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fac-$[Re(CO)_3(H_2O)_3]^+$ Nucleoside Monophosphate Adducts Investigated in Aqueous Solution by Multinuclear NMR Spectroscopy

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The *fac*-$[Re(CO)_3(H_2O)_3]^+$ cation, the putative DNA-binding species accounting for the biological activity of related Re(I) complexes, binds reversibly to N7 of 6-oxopurine nucleotide monophosphates (NMPs), in contrast to Pt(II) anticancer drugs. A relatively high amount of NMP is needed to convert all of the *fac*-$[Re(CO)_3(H_2O)_3]^+$ to adducts. The Re/nucleotide 1:1 adduct forms more rapidly and builds up to a higher concentration for guanosine 5′-monophosphate (5′-GMP) and inosine 5′-monophosphate (5′-IMP) than for the respective 3′-monophosphates (3′-GMP and 3′-IMP). These results are attributable to the 5′-positioning of the 5′-NMP phosphate group that allows it to approach the metal inner sphere for more favorable cation electrostatic and aqua ligand H-bonding interactions, both in the initial productive ion pair encounter complexes and in the N7-bound 1:1 adducts. A higher reactivity of 5′-GMP over 3′-GMP is known for cisplatin. In contrast, more Re/nucleotide 1:2 adduct was formed by 3′-GMP (and 3′-IMP) than by 5′-GMP (and 5′-IMP). Because the 3′-phosphate group cannot closely approach the metal inner coordination sphere, the greater stability for the 3′-GMP 1:2 adduct reflects the more favorable **G** N1H-phosphate interligand GMP–GMP interactions for 3′-GMP vs 5′-GMP (**G** = guanine base derivative). This type of interaction is known for platinum adducts. In 1:2 adducts the bound nucleotides are inequivalent, prompting us to perform mixed 5′-GMP/3′-GMP experiments, leading to the observation of major (***M***) and minor (***m***) mixed Re/5′-GMP/3′-GMP 1:1:1 adducts. The order of abundance at equilibrium in a typical experiment was ***M*** > bis 3′-GMP > ***m*** ≥ bis 5′-GMP. This stability order was rationalized by invoking the phosphate interactions described above. When methionine and 5′-GMP were allowed to compete for *fac*-$[Re(CO)_3(H_2O)_3]^+$, the Re/5′-GMP 1:1 adduct was the kinetic product and the S-bound Re/methionine adduct was the thermodynamic product, a result opposite to that typically found for cisplatin.

Introduction

The success of cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) in the treatment of various cancers has led to intensive efforts to determine its mode of action in order to guide development of other metal-based chemotherapeutic drugs.[1,2] The primary intracellular target of cisplatin is DNA, with the major DNA lesion being an intrastrand N7−Pt−N7 cross-link between two adjacent guanine residues.[3−6] The anticancer activity of *cis*-$[Pt\mathbf{A}_2X_2]$ compounds ($\mathbf{A}_2$ = two amines or a diamine carrier ligand; X = anionic leaving group) correlates with the number of NH groups. Models have suggested to us that this correlation could be related to the small size of the hydrogen atom rather than its hydrogen-bonding ability.[6] Indeed, as the bulk of the diamine carrier ligand increases, anticancer activity decreases.[5,7] Carrier-ligand bulk also influences the relative stability of single-strand vs duplex forms of oligonucleotides with the intrastrand cross-link.[8]

The great long-term clinical success of cisplatin makes it imperative to continue the search for other potential inorganic

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chemotherapeutic agents. This search has focused primarily on Pt(II), a square-planar, inert, and redox-stable metal center and one of a limited number of metal centers having all of these properties. Metal centers forming inert, redox-stable complexes typically have an octahedral geometry. Octahedral complexes are generally more bulky and more sterically crowded than square-planar complexes.[9] As mentioned, bulk decreases the anticancer activity of *cis*-[Pt**A**$_2$X$_2$] compounds.[5] Octahedral complexes do not bind so well as Pt(II) compounds to DNA and have a high propensity to bind to biomolecular targets other than cellular DNA; protein binding over nucleic acid binding can cause considerable toxicity, preventing the use of octahedral metal complexes as anticancer agents.[5] Several octahedral metal complexes do, however, have modest anticancer properties, with some of the most promising complexes containing Re(I), Ru(II), Ru(III), or dinuclear Rh(II)/Rh(II) metal centers.[9−21]

With the goal of gaining a better understanding of the coordination chemistry of octahedral metal complexes relevant to nucleic acid binding, we chose to investigate the *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ cation. Dinuclear complexes, such as [Re$_2$(CO)$_6$(μ-OH)$_3$]$^-$ and [Re$_2$(μ-OH)(μ-OPh)$_2$(CO)$_6$]$^-$, suppressed the growth of murine and human leukemias and lymphomas in cell culture studies.[19] Subsequent mass spectrometry studies demonstrated that [Re$_2$(CO)$_6$(μ-OH)$_3$]$^-$ is readily cleaved under protic conditions to yield the *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ cation, leading to a suggestion that this cation may be the species possessing anticancer properties.[22] The three water ligands in *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ are readily substituted by a variety of donor atoms, thus providing available sites for cross-linking interactions with DNA bases.[23,24] Notable similarities in the reactions/adducts of cisplatin and *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ include the following: First, both form M/**G** 1:1 and 1:2 adducts with 9-methylguanine (9-MeG), guanosine (Guo) and 2-deoxyguanosine (dGuo).[9,21] Second, both bind these guanine derivatives at N7, and the bases in 1:2 adducts adopt both head-to-head (HH) and head-to-tail (HT) orientations.[9,21] Third, the rate constant for the binding of Guo to *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ is very similar to that for Guo binding to *cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$.[21] Fourth, both readily bind N- and/or S-containing ligands such as Guo and thiourea.[9,21,24]

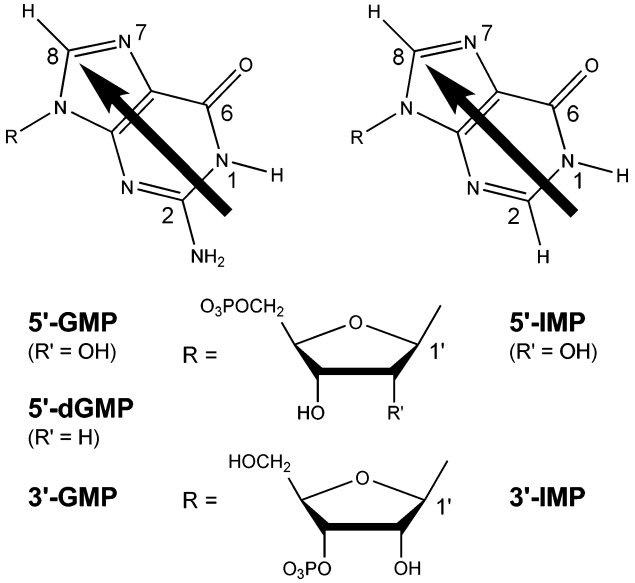

Figure 1. Guanine (left) and hypoxanthine (right) derivatives. The arrow and its head represent the base and the H8 atom, respectively.

However, compared to Pt(II) compounds, the *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ cation may have a higher affinity for oxygen donors,[23] and its binding equilibria are likely to be more dynamic and less complete.[25−30] Therefore, the likelihood that an investigation of the interaction of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ with 6-oxopurine nucleotide monophosphates (NMPs) would prove to be informative led us to study the binding of guanosine 5′-monophosphate (5′-GMP) and guanosine 3′-monophosphate (3′-GMP). We also performed studies with inosine monophosphates (5′-IMP and 3′-IMP) and 2′-deoxyguanosine 5′-monophosphate (5′-dGMP) to assess the effect of the C2−NH$_2$ substituent and/or N7 basicity (Figure 1).

Experimental Section

Materials and Sample Preparation. Stock solutions (50 and 200 mM) of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]OTf in water were prepared by

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published procedures and maintained at pH ~1.8.[31] 5′-GMP, 3′-GMP, 5′-IMP, 3′-IMP, 5′-dGMP (disodium salts), and D_2O (99.9%) (Sigma-Aldrich) were used as received. The H8 of 5′- or 3′-GMP was exchanged to D8 by incubating a solution of the nucleotide in D_2O at 100 °C for 5 h.[32] The solution was then filtered and taken to dryness by rotary evaporation, yielding solid D8−5′-GMP (*d*-5′-GMP) or D8−3′-GMP (*d*-3′-GMP) as the disodium salt.

Except as noted, a typical preparation involved treatment of an appropriate amount of NMP in 0.3 mL of H_2O with 0.4 mL of *fac*-$[Re(CO)_3(H_2O)_3]OTf$ (~50 or ~10 mM, depending on required final concentration); a small amount (0.1 mL) of D_2O was added to establish a lock signal. Dilute HCl or NaOH stock solutions (in H_2O) were used to adjust the pH (uncorrected) of the samples to ~3.6 in the NMR tubes as required.

NMR Spectroscopy. All NMR spectra were obtained on either a Bruker DPX400 spectrometer (400.1 MHz) or a Varian INO-VA500 spectrometer (500.1 MHz); both were equipped with a variable-temperature probe that was equilibrated at 25 °C unless otherwise indicated. 1D ^1H NMR spectra were referenced to the residual HOD peak, and presaturation was used to reduce the residual HOD peak. Each FID was accumulated for 64 transients, each containing 16K data points. Before Fourier transformation, an exponential apodization window function with a 0.2 Hz line broadening was applied. 1D proton-decoupled ^{31}P NMR spectra ($\{^1H\}-^{31}P$) were referenced to external trimethyl phosphate (TMP); each FID was accumulated for 64 transients, each containing 32K data points. Before Fourier transformation, an exponential apodization window function with a 2 Hz line broadening was applied. One hundred twenty-eight scans per block (256 blocks) were collected in a 2D rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiment conducted at 32 °C by using a spectral width of ~4000 Hz and a 500 ms mixing time. All NMR data were processed with either XWINNMR (Bruker) or VnmrJ (Varian) software.

Circular Dichroism (CD) Spectroscopy. CD samples were prepared from the respective NMR samples and diluted to ~1 mM GMP with deionized water (pH ~3.6). Three acquisitions, collected from 400 to 200 nm on a JASCO J-710 CD spectropolarimeter at a scan speed of 50 nm/min, were averaged to improve the signal-to-noise ratio.

Although NMR data indicate that the exchange rate is slow, we employed a set of additional experiments to determine if the re-equilibration after dilution would affect the CD results. For this, an aliquot (5 μL) of a Re/NMP NMR sample ([*fac*-$[Re(CO)_3(H_2O)_3]^+$] = 25 mM, r = 1:2) was added to 0.3 mL of H_2O (pH ~3.6) and the sample immediately transferred to the CD instrument. Ten acquisitions, collected from 280 to 230 nm, were averaged to improve the signal-to-noise ratio. CD spectra were recorded from 2 min to 2 days after dilution. The CD spectrum of the diluted sample did not change significantly from 2 min to 3 h after dilution. At 6 h after dilution, the CD spectrum had changed considerably. These results indicate that CD spectra measured promptly after dilution are representative of the NMR solution and can be used to assess the chirality of the dominant HT conformer.

Results and Discussion

Characteristic Features of Metal−NMP Adducts. Shift changes of the ^1H and ^{31}P NMR signals of the NMP upon coordination are informative. Extensive studies with *cis*-$[Pt\mathbf{A}_2\mathbf{G}_2]$ complexes indicate that, upon coordination via N7 to Pt, the **G** H8 singlet shifts downfield more for a Pt/NMP 1:1 adduct than for a 1:2 adduct with cis nucleotides.[3] This pattern can be explained by an inductive effect of the metal, causing H8 deshielding that is offset somewhat by H8 shielding from the anisotropic cis purine bases in 1:2 adducts.[3] Upon formation of a 1:1 adduct, the H1′ doublet shifts downfield slightly (inductive effect); conversely, the H1′ doublet shifts upfield upon formation of a 1:2 adduct (base anisotropic effect). An {N7, P_α} macrochelate, in which the 5′-nucleotide is coordinated via both N7 and P_α, has an H1′ singlet because the sugar pucker is forced to be virtually 100% N.[33−35] However, this {N7, P_α} macrochelate is rare, occurring for 5′- but not for 3′-monophosphates.[35−37] Direct inner-sphere coordination of phosphate oxygen to Pt causes the P_α ^{31}P NMR signal to shift ~4 to 12 ppm downfield.[24,34,35,38,39]

At pH ~3.6 (used here) the P_α group (Figure 1) of the free NMP is protonated and carries a single negative charge, the same as that of the phosphodiester group in DNA. Also, because deprotonation of *fac*-$[Re(CO)_3(H_2O)_3]^+$ begins around pH 4, the choice of pH ~3.6 avoids formation of *fac*-$[Re(CO)_3(H_2O)_2(OH)]$ and *fac*-$[Re(CO)_3(H_2O)(OH)_2]^-$ species.[23]

The fact that the guanine base bound to a metal is not C_2 symmetrical with respect to rotation about the M−N7 bond allows for rotamers. For square-planar metal geometries, there are two distinct rotamers at most for a given M/**G** 1:1 adduct. When the metal moiety lacks high symmetry, these rotamers are not equivalent, and thus there are two conformers. Introduction of a chiral sugar at the **G** N9 also influences the number of conformers. For octahedral or square-planar M/**G** 1:2 adducts with two cis N7-coordinated identical **G**'s, head-to-head (HH) and head-to-tail (HT) conformers are possible. HH conformers and HT conformers have the two H8 atoms on the same and opposite sides, respectively, of the N7−M−N7 plane. When the starting complex has high symmetry, one HH and two HT conformers are possible. An additional HH conformer can exist if the complex has lower symmetry (as in the adducts studied here, see Figure 2) or if the two **G**'s are not identical, such as in d(GpG) adducts.[40,41] If the starting complex has lower symmetry and the two **G**'s are not identical, two 1:2 adducts with two cis N7-coordinated nonidentical **G**'s can form, each having 2

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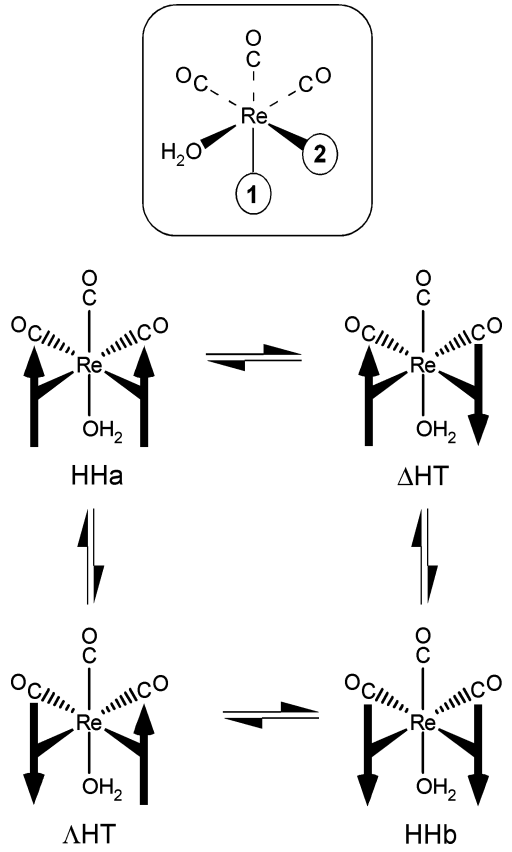

Figure 2. (top) Diagram showing coordination positions relative to each other; (bottom) HHa, ΛHT, HHb, and ΔHT orientations for an octahedral metal center. NMPs in coordination positions 1 and 2 are distinct, and all four conformers can interchange only if the type of NMP is identical in both coordination positions.

HH and 2 HT conformers; hence, a solution having a total of 4 HH and 4 HT conformers could be created.

In typical *cis*-[Pt**A**$_2$**G**$_2$] models with non-bulky amines, the rate of rotation about the Pt−N7 bonds is too rapid to detect distinct NMR signals for the conformers.[42] By using chiral carrier ligands with sufficient bulk to slow rotation, **G** H8 signals for the HH and HT rotamers could be observed, and the ΔHT and ΛHT absolute conformations could be established by NOE cross-peaks between the **G** H8 signals and the carrier ligand signals.[41,43,44] In turn, the CD signatures for these conformations were characterized.[44] For these adducts, the dominant HT conformer appears to be stabilized by hydrogen bonding of the phosphate group of one **G** with the N1H of the cis **G** and these "second-sphere" interactions, or SSC, have been identified as important factors that stabilize the ΛHT and ΔHT conformers in 5′-GMP and 3′-GMP complexes, respectively.[43,45−49] In addition, such studies with *cis*-[Pt**A**$_2$**G**$_2$] complexes with unlinked **G** nucleotides have shown that, while the HH conformer does form, the two HT conformers, ΔHT and ΛHT, are typically favored.[42,43,50−56] In addition, Pt/5′-GMP 1:2 adducts adopt the HH conformation more readily than do Pt/3′-GMP 1:2 adducts.[47,48,57]

Overview of Products Formed by *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ and NMPs. In describing the ^{1}H and ^{31}P NMR spectral results for NMP binding to *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$, we state the nature of the adducts formed before presenting all the evidence and the reasoning for such designations. In general for a given NMP, two principal adducts were formed: a 1:1 adduct, *fac*-[Re(CO)$_3$(H$_2$O)$_2$(NMP)], and a 1:2 adduct, *fac*-[Re(CO)$_3$(H$_2$O)(NMP)$_2$]$^-$ having inequivalent NMPs (Figure 2). We also found evidence for the formation of both a dinuclear 2:1 complex, *fac*-[Re$_2$(CO)$_6$(H$_2$O)$_4$(NMP)] (in which a phosphate oxygen and N7 are each bound to a *fac*-[Re(CO)$_3$(H$_2$O)$_2$]$^+$ moiety), and a trinuclear 3:1 complex, *fac*-[Re$_3$(CO)$_9$(H$_2$O)$_6$(NMP)]$^+$ (in which two phosphate oxygens and N7 are each bound to a *fac*-[Re(CO)$_3$(H$_2$O)$_2$]$^+$ moiety). When two different NMPs (NMP and N′MP) were present in the reaction mixture, two 1:2 adducts, *fac*-[Re(CO)$_3$(H$_2$O)(NMP)(N′MP)]$^-$, formed, as expected because the coordination positions are not equivalent (Figure 2).

5′-GMP Reaction Products. Upon treatment of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ with 5′-GMP ([Re] = 25 mM, r = 1:1), four new H8 singlets appeared (although one H8 singlet is very small, Figure 3). These signals are relatively downfield and are insensitive to pH from 3.6 to 1.4, properties indicating that the signals are from adducts with Re bound to 5′-GMP via N7. The predominant H8 signal appears immediately after mixing and dominates the spectrum, even at long reaction times. Because this major adduct signal has no partner H8 signal, it could arise from either a 1:1 or 1:3 Re/nucleotide adduct (Figure 4). The following observations demonstrate that this major product is the 1:1 adduct, *fac*-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)]: First, the intensity of the predominant H8 signal decreases upon addition of more 5′-GMP (vide infra), whereas the intensity of this signal could increase only if it were due to the 1:3 adduct. Second, the downfield shifts of the H8 singlet (∼0.4 ppm) and the H1′ doublet (∼0.1 ppm) of this major adduct relative to free 5′-GMP are consistent with a 1:1 adduct (Table 1). Third, the $^3J_{H1'-H2'}$ coupling constant decreases from ∼6 (free GMP) to ∼3.6 Hz, a feature consistent with metal coordination at N7 (Table 1 and

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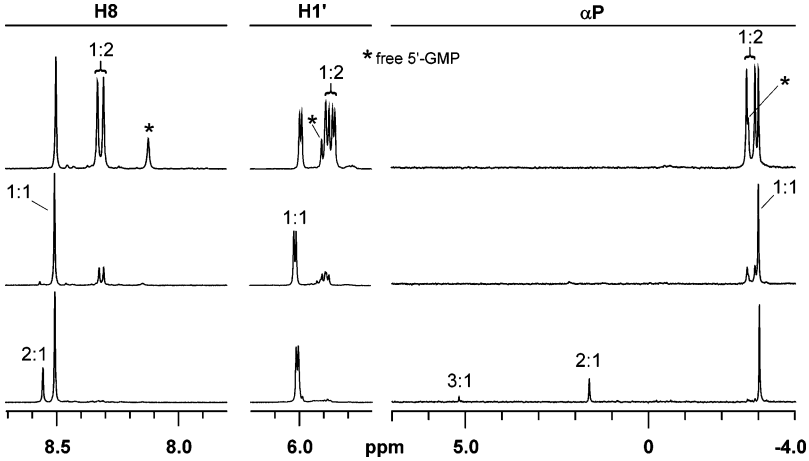

Figure 3. H8 (left), H1′(center), and P_α (right) NMR signals of equilibrated mixtures of *fac*-$[Re(CO)_3(H_2O)_3]^+$ (25 mM) and 5′-GMP at pH 3.6. (a) Re/GMP $r = 4{:}1$, (b) $r = 1{:}1$, (c) $r = 1{:}2$. The red arrow points to the small amount of 2:1 adduct in spectrum (b). See text for conditions and spectral assignments for signals not identified in the figure.

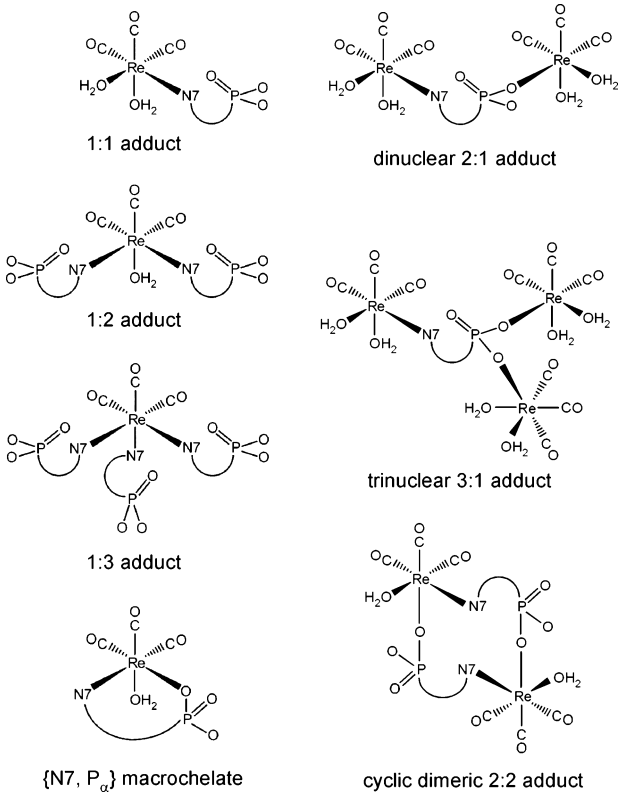

Figure 4. Possible Re/GMP adducts.

Supporting Information). Fourth, the chemical shift of the P_α signal of this adduct is similar (Table 1) to that for 5′-GMP, ruling out the only other 1:1 adduct with 5′-GMP bound via N7, a macrochelate (Figure 4) expected to have a P_α signal shifted downfield by 4−12 ppm.[24,34,35,38,39] These results, as well as those obtained at r values higher than 1:1 and with a mixture of nucleotides (vide infra), establish beyond question that when $r = 1{:}1$, the predominant adduct formed is *fac*-$[Re(CO)_3(H_2O)_2(5'\text{-GMP})]$, with 5′-GMP bound only through N7.

The next most abundant adduct in the $r = 1{:}1$ solution is *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})_2]^-$. This 1:2 adduct has two closely spaced H8 singlets of approximately equal intensity (Figure 3). Relative to free 5′-GMP, the H8 signals are shifted

Table 1. ^1H and ^{31}P NMR Shifts (ppm) and $^3J_{H1'-H2'}$ Coupling Constants (Hz, in Parentheses) of Nucleotides and Complexes Formed with *fac*-$[Re(CO)_3(H_2O)_3]^+$ [a]

	δ ^{1}H		δ ^{31}P
complex	H8	H1′	P_α
5′-GMP	8.12	5.90 (6.1)	−2.74
fac-$[Re(CO)_3(H_2O)_2(5'\text{-GMP})]$	8.50	5.99 (3.7)	−3.02
fac-$[Re(CO)_3(H_2O)(5'\text{-GMP})_2]^-$	8.32, 8.30	5.88 (5.1), 5.86 (3.9)	−2.69, −2.91[b]
fac-$[Re_2(CO)_6(H_2O)_4(5'\text{-GMP})]$	8.55	[c]	1.62[d]
fac-$[Re_3(CO)_9(H_2O)_6(5'\text{GMP})]^+$ in 5′-GMP and 3′-GMP	[c]	[c]	5.17
3′-GMP	8.00	5.93 (6.0)	−3.00
fac-$[Re(CO)_3(H_2O)_2(3'\text{-GMP})]$	8.52	6.00 (3.6)	−3.06
fac-$[Re(CO)_3(H_2O)(3'\text{-GMP})_2]^-$	8.40, 8.34	5.90 (3.8), 5.90 (3.8)	−3.04
fac-$[Re_2(CO)_6(H_2O)_4(3'\text{-GMP})]$	8.53	6.02 (4.0)	1.63[d]
fac-$[Re_3(CO)_9(H_2O)_6(3'\text{GMP})]^+$ in 5′-GMP and 3′-GMP	8.54	6.04 (3.9)	5.09
M-5′-GMP[e]	8.48	5.90[c]	−3.11[b]
M-3′-GMP	8.38	5.90[c]	−3.04[b]
m-5′-GMP	8.40	5.90[c]	−3.11[b]
m-3′-GMP	8.14	5.90[c]	−3.04[b]

[a] [Re] = 25 mM, pH 3.6, 32 °C. [b] Signal assigned to adduct only. [c] Signal obscured by other signals. [d] Shift highly dependent on pH (see text). [e] ***M*** and ***m*** = major and minor adducts, respectively, of *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})(3'\text{-GMP})]^-$.

ca. 0.2 ppm downfield, the two H1′ doublets are shifted slightly *upfield*, and neither of the two corresponding P_α signals is shifted significantly (Table 1, Figure 3). Two H8 singlets of equal intensity are expected for a 1:2 adduct. A 1:2 adduct lacks both a plane of symmetry (because of a chiral sugar) and a C_2 axis (because the *fac*-$[Re(CO)_3(H_2O)]^+$ moiety lacks C_2 symmetry). Addition of another equivalent of 5′-GMP ($r = 1{:}2$) resulted in a higher intensity for these H8 signals relative to that of *fac*-$[Re(CO)_3(H_2O)_2(5'\text{-GMP})]$; therefore, these signals undoubtedly arise from the 1:2 adduct, *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})_2]^-$.

The third most abundant adduct in the $r = 1{:}1$ solution is *fac*-$[Re_2(CO)_6(H_2O)_4(5'\text{-GMP})]$. This dinuclear adduct gives a small H8 singlet (Figure 3) downfield from the H8 singlet of *fac*-$[Re(CO)_3(H_2O)_2(5'\text{-GMP})]$; the H1′ signal of this adduct is obscured by the H1′ signals of other adducts. The corresponding P_α signal is ca. 5 ppm downfield from the P_α resonance of 5′-GMP. Taken together, these results indicate that both N7 and P_α are coordinated to Re in this minor product in the $r = 1{:}1$ solution (Table 1). Three conceivable

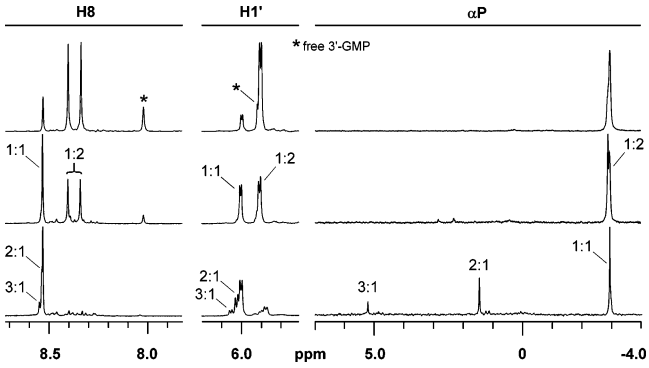

Figure 5. H8 (left), H1′(center) and P$_\alpha$ (right) NMR signals of equilibrated mixtures of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ (25 mM) and 3′-GMP at pH 3.6. (a) Re/GMP r = 4:1, (b) r = 1:1, (c) r = 1:2. See text for conditions and spectral assignments for signals not identified in the figure.

and reasonable adducts with 5′-GMP coordinated via both N7 and P$_\alpha$ can explain the observation of one H8 and one P$_\alpha$ signal for this third product. The first possibility is a C_2-symmetric cyclic dimeric 2:2 adduct with two 5′-GMPs linking two *fac*-[Re(CO)$_3$(H$_2$O)]$^+$ moieties (Figure 4). The abundance of this dimer relative to the 1:1 adduct should remain constant above r = 1:1 at a given GMP concentration because each contains one 5′-GMP per Re. The other two conceivable adducts have less than one 5′-GMP per Re. These are a dinuclear 2:1 adduct with N7 bound to one Re and P$_\alpha$ bound to a second Re, and a trinuclear 3:1 adduct with N7 bound to one Re and P$_\alpha$ bound to two different Re's (Figure 4). At a given GMP concentration, increasing the Re concentration above r = 1:1 would increase the abundance of both the di- and trinuclear adducts relative to the 1:1 adduct.

To distinguish among these possibilities for the third product, we performed the formation reaction twice more but by using different total concentrations and different r values. At high concentrations (100 mM) of both Re and 5′-GMP, the intensities of ^{1}H and ^{31}P NMR signals of the third product relative to those of the 1:1 adduct were the same as those found in the 25 mM r = 1:1 reaction. At the higher r = 4:1 ([Re] = 25 mM) the H8 and P$_\alpha$ signals of the third product had a higher intensity relative to the 1:1 adduct signals. Thus, more than one Re is bound per 5′-GMP. The third product with the ca. 5 ppm downfield P$_\alpha$ signal cannot be a cyclic dimeric 2:2 adduct.

The nature of the third product was established in this r = 4:1 experiment. A small additional P$_\alpha$ signal (Figure 3) was found ~8 ppm downfield from the P$_\alpha$ resonance of free 5′-GMP. Because the P$_\alpha$ signal of the fourth product appears only at higher r values and is one-fourth the size of the P$_\alpha$ signal of the third product, the fourth product contains more Re per 5′-GMP than the third product. Most reasonably, the third and fourth products are the 2:1 and 3:1 adducts. Although no H8 signal could be located for the 3:1 adduct, we should note that in 3′-GMP reactions at r = 4:1, both the H8 and P$_\alpha$ signals of this fourth product are evident (vide infra, Figure 5).

To assess our conclusions, we examined the effect of low pH on the ^{31}P NMR shifts (Supporting Information). When the pH of a solution containing the 1:1, 1:2, 2:1, and 3:1 adducts was decreased from 4 to 2, the P$_\alpha$ signal of the 1:1 adduct (with a shift similar to that of free 5′-GMP) did not shift significantly, consistent with the P$_\alpha$ group being uncoordinated and monoprotonated throughout the pH range. However, the P$_\alpha$ signal of the 2:1 adduct (shifted 5 ppm downfield from that of free 5′-GMP) shifted significantly upfield (ca. 3.5 ppm) over this pH range, consistent with a coordinated deprotonated P$_\alpha$ group becoming protonated. The pK_a of this P$_\alpha$ group is thus about 3.2, a value much lower than the pK_a for 5′-GMP (~6.3).[57−60] The much lower pK_a of the phosphate group in *fac*-[Re$_2$(CO)$_6$(H$_2$O)$_4$(5′-GMP)], compared to N7-bound Pt adducts,[61−64] is undoubtedly due to the direct binding of Re to this P$_\alpha$ group.[35,38,39] The very downfield P$_\alpha$ signal of the fourth adduct was insensitive to changes in pH between 4 and ~3 (this P$_\alpha$ signal disappeared below pH ~3); this lack of pH dependence, indicating a phosphate group pK_a below 2, is consistent with such a 3:1 adduct with two Re moieties bound to the P$_\alpha$ group. The binding of the second Re is expected to be weak, and thus it does not compete well with the proton as the pH drops below the pK_a of the 2:1 adduct. However, because two Re's are bound to it, the P$_\alpha$ group is always deprotonated in the 3:1 adduct.

The coordination of N7 of a nucleotide to inert metal centers can be demonstrated by addition of Cu^{2+} ions. The paramagnetic Cu^{2+} ion binds to N7 of the free nucleotide, causing the broadening and eventual disappearance of the H8 resonance.[65] Alternatively, if N7 of the nucleotide is bound to another metal, such as Pt or Re, Cu^{2+} coordination at N7 is blocked and the H8 resonance remains sharp.[35] Addition of a Cu^{2+} solution to 5 μM to an r = 1:1 solution ([Re] = 5 mM) caused the H8 and P$_\alpha$ signals of free 5′-GMP to disappear. The H8 signals of the 1:1 and 1:2 adducts remained sharp and the P$_\alpha$ signals of these adducts were still present but broadened considerably. Addition of Cu^{2+} solution to 50 μM did not cause broadening of the H8 signals of the 1:1 and 1:2 adducts; however, no ^{31}P signals were detected. These results thus confirm that N7 is bound to Re in both the *fac*-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)] 1:1 and *fac*-[Re(CO)$_3$(H$_2$O)(5′-GMP)$_2$]$^-$ 1:2 adducts.

3′-GMP Reaction Products. To evaluate the effect of phosphate group position on the adducts formed, we examined 3′-GMP reactions. Treatment of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ with 3′-GMP ([Re] = 25 mM, r = 1:1) led to the appearance of three new H8 singlets (Figure 5, Table 1); these signals can be attributed to the 1:1 adduct, *fac*-[Re(CO)$_3$(H$_2$O)$_2$(3′-

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Table 2. Concentrations (mM) of 1:1, 1:2, and Dinuclear Adducts of 5′-GMP, 3′-GMP, 5′-IMP, and 3′-IMP at Different Nucleotide Concentrations and Times after Mixing with *fac*-$[Re(CO)_3(H_2O)_3]^+$ [a]

	$r = 1:1$		$r = 1:2$	
complex	1 h	6 days	1 h	6 days
5′-GMP	2.6	0.9	6.2	3.6
fac-$[Re(CO)_3(H_2O)_2$(5′-GMP)]	2.3	3.2	3.0	2.9
fac-$[Re(CO)_3(H_2O)$(5′-GMP)$_2]^-$	0.1	0.5	0.4	1.8
3′-GMP	4.3	1.1	8.6	2.9
fac-$[Re(CO)_3(H_2O)_2$(3′-GMP)]	0.7	2.2	1.2	2.3
fac-$[Re(CO)_3(H_2O)$(3′-GMP)$_2]^-$	[b]	0.8	0.1	2.3
fac-$[Re_2(CO)_6(H_2O)_4$(3′-GMP)]	[b]	0.2	[b]	0.2
5′-dGMP	2.8	0.6	5.7	3.1
fac-$[Re(CO)_3(H_2O)_2$(5′-dGMP)]	2.1	3.5	3.1	3.5
fac-$[Re(CO)_3(H_2O)$(5′-dGMP)$_2]^-$	[b]	0.5	0.6	1.7
5′-IMP	3.7	1.7	5.8	4.7
fac-$[Re(CO)_3(H_2O)_2$(5′-IMP)]	1.3	3.0	4.0	4.0
fac-$[Re(CO)_3(H_2O)$(5′-IMP)$_2]^-$	*b*	0.2	b	0.7
fac-$[Re_2(CO)_6(H_2O)_4$(5′-IMP)]	[b]	[b]	0.2	[b]
3′-IMP	4.2	2.2	8.6	4.6
fac-$[Re(CO)_3(H_2O)_2$(3′-IMP)]	0.9	2.0	1.4	3.2
fac-$[Re(CO)_3(H_2O)$(3′-IMP)$_2]^-$	[b]	0.4	[b]	1.0
fac-$[Re_2(CO)_6(H_2O)_4$(3′-IMP)]	[b]	0.2	[b]	0.3

[a] [Re] = 5 mM. [b] Not observed.

GMP)], and the 1:2 adduct, *fac*-$[Re(CO)_3(H_2O)$(3′-GMP)$_2]^-$, for reasons given above for adducts of 5′-GMP. Signals arising from the 1:2 adduct were confirmed with an $r = 1:2$ experiment.

In solutions initially 25 mM in *fac*-$[Re(CO)_3(H_2O)_3]^+$, the downfield shoulder on the H8 signal of *fac*-$[Re(CO)_3(H_2O)_2$-(3′-GMP)] and the downfield P_α signal at ~5 ppm were relatively larger in an $r = 4:1$ reaction mixture than in an $r = 1:1$ reaction mixture (Figure 5). The third product is clearly the dinuclear 2:1 adduct, *fac*-$[Re_2(CO)_6(H_2O)_4$(3′-GMP)]. A fourth product with an H8 signal just downfield from the H8 signal of *fac*-$[Re(CO)_3(H_2O)_2$(3′-GMP)] and with a P_α signal ~8 ppm downfield from the P_α signal of free 3′-GMP is the 3:1 trinuclear adduct, *fac*-$[Re_3(CO)_9(H_2O)_6$(3′-GMP)$]^+$. As was found for 5′-GMP, a 100 mM, $r = 1:1$ experiment supported this interpretation by ruling out the cyclic dimeric 2:2 adduct. To summarize, for both 3′-GMP and 5′-GMP, four products were found: two abundant products, *fac*-$[Re(CO)_3(H_2O)_2$(GMP)] and *fac*-$[Re(CO)_3(H_2O)$(GMP)$_2]^-$, and two minor products, *fac*-$[Re_2(CO)_6(H_2O)_4$(GMP)] and *fac*-$[Re_3(CO)_9(H_2O)_6$(GMP)$]^+$.

Further Aspects of the GMP Reactions. Reactions at $r = 1:1$ and $r = 1:2$ of both 5′- and 3′-GMP with *fac*-$[Re(CO)_3(H_2O)_3]^+$ (at 5 mM, a concentration at which H8 signals did not overlap) at 1 h and at 6 days were compared (Table 2). The solutions were at equilibrium after 6 days, as no spectral changes occurred between 4 and 6 days. At 1 h, the 1:1 adduct was the predominant product at $r = 1:1$; however, more 1:1 adduct was present at 1 h for 5′-GMP than for 3′-GMP. In the normal nucleotide anti conformation, the 5′-phosphate group is closer to N7 than the 3′-phosphate group. In an initial ion pair interaction of the nucleotide with the metal cation, the stabilizing electrostatic and H-bonding interactions of the phosphate group with the cation place the N7 in a position closer to the metal center for a 5′-nucleotide than for a 3′-nucleotide. We attribute the faster reaction of 5′-GMP to this proximity.

At equilibrium (6 days), the 1:1 adduct remained the predominant product for 5′-GMP at both $r = 1:1$ and at $r = 1:2$, but for 3′-GMP this was the case only at $r = 1:1$. At $r = 1:1$, more 1:1 adduct was present in the 5′-GMP reaction than in the 3′-GMP reaction (Table 2). The factors (H-bonding and electrostatic interactions) facilitating the formation of *fac*-$[Re(CO)_3(H_2O)_2$(5′-GMP)] also stabilize the 1:1 adduct. These factors are either absent (H-bonding) or weaker (electrostatic) in *fac*-$[Re(CO)_3(H_2O)_2$(3′-GMP)], accounting for its lower abundance at $r = 1:1$. However, at equilibrium, the amount of *fac*-$[Re(CO)_3(H_2O)$(3′-GMP)$_2]^-$ formed was greater than the amount of *fac*-$[Re(CO)_3(H_2O)$(5′-GMP)$_2]^-$ formed under both $r = 1:1$ and $r = 1:2$ conditions (Table 2).

Factors favoring the Re/5′-GMP 1:1 adduct are less likely to be important in contributing to the stability of the Re/5′-GMP 1:2 adduct. First, *fac*-$[Re(CO)_3(H_2O)$(5′-GMP)$_2]^-$ has only one coordinated H_2O for H-bonding to phosphate. Second, the greater conformational freedom for the 5′-phosphate group will lead to some electrostatic repulsion between the bound nucleotides. In contrast, because the 3′-phosphate groups of *fac*-$[Re(CO)_3(H_2O)$(3′-GMP)$_2]^-$ are directed away from the center of the complex, electrostatic repulsion is less in this 1:2 adduct. Although the 3′-phosphate group cannot participate in H-bonding interactions with the coordinated H_2O, this group can form stabilizing H-bonds with N1H of the cis 3′-GMP.[66] Thus, differences in the relative abundance of the 1:1 adduct and 1:2 adduct between 5′-GMP and 3′-GMP are explained.

To understand better the role of phosphate group H-bonding to N1H of the cis GMP, we must consider which conformers are likely to be present. As mentioned above, the number of conformers depends on the symmetry of the complex. For a *fac*-$[Re(CO)_3(H_2O)$(NMP)$_2]^-$ adduct, two HH (HHa and HHb) and two HT conformers (ΔHT and ΛHT, Figure 2) are possible. Distinguishing between HH and HT conformers in solution is best accomplished by using NOESY techniques, as the two H8 protons are normally close enough to give an NOE cross-peak for an HH conformer but too far to give a cross-peak for an HT conformer.[41,51,67−69] No H8−H8 NOE cross-peaks were observed for *fac*-$[Re(CO)_3(H_2O)$(5′-GMP)$_2]^-$ or *fac*-$[Re(CO)_3(H_2O)$(3′-GMP)$_2]^-$, thus providing evidence that these adducts exist mainly as HT conformers.

Because of rapid rotation about the Re−N7 bond, the NMR signal detected for each coordinated NMP is a weighted average of the respective signals for all conformers but the signals reflect mainly the HT conformers. For a dynamic adduct, differentiating between the ΔHT and ΛHT conformers is best accomplished by using CD methods.[48] It has been established that the CD signals of both 1:1 adducts

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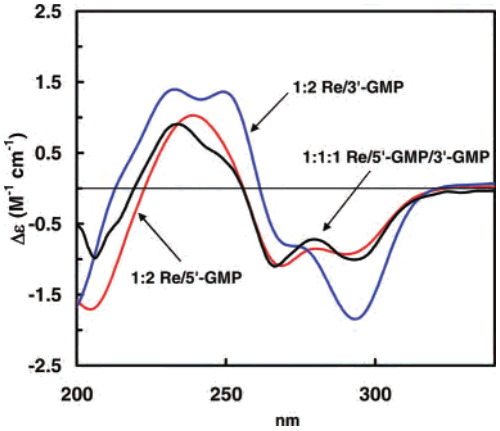

Figure 6. CD spectra recorded soon after dilution of equilibrated NMR solutions ([Re] = 25 mM, pH 3.6) of 5′-GMP (r = 1:2, red line), 3′-GMP (r = 1:2, blue line), and mixed 5′-GMP/3′-GMP (r = 1:1:1, black line).

and of HH conformers of 1:2 adducts are weak, while the CD signal of the HT conformers is much stronger.[69] Therefore, the sign of the CD spectrum reflects the conformation of the major HT conformer present. The CD signal of a ΔHT conformer has negative features at ∼227 and ∼285 nm and a positive feature at ∼252 nm, while the CD signal of a ΛHT conformer has positive features at ∼227 and ∼285 nm and a negative feature at ∼252 nm.[69] At pH ∼3.6, CD spectra of equilibrated solutions ([Re] = 25 mM, r = 1:2, pH ∼3.6) containing both the 1:1 and 1:2 adducts of 5′-GMP or 3′-GMP were recorded. The 5′-GMP solution exhibited a negative feature at ∼267 nm and a positive feature at ∼238 nm (Figure 6). A similar pattern was observed for the 3′-GMP solution (Figure 6). This pattern in both cases is indicative of the ΔHT conformer; therefore, we can conclude that *both fac*-[Re(CO)$_3$(H$_2$O)(5′-GMP)$_2$]$^-$ and *fac*-[Re(CO)$_3$(H$_2$O)(3′-GMP)$_2$]$^-$ exist primarily as ΔHT conformers. Past studies have found that the ΔHT conformation allows favorable phosphate interligand H-bonding to the carrier ligands for 5′-GMP adducts and to the N1H of the cis 3′-GMP for 3′-GMP adducts.[43,45−48] In the ΔHT conformation, only one phosphate group is well positioned to form H-bonds (to the coordinated H$_2$O) for 5′-GMP but both phosphate groups are positioned to form H-bonds (to the cis GMP) for 3′-GMP; thus, the preference for the ΔHT conformation for 3′-GMP is explained, as is the higher amount of 1:2 adduct for 3′-GMP than for 5′-GMP. However, the preference for the ΔHT conformation for the 5′-GMP 1:2 adduct was unexpected because two favorable N1H-5′-phosphate H-bonds are possible for the ΛHT conformer of 5′-GMP 1:2 adducts.

Mixed-Nucleotide Approach. Mixed 5′-GMP/3′-GMP experiments were performed to elucidate the properties of 1:2 adducts, particularly the preference for the ΔHT conformation by the 5′-GMP 1:2 adduct. In such mixtures, two Re/5′-GMP/3′-GMP 1:1:1 adducts are expected (Figure 7), in addition to the previously identified adducts containing 5′-GMP or 3′-GMP. Three r = 1:1:1 solutions at pH 3.6, all starting with [Re] = 5 mM, were studied. In one, 3′-GMP and 5′-GMP were added initially. In the other solutions, one GMP was added initially, the solution was allowed to

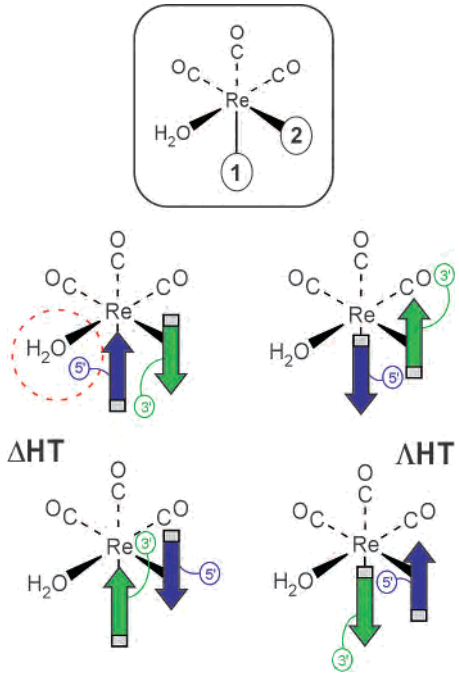

Figure 7. (top) Diagram showing coordination position numbering. (bottom) The pairs of HT conformers of each of the two possible *fac*-[Re(CO)$_3$(H$_2$O)(5′-GMP)(3′-GMP)]$^-$ adducts. In the pair on the left, the ΔHT conformer can form a favorable phosphate-to-coordinated water H-bond.

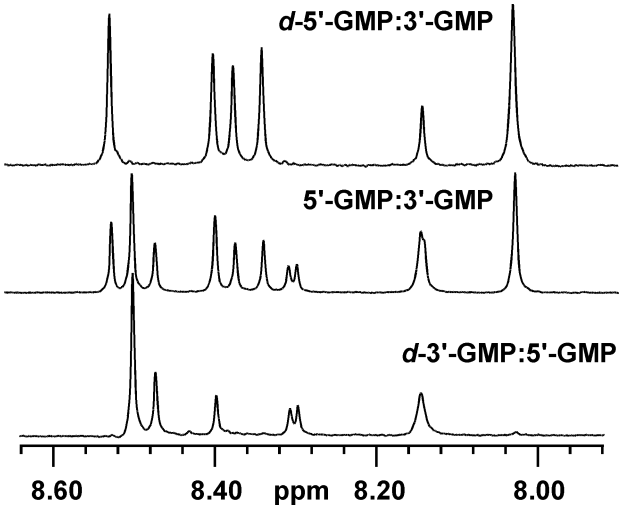

Figure 8. H8 NMR signals of equilibrated mixtures (r = 1:1:1, pH 3.6) of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ with the NMP's indicated. See text for signal assignments.

equilibrate, and then the other GMP was added. All three solutions gave identical NMR spectra. The H8 signals of previously discussed adducts were observed, as expected. In addition, three new H8 singlets (8.48, 8.38, and 8.14 ppm, Figure 8) were observed. Because the conformers of each adduct will interchange rapidly on the NMR time scale, four new H8 singlets are expected. We deduced that one new H8 singlet was overlapped with a previously discussed signal; consequently, we exchanged H8 with deuterium, preparing *d*-3′-GMP and *d*-5′-GMP.[32] The mixed-nucleotide experiment utilizing one of these deuterated nucleotides allows assignment of all H8 signals arising from the undeuterated nucleotide.

Table 3. Concentrations (mM) of 1:1, 1:2, and Mixed Bis Adducts of 5′- and 3′-GMP at Different Nucleotide Concentrations and Times after Mixing with *fac*-$[Re(CO)_3(H_2O)]^+$ [a]

	$r = 1:1$		$r = 1:2$	
complex	1 h	6 days	1 h	6 days
5′-GMP	2.0	0.4	3.9	1.4
fac-$[Re(CO)_3(H_2O)_2(5'\text{-GMP})]$	0.5	1.6	1.2	1.8
fac-$[Re(CO)_3(H_2O)(5'\text{-GMP})_2]^-$	[b]	0.1	[b]	0.4
3′-GMP	2.3	0.6	4.2	1.6
fac-$[Re(CO)_3(H_2O)_2(3'\text{-GMP})]$	0.3	1.1	0.7	1.0
fac-$[Re(CO)_3(H_2O)(3'\text{-GMP})_2]^-$	[b]	0.2	[b]	0.6
M [c]	[b]	0.3	[b]	0.8
m [c]	[b]	0.2	[b]	0.5

[a] [Re] = 5 mM. [b] Not observed. [c] ***M*** and ***m*** refer to the major and minor forms, respectively, of *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})(3'\text{-GMP})]^-$.

In a typical experiment, 1 equiv of 5′-GMP was added to an equilibrated sample containing equimolar concentrations of *d*-3′-GMP and *fac*-$[Re(CO)_3(H_2O)_3]^+$ (5 mM, $r = 1:1:1$) at pH 3.6. New H8 singlets (at 8.48 and 8.40 ppm, Figure 8) can be assigned to the 5′-GMPs in the two Re/5′-GMP/3′-GMP 1:1:1 adducts. In the competition experiment with *d*-5′-GMP, the H8 singlets (at 8.38 and 8.14 ppm, Figure 8) can be assigned to the 3′-GMPs in these two Re/5′-GMP/3′-GMP adducts. The relative intensity of the H8 signals from the Re/5′-GMP/3′-GMP adducts permits pairing of signals arising from the same complex. In this way, it was determined that the more abundant 1:1:1 adduct (***M***, 60%) has a 5′-GMP H8 signal at 8.48 ppm and a 3′-GMP H8 signal at 8.38 ppm, while the minor adduct (***m***) has a 5′-GMP H8 signal at 8.40 ppm and a 3′-GMP H8 signal at 8.14 ppm. At equilibrium (6 days, $r = 1:2$), the stability order was ***M***, *fac*-$[Re(CO)_3(H_2O)(3'\text{-GMP})_2]^-$, ***m***, and *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})_2]^-$ (Table 3).

The two Re/5′-GMP/3′-GMP 1:1:1 adducts can have a total of four HT conformers. Each adduct has a pair of ΔHT and ΛHT conformers (Figure 7). The conformers in each pair rapidly interchange. The CD pattern of an equilibrated solution containing the 1:1:1 adducts retains the ΔHT-type signal, indicating that ***M*** most likely favors the ΔHT conformation (Figure 6).

While for both *fac*-$[Re(CO)_3(H_2O)(5'\text{-GMP})(3'\text{-GMP})]^-$ adducts, the ΔHT conformer can be stabilized by an N1H-3′-phosphate H-bond between the cis nucleotides, only the ΔHT conformer of the 1:1:1 adduct with 5′-GMP in coordination position 1 can form a favorable H-bond to the coordinated water (Figure 7, left). An idealized model of the ΔHT conformer of this preferred adduct (Supporting Information) reveals that, in addition to these two H-bonds, an O6 to coordinated water H-bond is also possible. Therefore, we suggest that adduct ***M*** is the adduct in which 5′-GMP occupies coordination position 1 and 3′-GMP occupies coordination position 2. Because ***M*** is more stable than the 3′-GMP 1:2 adduct (which has two N1H-3′-phosphate H-bonds), the results suggest that phosphate−water H-bonding is more favorable than N1H−3′-phosphate H-bonding. The strength of this bond appears to be sufficient to overcome the normal preference of 5′-GMP 1:2 adducts to favor the ΛHT conformation.

Table 4. ^1H and ^{31}P NMR Shifts (ppm) and $^3J_{H1'-H2'}$ Coupling Constants (Hz, in Parentheses) of 5′-GMP, 3′-GMP, and 5′-dGMP and Complexes Formed with *fac*-$[Re(CO)_3(H_2O)_3]^+$ at Different Times after Mixing with *fac*-$[Re(CO)_3(H_2O)]^+$ [a]

	δ ^1H		
	H8	H1′	H2
5′-IMP	8.41	6.10 (6.0)	8.17
fac-$[Re(CO)_3(H_2O)_2(5'\text{-IMP})]$	8.78	6.21 (3.0)	8.26
fac-$[Re(CO)_3(H_2O)(5'\text{-IMP})_2]^-$	8.62, 8.49	6.09 (3.5), 6.08 (3.0)	8.17, 8.15
3′-IMP	8.30	6.07 (6.0)	8.16
fac-$[Re(CO)_3(H_2O)_2(3'\text{-IMP})]$	8.83	6.17 (3.5)	8.25
fac-$[Re(CO)_3(H_2O)(3'\text{-IMP})_2]^-$	8.68, 8.63	[b]	8.16, 8.14
5′-dGMP	8.16	6.31 (7.0)	–
fac-$[Re(CO)_3(H_2O)_2(5'\text{-dGMP})]$	8.45	6.37 (6.5)	–
fac-$[Re(CO)_3(H_2O)(5'\text{-dGMP})_2]^-$	8.34, 8.20	6.22 (6.0), 6.30 (6.5)	–

[a] [Re] = 5 mM, $r = 1:2$, pH 3.6, 25 °C. [b] Signal obscured by other signals.

Effects of Modifying the Nucleotides. To assess the effect of the exocyclic amino group at the C2 position of the guanine ring, we conducted some studies with 5′-IMP and 3′-IMP (Table 4, Figure 1). In general, we found products similar to those found above for 5′- and 3′-GMP. At equilibrium, the relative amounts of 1:1 vs 1:2 products formed with IMPs were similar to those with GMP. Therefore, we suggest that the exocyclic amino group of GMP does not contribute to the relative stability of the 1:1 vs 1:2 adducts. This conclusion agrees with a previous report with Pt adducts which indicates that the exocyclic amino group of GMP does not form H-bonds to the cis nucleotide.[48] The overall amount of 1:1 and 1:2 adducts formed with 5′-IMP and 3′-IMP was slightly less than the amount formed with 5′-GMP and 3′-GMP (data not shown). We believe that the less favorable formation of adducts by 5′- and 3′-IMP is related to the low N7 basicity of the hypoxanthine base of the IMPs versus that of 5′-GMP.[70,71]

The rate of reaction of 5′-dGMP and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ was reported to be ∼10 times faster than that of 5′-GMP and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$.[72] This "anomalous" behavior was explained by the lack of flexibility in the ribose ring as compared to the 2′-deoxyribose ring due to the presence of a bulky −OH group on the former.[72] If this explanation were correct, the difference between 5′-GMP and 5′-dGMP should be even larger for the reactions with the *fac*-$[Re(CO)_3(H_2O)_3]^+$ cation, which is somewhat bulkier than the *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ cation. Therefore, we decided to study the reaction of *fac*-$[Re(CO)_3(H_2O)_3]^+$ with 5′-dGMP at $r = 1:1$ and $r = 1:2$ (Table 4). We found types of products similar to those found for 5′-GMP. In addition, we performed a mixed experiment with both 5′-GMP and 5′-dGMP at $r = 1:1:1$ ([Re] = 5 mM, pH 3.6). The 5′-dGMP 1:1 adduct formed to about the same extent as the 5′-GMP 1:1 adduct at 1 h (Figure 9). Also, the 5′-GMP 1:2 adduct, the 5′-dGMP 1:2 adduct, and the two mixed 5′-GMP/5′-dGMP 1:1:1 adducts are present to about the same extent at equilibrium. These results are an indication that the 2′-substituent of the

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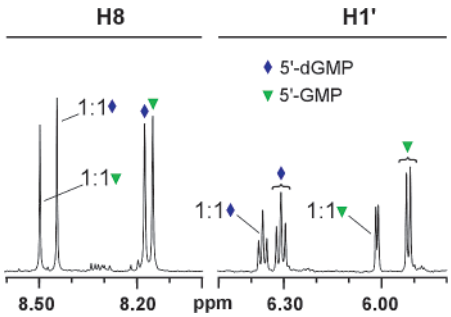

Figure 9. H8 and H1′ NMR signals of a mixture containing *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ (5 mM), 5′-GMP (5 mM), and 5′-dGMP (5 mM) at pH 3.6 and 1 h after mixing. The symbol alone denotes signals due to free nucleotide. In this r = 1:1:1 experiment, only trace amounts of 1:2 adducts are formed at 1 h; these adducts account for the weak signals observed just above the baseline.

Table 5. Concentrations (mM) of 1:1 and 1:2 Adducts of 5′-GMP and Methionine at Different Times after Mixing with *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ [a] in a Competition Reaction

	concn at 30 min	concn at 2 days	concn at 1 month
5′-GMP	2.6	1.6	3.5
fac-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)]	2.0	2.8	1.0
fac-[Re(CO)$_3$(H$_2$O)(5′-GMP)$_2$]$^-$	[b]	0.6	0.5
methionine	4.7	3.6	1.6
fac-[Re(CO)$_3$(H$_2$O)$_x$(methionine)]	0.3	1.4	3.4

[a] [Re] = 5 mM, r = 1:1:1. [b] Not observed; amount of 5′-GMP present at 30 min not equal to 5 mM due to formation of ca. 0.4 mM *fac*-[Re$_2$(CO)$_6$(H$_2$O)$_4$(5′-GMP)]; no *fac*-[Re$_2$(CO)$_6$(H$_2$O)$_4$(5′-GMP)] was detectable ca. 3 h after mixing.

ribose ring does not exert a great effect on the binding affinity of N7, and the results call into question the proposed[72] effect of the 2′-OH in the reactions of *cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ with 5′-dGMP and 5′-GMP.

Competition and Challenge Reactions Using Methionine and 5′-GMP. The well-known toxicity of Pt drugs has been attributed partially to reactions with sulfur-containing biomolecules.[73] In methionine vs GMP competition reactions with Pt complexes, Pt binds first to S of methionine, and over time this S-bound product converts to an N7-bound GMP adduct.[73,74] To assess the binding affinity of 5′-GMP for *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$, we used a competition reaction in which equimolar amounts of 5′-GMP, methionine, and *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ ([Re] = 5 mM, r = 1:1:1) were present in the reaction mixture. Shortly after mixing (~30 min), approximately half of the 5′-GMP had reacted with *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ to form *fac*-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)], while very little methionine had reacted to form products (Table 5). After 2 days the reaction mixture contained primarily *fac*-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)] (2.8 mM) and *fac*-[Re(CO)$_3$(H$_2$O)$_x$(methionine)] (1.4 mM, x = 1 or 2, Table 5). At 1 month after mixing, the reaction mixture contained mostly free 5′-GMP (3.5 mM) and *fac*-[Re(CO)$_3$(H$_2$O)$_x$(methionine)] (3.4 mM). No signals providing evidence for the formation of mixed 5′-GMP/methionine adducts were detected. Therefore, the kinetic product of such a competition reaction is *fac*-[Re(CO)$_3$(H$_2$O)$_2$(5′-GMP)]; however, the thermodynamic product is *fac*-[Re(CO)$_3$(H$_2$O)$_x$-(methionine)].

These results are the opposite of what occurs in similar competition reactions of typical Pt(II) complexes with methionine and 5′-GMP.[74] To compare the results found here to those of a typical Pt complex, we carried out a Pt/methionine/5′-GMP competition reaction[74] under the conditions used here ([Pt] = 5 mM, r = 1:1:1, pH 3.6). Again, the methionine adduct was the kinetic product, and this product converted over time to the N7-bound 5′-GMP thermodynamic product (unpublished data). Therefore, even under our low pH conditions, a typical Pt(II) complex has kinetic and thermodynamic preferences opposite to those found here for *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$.

Conclusions

For NMP = GMP or IMP, *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ forms the Re/5′-NMP 1:1 adduct more rapidly than the Re/3′-NMP 1:1 adduct. This result most likely arises from the stabilization of the 5′-NMP 1:1 adduct precursor, which we envision as being an encounter ion pair having inter-ion H-bonding between the 5′-phosphate and a coordinated water molecule. This finding agrees with results for reactions of aquated *cis*-[Pt**A**$_2$X$_2$] complexes with 5′- and 3′-NMPs.

In contrast to the normal situation for reactions of *cis*-[Pt**A**$_2$X$_2$] complexes with 5′- and 3′-NMPs, the reactions of *fac*-[Re(CO)$_3$(H$_2$O)$_3$]$^+$ with 5′- and 3′-NMPs do not go to completion under normal conditions in the presence of 2 equiv of NMP. At equilibrium both 1:1 and 1:2 adducts are present, indicating that the 1:2 adduct may be disfavored because of steric crowding. Adducts with IMP are less stable than those with GMP, a result undoubtedly related to the decreased electron-donating capability of the hypoxanthine base of the IMPs.

The NMR data confirm that all of these adducts have purine bases that rotate rapidly about the Re−N7 bond. This dynamic interchange of rotamers is attributed to the small size of the cis CO and H$_2$O ligands. NOESY data show that HH conformers are not present in significant amounts, and these data plus CD measurements suggest that the Re/NMP 1:2 adducts favor the ΔHT conformation.

Normally, 5′-GMP 1:2 adducts favor the ΛHT conformation.[45] However, it is proposed that favorable H-bonding interactions of the 5′-GMP phosphate with the coordinated water increases the stability of the ΔHT conformer over the ΛHT conformer. This interaction with coordinated water also explains why for 1:1 adducts, the 5′-NMP 1:1 adduct is favored over the 3′-NMP 1:1 adduct. In the latter, the 3′-phosphate group cannot interact with coordinated water.

In contrast, Re/3′-NMP 1:2 adducts are favored over Re/5′-NMP 1:2 adducts. This preference most likely stems from the presence in the Re/3′-NMP 1:2 adducts of stabilizing H-bonds between the N1H of each 3′-NMP with the phosphate group of the cis-bound 3′-NMP. This type of stabilizing interaction is most favorable in the ΔHT conformation.

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Mixed-nucleotide experiments, in which two different NMPs are present to react with *fac*-$[Re(CO)_3(H_2O)_3]^+$, reveal the formation of mixed 1:1:1 adducts; for example, two new mixed Re/5′-GMP/3′-GMP adducts were formed in a 60-(***M***):40(***m***) ratio. The most favored mixed species, ***M***, was more favored than even the Re/3′-GMP 1:2 adduct. This behavior is attributed to a favorable ΔHT conformer of the 1:1:1 adduct with 5′-GMP in coordination position 1. Only in this 1:1:1 adduct does one expect to have a ΔHT conformer with both phosphate groups participating in stabilizing H-bonds.

A 5′-GMP/methionine competition experiment indicated that *fac*-$[Re(CO)_3(H_2O)_3]^+$ binds faster to the harder 5′-GMP nitrogen atom, forming *fac*-$[Re(CO)_3(H_2O)_2$(5′-GMP)] as a kinetic product, but with time, the softer sulfur atom of methionine is preferred; thus, *fac*-$[Re(CO)_3(H_2O)_x$(methionine)] is the thermodynamic product. This relationship of kinetic and thermodynamic preferences is opposite to that for typical Pt complexes. The results offer hope that an anticancer drug based on Re(I) compounds could be developed with lower toxicity than drugs based on Pt.

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Supporting Information Available: Discussion of sugar pucker of nucleotides coordinated to *fac*-$[Re(CO)_3(H_2O)_3]^+$, discussion of exclusion of the presence of the cyclic dimeric 2:2 adduct, pH dependence of the ^{31}P NMR signals of the 1:1 5′-GMP, dinuclear, and trinuclear 5′-GMP adducts, and a model of the likely favored *fac*-$[Re(CO)_3(H_2O)$(5′-GMP)(3′-GMP)]$^-$ ΔHT conformer. This material is available free of charge via the Internet at http://pubs.acs.org.

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