Heavy Metal Nucleotide Interactions. 12. Competitive Reactions in Systems of Four Nucleotides with *cis*- or *trans*-Diammineplatinum(II). Raman Difference Spectrophotometric Determination of the Relative Nucleophilicity of Guanosine, Cytidine, Adenosine, and Uridine Monophosphates as Well as the Analogous Bases in DNA[1,2]

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Abstract: Raman difference spectrophotometry was used to establish that *cis*- and *trans*-$(H_3N)_2Pt^{II}$ react extensively at pH 7, 25 °C, with a mixture of 5′-GMP, 5′-CMP, 5′-AMP, and 5′-UMP at low r ($(H_3N)_2Pt^{II}$:phosphate) values. At $0.05 \leq r \leq 0.2$, the cis isomer binds to 5′-GMP and, to a lesser extent, to 5′-AMP, while the trans isomer is somewhat more selective for 5′-GMP. Neither the cis nor the trans complex reacts measurably with the pyrimidine nucleotides at these low r values. The Raman studies also confirm that the site of binding on GMP is N(7). Similar results are obtained at 37 °C. ^1H NMR spectra identify *cis*-$[(H_3N)_2Pt(GMP)_2]^{2-}$, *cis*-$[(H_3N)_2Pt(AMP)_2]^{2-}$, and the ternary complex *cis*-$[(H_3N)_2Pt(GMP)(AMP)]^{2-}$ as significant species in these solutions. This selectivity is kinetically controlled and is similar to that observed with alkyl sulfates, alkylsulfonates, mustards, epoxides, and nitroso compounds, while CH_3Hg^{II}, where the reactions are thermodynamically controlled, and diazoalkanes react readily with Guo, Urd, and dThd with substitution of N-bonded protons. In reactions with calf-thymus DNA, *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ at pH 7, $r = 0.2$, shows no selectivity and little binding to the bases. The trans isomer shows slight preferential binding to the guanine bases. In contrast, *cis*-$[PtCl_2(NH_3)_2]$ shows high selectivity, and the guanine bases are completely platinated at N(7) with $r = 0.2$. Extensive denaturation of the double helix occurs. Reaction with the adenine bases also occurs, and this is extensive at $r = 0.4$. No reaction occurs with either pyrimidine under these conditions. The lack of binding selectivity of the aquated complexes suggests that a direct nucleophilic displacement of chloride or some other carrier ligand occurs in vivo. *cis*-$[PtCl_2(NH_3)_2]$ shows considerably higher reactivity with the guanine bases of calf-thymus DNA than the nitrogen mustard *N*-methylbis(2-chloroethyl)amine does.

There are several experiments that indicate electrophilic attack at the guanine base occurs when a native polynucleotide is reacted with *cis*-$(H_3N)_2Pt^{II}$ compounds at low values of r (r = $[(H_3N)_2Pt^{II}]$:total base (monomer) = $[(H_3N)_2Pt^{II}]$:total phosphate). Mansy,[4] using the UV absorption, observed that the rate of reaction of *cis*-$[PtCl_2(NH_3)_2]$ with DNAs, $r = 1$, increased with increasing G + C content; Stone et al.[5] found the increases in buoyant density upon platination also were proportional to the G + C content. Using ^{195m}Pt, Munchausen and Rahn[6] found that the extent of platinum binding increased with G + C content. When the DNA was subjected to mild acid hydrolysis, platinum was found bound to both guanosine and adenosine. Roos[7] has found a saturation binding value of 0.57 platinum per phosphate using equilibrium dialysis to study the binding of $[PtCl_2en]$ to *Escherichia coli* DNA. He also noted that this saturation value does not determine the bases to which binding occurs as had been claimed earlier.[8]

Robins[9] found that $[PtCl_2en]$ reacted more rapidly with guanosine than with adenosine or cytidine. On the other hand, Schovell and O'Connor[10] have obtained values for the conditional equilibrium constants for binding of *cis*-$(H_3N)_2Pt^{II}$ to guanosine, adenosine, and cytidine at pH 6.5, 25 °C, which are very nearly the same. The UV spectrophotometric data could be fitted adequately assuming formation of only the monocomplexes: log K values—Guo, 3.7; Ado, 3.6; Cyd, 3.5. On the basis of these results, together with the comparisons of the reactions of *cis*-$(H_3N)_2Pt^{II}$ and H_3CHg^{II} with cytidine,[11] uridine,[11] inosine,[12] and guanosine,[13] it appears that the binding specificity in the reactions of *cis*-$(H_3N)_2Pt^{II}$ favoring the guanine base is probably kinetic rather than thermodynamic in origin.

There are very few techniques that can be used to determine directly the binding reaction(s) that predominate in an aqueous system with many competing processes, e.g., in the reaction of a strong electrophile with four nucleotides. In the systems under consideration here, we are concerned with the binding of *cis*- and *trans*-$(H_3N)_2Pt^{II}$. In these cases, there is competition not only among the four nucleotides in the nucleophilic attack at platinum but also competition among different donor sites on the same nucleotide. Raman difference spectrophotometry (RADS) is one technique that can be used to establish at which site(s) on which nucleotide(s) binding occurs preferentially.

Recently we have tested the applicability of the RADS method in a study of the reactions of H_3CHg^{II} with DNA models and native DNA.[14] In this article, we report the use of RADS to study the reactions of *cis*- or *trans*-$(H_3N)_2Pt^{II}$ with a mixture of 5′-GMP, 5′-CMP, 5′-AMP, and 5′-UMP. Since 5′-UMP and 5′-dTMP bind similarly to the platinum electrophiles[15] and UMP gives a simpler Raman spectrum, UMP was used in the model system. This nucleotide mixture serves as a reasonable binding model for denatured polynucleotides. It excludes any conformationally dependent effects of a double helical structure, and it is completely protein free, thus avoiding any problem of histone contamination which may occur with native DNAs.

In order to compare the competitive reactions in a system where the bases are fixed to a phosphate diester backbone, we have carried out comparable studies with native calf-thymus DNA.

Experimental Section

Platinum Complexes. *cis*-Dichlorodiammineplatinum(II) and aqueous solutions of *cis*- and *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ as the nitrate and perchlorate were prepared as described previously.[11-13] For

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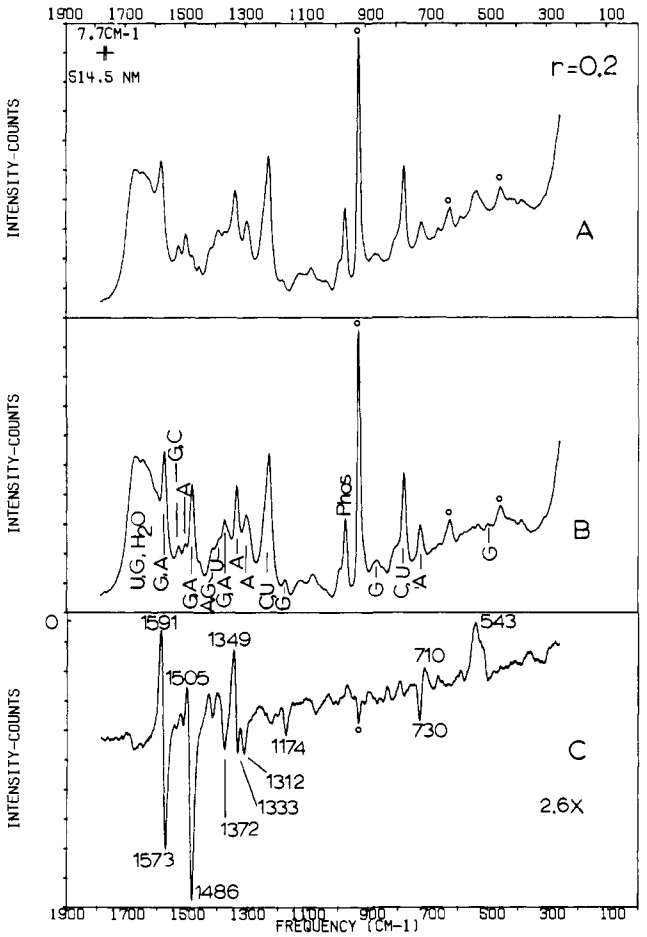


Figure 1. RADS for the study of the competitive reactions of *cis*-$(H_3N)_2Pt^{II}$ with a mixture of GMP, CMP, AMP, and UMP and H_2O at 25 °C, pH 7, 0.1 M ClO_4^-: (A) 20 mM $(H_3N)_2Pt^{II}$ + 20 mM GMP + 20 mM CMP + 30 mM AMP + 30 mM UMP; (B) 20 mM GMP + 20 mM CMP, + 20 mM AMP + 30 mM UMP; contributions of the individual nucleotides are indicated; (C) A − B, ordinate expanded by a factor of 2.6. Scan conditions: 10 s/step, 1-cm^{-1} steps. The presence of circles (o) above a band indicates that it arises from the internal reference, here ClO_4^-. The spectral slit widths are indicated in the upper left of each spectrum.

the NMR studies, $[Pten(OH_2)_2](F_3CSO_3)_2$ was used, and its synthesis also has been described.[13]

Nucleotide and DNA Solutions. The DNA model solutions were prepared from stock solutions of the nucleotides. These are obtained as follows: 5′-AMP, 5′-GMP, and 5′-UMP, Aldrich Chemical Co., Milwaukee, Wis.; 5′-CMP, Cyclo Chemical, Los Angeles, Calif., and Sigma Chemical Co., St. Louis, Mo. Samples of [8-^{2}H]AMP and [8-^{2}H]GMP were prepared by heating 5′-AMP and 5′-GMP in D_2O at ca. 90 °C for 2 days as described by Elvidge et al.[16] The products were isolated, redissolved in the minimum quantity of D_2O, and precipitated with ether–acetone. This was repeated, and the products were dried under vacuum over P_4O_{10}. Solutions containing calf-thymus DNA, sodium salt, were prepared from Sigma type I. The fibrous DNA was placed in 70% ethanol–water in a refrigerator, the solvent was decanted and replaced by fresh ethanol–water after a few hours, and the process was repeated several times to remove salts from the polynucleotide. The fibers then were lyophilized.

Binding Reaction Studies. Solutions for the studies with the four nucleotide model system were prepared by mixing a slightly acidic stock solution of the aquo-platinum(II) complex with a solution containing the four nucleotides to give the desired *r* value. The pHs then were adjusted with $HClO_4(HNO_3)$ or NaOH solutions using a Radiometer PHM-4 pH meter. For the ^{1}H NMR studies, the solvent was D_2O (99.8% Columbia Organic Chemicals, Columbia, S.C.), and $DClO_4$ or NaOD was used to adjust the p^{2}H. The meter readings were corrected as described by Glascoe and Long.[17] Before determining the Raman spectra, the solutions were passed through 250-nm pore size ultra filters.

The DNA solutions were prepared by weighing out a sample of the lyophilized calf-thymus DNA and adding it to a solution of $NaNO_3$ to give the appropriate nitrate concentration and a solution of the platinum complex to give the desired *r* value. The total phosphate concentrations were ca. 5 mM. The pH then was adjusted. These relatively nonviscous solutions were incubated for a measured period of time, normally 3 days at 37 °C, and then passed through a Millipore AP 2002500 borosilicate microfiber glass prefilter to remove particulate impurities. The polynucleotide concentration then was increased at constant pH and electrolyte concentration by forcing the excess solvent through a membrane filter (Amicon Diaflo UM10, UM20E, or PM30, in an Amicon Model 8MC Micro-ultrafiltration system). This viscous solution was transferred to a rectangular Raman cell with optically flat windows.

After addition of the platinum complex, all solutions were stored in the dark.

Raman Spectra. The general procedures for obtaining Raman and Raman difference spectra (RADS) have been described previously.[11-14,18] The model system difference spectra were obtained with the computerized Raman difference spectrophotometer that has been described previously.[19,20] Laser power at the sample was ca. 700 mW. The DNA spectra were obtained with a difference spectrophotometer built around a Jobin-Yvon Ramanor HG-2 monochromator interfaced to the same computer system that controls the other instrument. Spectra were processed off-line using PROGRAM RAMAN. They were subjected to a 25 point quartic smooth.

Nuclear Magnetic Resonance Spectra. The ^{1}H NMR spectra were determined with a Varian XL-100 (100 MHz) spectrometer using a CAT, and the general procedure has been described previously.[11-13,18] The internal reference was $N(CH_3)_4^+$, and the D_2O solvent provided the lock signal. Probe temperature was ca. 30 °C.

Data and Results

Raman Difference Studies on the Competitive Reactions of 5′-GMP + 5′-CMP + 5′-AMP + 5′-UMP Mixtures with *cis*- or *trans*-$(H_3N)_2Pt^{II}$ in H_2O at pH 7, 25 °C. Selectivity. Solutions were prepared with 20 mM GMP, 20 mM CMP, 30 mM AMP, and 30 mM UMP. This approximates the base composition of calf-thymus DNA with UMP substituted for dTMP. As noted above, these have identical binding reactions with the platinum(II) complexes, but UMP gives better quality spectra. Previous studies indicated dTMP samples tend to exhibit fluorescence,[14] and the spectral changes upon metallation at N(3) of Urd are somewhat more pronounced than for the corresponding reaction with dThd. Spectra were determined for solutions with $r = 0.05$, 0.10, and 0.20. In this discussion $(H_3N)_2Pt^{II}$ or $[(H_3N)_2Pt(OH_2)_2]^{2+}$ indicate that the solutions were prepared with the perchlorate, nitrate, or trifluoromethanesulfonate salts. These will be extensively hydrolyzed at pH 7, and a species distribution diagram is given in ref 11. With freshly prepared solutions, polynuclear hydrolysis products should not be present to any significant extent.

Figure 1 illustrates the set of parent spectra and the difference spectrum for the solution containing the cis isomer, $r = 0.20$. Prominent features of the spectrum of the four-nucleotide mixture are assigned to the appropriate nucleotide. Figure 2 illustrates trends in the difference spectra, for $r = 0.05$, 0.10, and 0.20. For the interpretation of these difference spectra, it is helpful to have the difference fingerprints for reactions of *cis*-$(H_3N)_2Pt^{II}$ with each of the nucleosides involved. These are illustrated in Figure 3. Since there is no significant reaction with the phosphate at the *r* values involved in this study, the difference spectra of nucleosides and nucleotides are identical, and difference spectra for uridine and cytidine are used in Figure 3. Relative ordinate expansions based on the internal perchlorate reference are included, so the intensities may be compared.

Comparison of the difference spectrum for the solution with $r = 0.05$, Figure 2, with the spectra in Figure 3 shows that the difference spectrum is almost identical with the difference

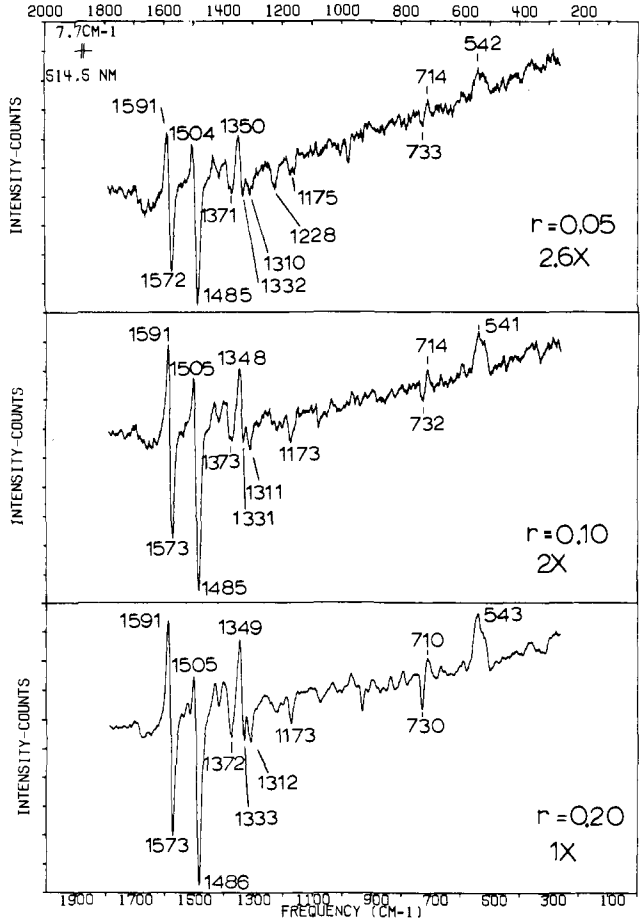


Figure 2. RADS as a function of r for the *cis*-$(H_2N)_2Pt^{II}$-GMP + CMP + AMP + UMP system, H_2O, 25 °C, pH 7. Nucleotide concentrations are the same as in Figure 1. Relative ordinate expansions are indicated. Ordinate ranges in counts are $r = 0.05$, 14×10^3; $r = 0.10$, 18×10^3; $r = 0.20$, 36×10^3 counts.

fingerprint for binding of *cis*-$(H_3N)_2Pt^{II}$ to GMP alone. As r is increased to 0.10 and then 0.20, there are small systematic changes. The relative intensity of the maximum at 1505 cm^{-1} decreases, there are weak minima at 1333 and 1312 cm^{-1}, and a weak derivative feature with a minimum at 730 and a maximum at 710 cm^{-1} increases in intensity relative to the prominent features due to GMP binding. These features clearly are due to increasing binding to AMP which has an intense minimum at 1509 cm^{-1}, sharp minima at 1333 and 1311 cm^{-1}, and a derivative feature with a minimum at 731 cm^{-1} and a maximum at 713 cm^{-1}. This can be seen by referring to the *cis*-$(H_3N)_2Pt^{II}$-AMP fingerprints in Figure 3.

When r has been increased to 0.20, the spectrum indicates considerable binding to AMP. It should be noted in Figure 3 that the Raman scattering of GMP is considerably more intense than that of the other bases. In order to give comparable displays in Figure 3, the AMP spectrum ordinate has been expanded four times compared to the GMP spectrum. With this in mind, it is seen that the intensity of the negative feature at 730 cm^{-1} in the model system with $r = 0.2$ indicates about as much binding to AMP as to GMP.

Difference spectra also were recorded for the corresponding reactions with *trans*-$(H_3N)_2Pt^{II}$ at 25 and 37 °C. The 25 °C spectra are illustrated in Figure 4 and the 37 °C spectra in the microfilm edition (sse paragraph at end of article), and they are essentially indistinguishable from the RADS fingerprint for *cis*-$(H_3N)_2Pt^{II}$ bound to GMP. There appears to be less binding to AMP with *trans*- than with *cis*-$(H_3N)_2Pt^{II}$.

Reaction with *cis*-$(H_3N)_2PtCl_2$. In order to compare the reactions of *cis*-$[PtCl_2(NH_3)_2]$ with those of *cis*-$[(H_3N)_2$-

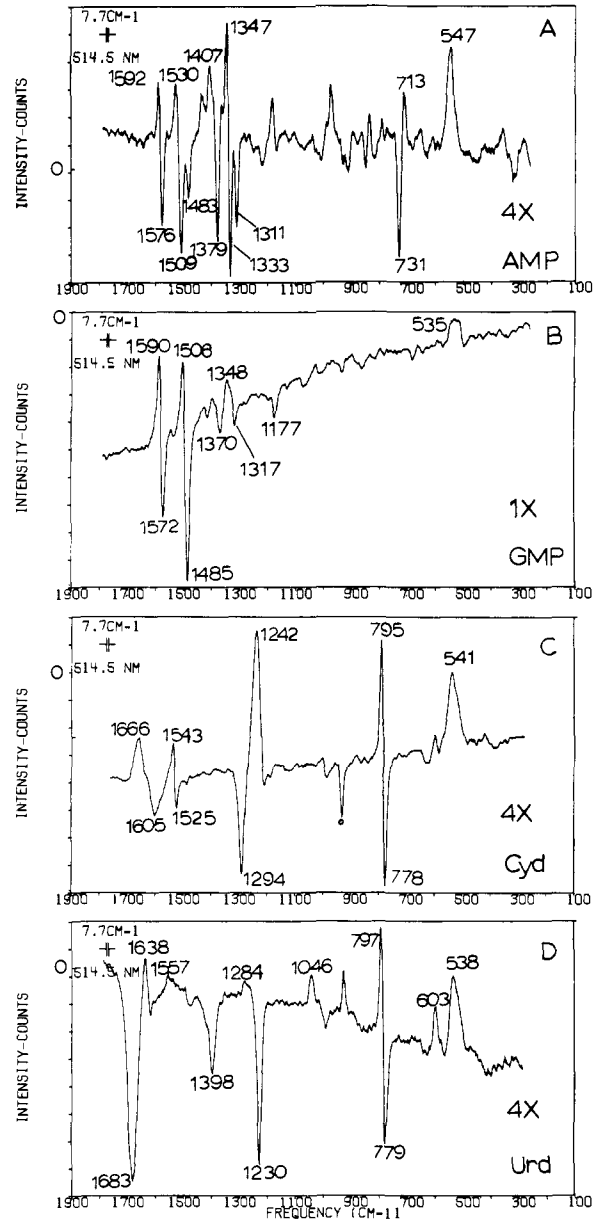


Figure 3. RADS fingerprints for reaction of *cis*-$(H_3N)_2Pt^{II}$ with nucleotides: (A) 25 mM AMP + 12.5 mM *cis*-$(H_3N)_2Pt^{II}$; (B) 25 mM GMP + 12.5 mM *cis*-$(H_3N)_2Pt^{II}$; (C) 25 mM Cyd + 25 mM *cis*-$(H_3N)_2Pt^{II}$; (D) 25 mM Urd + 25 mM *cis*-$(H_3N)_2Pt^{II}$. All solutions are 0.1 M in ClO_4^-. Relative ordinate expansions are indicated.

$Pt(OH_2)_2]^{2+}$, a solution was prepared by reacting the solid dichloro complex with the four-nucleotide model system at 25 °C, pH 7, to give $r = 0.2$. The Raman spectrum of this solution shows the same binding specificity as the diaquo complex. The extent of the reaction is slightly less with the dichloro compared to the diaquo complex. The spectrum is illustrated together with the corresponding one for *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, $r = 0.2$, 25 °C, pH 7, in Figure 5.

Extent of the Reactions. Knowing that the binding reactions involve only GMP and AMP with $r \leq 0.2$, one can select bands due to vibrations of one nucleotide or another to monitor the extent of the nucleotide platination. For example, the band at 1486 cm^{-1} of the model system derives ca. 95% of its intensity from GMP, 5% from AMP. This shifts to 1500 cm^{-1} and decreases in intensity as GMP is platinated at N(7), and the two bands are well resolved.[13] The model system band at 727 cm^{-1} is due to AMP, and this shifts to 718 cm^{-1} when AMP is platinated.

Examination of the data in Figure 5 for the titration of the

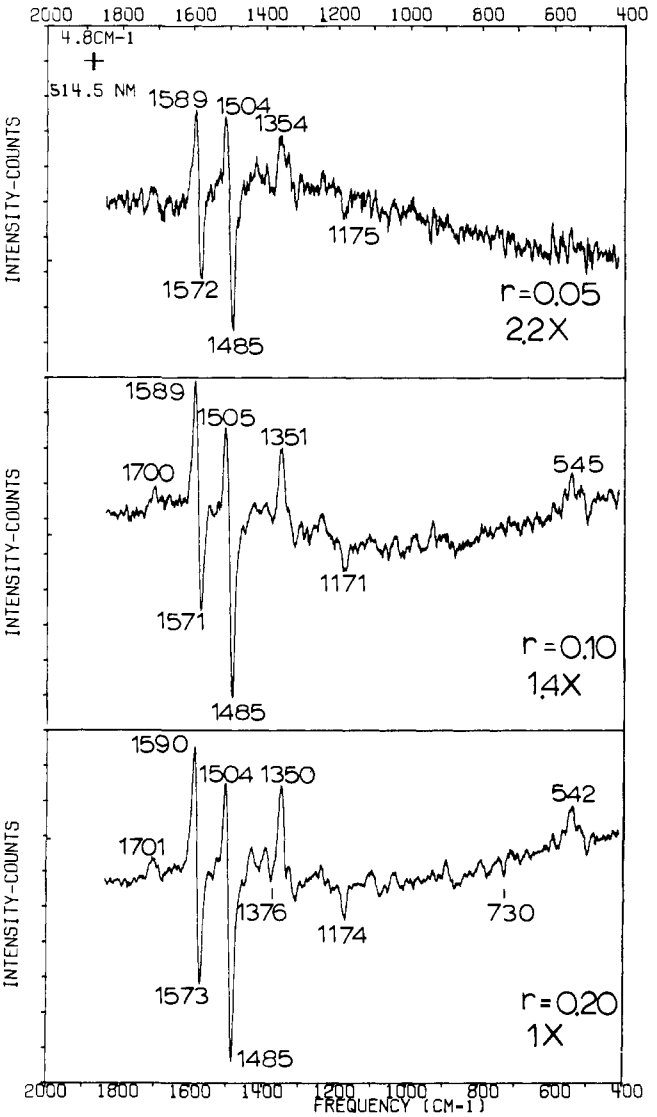


Figure 4. RADS as a function of r for the *trans*-$(H_3N)_2Pt^{II}$-GMP + CMP + AMP + UMP system, H_2O, 25 °C, pH 7. Nucleotide concentrations are the same as in Figure 1. Relative ordinate expansions are indicated.

GMP + CMP + AMP + UMP system with *cis*-$(H_3N)_2Pt^{II}$ in H_2O, pH 7, 25 °C, indicates that although the reaction involves binding to GMP, the stoichiometry is not 2:1 for GMP:Pt. As r increases, the intensity of the band at 1486 cm^{-1} decreases to ca. 67, 50, and 5%, r = 0.05, 0.10, and 0.20, respectively, of the initial value at r = 0. This corresponds to complexing of ca. 8, 12, and all 20 mM GMP with $(H_3N)_2Pt^{II}$. Judging from the behavior of the 726-cm^{-1} band, particularly as observed in the difference spectra in Figure 2, it appears that the remaining coordination sites at platinum are probably occupied largely by AMP. This corresponds only to a slight selectivity of GMP over AMP binding.

These data indicate that the following reactions are important at low r values:

$$cis\text{-}[(H_3N)_2Pt(OH)(OH_2)]^+ + 2GMP^{2-} \underset{}{\overset{\text{pH 7}}{\rightleftharpoons}} cis\text{-}[(H_3N)_2Pt(GMP)_2]^{2-} + H_2O + OH^- \quad (1)$$

$$cis\text{-}[(H_3N)_2Pt(OH)(OH_2)]^+ + GMP^{2-} + AMP^{2-} \overset{\text{pH 7}}{\rightleftharpoons} cis\text{-}[(H_3N)_2Pt(GMP)(AMP)]^{2-} + H_2O + OH^- \quad (2)$$

At r = 0.2 where the platinum complex theoretically is capable of binding 100% of the GMP and 67% of the AMP if both oxygen donor ligands are displaced from *cis*-$[(H_3N)_2Pt(OH)(OH_2)]^+$, it is to be expected that reaction 3 also will be important.

$$cis\text{-}[(H_3N)_2Pt(OH)(OH_2)]^+ + 2AMP^{2-} \overset{\text{pH 7}}{\rightleftharpoons} cis\text{-}[(H_3N)_2Pt(AMP)_2]^{2-} + H_2O + OH^- \quad (3)$$

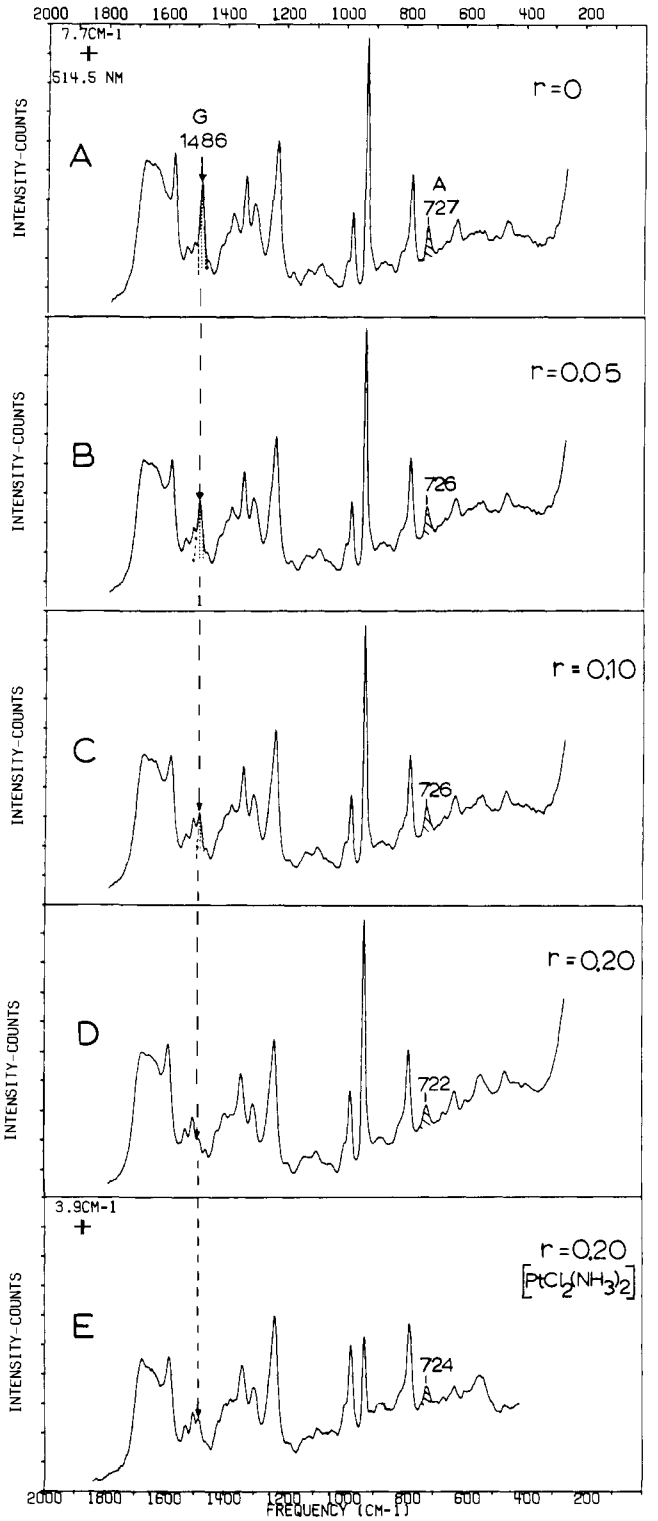


Figure 5. Raman titration: spectra as a function of r for the *cis*-$(H_3N)_2Pt^{II}$-GMP + CMP + AMP + UMP system, H_2O, 25 °C, pH 7. Nucleotide concentrations are the same as in Figure 1. The internal reference is 0.1 M ClO_4^-: (A to D) *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$; (E) *cis*-$[PtCl_2(NH_3)_2]$. Ordinate ranges: (A) 69 × 10^3; (B) 72 × 10^3; (C) 72 × 10^3; (D) 94 × 10^3; (E) 77 × 10^3 counts.

Similar measurements were carried out with the trans isomer at 25 °C, r = 0.05, 0.10, and 0.20, and at 37 °C, r = 0.05 and 0.10, and the spectra are illustrated in the microfilm edition. The 25 °C spectra are almost indistinguishable from the data for the cis isomer. There appears to be considerably less AMP binding in the case of the trans isomer, because the 727-cm^{-1} band decreases significantly in intensity only at r = 0.2. The data for 37 °C indicate a more quantitative reaction to form *trans*-$[(H_3N)_2Pt(GMP)_2]^{2-}$. This is to be expected, because the reactions of the platinum complexes with GMP are especially slow at pH ca. 7.[13] In this case the intensity at 1486 cm^{-1} indicates ca. 50% of the GMP is bound with r = 0.05 and almost 100% is bound at r = 0.10. Examination of the AMP band at 727 cm^{-1} shows no evidence for significant AMP binding at r = 0.1 where the GMP is theoretically equivalent to the platinum complex.

^{1}H NMR Studies of the Competitive Reactions of the GMP + CMP + AMP + UMP Mixture with enPt^{II} in D_2O, pD 7, 25 °C. For the NMR experiments, the ethylenediamine complex was used, because its complexes also have resonances due to the electrophile. Data for r = 0.10 and 0.20 are illustrated in Figure 6. Lower platinum-complex concentrations could not be employed, because the signal:noise ratio of the best NMR spectrum we could obtain was decidedly inferior to that of the Raman spectra.

At first glance, the results appear somewhat different than those of the Raman experiment. As we have discussed previously, the perturbations of the nucleotide Raman spectra depend almost entirely on electronic changes caused by binding the heavy metal electrophile.[11,12] The ^{1}H NMR shifts are due not only to electronic changes arising from metal binding but also to remote atom anisotropy. Consequently a complexed nucleotide has essentially the same Raman spectrum regardless of what other ligands are bound to the metal center, while the ligand chemical shifts usually are sensitive to the other ligands present and the overall molecular conformation.

In the spectrum of the system with r = 0.1, the most intense resonance other than that of unreacted nucelotide is at 5.52 ppm downfield from internal $(CH_3)_4N^+$. This signal is characteristic of H(8) of $[enPt(GMP)_2]^{2-}$.[13] In addition, very weak signals are observed at 6.42 and 6.34 ppm, and these are characteristic of $[enPt(AMP)_2]^{2-}$.[21] Finally, three additional signals are observed at ca. 6.21, 6.04, and 5.71 ppm. Similar resonances are observed at r = 0.2. No resonances corresponding to these last three signals are observed either with enPt(II)-GMP or enPt(II)-AMP systems, but they do appear when enPt(II) is added to mixtures containing 20 mM GMP + 30 mM AMP. This spectrum is illustrated in Figure 7.

The signal at 5.71 ppm is assigned to H(8) of GMP and the pair of signals at 6.21 and 6.04 ppm is assigned to H(8) of AMP in the mixed complex $[enPt(GMP)(AMP)]^{2-}$. The correctness of these assignments was verified by examining 10 mM enPt^{II} + 20 mM [8-^{2}H]GMP + 30 mM AMP and 10 mM enPt^{II} + 20 mM GMP + 30 mM [8-^{2}H]AMP solutions. The former does not exhibit either the 5.52 resonance characteristic of $[enPt(PMP)_2]^{2-}$ (5.48 ppm in the two-nucleotide mixture) or the 5.71 (5.72 ppm in the two-nucleotide mixture) resonance but does exhibit signals at 6.03, 6.20, 6.34, and 6.42 ppm. The solution with deuterated AMP has resonances at 5.48 and 5.70 ppm but none at lower field. These spectra also are illustrated in Figure 7.

The solutions containing only enPt^{II} + GMP + AMP all show an ethylenediamine resonance at -0.30_5 ppm (upfield from $(CH_3)_4N^+$) with weak shoulders at −0.25 and −0.34 ppm. The resonances due to $[enPt(GMP)_2]^{2-}$ and $[enPt(AMP)_2]^{2-}$ occur at −0.34[13] and −0.25[21] ppm, so the -0.30_5-ppm resonance is assigned to the mixed complex.

The intensity of the signal due to unreacted AMP in the GMP + CMP + AMP + UMP mixture can be used to estimate the extent of reaction using the CMP bands as an internal reference. On this basis it appears that ca. 18 and 8 mM AMP remain, corresponding to reaction of ca. 12 and 22 mM at r = 0.1 and 0.2, respectively. These data indicate slightly more reaction with AMP than with GMP, and this is in accord with the relative intensities of the signals at −0.24 and − 0.34 ppm assigned to the bis AMP and GMP complexes. The Raman data suggested binding of ca. 12 and 20 mM GMP at r = 0.10 and 0.20. Since there seems to be relatively little selectivity between the two purine bases, this may be affected slightly by the change from $(H_3N)_2Pt^{II}$ to enPt^{II}, the change in electrolyte concentration, or to the exact method of solution preparation.

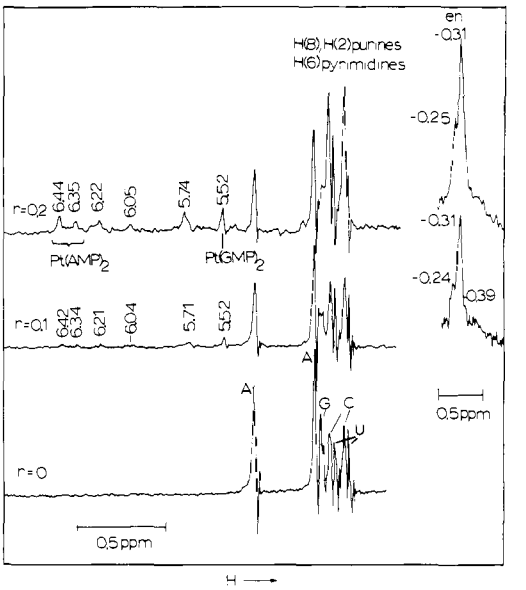


Figure 6. ^{1}H NMR titration: spectra (100 MHz) as a function of r for the enPt^{II}–GMP + CMP + AMP + UMP system, D_2O, 25 °C, pD 7. The internal reference is $N(CH_3)_4^+$. Nucleotide concentrations are the same as in Figure 1.

Raman Studies on the Reaction of *cis*- and *trans*-$(H_3N)_2Pt^{II}$ with Calf-Thymus DNA at pH 7. Figure 8 illustrates the spectrum of calf-thymus DNA at pH 7, 25 °C, in the presence of $NaNO_3$ and with ca. 100 mM total phosphate. The contributions to the various bands by the individual bases are indicated, and the spectrum is essentially the same as that reported by Erfurth and Peticolas.[22] Figure 8 also illustrates the spectrum of a solution prepared by reacting *cis*-$[(H_3N)_2Pt(OH_2)_2][NO_3]_2$ with the DNA to give r = 0.2, total phosphate 5 mM, for 3 days at 37 °C. This solution then was concentrated to give ca. 100 mM total phosphate, and the spectrum was recorded. These solutions correspond approximately in concentration and composition, except for the presence of the thymine rather than the uracil base, to the model system studies illustrated in Figure 5. The spectral changes are much less pronounced with the native polynucleotide than with the model system. Nitrates rather than perchlorates were used, since nitrate ion has little effect on the DNA structure.

The spectrum of the solution of DNA reacted with *cis*-$(H_3N)_2Pt(OH_2)_2]^{2+}$ is almost identical with that of the unreacted DNA. Slight decreases in the relative intensities of the bands at 1374 and 1490 cm^{-1} occur and the width at half-maximum of the latter band decreases slightly. These changes could arise from binding to the adenine and guanine bases; see Figure 3.

Figure 9 illustrates a series of spectra obtained with samples of DNA that have been reacted with *cis*- and *trans*-$(H_3N)_2Pt(OH_2)_2^{2+}$ and with *cis*-$(H_3N)_2PtCl_2$ at pH 7. The spectrum for the solution with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, r =

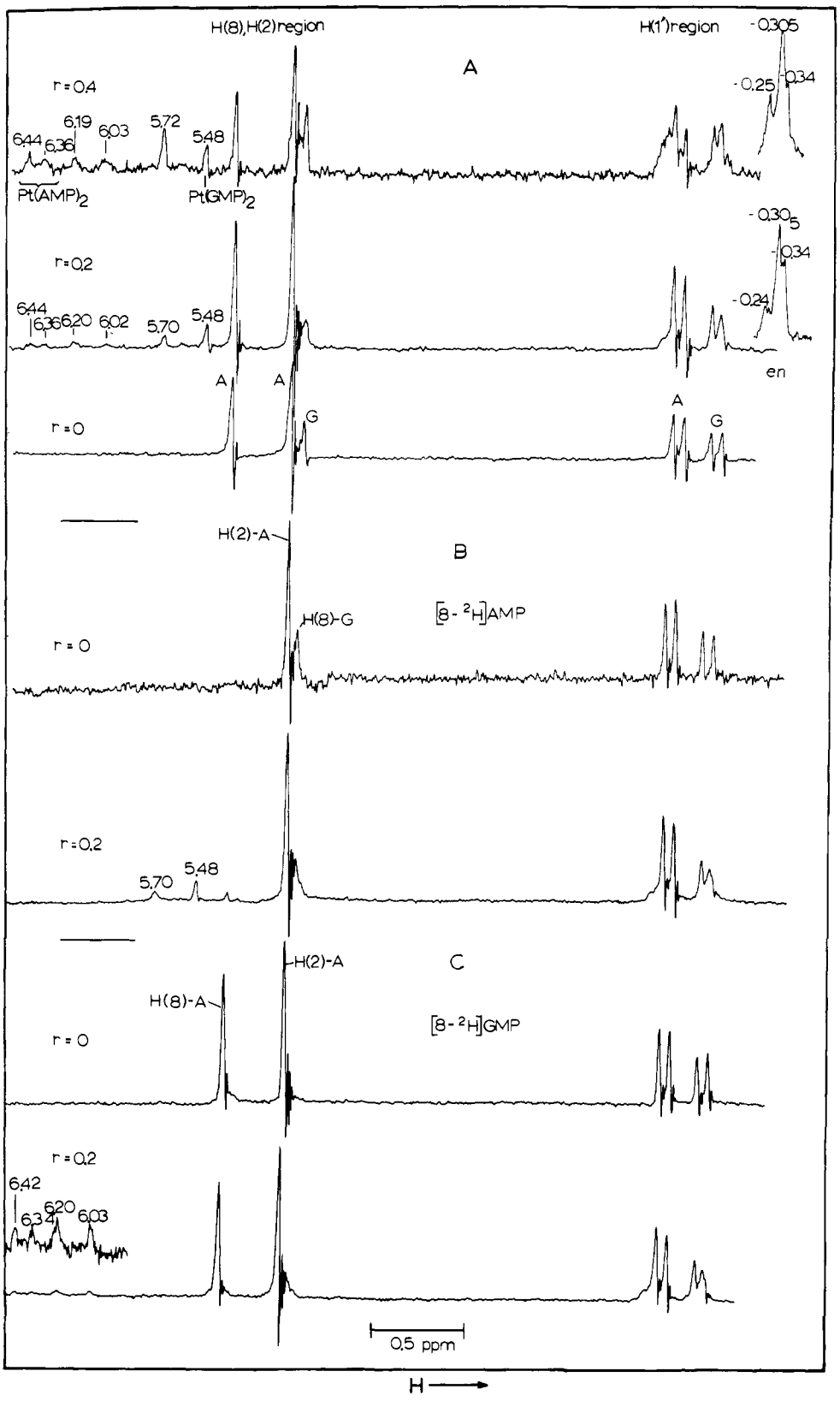


Figure 7. ^{1}H NMR titrations: spectra (100 MHz) as a function of r for the enPt^{II}–20 mM GMP + 30 mM AMP system, D_2O, 25 °C, pD 7. (A) GMP + AMP; (B) GMP + [8-^{2}H]AMP; (C) [8-^{2}H]GMP + AMP.

0.2, is essentially the same as that in Figure 8, and this shows that the small changes are reproducible. The corresponding spectrum for the solution containing *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, r = 0.2, shows evidence of more reaction. The solutons prepared by reaction with *cis*-$[(H_3N)_2PtCl_2]$ show much greater changes. These will be discussed systematically below.

Changes in the band at 1576 cm^{-1} are diagnostic of reaction with the guanine and adenine bases. The frequency of this band increases slightly in the solution containing *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and increases by ca. 10 cm^{-1} in the solutions containing *cis*-$[PtCl_2(NH_3)_2]$. This latter value is the same shift observed in the model system binding studies illustrated in Figures 1 and 5.

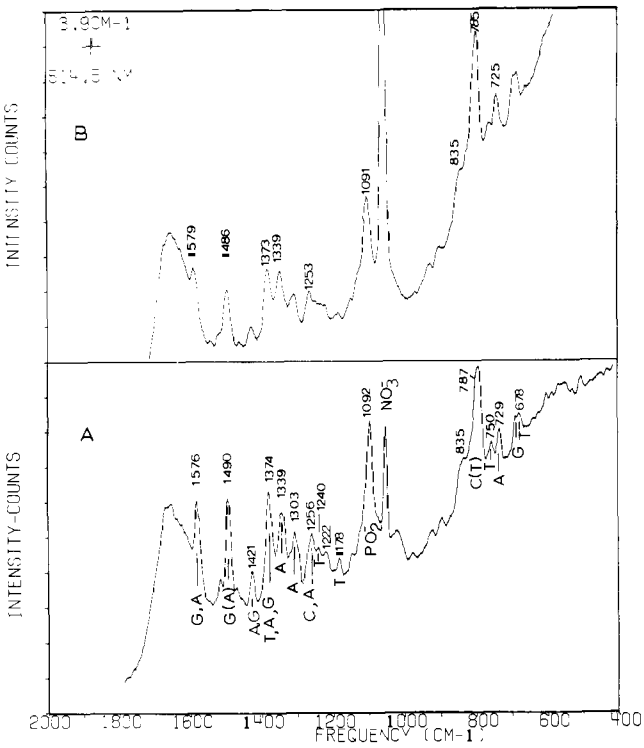


Figure 8. Raman spectra of calf-thymus DNA + *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ reacted at pH 7. (A) Calf-thymus DNA. The internal reference is NO_3^-. The bases contributing to bands are indicated; minor contributions are in parentheses. (B) DNA + *cis*-$(H_3N)_2Pt^{II}$, $r = 0.2$.

The solution containing *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.4$, shows a new band at ca. 1540 cm^{-1}. The only reaction at low r values which has been observed to produce scattering in this region is platination of AMP; see Figure 3. This gives a band at ca. 1530 cm^{-1}. At $r = 1$, the deprotonated GMP complex also gives a band at ca. 1540 cm^{-1}.[13]

Changes in the band at 1490 cm^{-1} are mainly diagnostic of reaction with the guanine base. The solution with *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ develops a high frequency shoulder, while those containing *cis*-$[PtCl_2(NH_3)_2]$ show complete disappearance of this band. The changes are very similar to those exhibited by the model system at $r = 0.2$; see Figure 5. This indicates complete reaction with the guanine base at $r = 0.2$.

Reaction with *cis*-$[PtCl_2(NH_3)_2]$ leads to a marked decrease in the scattering at 1374 cm^{-1}, a band due to the thymine, adenine, and guanine bases. Platination of guanine has little effect on this band, while reaction with adenine causes a decrease in intensity; see Figure 3. Reaction at the thymine base also has only a minor effect.[15] This indicates binding to the adenine base occurs, mainly in the solution with $r = 0.4$.

Platination leads to a rather large increase in intensity at ca. 1340 cm^{-1}, particularly in the solution containing *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.4$. The scattering at this position is due to the adenine base, and the intensity increases upon platination; see Figure 3. Scattering at 1303 cm^{-1} also is due to the adenine base. It disappears upon platination, and it is absent from the spectrum of the solution with *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.4$.

The scattering at 1240 cm^{-1} which is due to the pyrimidine bases shows no increase in the solution with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, $r = 0.2$, a slight increase in the solutions with *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and with *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.2$, and still a larger increase in the spectrum with the chloro complex, $r = 0.4$. The behavior of the 787-cm^{-1} band, vide infra, indicates there is no binding to the cytosine or thymine bases. The intensity at 1240 cm^{-1}, however, is the most sensitive to unwinding of the DNA double helix. A large increase in intensity is observed as DNA melts.[22] The increase observed here indicates that the DNA is partially denatured in the solutions with *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.2$, probably completely denatured in the solution with *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.4$, but still in the double

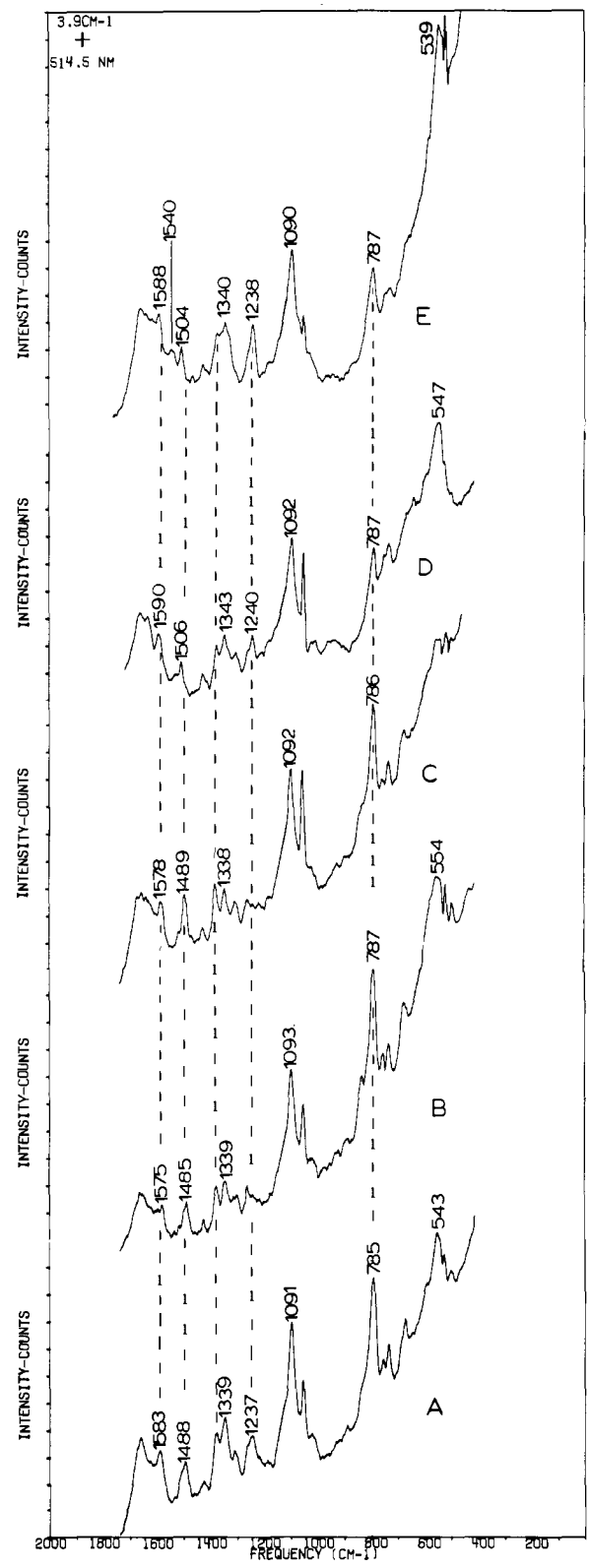


Figure 9. Raman spectra of calf-thymus DNA + *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, and *cis*-$[PtCl_2(NH_3)_2]$ in H_2O. The internal reference is NO_3^-: (A) *trans*-$[(H_3N)_2Pt(OH)_2]^{2+}$, $r = 0.2$; (B) *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, $r = 0.2$; (C) DNA, $r = 0$; (D) *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.2$; (E) *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.4$.

helical form in the solution with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, r = 0.2.

The phosphate mode at 1042 cm^{-1} shows no significant shift in the solutions containing the platinum species. Calf-thymus DNA solutions exhibit a weak band at ca. 835 cm^{-1} which occurs with solutions as a shoulder on the scattering at 787 cm^{-1}. It appears with much better definition in spectra of fibers[23] and appears to be indicative of the normal B conformation of the polynucleotide. Upon melting, it disappears. This band occurs in the sample reacted with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, it appears with slightly reduced intensity in the solution containing *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, but it is completely absent from the solutions containing *cis*-$[PtCl_2(NH_3)_2]$. These changes parallel those at 1240 cm^{-1} which also indicate disruption of the B conformation.

The 787-cm^{-1} band which is sensitive to binding to the pyrimidine bases shows no significant shift in any of the spectra. The decrease in intensity in the solutions with *cis*-$[PtCl_2(NH_3)_2]$ is an unusual effect, because the intensity of this band is normally insensitive to conformational changes. At the moment, we have no explanation to offer for it.

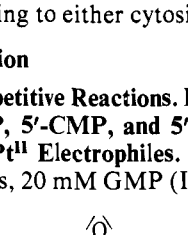
The band at 750 cm^{-1} due to thymine shifts to slightly lower frequency in the solutions containing the chloride, an effect which also is observed upon melting DNA. [22]

The (Pt-N) frequency for the solution with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ is 554 cm^{-1} and significantly higher than observed for the complex bound to the mononucleotide complexes. For example, the *cis*-$[PtCl_2(NH_3)_2]$ + four-mononucleotide mode, r = 0.2, has ν(Pt-N) at 545 cm^{-1}, while the DNA sample, r = 0.2, has ν(Pt-N) at 547 cm^{-1}. This indicates that binding occurs in both cases to sites of similar donor strength.

To summarize these results, there is very little selective binding when *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ is reacted with calf-thymus DNA at pH 7. The corresponding trans isomer shows some binding to the bases and incomplete unwinding of the DNA. Reaction of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ with calf-thymus DNA leads to a selective binding to the purine bases very much as observed in the studies with the model systems containing four mononucleotides. At r = 0.2, essentially all the guanine bases have been platinated at N(7), and the DNA appears to be extensively denatured. At r = 0.4, there appears to be extensive reaction with the adenine bases as well but essentially no binding to either cytosine or thymine.

Discussion

Competitive Reactions. Relative Nucleophilicity of 5′-GMP, 5′-AMP, 5′-CMP, and 5′-UMP toward the *cis*- and *trans*-$(H_3N)_2Pt^{II}$ Electrophiles. When a mixture of four mononucleotides, 20 mM GMP (I), 20 mM CMP (II), 30 mM AMP (III), and 30 mM UMP (IV), is allowed to compete for a limited amount of either *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, *cis*-$[PtCl_2(NH_3)_2]$, or *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ considerable specificity in the binding is observed. Both purines react at r = 0.05, but there is no reaction with either of the pyrimidine nucleotides even at r = 0.20. At the latter value of r, the platinum species are capable of binding up to 40% of the total nucleotide mixture. The trans isomer shows a somewhat higher selectivity for GMP than the cis isomer does. In both cases, the Raman shifts are characteristic of platination at N(7) of the guanine base.[13]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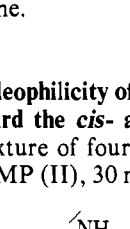
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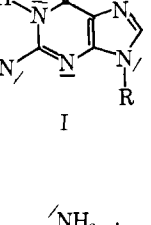
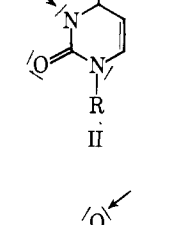

III


IV, X = H
V, X = CH_3

The ^1H NMR results in the competitive reaction studies are qualitatively in agreement with the Raman data. At r = 0.1 and 0.2, signals assigned to $[enPt(GMP)_2]^{2-}$, $[enPt(AMP)_2]^{2-}$, as well as the ternary complex $[enPt(GMP)(AMP)]^{2-}$ are observed. It is possible that AMP competes as well as it does with GMP in part because it can coordinate through either N(7) or N(1) while GMP at pH 7 under these conditions of excess nucleotide uses only N(7).[13]

The order of the nucleotides in terms of their nucleophilicity toward *cis*- or *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ or *cis*-$[PtCl_2(NH_3)_2]$, GMP > AMP ≫ CMP ≫ UMP, shows no relation to the basicity for protons and is, in fact, the inverse of this order.[24] Data obtained by reacting Pt($[^{14}C]$en)Cl_2 and ^3H-labeled nucleosides followed by chromatographic separation of the reaction mixture suggested that the reaction rates were in the order guanosine > adenosine ≃ deoxycytidine[9] and indicated a high nucleophilicity for guanosine. This ordering is not valid for the experiments reported here. The fact that the equilibrium constants are about the same[10] for binding of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ to guanosine, adenosine, and cytidine indicates that the binding reactions are kinetically controlled.

These platinum electrophiles show approximately the same specificity as alkyl sulfates, alkylsulfonates, mustards, epoxides, and nitroso compounds, i.e., G > A > C ≫ U, T.[25] Under neutral conditions these reagents alkylate preferentially the N(7) of G, N(1) of A, N(3) of C, and N(3) of U and T.

The heavy metal electrophile CH_3HgOH exhibits very different specificity than the platinum(II) complexes. When a mixture of 20 mM 5′-GMP, 20 mM 5′-CMP, 30 mM 5′-AMP, and 30 mM 5′-dTMP was allowed to compete for a limited amount of CH_3Hg^{II} in H_2O at pH 7, the principal reaction observed by Raman difference spectrophotometry was substitution of the proton at N(3) of dTMP by CH_3Hg^{II}.[14] A secondary, competing reaction involved binding of CH_3Hg^{II} at N(1) of GMP, again with substitution of a proton. This specificity is the same as that exhibited by diazoalkanes.[25] These are presumed to react by abstraction of an acidic proton with formation of a reactive alkyldiazonium ion.[26]

In comparison with alkylating agents such as alkyl iodides, platinum(II) complexes tend to react relatively more rapidly with polarizable reagents and less rapidly with less polarizable ones.[26] Thus, *trans*-$[Ptpy_2Cl_2]$ reacts considerably more rapidly with imidazole or pyridine (nucleophilicity (n_{Pt}) = 3.44 and 3.19, respectively) in methanol than with methoxide, n_{Pt} = 2.4, while the reverse is true for CH_3I, n_{CH_3I} = 6.29 for CH_3O^-, 5.25 for pyridine, and 4.97 for imidazole.[27,28] Consequently, reactions of the platinum electrophiles with an exocyclic oxygen, e.g. of GMP, would be expected to be quite slow even with the conjugate base deprotonated at N(1).

In the binding reactions of CH_3HgOH, nucleophilicity is relatively unimportant, because its reactions are very rapid. Equilibrium should be attained within the time of mixing, while the platinum ammines are substitutionally inert and require, with the concentrations involved here, hours for attainment of equilibrium at pH 7 with a ligand such as cytidine or weeks with uridine. While it might be argued that the equilibrium binding sites of *cis*- or *trans*-$(H_3N)_2Pt^{II}$ and H_3CHg^{II} are

different, this is unlikely. Both are very soft electrophiles. An estimate of the equilibrium constant for reaction 4:

$$cis\text{-}[(H_3N)_2Pt(OH_2)_2]^{2+} + Urd \rightleftharpoons cis\text{-}[(H_3N)_2Pt(UrdH_{-1})(OH_2)]^{+} + H_3O^{+} \quad (4)$$

obtained from Raman and ^1H NMR spectra gave the value $\log K \geq 0.6$ (0.1 M ClO_4^-, 25 °C)[11] comparable to the value for reaction 5, $\log K = 0.2$:[29]

$$[H_3CHgOH_2]^{+} + Urd \rightleftharpoons [CH_3HgUrdH_{-1}] + H_3O^{+} \quad (5)$$

Both reactions involve coordination of the heavy metal electrophile to N(3) with displacement of the proton. A similar estimate for reaction 6 gave the value $\log K = 2.9$ (0.1 M ClO_4^-, 25 °C):

$$cis\text{-}[(H_3N)_2Pt(OH_2)_2]^{2+} + Cyd \rightleftharpoons cis\text{-}[(H_3N)_2PtCyd(OH_2)]^{2+} + H_2O \quad (6)$$

while O'Connor and Scovell[10] found $\log K = 3.5$ under rather different experimental conditions. These are somewhat smaller than the value for CH_3Hg^{II}, reaction 7, $\log K = 4.6$:[30]

$$H_3CHgOH_2^{+} + Cyd \rightleftharpoons [H_3CHgCyd]^{+} + H_2O \quad (7)$$

In both cases binding to N(3) of Urd with displacement of the proton is greatly favored thermodynamically at pH 7 over coordination to N(3) of Cyd. Indeed, a rough correlation is observed between the nucleophilic reactivity constant for platinum, n_{Pt}, and the stability constants for binding to CH_3Hg^{II}.[27]

The behavior exhibited by *cis*- and *trans*-$(H_3N)_2Pt^{II}$ on the one hand and CH_3Hg^{II} on the other probably represents the extremes in behavior of heavy metal electrophiles. Substitutionally inert complexes can be expected to behave as the platinum electrophiles and show considerable selectivity for good nucleophiles. As the complex lability increases, the binding reactions can be expected to shift to those exemplified by the mercurial.

Electrophilic Attack on Native Calf-Thymus DNA. The reactions of the platinum(II) complexes with the native DNA were carried out with solutions containing 5 mM total phosphate. Roos[7] has studied the reaction between $[PtCl_2en]$ and *E. coli* DNA at ca. 1 mM concentrations. Measurements of the binding reaction using the ratios of the solution absorbance at 260 and 280 nm or 250 and 270 nm indicated no further reaction after 12 h at 20 °C. Measurement of the bound platinum as a function of time in equilibrium dialysis experiments showed no consistent variation after 3 days. The binding was reversible, and the saturation binding to the *E. coli* DNA was found to be ca. 0.55 Pt/base residue. This material is slightly richer in G and C than calf-thymus DNA. Munchausen and Rahn[6] found $r_b = 0.26$ for *E. coli* and 0.27 for calf-thymus DNA. The basis for the difference in the *E. coli* values has been suggested[7] to arise from the use of buffers and the presence of NaCl in the latter experiments. Measurements of this type are probably very dependent upon the experimental conditions. On the basis of these experiments, it can be concluded that equilibrium should have been attained in all the solutions employed for the Raman studies.

The Raman spectra show first that neither of the aquo cations *cis*- or *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ shows much selectivity in its binding reactions. For example, the solution containing the cis isomer, $r = 0.2$, shows none of the characteristic perturbations caused by platination of the guanine base. A weak shoulder to higher frequency on the 1489-cm^{-1} band due to the guanine base upon reaction with the trans isomer suggests some perferential binding to the guanine base. Changes from the B conformation can be followed easily by the increase in scattering at ca. 1238 cm^{-1}. This is caused by reversal of the Raman hypochromism effect which arises from base stacking. This effect has its origin in the hypochromism of the absorption in the near UV which, as noted by Munchausen and Rahn,[6] is lost upon binding of *cis*-$[PtCl_2(NH_3)_2]$ to DNAs. No increase in the scattering at 1238 cm^{-1} occurs in the reaction with *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, while a significant increase is observed with the trans isomer. Loss of the B conformation also is signaled by loss of the weak scattering at ca. 835 cm^{-1}. This decreases in intensity somewhat upon reaction with *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$, $r = 0.2$, but appreciable material still appears to exist in the B conformation.

In contrast to the lack of specificity shown by the aquo cation, *cis*-$[PtCl_2(NH_3)_2]$ binds with high selectivity to the purine bases. At $r = 0.2$, complete reaction with the guanine residues is indicated by the characteristic shifts associated with platination at N(7) of the guanine residue just as occurred in the four-nucleotide model system. Appreciable scattering is observed at 1240 cm^{-1} indicating extensive unstacking of the bases. This denaturation is confirmed by complete disappearance of the scattering at 835 cm^{-1} diagnostic of the B conformation. These changes are very similar to those caused by heating calf-thymus DNA to 98 °C at pH 7 which completely denatures the biopolymer.[22] The solution with $r = 0.4$ shows additional spectral changes indicating platination of the adenine bases but still not of either of the pyrimidines.

Studies with the bifunctional methyl nitrogen mustard HN_2, *N*-methylbis(2-chloroethyl)amine hydrochloride, at pH 7, $r = 0.2$, using Raman spectroscopy also indicated that reaction occurred at the guanine residues.[33] The reaction was not nearly so extensive as with *cis*-$[PtCl_2(NH_3)_2]$ as judged from the residual intensity at 1490 cm^{-1}, the absence of a significant increase in scattering intensity at 1240 cm^{-1}, and the presence of scattering at ca. 830 cm^{-1} indicative of the B conformation. The alkylated DNA was observed to melt at 52 °C in D_2O, p^2H 7, compared to the melting of the normal DNA at 85-87 °C. Clearly *cis*-$[PtCl_2(NH_3)_2]$ is a more reactive compound, probably with higher specificity for the endocyclic nitrogens of the purines.

Since the studies on the four-mononucleotide model system indicate that the binding specificity is kinetically rather than thermodynamically controlled, it is not surprising that *cis*- and *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ react differently with the native DNA than they do with the model system. The secondary and tertiary structures of the polynucleotide can be expected to have a significant effect on the availability of reactive sites.

It appears that the aquated species are too reactive to exhibit much specificity in binding to a native polynucleotide. While the distribution among sites has not been established, it is likely to include the phosphate as well as other weakly nucleophilic but readily accessible sites. Rates of substitution in platinum(II) complexes that involve displacement of an aquo ligand usually are about two orders of magnitude faster than the corresponding displacement of chloride,[28] although the hydrolyzed species at pH 7 will react somewhat more slowly. A similar lack of specific base binding was observed in the reaction of $[(H_3N)_2Pt(OH_2)_2]^{2+}$ with poly(rG), and it was attributed to the effect of the extensive secondary structure on the availability of the N(7) site.[13] Although the $[(H_3N)_2Pt(OH_2)_2]^{2+}$ will be extensively hydrolyzed at pH 7 (a species distribution diagram is illustrated in ref 11) so the average charge per platinum(II) should be slightly less than +1, it is possible that charge effects tend to cause more interaction with the phosphate than in the case of the dichloro complex. There also is an inverse correlation between the observed rate constant for substitution in platinum(II) complexes and the nucleophilic discrimination factor, i.e. compounds for which substitution occurs more slowly show a greater dependence of the rate on the nucleophilic character of the incoming ligand. Consequently, *cis*-$[PtCl_2(NH_3)_2]$ reacts extensively with the

guanine bases and is quite selective in binding to the purines.

Because water is a reasonable nucleophile it has been presumed that *cis*-$[PtCl_2(NH_3)_2]$ in vivo first undergoes aquation and then the aquated species react with the nuclear DNA.[34] The aquation has been presumed to occur in the intracellular fluid after transport through the membrane of the *cis*-$[PtCl_2(NH_3)_2]$ has taken place. On the basis of the poor selectivity exhibited by the cis and trans aquo species, it would appear that a direct bimolecular displacement of chloride or some even more strongly bound carrier ligand would be necessary to have the high selectivity that has been observed.

Acknowledgments. We wish to thank Engelhard Industries and Matthey Bishop, Inc. for loans of platinum.

Supplementary Material Available: Raman spectra, 3 pages. Ordering information is given on any current masthead page.

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Communications to the Editor

Preparation and Crystal Structure of $Ir_7(CO)_{12}(C_8H_{12})(C_8H_{11})(C_8H_{10})$, a Heptanuclear Iridium Cluster Showing Three Stages of Cyclooctadiene Coordination and Dehydrogenation

Sir:

Recent interest in the preparation and properties of large metal cluster compounds[1] has resulted in the structural characterization of large clusters involving cobalt,[2] nickel,[3,4] ruthenium,[5] rhodium,[6,7] osmium,[8,9] and platinum,[10] but the corresponding chemistry of iridium is underdeveloped. The hexanuclear compounds $Ir_6(CO)_{15}^{2-}$ and $Ir_6(CO)_{16}$ are moderately well characterized by infrared and powder x-ray comparison with the known cobalt and rhodium compounds, but $Ir_8(CO)_{20}^{2-}$ and $Ir_8(CO)_{22}^{2-}$ have been formulated solely on the basis of elemental analyses.[11] We wish to report the preparation and first x-ray structural characterization of a large iridium cluster, $Ir_7(CO)_{12}(C_8H_{12})(C_8H_{11})(C_8H_{10})$. The compound also is a rare example of a large cluster with hydrocarbon ligands. Previous examples include the arene derivatives $Ru_6(CO)_{14}(arene)C$,[12] of which the mesitylene complex has been structurally characterized,[13] and the compounds formulated as $Rh_6(CO)_{14}(diene)$,[14] in which, for example, diene = 1,5-cyclooctadiene. The title compound contains not only coordinated cyclooctadiene, but also singly and doubly dehydrogenated cyclooctadiene moieties.

The reaction of $Ir_4(CO)_{12}$ with 1,5-cyclooctadiene in refluxing chlorobenzene (18 h) provided a complex mixture of products.[15] Column chromatography of the mixture (Florisil/pentane) developed a green fraction, which after further purification by TLC (silica gel/pentane) afforded a green-black crystalline solid (yield ~3%).[16] The field desorption mass spectrum (*m/e* 2008 (^{193}Ir), M^+)[17] and ^1H NMR data[18] obtained for this compound are consistent with the formulation $Ir_7(CO)_{12}(C_8H_{12})(C_8H_{11})(C_8H_{10})$. A single-crystal x-ray diffraction study was undertaken to determine the geometry of the metal framework together with the binding modes of the organic ligands.[19]

As shown in Figure 1, the seven iridium atoms adopt a capped octahedral geometry with Ir(7) above the Ir(2)-Ir(5)-Ir(6) face. Eleven of the twelve carbonyl ligands are bound terminally to the six metal atoms of the octahedral fragment, two per metal except for Ir(6), which has one terminal carbonyl and shares the twelfth carbonyl with Ir(7). In addition to the bridging carbonyl, the apical iridium atom coordinates a normal, chelating 1,5-cyclooctadiene ligand. Shown at the lower left of Figure 1 is a cyclooctadiene ligand missing one olefinic proton; the bridging vinyl moiety is π bonded to Ir(4) and σ bonded to Ir(6). The Ir(6)-C(21) σ-bond length is 2.02 (3) Å, the distances in the π bond are Ir(4)-C(21) = 2.18 (3) Å and Ir(4)-C(22) = 2.29 (4) Å, and the C(21)-C(22) bond length is 1.51 (5) Å. Located beneath the Ir(1)-Ir(2)-Ir(3) face of the cluster in the center background of the view is the acetylenic portion of a cycloocta-1-en-5-yne ligand, coordinated as a $[2\sigma + \pi]$ four-electron donor. The Ir(2)-C distances are both 2.20 (4) Å, the σ-bond distances are 2.08 (3) and 2.04 (4) to Ir(1) and Ir(3), respectively, and the C(13)-C914) length is 1.32 (5) Å. The additional olefinic portions of the C_8H_{11} and C_8H_{10} ligands are uncoordinated.

Only three heptanuclear cluster complexes have been characterized previously and each has a capped octahedral geometry. Both $Rh_7(CO)_{16}^{3-}$ [21] and $Os_7(CO)_{21}$[9] have the

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