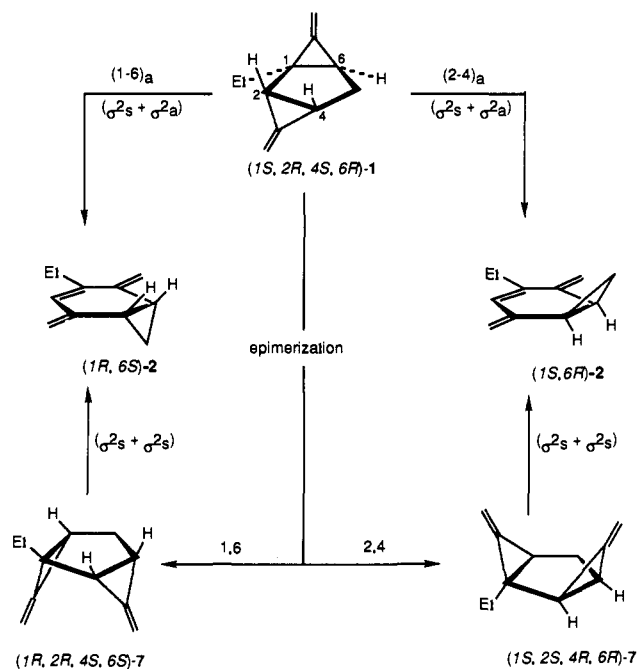
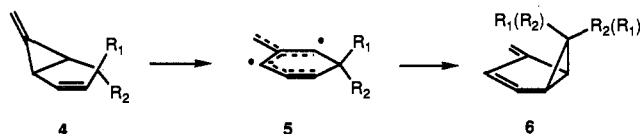


Scheme I



two mutually reinforcing factors produce the contrast in rearrangement mechanisms of **4** and **1**.



The first is that in the hypothetical "half-planar" geometry resulting from opening of one of the methylenecyclopropane units of **1**, the orbitals of the trimethylenemethane (TMM) are out of alignment with the canted orbitals of the remaining bridge bond. Not until the exocyclic CH<sub>2</sub> group of the TMM unit has passed through the plane of the six-membered ring, giving a syn geometry, does overlap of the reacting orbitals become favorable. From **4**, however, formation of the TMM **5** directly generates a fully planar carbon skeleton with good overlap of the p orbitals.

The second is that the geometry of **1** holds the bent bridge bond orbitals in a nearly perpendicular relationship conducive to an orbital symmetry allowed ( $\sigma^{2s} + \sigma^{2a}$ ) cycloaddition, which would produce the observed cis fusion of the rings and a cis endocyclic double bond in the product **2** (Scheme I). For this mechanism, two competing allowed ( $\sigma^{2s} + \sigma^{2a}$ ) reactions passing over diastereomeric transition states to enantiomeric products are expected, in accord with the observed partially racemized **2**. Which  $\sigma$ -bond prefers to participate antarafacially will determine the dominant enantiomeric configuration of **2** in the product.

Alternatively, double epimerizations of reactant **1** at the two bridge bond sites could give the enantiomers of the (at present unknown) syn tricyclic compound **7** at unequal rates, which eventually in formally forbidden ( $\sigma^{2s} + \sigma^{2s}$ ) reactions would give the enantiomers of **2** in unequal amounts.<sup>13</sup>

**Acknowledgment.** We thank the National Science Foundation and the National Institutes of Health for support of this research.

**Supplementary Material Available:** Details of synthesis and characterization of reactant **1** and product **2** (5 pages). Ordering information is given on any current masthead page.

(13) A subtly different pathway would involve conversion of the syn biradical derived from **1** directly to **2** before cyclization to **7**. The formal reaction **7** → **2** might also proceed through the same biradical. Note that a hypothetical alternative ( $\sigma^{2s} + \sigma^{2s}$ ) reaction from anti reactant **1** not only would be formally forbidden but also would lead to a much more strained, trans-fused rearrangement product.

## Proton-Proton Overhauser Effects of Receptor-Bound Cyclosporin A Observed with the Use of a Heteronuclear-Resolved Half-Filter Experiment

G. Wider, C. Weber, and K. Wüthrich\*

Institut für Molekularbiologie und Biophysik  
Eidgenössische Technische Hochschule-Hönggerberg  
CH-8093 Zürich, Switzerland  
Received January 2, 1991

This communication presents a novel combination of the use of isotope labels, heteronuclear NMR<sup>1</sup> correlation spectroscopy, and heteronuclear editing<sup>2-4</sup> for structure determinations<sup>5</sup> of receptor-bound bioactive molecules. The new experiment complements homonuclear 2D <sup>1</sup>H NMR with an X( $\omega_1, \omega_2$ )-double-half-filter (X is usually <sup>13</sup>C or <sup>15</sup>N) that yields subspectra containing exclusively intramolecular cross peaks between different hydrogen atoms of the isotope-labeled ligand molecule in the complex or the unlabeled receptor molecule, respectively.<sup>2,4,6</sup> Limitations for the use of the latter, homonuclear 2D NMR experiment may arise with increasing size of the individual molecules in the complex, when there is spectral overlap even in the edited subspectra. The presently introduced heteronuclear-resolved half-filter experiment alleviates the aforementioned limitations for the isotope-labeled component in the complex. A practical application is described with studies of fully <sup>13</sup>C labeled cyclosporin A (CsA) (MW 1265) bound to unlabeled cyclophilin (MW 17900). CsA is an immunosuppressive cyclic undecapeptide that has found widespread use in the treatment of allograft rejection following organ transplantations.<sup>7</sup> The protein cyclophilin is the presumed cellular receptor of CsA.<sup>8</sup>

The presently used heteronuclear-resolved half-filter experiment consists of a <sup>1</sup>H NOE relayed [<sup>13</sup>C,<sup>1</sup>H] COSY measurement recorded with a <sup>13</sup>C( $\omega_2$ )-half-filter (Figure 1). The delay  $\tau_1$  for coherence transfer is chosen as  $\tau_1 = 1/[2J(^{13}\text{C}, ^1\text{H})]$  or slightly shorter, and the delay  $\tau_2$  in the half-filter element<sup>2</sup> is set to  $\tau_2 = 1/[J(^{13}\text{C}, ^1\text{H})]$ . The  $\pi$  pulses are always in the middle of the respective time period. The  $\pi(^1\text{H})$  pulse in the middle of the mixing time prevents the unwanted evolution of heteronuclear antiphase magnetization present at the beginning of the mixing time into in-phase magnetization. Two data sets are recorded with and without application of the  $\pi(^{13}\text{C})$  editing pulse. The spectrum obtained as the difference of these two recordings (<sup>13</sup>C-selected subspectrum) contains exclusively NOEs between different <sup>13</sup>C-bound protons. The sum spectrum (<sup>13</sup>C-filtered subspectrum) exhibits only NOEs between <sup>13</sup>C-bound and <sup>12</sup>C-bound protons.

We applied the experiment of Figure 1 to a complex containing one molecule each of 99% <sup>13</sup>C-labeled CsA and unlabeled cyclophilin. To collect the data for a three-dimensional structure determination of CsA bound to cyclophilin,<sup>9</sup> we used primarily 2D [<sup>1</sup>H,<sup>1</sup>H] NOESY with a <sup>13</sup>C-double-half-filter.<sup>6</sup> In the subspectrum that contains the intramolecular <sup>1</sup>H-<sup>1</sup>H NOEs of the <sup>13</sup>C-labeled CsA, some of these NOEs could not be unambiguously

(1) Abbreviations and symbols used: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; 2D, two-dimensional; 3D, three-dimensional; NOESY, 2D NOE spectroscopy; COSY, 2D correlated spectroscopy; ppm, parts per million; CsA, cyclosporin A; MeVal, *N*-methylvaline; MeLeu, *N*-methylleucine.

(2) Otting, G.; Wüthrich, K. *Q. Rev. Biophys.* 1990, 23, 39-96.

(3) Otting, G.; Senn, H.; Wagner, G.; Wüthrich, K. *J. Magn. Reson.* 1986, 70, 500-505. Fesik, S. W.; Gampe, R. T., Jr.; Rockway, T. W. *J. Magn. Reson.* 1987, 74, 366-371. McIntosh, L. P.; Dahlquist, F. W.; Redfield, A. G. *J. Biomol. Struct. Dyn.* 1987, 5, 21-34. Bax, A.; Weiss, M. *J. Magn. Reson.* 1987, 71, 571-575. Griffey, R. H.; Redfield, A. G. *Q. Rev. Biophys.* 1987, 19, 51-82.

(4) Otting, G.; Wüthrich, K. *J. Magn. Reson.* 1989, 85, 586-594.

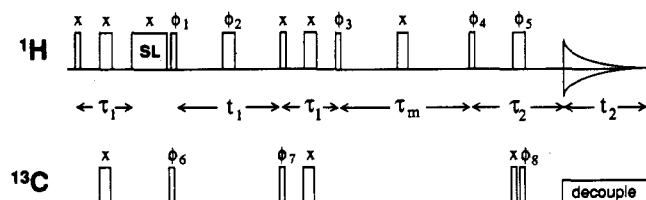
(5) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

(6) Wider, G.; Weber, C.; Traber, R.; Widmer, H.; Wüthrich, K. *J. Am. Chem. Soc.* 1990, 112, 9015-9016.

(7) Kahan, B. D., Ed. *Cyclosporin*; Grune and Stratten: Philadelphia, 1988.

(8) Handschuhmacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. *J. Science* 1984, 226, 544-547.

(9) Weber, C.; Wider, G.; von Freyberg, B.; Traber, R.; Braun, W.; Widmer, H.; Wüthrich, K. *Biochemistry*, in press.



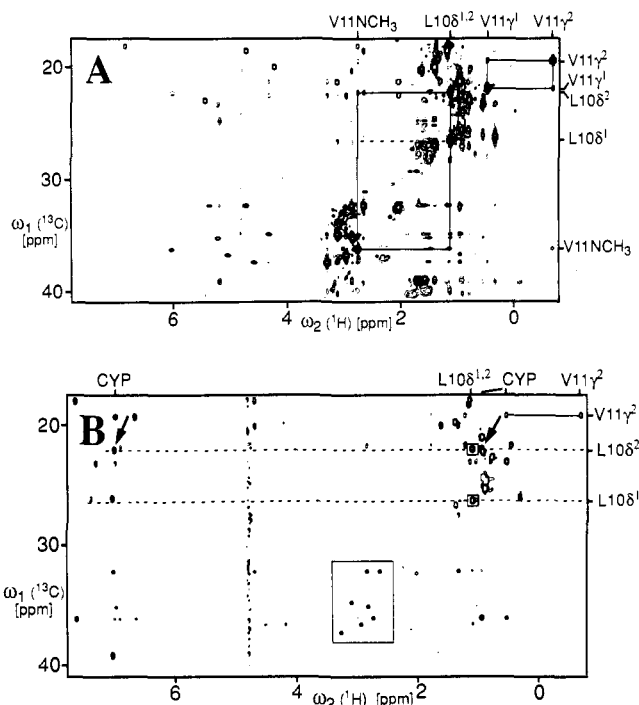
**Figure 1.** Experimental scheme for NOE-relayed  $^{13}\text{C},^1\text{H}$  COSY with  $^{13}\text{C}(\omega_2)$ -half-filter (*heteronuclear-resolved half-filter experiment*). Heteronuclear couplings are refocused during the evolution period,  $t_1$ , by the  $\pi(^1\text{H})$  pulse. During the detection period,  $t_2$ , broad-band decoupling of  $^{13}\text{C}$  is used. The vertical bars represent radio-frequency pulses, where the different pulse lengths ( $\pi/2$ ,  $\pi$ , spin lock pulse SL) are distinguished by the width of the bars. The phases of the pulses are indicated above the pulse symbols. The phases  $\phi_1$  and  $\phi_3$  were independently alternated between  $y$  and  $-y$ , and the phases  $\phi_2$ ,  $\phi_4$ ,  $\phi_5$ ,  $\phi_6$ , and  $\phi_7$  between  $x$  and  $-x$ . In addition, a two-step CYCLOPS<sup>2</sup> was applied. The receiver phase was inverted whenever the phase of a  $(\pi/2)(^1\text{H})$  pulse was changed. The basic phase cycle was repeated twice, once with the phase  $\phi_8 = x$  and once with  $\phi_8 = -x$ , and the two resulting spectra were stored in different memory locations. The desired subspectra were obtained as the sum ( $^{13}\text{C}$ -filtered) or the difference ( $^{13}\text{C}$ -selected) of these two data sets.

assigned because of spectral overlap. The  $^{13}\text{C}(\omega_2)$ -selected subspectrum from the experiment of Figure 1 enabled assignment of all these previously uncertain intramolecular NOEs, thanks to the large dispersion of the  $^{13}\text{C}$  chemical shifts.

The  $^{13}\text{C}(\omega_2)$ -selected subspectrum (Figure 2A) obtained with the experiment of Figure 1 contains intramolecular  $^1\text{H}$ - $^1\text{H}$  NOEs of CsA and intense direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks originating also from CsA. These two types of cross peaks form typical patterns, as is exemplified in the upper right corner of Figure 2A for the  $\gamma^1$  and  $\gamma^2$  methyl groups of *N*-methylvaline 11 (note that seven of the 11 amino acid residues in CsA are *N*-methylated). Similar rectangles relating the *N*-methyl group of MeVal 11 and the  $\delta$ -methyl groups of MeLeu 10 show that there is a NOE with MeLeu 10 $\delta^2$  (solid lines), but not with MeLeu 10 $\delta^1$  (broken line). This information could not be obtained from  $^{13}\text{C}(\omega_1, \omega_2)$ -double-half-filtered [ $^1\text{H}, ^1\text{H}$ ] NOESY because the proton chemical shifts of the two  $\delta$ -methyl groups of MeLeu 10 are nearly identical. In addition to the resonances originating from CsA, direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks from methyl groups in the unlabeled cyclophilin appear in the  $^{13}\text{C}$ -selected subspectrum. Although these peaks have intensity similar to that of the intramolecular NOE relay peaks, they do not show a  $^{13}\text{C}$ - $^{13}\text{C}$  coupling and usually do not match with two chemical shift positions of CsA, so that there is little danger of misinterpretation.

The  $^{13}\text{C}(\omega_2)$ -filtered subspectrum (Figure 2B) was used to investigate intermolecular NOE cross peaks between CsA and cyclophilin. In principle, this subspectrum should contain *only* intermolecular NOE cross peaks. In practice, however, the direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks are not completely suppressed, and their residual intensity of about 3% is comparable to the intensity of the NOE cross peaks. (Contributions to these residual peaks may come from imperfect matching of the delay  $\tau_2$ , imperfect editing pulses, and instrumental instabilities.) As an illustration, the well-separated direct correlation peaks of the seven *N*-methyl groups in CsA are identified in Figure 2B. These residual direct peaks are readily identified by comparison with the  $^{13}\text{C}(\omega_2)$ -selected subspectrum (Figure 2A) and may be used as reference points for the assignment of the  $^1\text{H}$ - $^1\text{H}$  NOE cross peaks in horizontal two-peak patterns. This is illustrated in Figure 2B for a NOE between MeVal 11 $\gamma^2$  and a cyclophilin proton. The broken horizontal lines in Figure 2B help one to visualize that there are NOEs from the  $\delta^2$ -methyl of MeLeu 10 to a methyl group and an aromatic proton of cyclophilin, whereas no intermolecular NOEs are present for the  $\delta^1$ -methyl. Because of the  $^1\text{H}$  chemical shift degeneracy for the two methyl groups of MeLeu 10 (see Figure 2A), the assignment of these NOEs to an individual methyl group could not be made in a  $^{13}\text{C}(\omega_1, \omega_2)$ -double-half-filtered NOESY spectrum.

In conclusion, the presently introduced heteronuclear-resolved half-filter experiment enabled the identification of numerous



**Figure 2.**  $^1\text{H}$  NOE relayed  $^{13}\text{C},^1\text{H}$  COSY spectrum recorded with  $^{13}\text{C}(\omega_2)$ -half-filter (Figure 1) of fully  $^{13}\text{C}$  labeled CsA bound to unlabeled cyclophilin. (Concentration of the cyclophilin-CsA complex 0.7 mM; solvent  $\text{D}_2\text{O}$ ; pD = 6.0;  $T = 26^\circ\text{C}$ ;  $^1\text{H}$  frequency 600 MHz;  $\tau_m = 50$  ms,  $\tau_1 = 3.1$  ms,  $\tau_2 = 7.1$  ms;  $t_{1\text{max}} = 29.4$  ms,  $t_{2\text{max}} = 92$  ms; sweep width for  $^1\text{H}$ , 11111 Hz, for  $^{13}\text{C}$ , 7812 Hz). (A)  $^{13}\text{C}(\omega_2)$ -selected spectrum showing intramolecular  $^1\text{H}$ - $^1\text{H}$  NOEs of CsA. Rectangular patterns of direct  $^{13}\text{C}$ - $^1\text{H}$  heteronuclear correlation peaks and  $^1\text{H}$ - $^1\text{H}$  NOE peaks are indicated with solid lines for the two  $\gamma$ -methyl groups of MeVal 11 ( $\text{V11}\gamma^1$ ,  $\text{V11}\gamma^2$ ) and for the  $\delta^2$ -methyl group of MeLeu 10 ( $\text{L10}\delta^2$ ) and the *N*-methyl group of MeVal 11 ( $\text{V11NCH}_3$ ). The direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks of the  $\delta^1$ -methyl of MeLeu 10 and the expected position of the NOE cross peak relating MeLeu 10 $\delta^1$  and MeVal 11 $\gamma^2$  are where the broken horizontal line intersects the vertical lines. (B)  $^{13}\text{C}(\omega_2)$ -filtered subspectrum showing intermolecular  $^1\text{H}$ - $^1\text{H}$  NOEs between CsA and cyclophilin. The box in the lower center of the spectrum contains the residual direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks of the seven *N*-methyl groups of CsA. The horizontal line in the upper right corner connects the direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks of the  $\gamma^2$ -methyl group of MeVal 11 ( $\text{V11}\gamma^2$ ) with a NOE cross peak between this methyl group of CsA and a methyl resonance of cyclophilin (CYP). The two broken horizontal lines have been drawn through the residual direct  $^{13}\text{C}$ - $^1\text{H}$  correlation peaks of the two  $\delta$ -methyl groups of Leu 10 ( $\text{L10}\delta^1$ ,  $\text{L10}\delta^2$ ), which are identified by a square box. The two arrows point to two intermolecular NOE cross peaks between MeLeu 10 $\delta^2$  and protons of cyclophilin.

intramolecular  $^1\text{H}$ - $^1\text{H}$  NOEs in fully  $^{13}\text{C}$  labeled CsA bound to cyclophilin. These NOEs, which could not be resolved and assigned with other available 1D or 2D NMR experiments, were an important part of the input for the structure determination of bound CsA.<sup>9</sup> The technique should be quite generally useful for studies of receptor-bound isotope-labeled peptides and related compounds in complexes with molecular weights up to at least 20000, since  $^1\text{H}$  chemical shift degeneracies of molecular fragments with identical chemical structure are common in nonglobular polypeptides.<sup>5</sup> A limitation may arise from accidental overlap of  $^1\text{H}$ - $^1\text{H}$  NOE cross peaks with the much more intense direct heteronuclear correlation peaks (Figure 2A). Therefore, the heteronuclear-resolved half-filter experiment will foreseeably complement rather than replace the use of  $^1\text{H}$  NOESY experiments with heteronuclear double-half-filters.<sup>2,4,6</sup> Another alternative would be the use of a 3D heteronuclear correlated [ $^1\text{H}, ^1\text{H}$ ] NOESY experiment,<sup>10</sup> but for studies of relatively small recep-

(10) Fesik, S. W.; Zuiderweg, E. R. P. *J. Magn. Reson.* **1988**, *78*, 588-593. Kay, L. E.; Marion, D.; Bax, A. *J. Magn. Reson.* **1989**, *84*, 72-84. Ikura, M.; Kay, L. E.; Tschudin, R.; Bax, A. *J. Magn. Reson.* **1990**, *86*, 204-209.

tor-bound ligand molecules the combined use of the two aforementioned 2D NMR experiments appears preferable on various practical grounds.

**Acknowledgment.** Support by the Schweizerischer Nationalfonds (Project 31-25174.88) and by Sandoz Pharma Ltd. is gratefully acknowledged. We thank Mr. R. Marani for the careful processing of the manuscript.

### Selective Recognition and Coordination by the $[\text{Rh}^{\text{III}}(\text{tris}(\text{aminoethyl})\text{amine})(\text{H}_2\text{O})_2]^{3+}$ Cation of the Adenine Nucleobase Only When It Is a Constituent of a 5'-Nucleotide

Lourdes M. Torres and Luigi G. Marzilli\*

Department of Chemistry, Emory University  
Atlanta, Georgia 30322

Received February 12, 1991

We report the unusual observation of highly selective coordination of a nucleobase when it is a constituent in a 5'-nucleotide but not when it is in a nucleoside or in a 3'- or 2'-nucleotide. Such an example of molecular recognition is unprecedented. Several recent elegant syntheses of organic compounds have made possible the discovery of selective interactions with nucleobase derivatives in organic solvents.<sup>1-7</sup> In the present case, we demonstrate the use of an octahedral metal center in aqueous solution to arrange groups for the recognition of both base and phosphate groups. The tripodal tren (tris(aminoethyl)amine) ligand was selected because it can adopt only one geometry in octahedral complexes. The size and charge of the central metal can be varied; the resulting versatility should allow fine tuning of interactions of octahedral complexes with the molecular target not readily achieved with typical organic compounds containing a limited number of atom types.

An aqueous solution of  $[\text{Rh}^{\text{III}}(\text{tren})(\text{H}_2\text{O})_2]^{3+}$  (0.056 M) and adenosine (Ado) in a 1:1 ratio was brought to 60 °C. After 48 h, essentially no evidence of reaction was observed by <sup>1</sup>H NMR (Figure 1). On the other hand, both guanosine (Guo) and inosine (Ino) react with  $[\text{Rh}(\text{tren})(\text{H}_2\text{O})_2]^{3+}$  ( $t_{1/2} < 2$  h) under the same conditions to form complexes with the H8 signal shifted strongly downfield. For example, the H8 signal of Guo shifts from 8.05 ppm to 8.49 ppm; for Ino, from 8.36 ppm to 8.75 ppm. Such ~0.5 ppm downfield shifts are characteristic of N7 coordination.

These differences in reactivity for the nucleosides can be understood by the nature of the tren-nucleobase interactions, which are unfavorable and repulsive between tren and the exocyclic 6-NH<sub>2</sub> group of Ado but favorable for H-bonding interactions between tren and the 6-oxo group of Guo and Ino.<sup>8</sup>

In contrast to Ado, 5'-AMP does react with  $[\text{Rh}(\text{tren})(\text{H}_2\text{O})_2]^{3+}$  ( $t_{1/2} \sim 2$  h at 60 °C) to form a complex (I-5'-AMP) with the following spectral characteristics: H8 signal at 9.10 ppm, H2 at 8.26 ppm, and H1' (doublet) at 6.26 ppm (Figure 1). Compared to free 5'-AMP (H8 at 8.52 ppm, H2 at 8.15 ppm, and H1' (doublet) at 6.09 ppm), the large downfield shift of the H8 signal

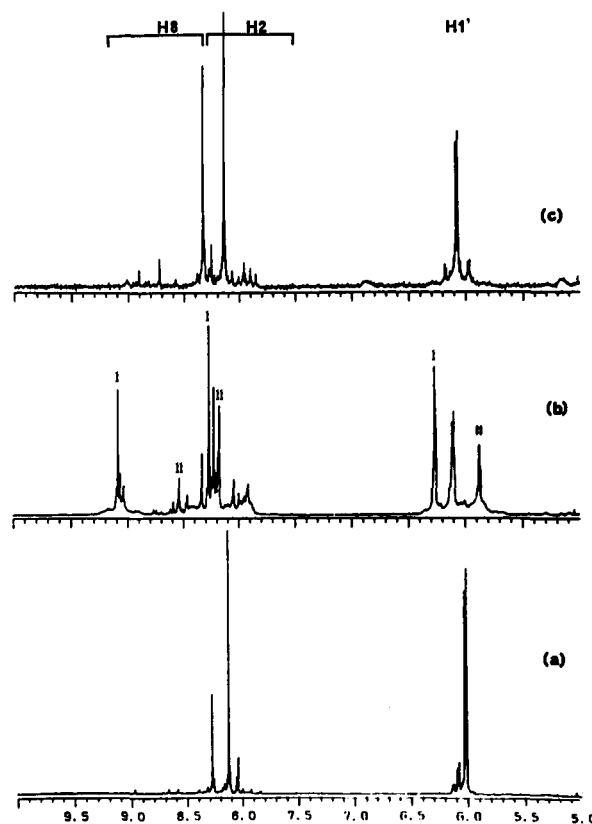


Figure 1. H8, H2, H1' region of the 360-MHz spectra of 1:1 mixtures of  $[\text{Rh}(\text{tren})(\text{H}_2\text{O})_2]^{3+}$  with (a) Ado,  $t = 48$  h, 60 °C; (b) 5'-AMP,  $t = 2$  h, 60 °C (signals for I-5'-AMP and II-5'-AMP are labeled I and II, respectively); and (c) 3'-AMP,  $t = 48$  h, 60 °C (Nicolet 360-MHz spectrometer, 99.98% D<sub>2</sub>O, sodium 3-(trimethylsilyl)propionate-*d*<sub>4</sub> internal reference, pD = 6.9, 23 °C).

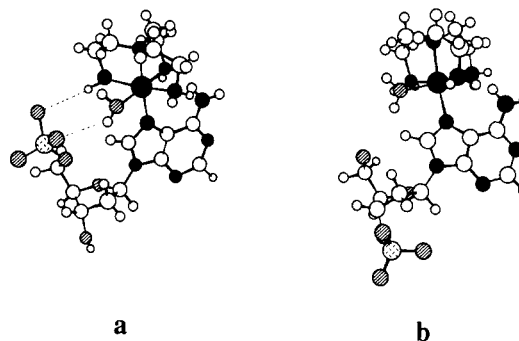


Figure 2. (a) Graphic depictions based on crystallographic data<sup>19-21</sup> indicating potential favorable interligand H bonding of the phosphate group in  $[\text{Rh}(\text{tren})(\text{H}_2\text{O})_2(5'\text{-AMP-N7})]^{3+}$ . (b) The absence of such interactions in a hypothetical  $[\text{Rh}(\text{tren})(\text{H}_2\text{O})_2(3'\text{-AMP-N7})]^{3+}$ .

and smaller shifts of H2 and H1' are characteristic of N7 binding.<sup>9-13</sup> Further evidence for N7 coordination was obtained from experiments using a paramagnetic probe. Addition of Cu<sup>2+</sup> to a solution containing free 5'-AMP and I-5'-AMP resulted in line broadening of the H8 and H2 signals of the free ligand, while the H8 signal of I-5'-AMP was not affected.<sup>9,10</sup> The <sup>31</sup>P signal corresponding to complex I-5'-AMP appears at 1.1 ppm, only 1.4 ppm downfield from the free ligand. A similar behavior was observed with 5'-dAMP.

(1) (a) García-Tellado, F.; Goswami, S.; Chang, S.-K.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1990**, *112*, 7393. (b) Hirst, S. C.; Hamilton, A. D. *Tetrahedron Lett.* **1990**, *31*(17), 2401. (c) Hamilton, A. D.; Little, D. *J. Chem. Soc., Chem. Commun.* **1990**, 297.

(2) (a) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245. (b) Keong, K. S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 3327. (c) Rebek, J., Jr.; Williams, K.; Parris, K.; Ballester, P.; Jeong, K. S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1244.

(3) Echevarren, A.; Galán, A.; Lehn, J.-M.; de Mendoza, J. *J. Am. Chem. Soc.* **1989**, *111*, 4994.

(4) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397.

(5) Zimmerman, S.; Weiming, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054.

(6) Ebmeyer, F.; Vogtle, F. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 7.

(7) Slavín, L. L.; Bose, R. N. *J. Chem. Soc., Chem. Commun.* **1990**, 1256.

(8) (a) Marzilli, L. G.; Kistenmacher, T. *J. Acc. Chem. Res.* **1977**, *10*, 146. (b) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32.

(9) Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 2421.

(10) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 8299.

(11) Reily, M. D.; Hambley, T. W.; Marzilli, L. G. *J. Am. Chem. Soc.* **1988**, *110*, 2999.

(12) Alessio, E.; Xu, Y.; Cauci, S.; Mestroni, G.; Quadrioglio, F.; Viglino, P.; Marzilli, L. G. *J. Am. Chem. Soc.* **1989**, *111*, 7068.

(13) Kuo, L. Y.; Kanatzidis, M. G.; Marks, T. J. *J. Am. Chem. Soc.* **1987**, *109*, 7207.