Bioorganometallic Chemistry. 3. Role of the Phosphate Group during Reactions of Adenosine Monophosphate Derivatives with an (η^5-Pentamethylcyclopentadienyl)rhodium Aqua Complex in the Diastereoselective Formation of Cyclic Trimers, [Cp*Rh(AMP)]$_3$

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The reactions of adenosine 5′-monophosphate (5′-AMP) in water (pH 5–8) with a (η^5-pentamethylcyclopentadienyl)rhodium aqua complex, [Cp*Rh(H$_2$O)$_2$(OTf)$_2$]$_x$ (**1**), showed, by ^{1}H and ^{31}P NMR spectroscopy, that the 5′-P(O)$_2$=O group significantly inhibited cyclic trimer formation, [Cp*Rh(μ-η^1(N1):η^2(N6,N7)-5′-AMP)]$_3$, via competition with N1 and NH6 for the Cp*Rh site and, as well, provided other mononuclear and dinuclear Cp*Rh–O–P complexes. In contrast, both the phosphate methyl ester of 5′-AMP and 3′-AMP exclusively formed the cyclic trimer structures [Cp*Rh(μ-η^1(N1):η^2(N6,N7)-methyl-5′-/3′-AMP)]$_3$. The consequence of steric effects, as demonstrated by the position and substitution of the phosphate group attached to the ribose, on the diastereoselectivity of cyclic trimer formation, as observed by ^{1}H and ^{31}P NMR spectroscopy and circular dichroism analysis over time, shows that the phosphate methyl ester of 5′-AMP provides a greater diastereoselectivity (6:1) after 1 week of equilibration compared to 5′-AMP (1.5:1) and 3′-AMP (1.2:1).

Introduction

The bonding modes of metal complexes with DNA/RNA nucleobases, nucleosides, nucleotides, and oligonucleotides have been extensively studied in order to determine the mode of action of these metal complexes with regard to their drug activity, as useful tools for molecular biology, and as regulators of gene expression.[1] Relatively few of these bonding studies have focused on organometallic compounds with these biological ligands.[2,3]

Recently, we reported on the reactions of an (η^5-pentamethylcyclopentadienyl)rhodium aqua complex, [Cp*Rh(H$_2$O)$_2$(OTf)$_2$]$_x$, (**1**), with 9-methyladenine (9-MA) and adenosine (Ado) in water (pH 6–9) that provided unusual and unprecedented nucleobase cyclic trimer structures, [Cp*Rh(μ-η^1(N1): η^2(N6,N7)-9-MA/Ado)]$_3$(OTf)$_3$,[4a] and also found an extensively hydrogen-bonded structure with **1** and 1-methylcytosine (MC), *trans*-[Cp*Rh(η^1(N3)-MC)(μ-OH)]$_2$(OTf)$_2$.[4b] In order to evaluate the scope of cyclic trimer formation, we have studied the reactions of several nucleotides, including adenosine 5′-monophosphate (5′-AMP), the phosphate methyl ester of 5′-AMP, and 3′-AMP, with **1** in water (pH 5–7) and have found a significant inhibitory effect of the –P(O)$_2$=O group on cyclic trimer formation when it is in the 5′-position. In this paper, we report on ^{1}H and ^{31}P NMR studies concerning the role of the 5′-phosphate group as it influences cyclic trimer formation in comparison to the phosphate methyl ester of 5′-AMP and a positional isomer, 3′-AMP, as well as the diastereoselectivity that is involved in cyclic trimer formation using ^{1}H and ^{31}P NMR and circular dichroism (CD) analysis as a function of time.

Results and Discussion

^{1}H and ^{31}P NMR and CD Spectroscopy Studies on Cp*Rh–AMP Interactions. The ^{1}H NMR spectrum of free 5′-AMP at pD 5.65 is shown in Figure 1 E, while the spectrum of 5′-AMP (pD 5.74) in the presence of 1.2 equiv of the aqua complex, **1** (Figure 1F), clearly is very complicated and shows broadened H8 and H2 signals at 8.49 and 8.28 ppm and sharp H8 and H2 singlets at 8.36 and 8.19 ppm, respectively. In addition, the dramatic chemical shifts observed previously for cyclic trimers of 9-MA and Ado[4a] are also evident for the H8 (singlets at 8.85 and 8.78 ppm in a ratio of 1.5:1) and H2 (singlets at 7.72 and 7.69 in a ratio of 1.5:1) protons and signify the formation of [Cp*Rh(μ-η^1(N1):η^2(N6,N7)-5′-AMP)]$_3$ (**2**) (25%, by NMR integration of H8 and H2 protons; the overall charge on **2**, not designated, will be dependent on the pH). The fact that we observed two signals for both H8 and H2 was an indication that **2** was a mixture of two diastereomers (C_3 symmetry); a similar observation was noted previously for the cyclic trimer of Ado (1:1, slight NMR peak separation) but was not commented upon at that time.[4a]

Additionally, the ^{31}P NMR spectrum corroborates that there are at least five species in solution and includes several possible Cp*Rh–OP complexes with singlets at 8.62 (broadened at ambient temperature), 7.11 (sharp), and 5.76 ppm (broadened at ambient temperature) and a diastereomeric mixture for cyclic trimer **2** (–2.48 and –2.57 ppm (sharp); –2.73 ppm for free 5′-AMP). These 8–12 ppm downfield shifts of the ^{31}P signals are reminiscent of N7/PO5′ macrochelate structures as found for nucleotide complexes of Cp$_2$MoCl$_2$,[2d] *cis*-[Pt(Cl)$_2$(NH$_3$)$_2$],[5a] [Rh(tren)(H$_2$O)$_2$]$^{3+}$ (tren = tris(aminoethyl)amine),[5b] *trans*-Ru(Cl)$_2$(DMSO)$_4$,[5c] and a [Cp*Rh(P$_3$O$_9$)]$^-$ complex with discrete Cp*Rh–OP bonding.[6] Interestingly, a 2-D EXSY experiment (Figure 2)

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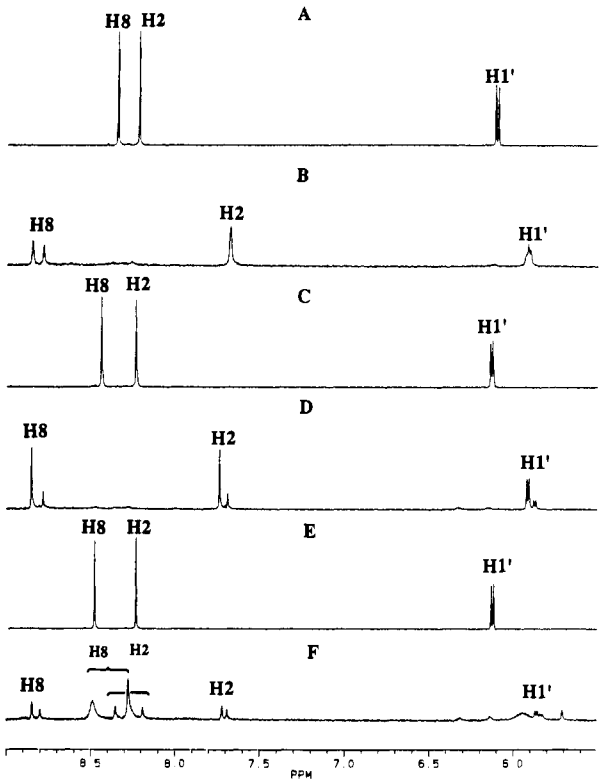


Figure 1. 400-MHz ^{1}H NMR spectra at room temperature of free and bound adenosine monophosphates (0.01 M). (A) 3′-AMP, pD 5.66; δ (ppm) 8.34 (H8), 8.22 (H2) 6.10 (d, H1′). (B) 3′-AMP + [Cp*Rh-$(H_2O)_2(OTf)_2]_x$ (1:1.3), pD 5.64; δ (ppm) 8.85, 8.78 (H8), 7.67 (H2), 5.91 (m, (H1′). (C) Me-5′-AMP, pD 5.67; δ (ppm) 8.44 (H8), 8.23 (H2), 6.13 (d, H1′). (D) Me-5′-AMP + $[Cp^*Rh(H_2O)_2(OTf)_2]_x$(1:1.1), pD 5.72; δ (ppm) 8.85, 8.78 (H8), 7.74, 7.69 (H2), 5.91, 5.87 (d, H1′). (E) 5′-AMP, pD 5.65; δ (ppm) 8.47 (H8), 8.23 (H2), 6.12 (d, H1′). (F) 5′-AMP + $[Cp^*Rh(H_2O)_2(OTf)_2]_x$(1:1.2), pD 5.74; δ (ppm) 8.85, 8.79, 8.49, 8.36 (H8), 8.28, 8.19, 7.72, 7.69 (H2), 6.32 (b), 6.11 (b), 5.93 (b), 5.86 (d), 5.82 (d), 5.72 (s) (H1′).

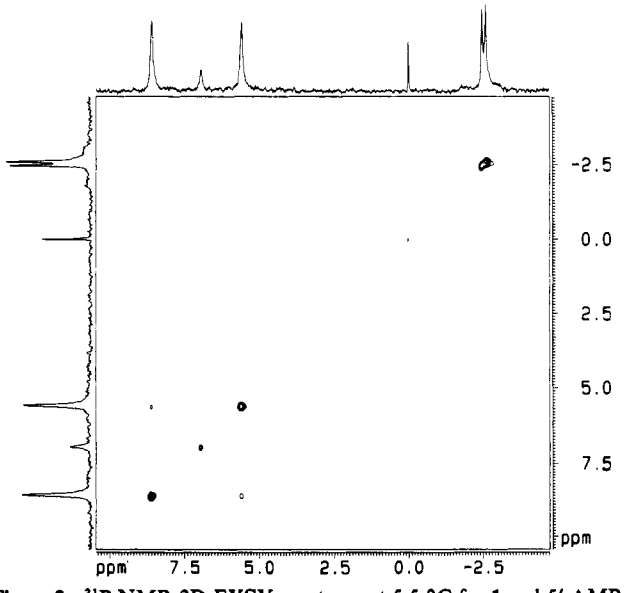


Figure 2. ^{31}P NMR 2D-EXSY spectrum at 5.5 °C for **1** and 5′-AMP.

performed at 5.5 °C verified exchange of the 8.62 and 5.76 ppm signals but showed, under these experimental conditions, no exchange between the upfield and downfield resonances. However, by raising the temperature to 70 °C, where the up- and downfield ^{31}P signals are broad singlets, and by using longer mixing times (70 ms), we indeed see exchange between phosphate-coordinated (downfield) and cyclic trimer nitrogen coordinated (upfield)

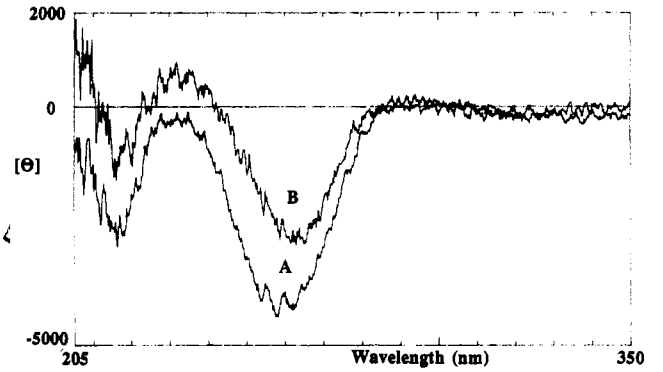
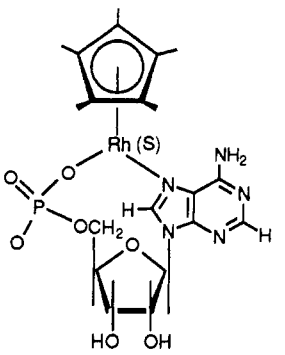


Figure 3. CD spectra, [θ] (deg $M^{-1}cm^{-1}$) vs nm: (A): 2.9×10^{-5} M solution of Me-5′-AMP in H_2O at pH 7.6; (B) Me-5′-AMP and 1.1 equiv of $[Cp^*Rh(H_2O)(OTf)_2]_x$ after 7 days at pH 7.4

Cp*Rh-5′-AMP complexes. This latter 2-D EXSY (supplementary material) result confirms that the phosphate–Rh interactions compete with N–Rh, cyclic trimer formation (N7, NH6, and N1) when the $-P(O)_2{=}O$ group is in the 5′-position.

Therefore, the ^{31}P NMR data strongly suggest that the Cp*Rh-bonded 5′-AMP complex, with the 7.11 ppm sharp signal that represents a 9.83 ppm downfield shift from that of free 5′-AMP and the ^{1}H NMR shifts at 8.36 and 8.19 ppm (sharp signals), in comparison to those of free 5′-AMP, for both the H8 and H2 protons, has an N7/PO5′ macrochelate structure and allows its possible formulation as the monomeric complex, [Cp*Rh-(η^2(N7,PO5′)-5′-AMP(S)] (**3**), where S = solvent.



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However, the other unknown Cp*RhOP complex or complexes, with ^{31}P NMR signals at 8.62 and 5.76 ppm, that are undergoing site exchange, are tentatively postulated to be a dinuclear complex or complexes with N7 bonding to one Cp*Rh center and a intermolecular 5′-phosphate coordination from another Cp*Rh-5′-AMP complex. This type of nucleotide dimer structure has precedence with the solution and solid state determination of a $[Cp_2Mo(5'\text{-dGMP})]_2$ dimer complex.[2d]

To further define the role of the 5′-phosphate group interaction with Cp*Rh and its ability to inhibit cyclic trimer formation, we reacted the Cp*Rh aqua complex, **1**, with the phosphate methyl ester of 5′-AMP at pD 5.67. Methylation of one of the oxygens on the 5′-$P(O)_2{=}O$ group considerably weakens the nucleophilicity of the other oxygen anion and should quench the ability of the 5′-phosphate group to compete with NH6 and N1 for Cp*Rh. Therefore, a similar ^{1}H NMR experiment with methyl-5′-AMP (Figure 1C) clearly shows exclusive cyclic trimer formation, $[Cp^*Rh(\mu\text{-}\eta^1(N1){:}\eta^2(N6,N7)\text{-methyl-5}'\text{-AMP})]_3$ (**4**), with ^{1}H NMR signals for H8 at 8.85 and 8.78 ppm (3:1 ratio) and for

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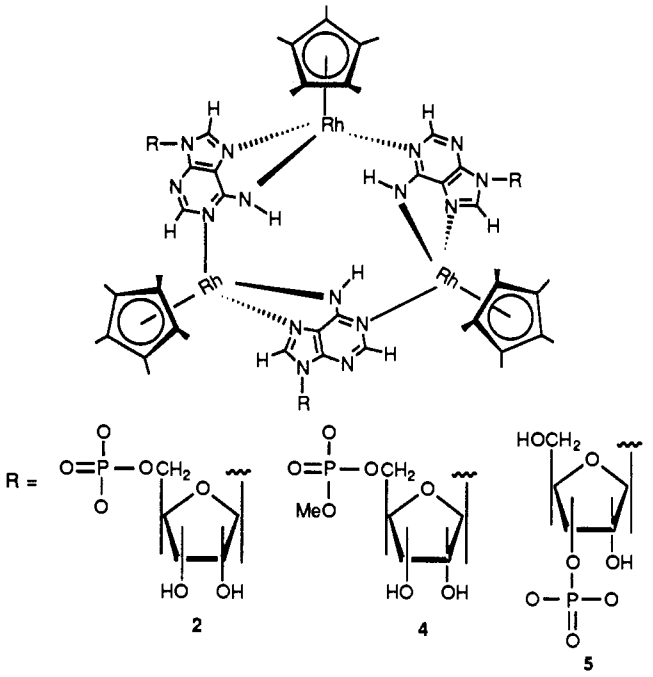
H2 at 7.74 and 7.69 ppm (3:1 ratio, Figure 1D). The ^{31}P NMR spectrum of the reaction of methyl-5′-AMP with 1.1 equiv of **1** (pD 5.72) shows two peaks at −1.61 and −1.76 ppm (−1.51 ppm for free methyl-5′-AMP) with no downfield ^{31}P signals as was observed with 5′-AMP. *Dramatically, as predicted, methylation of the 5′-phosphate group allows exclusive cyclic trimer formation.*

Furthermore, we interpret the 3:1 ratio of signals for H8 and H2 as being indicative of a more pronounced diastereoselectivity for the formation of **4** in comparison to **2**. The CD spectrum of



the diastereomers of **4**, after 7 days of equilibration at pH 7.7 (Figure 3), was studied and compared to the CD spectrum of methyl-5′-AMP. The CD spectrum of **4** shows a slight change in comparison to that of methyl-5′-AMP. However, this change in diastereoselectivity is further corroborated by time-dependent ^{1}H NMR studies, which show an initial 1:1 ratio of [Cp*Rh(methyl-5′-AMP)$_3$] diastereomers at time 0. After 24 h, the diastereomer ratio of the trimers is 3:1 and further equilibration over 7 days gives a final diastereomer ratio of 6:1. We attribute the diastereoselectivity differences among methyl-5′-AMP, 5′-AMP, and Ado to a more pronounced steric effect of the –P(OMe)(O)=O group on a ribose, which allows a resolution of one cyclic trimer diastereomer in preference to the other to occur over time by equilibration between the two possible forms.[5c]

Moreover, we reacted a positional isomer of 5′-AMP, 3′-AMP (Figure 1A), with 1.3 equiv of **1** and again found cyclic trimer formation, complex **5**,containing two diastereomers in the ratio of 1.2:1 (Figure 1B). The ^{31}P NMR spectrum of this sample also revealed a minor product (5%) with a signal at 4.01 ppm (−2.82 ppm for free 3′-AMP), in addition to the broad resonance at −2.80 ppm attributed to the cyclic trimer. This minor product provides further evidence for dinuclear [Cp*Rh–AMP]$_2$ species, since monomeric [Cp*Rh(η^2(N7,OP)-3′-AMP)] coordination is not possible. Clearly, methylation of one of the oxygens or moving the –P(O)$_2$=O group so that it is not in proximity to the N7 and NH6 groups prevents the phosphate group from competing for the Rh metal center and, therefore, inhibiting cyclic trimer formation.

To further understand the equilibrium between the possible diastereomers, we studied the reaction of **5** with 5′-AMP. An ^{1}H NMR experiment was conducted with complex **5** and approximately 3 equiv of 5′-AMP (pD 5.73), and after 24 h, new resonances that were different from those found for either **5** or **2** were present at 8.8–8.9 and 7.6–7.7 ppm in the ^{1}H NMR spectrum. We attribute these signals to mixed cyclic trimers, [Cp*Rh(5′-AMP)$_x$(3′-AMP)$_y$]$_3$, and this result strengthens the equilibrium concept and the plausible reason that the diastereoselectivity of **4** increases with time.

Other studies on the reactions of metal complexes and nucleotides have suggested a directional influence of the 5′-phosphate group, which encourages selective interactions or stabilizes complex formation.[5b,7] In particular, an [Rh(tren)(H$_2$O)$_2$]$^{3+}$ system has shown selective recognition and coordination of 5′-AMP in comparison to 3′-AMP.[5b] Formation of an intramolecular hydrogen bond between the phosphate and the coordinated tren ligand stabilizes the N7–Rh-bound 5′-AMP (not available in 3′-AMP) and limits repulsion between H$_2$N(6) of 5′-AMP and the tren ligand.

In our example, the role of the 5′-P(O)$_2$=O group inhibits cyclic trimer formation and this directional influence interferes with the condensation reaction between the exocyclic amine of 5′-AMP and the reactive Cp*Rh–hydroxy species. Thus, with the less nucleophilic phosphate, methyl-5′-AMP, or the positional isomer, 3′-AMP, minimal interference was observed for cyclic trimer formation. Therefore, Cp*Rh–OP interactions effectively compete with cyclic trimer formation only in the 5′-AMP example and represent a new role of the 5′-P(O)$_2$=O group in directing nucleotide reactions.

In future publications, we will report on other interesting chemistry of the chiral cyclic trimers as well as the reactions of **1** with sequence-specific oligonucleotides and the utility of **1** as a tether to anchor single DNA molecules to microscopic surfaces for applications in mapping and sequencing the human genome.[8]

Experimental Section

Materials. Adenosine 3′-monophosphate (3′-AMP), adenosine 5′-monophosphate (5′-AMP) hydrate, Me$_4$NOH·5H$_2$O, trimethyl phosphate (99+%), and trifluoromethanesulfonic acid (99+%) were purchased from Aldrich and used as received. The monomethyl phosphate ester of adenosine 5′-monophosphate (Me-5′-AMP) was synthesized by the procedure of Khorana[9] and passed through the sodium form of a Dowex 50 ion-exchange column to convert it to the sodium salt. Its purity was verified by ^{1}H and ^{31}P NMR spectroscopy. [Cp*Rh(OTf)$_2$]$_x$was prepared by the previously published method.[4a] The 40% NaOD in D$_2$O was purchased from Bio-Rad and diluted with D$_2$O for pH adjustments. D$_2$O (99.8%) was purchased from MSD Isotopes.

Sample Preparation. Stock solutions of the adenosine nucleotides were prepared by dissolving the appropriate nucleotide in D$_2$O with the addition of a minimal amount of a NaOD solution. A stock solution of [Cp*Rh(D$_2$O)$_2$(OTf)$_2$]$_x$ was prepared by dissolving an appropriate amount of [Cp*Rh(OTf)$_2$]$_x$in D$_2$O. The relative concentrations were verified by the ^{1}H NMR spectra of the stock solutions with 10-μL aliquots of an internal standard solution of Me$_4$NOH·5H$_2$O (10 mg/mL of D$_2$O). Samples were prepared from fresh stock solutions of the appropriate adenosine nucleotide and [Cp*Rh(D$_2$O)$_2$(OTf)$_2$]$_x$ with no precautions to exclude air. Thus, 10-μL aliquots of D$_2$O internal standard solutions Me$_4$NOH (10 mg/mL) and (MeO)$_3$PO (10 μl/mL) were added, and the sample was diluted to a final volume of 0.75 mL with a nucleotide concentration of 0.01 M. The pH was adjusted by D$_2$O solutions of NaOD and HOTf and was measured after a 3-h equilibration period. Spectra were typically recorded 24 h after sample preparation.

Physical Measurements. The pH was measured with an Orion 601A pH meter equipped with an Orion semimicro combination pH electrode. The ^{1}H NMR spectra were obtained on a Bruker AM400 FT NMR spectrometer with solvent presaturation. The following spectral parameters were used: 90° pulse, 5-s relaxation delay, 16K data points, 0.2-Hz LB,

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16 scans. Chemical shifts are reported relative to trimethylsilyl propionate with Me_4N^+ used as an internal reference. The ^{31}P NMR spectra were obtained on a Bruker AM400 FT NMR spectrometer with composite pulse ^1H decoupling. The following spectral parameters were used: 90° pulse, 7-s relaxation delay, 16K data points, 6000-Hz sweep width, 3-Hz LB, 200–500 scans. Spectra are reported relative to trimethyl phosphate used as either an internal or external standard. The ^{31}P 2-D EXSY experiment was performed on a Bruker AMX400 FT NMR spectrometer with composite pulse ^1H decoupling on a 0.01 M solution of 5′-AMP with 1.2 equiv of $[Cp^*Rh(D_2O)_2(OTf)_2]_x$, pD 5.74. The standard Bruker NOESY pulse sequence was used with composite pulse ^1H decoupling. The following spectral parameters were used: 15.2 ppm spectral width, 90° pulse, 0.208-s acquisition time, 1.7-s relaxation delay, 0.01-s mixing time, 5.5 °C. The mixing time was chosen to be fast relative to the rate of exchange and 8 scans were collected for each of 128 t_1 increments. Four steady-state scans were used prior to data collection. The F1 dimension was zero-filled to 512 data points, and a 45° shifted sine squared function and exponential multiplication with LB = 10 Hz was performed on the F2 dimension prior to Fourier transformation. The CD spectra were recorded on a Jasco J600 spectropolarimeter at ambient temperature.

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Supplementary Material Available: 2D-EXSY spectrum recorded at 70 °C (1 page). Ordering information is given on any current masthead page.