances are well

<table>
<thead>
<tr>
<th>Compound</th>
<th>Purine carbon atoms</th>
<th>DToF carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-EtGuaH</td>
<td>C6</td>
<td>169.88</td>
</tr>
<tr>
<td>[Rh₂(DTolF)₂(9-EtGuaH)₂]</td>
<td>C2</td>
<td>148.62</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>127.29</td>
</tr>
<tr>
<td></td>
<td>C8</td>
<td>127.27</td>
</tr>
<tr>
<td></td>
<td>C5</td>
<td>127.07</td>
</tr>
<tr>
<td>[Rh₂(DTolF)₂(9-EtGuaH)₂]</td>
<td>N-CH-N</td>
<td>135.19</td>
</tr>
<tr>
<td></td>
<td>4*</td>
<td>129.68</td>
</tr>
<tr>
<td></td>
<td>1*</td>
<td>28.14</td>
</tr>
</tbody>
</table>

[a] ¹³C NMR spectra collected in D₂O. [b] ¹³C NMR data collected in CD₂CN. [c] Both isomers. [d] The ¹³C NMR resonances for the CH₃ and -CH₂-groups of 9-EtGuaH appear at δ = 15.17 and 40.79 ppm, respectively (for both isomers). [e] ¹³C NMR data collected in CD₃CN/D₂O 80/20%. [f] The resonances could not be assigned accurately due to the broad peak at δ = 118.2 ppm from the residual metal impurity in CD₂CN.
H8/H8 region of the 2D ROESY NMR spectrum for [Rh2(DTolF)2(d(GpG))] in CD3CN/D2O 80:20% at 10°C.

Figure 3.

Figure 4. H1' and H2'/H2'' region of the 2D [1H-2H] DQF-COSY NMR spectrum for [Rh2(DTolF)2[d(GpG)]] in CD3CN/D2O 80:20% at 10°C. The 5'-G and 3'-G cross-peaks are indicated with a medium dash (---) and a dotted line (--), respectively.

(GpG) HH cross-linked adduct to have both deoxyribose residues in the C3'-endo (N-type) conformation.[22,25]

31P NMR spectroscopy

[Rh2(DTolF)2(d(GpG))]: The 1D 31P NMR spectrum displays a resonance at $\delta = -2.38$ ppm (Table 1), which is located downfield from that of the unbound dinucleotide d(GpG) ($\delta = -4.00$ ppm). Typically, for HH isomers, the phosphate groups resonate about 1 ppm downfield from the unbound dinucleotide.[22,25,33] Downfield shifts of the 31P NMR resonances in DNA usually indicate an increase in the unwinding angle characterized by changes in the R-O-P-OR' torsion angles.[48] The downfield 31P NMR chemical shifts, observed for d(GpG) containing oligonucleotide adducts with platinum and other metals imply that, when adjacent guanine residues bind to the metal, an extension of the conformation about the diester bond between the G bases occurs.[49]

Molecular modeling

Models of the dirhodium bis-formamidinate adducts were constructed and subjected to simulated annealing calculations. The conformational features of the adducts determined by NMR spectroscopy were reproduced well by the calculations. The differences in energy values have been interpreted in conjunction with the NMR spectroscopic data. The HH and HT models constructed for [Rh2(DTolF)2(9-EtGuaH)2]3+ are nearly isoenergic (199.6 and 199.2 kcal mol$^{-1}$, respectively), a result that supports their presence in 1:1 ratio in solution (as inferred from the 1H NMR spectroscopic data).

Initial HH1R, HH1L, HH2L conformers for the tethered adduct [Rh2(DTolF)2[d(GpG)]] were independently con-
Table 3. Summary of lowest energy dirhodium adducts with d(GpG) and d(pGpG).

| Model | Percent [%] | Energy [kcal mol⁻¹] | $\chi$ [%] | $P$ [%] | Dominant 3'-G
|-------|-------------|----------------------|------------|--------| sugar type
|       | $5'$-G | $3'$-G | $5'$-G | $3'$-G | $5'$-G H8/3'-G H8 [Å] | 3'-G/5'-G Dihedral angle [%]
| [Rh₂(DTolF)₂[d(GpG)]] HH1R[a] | 100 | 300.7 | −137 | −146 | 20 | 12 | N | 3.30 | 75.9 | 82.1 |
| [Rh₂(DTolF)₂[d(GpG)]] HH2L | 0 | 303.1 | −1.2 | −166 | 5 | 14 | S | 3.51 | 73.7 | 75.0 |
| [Rh₂(O₂CC₂H₄)₂[d(GpG)]] HH1R[b] | 74 | 255.6 | −129 | −125 | 28 | 126 | S | 2.97 | 75.0 | 75.0 |
| [Rh₂(O₂CC₂H₄)₂[d(pGpG)]] HH1L[c] | 100 | 329.5 | −134 | −45 | 27 | 144 | S | 3.12 | 81.3 | 81.3 |

[a] Experimentally observed. [b] $\chi = O4'$-C1'-N9'-C4': $|\chi| > 90^\circ$ and $|\chi| < 90^\circ$ correspond to the anti and syn range respectively, for torsion angles $-180^\circ < \chi < +180^\circ$. [c] $P$-pseudorotation phase angle calculated from the equation $tanP = (v_1 + v_5 - v_2 - v_4)/(2v_2(sin36^\circ + sin72^\circ))$ (P=4 are endocyclic sugar torsion angles: $0^\circ \leq P \leq 36^\circ$ (±18°) corresponds to a N sugar, while $144^\circ \leq P \leq 190^\circ$ (±18°) indicates an S sugar; if $v_1 < 0$, $P = P + 180$. [d] Dihedral angles between 5'-G and 3'-G were calculated by using atoms N1, N3, N7, of each purine ring. [e] Reference [22]. [f] These angles are in the range to be considered syn; however, the H8-H2' distance is less than the H8-H1' distance and other low-energy structures have $\chi$ angles in the anti range. [g] In most other low energy structures of these variants, the 3'-G sugar rings have an S-type conformation. [h] Reference [25].

structed and subjected to simulated annealing calculations, because starting right- and left-handed models produced minimized conformers with the same canting as the original models only. The lowest energy HH1R variant (Figure 5) is 2.4 kcal mol⁻¹ more stable than the lowest energy HH2L variant (Table 3). The lowest energy HH1R variant (Figure 5) is lower than the lowest energy HH1L conformer by 2.0 kcal mol⁻¹ (the HH1L conformers were not further considered because their presence is not supported by the NMR data). For the HH1R conformers, the H8/H8 distances are in the range 3.2–3.4 Å (Table 3), thus corroborating relatively intense H8/H8 cross-peaks in the 2D ROESY NMR spectrum (Figure 3; vide supra). Both the 5'-G and 3'-G sugar residues are in the anti-orientation with respect to the glycosyl bonds, in accordance with the NMR data (vide supra). The measured interproton H8/H3' distances (2.34 and 3.09 Å for 5'-G and 3'-G, respectively), for the lowest energy HH1R conformer, are in accord with the strong H8/ H3' ROE NMR cross-peaks and thus N-type conformations for both the 5'-G and 3'-G deoxyribose rings. Since an N-type conformation for the 3'-G deoxyribose is rarely encountered in platinum complexes,[19,40] the conformations of the 3'-G residues for 500 minimized HH1R [Rh₂(DTolF)₂[d-(GpG)]] models were considered. It was found that 57% of the 3'-G sugars are in an N-type conformation. If the HH1R [Rh₂(NHCHNH₂)₂[d(GpG)]] model is constructed (by replacing the tolyl groups with hydrogen atoms) and minimized, 53% of the 3'-G sugars are in an N-type conformation.

Discussion

As in the case of [Rh₂(O₂CC₂H₄)₂][19,20,22] reaction of [Rh₂(DTolF)₂(CH₃CN)₂][BF₄] with 9-EtGuaH proceeds by formation of HH to HT isomers in 1:1 ratio, wherein 9-EtGuaH adopts equatorial bridging interaction through the N7/O6 pseudorotation phase angle calculated from the equation $tanP = (v_1 + v_5 - v_2 - v_4)/(2v_2(sin36^\circ + sin72^\circ))$ (P=4 are endocyclic sugar torsion angles: $0^\circ \leq P \leq 36^\circ$ (±18°) corresponds to a N sugar, while $144^\circ \leq P \leq 190^\circ$ (±18°) indicates an S sugar; if $v_1 < 0$, $P = P + 180$. [d] Dihedral angles between 5'-G and 3'-G were calculated by using atoms N1, N3, N7, of each purine ring. [e] Reference [22]. [f] These angles are in the range to be considered syn; however, the H8-H2' distance is less than the H8-H1' distance and other low-energy structures have $\chi$ angles in the anti range. [g] In most other low energy structures of these variants, the 3'-G sugar rings have an S-type conformation. [h] Reference [25].

As in the case of [Rh₂(O₂CC₂H₄)₂][19,20,22] reaction of [Rh₂(DTolF)₂(CH₃CN)₂][BF₄] with 9-EtGuaH proceeds by formation of HH to HT isomers in 1:1 ratio, wherein 9-EtGuaH adopts equatorial bridging interactions through the N7/O6 atoms spanning the dirhodium unit in a cis disposition.[23] The presence of the two isomers in equal amounts (a finding reproduced by the simulated annealing calculations) is based on the ¹H NMR spectroscopic data. The pH-dependence study of the H8 ¹H NMR resonance for [Rh₂(DTolF)₂(9-EtGuaH)₂][BF₄] in CD₃CN (Figure 1), indicates the absence of N7 protonation at low pH (due to N7 binding to the metal) and a substantial enhancement in the acidity of N1-H, (the pKₐ value has decreased to ~7.3 as compared to ~9.5 for unbound 9-EtGuaH and ~8.5 for N7 only bound adducts), due to the O6 binding of the base to the dirhodium unit.[22,23] The pH-dependence ¹H NMR titration curves for the H8 resonances of [Rh₂(DTolF)₂[d(GpG)]] (see Figure 5. Lowest energy conformer for the experimentally observed HH1R variant of [Rh₂(DTolF)₂[d(GpG)]]), resulting from simulated annealing calculations. The 5'-G residue is positioned to the left and 3'-G is the more canting base. Color code: Rh green, N blue, O red, P yellow, C gray, H white.
Figure S1 in the Supporting Information) closely resemble those of [Rh₂(DTolF)₂(9-EtGuaH₂)](BF₄)₂ (Figure 1), that is, no N7 protonation is observed at low pH and the pKₐ values of N1-H deprotonation have decreased to ~7.5 for both 5'-G H8 and 3'-G H8. The latter effects are induced by purine binding to the rhodium centers through N7/O6.[22,25] For both the [Rh₂(DTolF)₂(9-EtGuaH₂)](BF₄)₂ and [Rh₂-(DTolF)₂(d(GpG))] adducts, the downfield shifts by Δδ ~ 4.0 ppm of the ¹³C NMR resonances for the C6 nuclei as compared to the corresponding resonances of the unbound ligands, further corroborate N7/O6 binding.[22,25] 

In [Rh₂(DTolF)₂(d(GpG))] the two dinucleotide H8 protons are nonequivalent. In addition to the deshielding effect of the metal on both rings, the more canted base experiences an upfield shifting effect due to the ring-current anisotropy of the other cis base. The guanine bases are not oriented exactly perpendicular to the coordination plane, and the degree and direction of canting depends on the carrier only spectroscopic data as well as the molecular modeling results. 

The N-pucker for the 5'-G is universal in such cross-links.[33] The 3'-G sugar is also in the N (C3'-endo) conformation. In the case of platinum adducts, N-type conformations for both deoxyribose residues have only been reported for HT (S,R,R,S)-[BipPt(d(GpG))][29] and trans-[PtCl(NH₃)₃][μ-NH₂-(CH₂)₆NH₂][d(GpG)]).[30] It is unprecedented for a dirhodium-d(GpG) HH cross-linked adduct to have both deoxyribose residues in the C3'-endo (N-type) conformation (Table 3).[22,25] It is known that several sterically and electronically[32] affect the preferred furanose puckering modes, such as the C3' furanose substituent, as well as the modifications of the base.[33,34] In the case of [Rh₂(DTolF)₂(d(GpG))], most likely electronic changes to the base and steric factors, due to the formamidinate bridging groups, are responsible for the dominance of an N-type 3'-G deoxyribose ring. 

Conclusion 

The present study supports equatorial N7/O6 binding of GG containing DNA fragments spanning the Rh–Rh bond in the [Rh₂(DTolF)₂(d(GpG))] adduct. The guanine bases are almost completely deprotonated upon coordination to the metal centers (3'-G/5'-G dihedral angle 75.9°) and favorably poised to accommodate the bidentate N7/O6 binding to the dirhodium core. The tethers of the guanine bases dictates the HH nature of the [Rh₂(DTolF)₂(d(GpG))] adduct, whereas the formamidinate bridging groups favor one right-handed HH1R conformer in solution, as opposed to two conformers HH1R (75%) and HH2R (25%) in the case of [Rh₂(O₂CCH₃)₃(d(GpG))].[22] In [Rh₂(DTolF)₂(d(GpG))], the presence of the bulky, nonlabile and electron-donating formamidinate bridging groups induces electronic and conformational changes to the dirhodium d(GpG) adduct that are different, in some aspects, from those of the acetate. Clearly, tailoring the bridging groups on the dirhodium core affects the nature of the preferred DNA adducts. A notable point of this study relates to the N7/O6 binding to the dirhodium core with the consequential large decrease of the pKₐ value for N1-H deprotonation. This N7/O6 binding mode influences the Watson–Crick hydrogen bonding of the bases and shifts the pKₐ value into the physiological pH range.[35,36] In light of the biological activity of cis-[Rh₂-
(DTolF)2(O2CCF)2(H2O)1], the implications of its interactions with DNA fragments remain to be determined.

**Experimental Section**

**Materials.** The reagent 9-ethylguanine (9-EtGuaH) was purchased from Sigma. The starting material RhCl3·xH2O was obtained from Pressure Chemical Co. (Pittsburgh, PA) and was used without further purification. The compound \( \text{RhCl}(\text{O}2\text{CCF})2(\text{H}2\text{O})1 \) was prepared according to literature procedures.[1,2] The dinucleotide \( \text{d(GpG)} \) was purchased that the crude 5′-O-dimethoxytrityl(DMT)-protected material from the Gene Technologies Laboratory at Texas A&M University, was purified by reverse-phase HPLC and was used as the sodium salt. Concentrations of the dinucleotide were determined by UV spectroscopy (Shimadzu UV 1601PC spectrophotometer) at 252 nm \( (ε_252=2.5×10^4 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L}^{-1}) \). Deuterium oxide (\( \text{D}_2\text{O} \), 99.96%), deuterated acetoneitrile (CD3CN, 99.8%), deuterium chloride (\( \text{D}_2\text{HCl} \), 99.5%), and sodium deuterdeoxructose (NaOD, 99.5%) were purchased from Cambridge Isotope Laboratories, Sigma. The starting material \( \text{RhCl}(\text{O}2\text{CCF})2(\text{H}2\text{O})1 \) was purchased from Aldrich.

**Syntheses**

The compound was synthesized by an aqueous reaction of \( \text{RhCl}3·x\text{H}2\text{O} \) with \( \text{Cp}2\text{Co} \) to give \( \text{RhCl}(\text{O}2\text{CCF})2(\text{H}2\text{O})1 \) in a typical reaction, a slurry of \( \text{RhCl}(\text{O}2\text{CCF})2(\text{H}2\text{O})1 \) (225 mg, 0.21 mmol) in \( \text{CH}_2\text{CN} \) (15 mL) and the mixture was heated under reflux for a few hours (during this time the solution turned to green). The reaction solution was filtered and the filtrate were washed with an aqueous solution of NaOD. A green solid resulted. 1H NMR (500 MHz, CD3CN, 20°C) \( δ=8.08 \text{ (s, 2H; H8)}, 8.05 \text{ (s, 2H; H8)}, 7.55 \text{ (t, } \text{J}1\text{=3.8Hz, 2H; N-Ch}), 7.41 \text{ (t, } \text{J}1\text{=3.8Hz, 2H; N-Ch}), 6.99 \text{ (m, tolyl)}, 4.07 \text{ (q, overlapping, CH)}, 2.24 \text{ (s, 12H; CH3(tolyl))}, 2.21 \text{ (s, 12H; CH3(tolyl))}, 1.95 \text{ (s, ax-CH-CN)}, 1.39 \text{ (t, 6H; CH)}, 1.31 \text{ ppm (t, 6H; CH; MS-ESI: m/z (%) on 1000.1254 (103Rh,1H)} \) 1H NMR spectra were recorded on a 500-MHz Varian spectrometer operating at 125.76 MHz for 13C. The 1D 31PNMR test[57,58] (13C proton impurity of CD3CN, whereas the 13CNMR spectra were referred to the residual proton impurity of CD3CN, whereas the molecules in the present study are metal–metal bonded rhodium(III) compounds with a paddlewheel structure. In order to account for the difference in the oxidation state and the coordination environment of the metal for this type of complexes, the appropriate valence bond parameter was previously developed.[22,23]

**Molecular modeling:** Molecular modeling results were obtained by using the software package Cerius 4.6 (Accelrys Inc., San Diego). To sample the conformational space of each compound, simulated annealing calculations in the gas phase were performed by using the open force field (OFF) program, with a modified version of the universal force field (UFF).[60] The simulated annealing was carried out for 800 ps, over a temperature range of 300–500 K, with \( \Delta T=50 \text{ K} \), using the Nosé temperaturermostat, a relaxation time of 0.05 ps and a time step of 0.003 ps. The compounds were minimized (quenched) after each annealing cycle, producing 500 minimized structures. UFF is parameterized for octahedral rhodium(III), whereas the molecules in the present study are metal–metal bonded rhodium(II) compounds with a paddlewheel structure. In order to account for the difference in the oxidation state and the coordination environment of the metal for this type of complexes, the appropriate valence bond parameter was previously developed.[22,23]

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