## Experimental Section

$^{31}P\{^1H\}$ Fourier mode NMR spectra (36.43 MHz) were recorded as described in previous publications of this series.[2] All preparations were carried out in the inert atmosphere of a nitrogen drybox using standard procedures. Tetrahydrofuran was distilled under argon from sodium/benzophenone just prior to use. All other solvents were dried over molecular sieves and purged with nitrogen. Reagents were commercially available and used without further purification. Chromatography was done on neutral grade alumina (activity I or II), eluting with pentane.

**I. Iron.** (A) **Pentakis(trimethyl phosphite)iron(0).** A 1 l. three-necked flask equipped with mechanical stirrer and nitrogen flush was charged with anhydrous $FeBr_2$ (12.9 g, 60 mmol), THF (500 ml), $P(OMe)_3$ (62 g, 500 mmol), and sodium amalgam (Na, 2.8 g, 120 mmol in 60 ml of Hg). The yellow-orange mixture turned brown as the reduction took place. The brown suspension was filtered through Celite and the filtrate was stripped to dryness on a vacuum line at room temperature. The dark solids were extracted with several 50-ml portions of pentane, chromatographed, and the resultant yellow solution was stripped to dryness, yielding $Fe[P(OMe)_3]_5$ in analytical purity (yields as high as 40%). The extreme solubility of the product makes recrystallization impractical. The material can be sublimed onto a liquid nitrogen cold finger under high vacuum with loss in yield: mp 158–160 °C dec.

Anal. Calcd for $FeP_5O_{15}C_{15}H_{45}$: Fe, 8.3; P, 22.9; O, 35.5; C, 26.7; H, 6.7. Found: Fe, 9.0; P, 24.3; O, 33.1; C, 27.0; H, 6.8.

(B) **Pentakis(triethyl phosphite)iron(0).** This compound is prepared and isolated in a manner analogous to that described in IA. The material can be recrystallized from acetone: mp 150–152 °C.

Anal. Calcd for $FeP_5O_{15}C_{30}H_{75}$: P, 17.5; O, 27.1; C, 40.7; H, 8.53. Found: P, 18.8; O, 29.0; C, 40.1; H, 8.28.

**II. Ruthenium.** (A) **Pentakis(trimethyl phosphite)ruthenium(0).** A flask was charged with $Ru[P(OCH_3)_3]_4Cl_2$[11] (2.0 g, 3.0 mmol), THF (40 ml), $P(OCH_3)_3$ (4 g, 32 mmol), and sodium amalgam (Na, 0.14 g; 6 mmol in 25 ml of Hg). The clear yellow solution became turbid as stirring was continued. The suspension was stripped under vacuum and the solids were extracted with pentane. The solution was chromatographed on alumina before reduction of volume and cooling to yield a white crystalline solid: mp 190 °C.

Anal. Calcd for $RuP_5O_{15}C_{15}H_{45}$: C, 25.0; H, 6.29; P, 21.5; O, 33.3. Found: C, 25.2; H, 6.13; P, 21.0; O, 32.1.

**III. Osmium.** (A) $OsCl_4[P(OCH_3)_3]_2$. Amalgamated zinc (excess) and $Na_2OsCl_6$ (4.5 g, 10 mmol) are added with stirring to a solution of 40 ml of tetrahydrofuran and $P(OCH_3)_3$ (10.0 g, 80 mmol). After stirring for 1 day, the entire solution was filtered through alumina and washed with tetrahydrofuran. The resulting solution was stripped to dryness to give a 25% yield (1.5 g, 2.5 mmol) of yellow powder of the stoichiometry $OsCl_4[P(OCH_3)_3]_2$. The material does not melt up to 300 °C.

Anal. Calcd for $OsP_2O_6C_6H_{18}Cl_4$: P, 10.7; C, 12.4; H, 3.13; Cl, 24.4. Found: P, 10.4; C, 12.73; H, 3.28; Cl, 23.46.

(B) **Pentakis(trimethyl phosphite)osmium(0).** In a nitrogen flush box, a 25-ml flask was equipped with a magnetic stirrer and charged with $OsCl_4[P(OCH_3)_3]_2$ (290 mg, 0.5 mmol), $P(OCH_3)_3$ (350 μl, 3.0 mmol), 10 ml of tetrahydrofuran, and 16 ml of sodium amalgam (0.125 M in sodium). After 4 h of stirring, the tetrahydrofuran solution was decanted off, stripped to dryness, and the resulting white solids were extracted with pentane. Stripping to dryness yielded white tacky solids which were used for the NMR studies without further purification.

**Acknowledgment.** We gratefully acknowledge Mr. M. A. Cushing for assistance in the preparation of some of the complexes and Messrs. G. Watunya, F. N. Schoch, and R. O. Balback for obtaining many of the NMR spectra.

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# Interaction of Aquated *cis*-$[(NH_3)_2Pt^{II}]$ with Nucleic Acid Constituents. 1. Ribonucleosides

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**Abstract:** The extent of interaction of *cis*-$(NH_3)_2Pt^{II}$ with the ribonucleosides guanosine, adenosine, and cytidine at pH 6.5 has been studied. The apparent formation constants for the 1:1 complexes were determined at 25 °C utilizing ultraviolet difference spectroscopy. The log $K$ values obtained are 3.7, 3.6, and 3.5, respectively. In all cases, the data are consistent with the nucleoside functioning as a monodentate ligand with no net deprotonation involved in the binding process. Metal binding at multiple sites on the nucleoside may occur at high *cis*-$(NH_3)_2Pt^{II}$/nucleoside mole ratios and also, for the extended incubation periods required in these studies, elevated temperatures appear to promote secondary reactions. Sites of binding are suggested and the implications of these results to the binding process at the polynucleotide level are discussed.

The study of the interaction of heavy metal complexes with biological moieties has recently seen an upsurge of interest due to studies concerned with methylation of heavy metals in aqueous environments,[1,2] investigations exploring the potential use of heavy metals to sequence nucleic acids using electron microscopy,[3] and recent attempts to understand the chemotherapeutic action of inorganic coordination complexes.[4,5]

The impetus for much of the present interest in the chemistry of *cis*-$(NH_3)_2PtCl_2$ came from the initial discovery by Rosenberg et al. that this complex not only inhibited replication in *E. coli* cells,[6,7] but also exhibited a broad spectrum of antitumor activity.[5,8-12] Although it has been generally realized that heavy metals do bind to nucleic acids, proteins, and the respective monomeric constituents, a number of studies have

indicated that at low concentrations of *cis*-$(NH_3)_2PtCl_2$, the major effect in mammalian cells is to selectively and significantly inhibit DNA synthesis, both in vitro[13,14] and in vivo.[15] Many studies have therefore subsequently focused on the interaction of *cis*-$(NH_3)_2PtCl_2$ with nucleic acids and the constituent nucleosides and/or nucleotides. Similarities between *cis*-$(NH_3)_2PtCl_2$ and the classical alkylating agents are apparent[16] and the possibility that a similar interstrand cross-linking is responsible also for the activity of the *cis*-$(NH_3)_2PtCl_2$ has been investigated.[17,18] Although interstrand cross-links do occur to a small extent, it has been concluded in a number of studies that these cross-links are not responsible for either the inactivating lesion for bacteriophage or the inactivation of transforming DNA.[19] Recent work also suggests that *cis*-$(NH_3)_2PtCl_2$ possibly undergoes a two-step process to yield the biologically active species. This moiety is presumed to be the aquated *cis*-$(NH_3)_2Pt^{II}$ in which the chlorides have dissociated.[14]

Mansy et al.[20] have reported solution studies with both the *cis*- and *trans*-$(NH_3)_2PtCl_2$ interacting with the nucleosides and the methylated derivatives. On the basis of spectrophotometric differences as a function of pH, they have made suggestions as to the binding sites on the nucleoside. For complexation of the cis isomer, they suggest cytidine acts as a bidentate ligand, binding at the N-3 and the 4-$NH_2$ positions, adenosine chelates through N-7 and 6-$NH_2$ or N-1 and 6-$NH_2$, while the guanosine binding reaction appears complex. No observable reaction occurred with uridine or thymidine. Robins, however, suggested that the nucleic acid constituents act as monodentate ligands in the analogous complexation with dichloroethylenediamineplatinum(II).[21] Roos et al.[22] have investigated the interaction of platinum complexes with various homo- and heterodinucleoside monophosphates with both the (3′–5′) and (2′–5′) phosphodiester linkages as models for the double-stranded structure of the DNA helix. The authors interpret the circular dichroism results to indicate that the cis isomer reacts with A3′p5′A and A3′p5′C to form a "cross-link" of the two bases, since the complexes formed exhibit the characteristic biphasic CD spectrum. The binding in A3′p5′A is thought to be at the 6-$NH_2$ group with proton displacement, while with A3′p5′C binding is at 6-$NH_2$ in adenosine and N-3 in cytidine.

Theophanides et al. have studied isolated complexes formed by various platinum species with nucleosides and modified bases.[23-25] Reports on the crystal structures of $[Cl_3Pt(9\text{-}MeAH^+)]$[25] (9-MeAH = 9-methyladenine $H^+$), *cis*-$(NH_3)_2Pt[5'\text{-}IMP]_2$,[26] and (en)Pt[5′-GMP]$_2$[27] indicate that the platinum interaction is at the N-7 position in each complex with no observed chelation. Recently, investigations examining the specificity of interaction between DNA and a number of platinum complexes has also been presented.[28]

In concert with an interest in the site of interaction, the extent of interaction in solution, as defined by thermodynamic parameters, is of primary concern not only in discerning the driving force for complexation, but also in revealing if any degree of thermodynamic selectivity exists with respect to *cis*-$(NH_3)_2Pt^{II}$ binding to any of the nucleosides.

In this report we wish to report the conditional formation constants for the interaction of aquated *cis*-$(NH_3)_2Pt^{II}$ with the ribonucleosides guanosine, adenosine, and cytidine.

## Experimental Section

The nucleosides were obtained from Sigma Chemical Co. and sodium perchlorate from G. Frederick Smith Chemical Co. The *cis*-$(NH_3)_2PtCl_2$ was obtained from the Matthey Bishop Co. and used without further purification.

All solutions were prepared from deionized, distilled water and contained 0.1 M $NaClO_4$ as the inert electrolyte. To determine the concentration of $NaClO_4$ initially, a portion of the solution was diluted and passed through a Dowex-50 W Exchange Resin (Sigma) and standardized by titration with standard base.

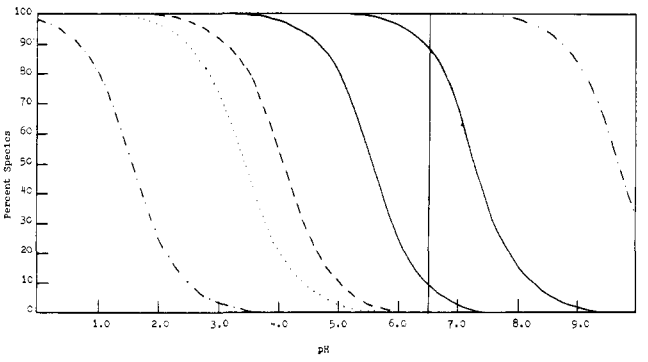


**Figure 1.** Percent species vs. pH plot for *cis*-$[(NH_3)_2Pt(OH_2)_2]^{2+}$ (p$K$ = 5.6, 7.3, —), cytidine (p$K$ = 4.22, - - -), adenosine (p$K$ = 3.45, ···), and guanosine (p$K$ = 1.6, 9.16, ·····). The vertical line at pH 6.5 indicates the pH of the studies.

The aquated *cis*-$(NH_3)_2Pt^{II}$ solutions were prepared by reacting the dissolved *cis*-$(NH_3)_2Pt^{II}$ with a stoichiometric amount of standard $AgNO_3$ solution. The mixture was stirred overnight, suction filtered through a Millipore filter (0.22 μm), and diluted to the final stock concentration ($1.0 \times 10^{-3}$ M aquated *cis*-$(NH_3)_2Pt^{II}$). The pH adjustment of all solutions to pH 6.5 was made by addition of standard NaOH solution. All pH measurements were obtained using a Fisher Accumet Model 420 pH meter; the combination pH electrode was standardized against pH 6 and 8 buffers. The nucleoside solutions were prepared, the pH adjusted similarly, and the concentration determined using the reported extinction coefficients of $\epsilon_{271}$ $8.9 \times 10^3$ (Cyd), $\epsilon_{259}$ $15.4 \times 10^3$ (Ado), and $\epsilon_{252}$ $13.7 \times 10^3$ (Guo).[29] All solutions containing aqueous *cis*-$(NH_3)_2Pt^{II}$ were stored and worked with in the dark. Buffer solutions were not used, since they may interact with the platinum complex and further complicate the study. Also, by monitoring the solution pH initially and at equilibrium, it was possible to further elucidate the nature of the interaction.

The concentration of the aquated *cis*-$(NH_3)_2Pt^{II}$ solution was determined by making an aliquot of the stock solution 2 M in NaCl (by addition of the appropriate amount of solid NaCl) and comparing the equilibrium spectrum (using 5-cm cells) with that of a solution of known concentration of *cis*-$(NH_3)_2PtCl_2$ in 2 M NaCl, prepared directly. We determined and used $\epsilon_{300}$ 128 at pH 7.0 as our standard.[26a] The comparative spectra verified that our preparation of aquated *cis*-$(NH_3)_2Pt^{II}$ was nearly quantitative. Beer's law was obeyed for all freshly prepared solutions.

Solutions were initially deaerated with argon. The *cis*-$(NH_3)_2Pt^{II}$/nucleoside reaction mixtures and reactant blanks were further prepared under an argon atmosphere, sealed in vials, and incubated under an argon atmosphere in a sealed container placed in a water bath at 25 °C. After 4 and 5 weeks, aliquots of the solutions were removed, the pH was obtained, and then the difference spectra recorded. Solutions were then incubated further to 8 weeks and 12 weeks time, after which the difference spectra were again recorded.

The difference spectra were obtained using four 1-cm path-length cells; the sample compartment contained two cells, one containing the reaction solution (*cis*-$(NH_3)_2Pt^{II}$/nucleoside) and the other containing only the inert electrolyte solution (0.1 M $NaClO_4$), whereas the reference compartment contained individual cells of the nucleoside and the aquated *cis*-$(NH_3)_2Pt^{II}$ solutions, respectively, at the same conditions (0.1 M $NaClO_4$, pH, temperature, and initial concentration). The difference spectra reported at 25 °C were obtained on a 0.500 expanded absorbance scale on a Beckman Acta MIV recording spectrophotometer.

**Calculation.** The equilibrium may be formally and properly represented in terms of an apparent or conditional formation constant, $K$, after the work of Ringbom.[30] Figure 1 shows the percent species vs. pH plot for the nucleosides and the *cis*-$[(NH_3)_2Pt(OH_2)_2]^{2+}$ species and points out the necessity for a conditional formation constant. Although, at the reaction pH (6.5), the nucleosides exist completely in the neutral form (p$K_{Cyd}$ = 4.22, p$K_{Ado}$ = 3.45, p$K_{Guo}$ = 1.6, 9.16),[31] the *cis*-$((NH_3)_2Pt(OH_2)_2]^{2+}$ is deprotonated (p$K_1$ = 5.6,

$pK_2$ = 7.3 at 20 °C)[32] to produce the hydroxo containing species and therefore although the *cis*-$[(NH_3)_2Pt(H_2O)(OH)]^+$ is the predominant species (>75%) at pH 6.5, it is not the only species. In writing out the general reaction (1) and the equilibrium expression (2), one must include all forms of not only the reactant *cis*-$(NH_3)_2Pt^{II}$ species, but also all possible products. The equilibrium pH measurements were consistent with and support the assumption that a water is displaced from the platinum coordination sphere by the nucleoside in the complex formation (vide infra).

$$cis\text{-}(NH_3)_2Pt^{II}{}_{aq} + Nu \overset{K}{\rightleftharpoons} cis\text{-}[(NH_3)_2Pt(Nu)] + H_2O \quad (1)$$

where the equilibrium expression is

$$K = \frac{[cis\text{-}(NH_3)_2PtNu]}{[cis\text{-}(NH_3)_2Pt^{II}{}_{aq}][Nu]} = \frac{(c)}{(a-c)(b-c)} \quad (2)$$

in which

$$[cis\text{-}(NH_3)_2Pt^{II}{}_{aq}] = (a-c)$$

$$= \sum_{n=0}^{2} [cis\text{-}(NH_3)_2Pt(H_2O)_n(OH)_{2-n}]^{n+} \quad (3)$$

and

$$[cis\text{-}(NH_3)_2PtNu]$$

$$= \sum \left\{ \begin{array}{c} \text{All corresponding 1:1 complexes formed} \\ \text{from the } cis\text{-}(NH_3)_2Pt^{II}{}_{aq} \text{ species with} \\ \text{the nucleoside} \end{array} \right\} \quad (4)$$

That an isosbestic point is observed in all the difference spectra is consistent with only one reaction occurring and only two absorbing species. This both simplifies the calculations and also indicates that although the free aquated *cis*-$(NH_3)_2Pt^{II}$ species *do* absorb somewhat ($\epsilon_{280}$ 90 at 25 °C),[33] it is not, in effect, observable or significant.

Another factor which further simplifies the calculation is that all *cis*-$[(NH_3)_2Pt(H_2O)_n(OH)_{2-n}]^{n+}$ species (≡ CPD) can be assumed to exhibit essentially the same low extinction coefficient[33] ($\epsilon_{280}$ 90 ± 10).

The $\Delta A$ values used in the calculations of $K$ were obtained at the wavelength maximum in the difference spectra for each system, corresponding to 289, 282, and 291 nm for cytidine, adenosine, and guanosine, respectively.

In the difference spectra, the $\Delta A$ expression can be reduced to the form

$$\Delta A = \epsilon^* c \quad (5)$$

where

$$\epsilon^* = [\epsilon_{CPDNu} - \epsilon_{CPD} - \epsilon_{Nu}] \quad (6)$$

Incorporation of these quantities into eq 2 leads to the final linear expression

$$\left[\frac{[b(a-c)]}{\Delta A}\right] = \frac{1}{\epsilon^*}\left[\frac{(a-c)}{1}\right] + \left[\frac{1}{K\epsilon^*}\right] \quad (7)$$

By making use of the process of iteration with respect to $c$ and $\epsilon^*$, the best value for $\epsilon^*$ and $K$ both converge rapidly. The extinction coefficient for the complex, $\epsilon_{CPDNu}$, can then be determined directly.[49]

## Results

After the 4- and 5-week incubation periods, the pH of each solution was obtained to determine the degree of protonation or deprotonation that had resulted from the complexation reaction. In the case of cytidine and adenosine, the pH of the solutions was virtually the same as the initial pH (pH 6.4 ± 0.2), while for the guanosine reaction, the equilibrium pH was slightly higher (pH 6.4–6.9) with no discernible correlation between the final pH and the *cis*-$(NH_3)_2Pt^{II}$/Guo mole ratio. To additionally determine if any secondary reaction (self-condensation possibly) had occurred in the *cis*-$(NH_3)_2Pt^{II}$ blanks during the incubation period, the Beer's law plot obtained for the incubated *cis*-$(NH_3)_2Pt^{II}$ solutions (5 weeks) was compared to that of freshly prepared solutions, both in 2 M NaCl. Figure 2 displays the least-squares fit of the results

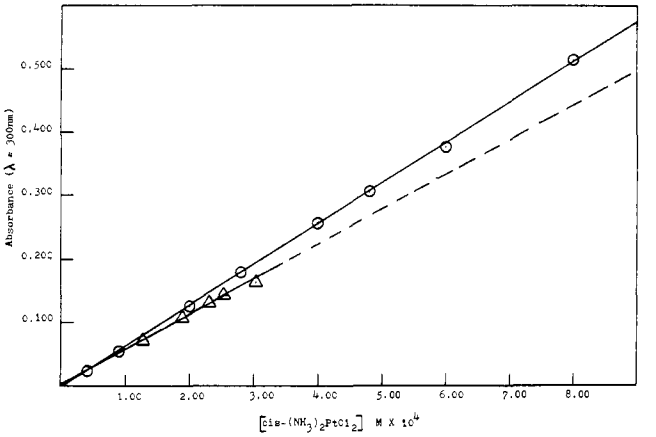


**Figure 2.** Beer's law plot for *cis*-$(NH_3)_2PtCl_2$ in 2 M NaCl at pH 6.5 (25 °C), λ 300 nm: (a) freshly prepared directly from *cis*-$(NH_3)_2PtCl_2$ (⊙), ε 128; (b) prepared from aquated *cis*-$(NH_3)_2Pt^{II}$ incubated for 5 weeks at 25 °C (△), ε 115.

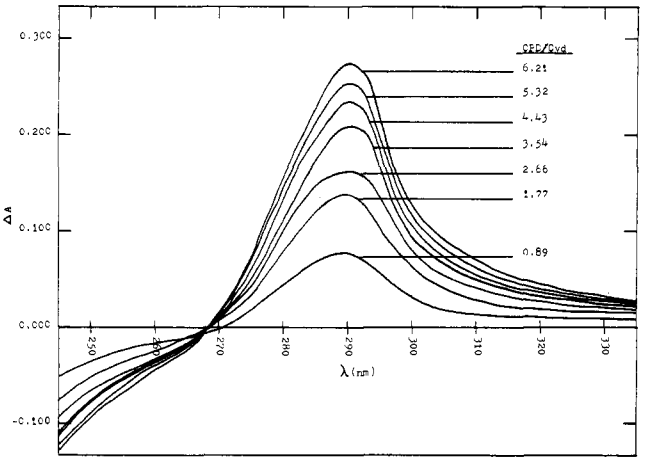


**Figure 3.** UV difference spectra resulting from aquated *cis*-$(NH_3)_2Pt^{II}$–cytidine interaction (pH 6.5 ± 0.2, $T$ = 25 °C, $t$ = 5 weeks). The *cis*-$(NH_3)_2Pt^{II}$/cytidine mole ratios are as indicated.

and it is clear that a detectable (<11%) decrease in the extinction coefficient (λ 300 nm) had resulted from incubation under the conditions of the study.

The difference spectra for the *cis*-$(NH_3)_2Pt^{II}$ interaction in the cytidine, adenosine, and guanosine reaction solutions after 5 weeks incubation at 25 °C are shown in Figures 3, 4, and 5, respectively. Table I summarizes the CPD/nucleoside mole ratios, together with the experimental and calculated results. All tabulated values were used in the calculation of the $K$ and $\epsilon^*$ values (Figure 6), while only the solutions indicated with a prime are displayed in Figures 3–5. As evidenced in the figures, complexation produces a red shift in the position of the UV absorption band of the nucleoside. This observed wavelength shift is similar to, but of greater magnitude than, that produced by protonation or methylation of the corresponding nucleoside (except in the case of adenosine) as the comparison shows in Table II.

In each system, one or more isosbestic points ($\lambda_{iso}$) are clearly evident (Figures 2–5, Table II), consistent with only one reaction taking place; it is therefore assumed that a 1:1 *cis*-$(NH_3)_2Pt^{II}$–nucleoside complex is the product. However, other studies in our laboratory indicate that the adenosine and guanosine systems become complex at higher temperatures and under other conditions. Therefore these systems are interesting in that they also pointedly emphasize the importance

**Table I.** Experimental and Calculated Results for CPD-Nucleoside Interactions

| | Cytidine, $\lambda_{calcd}$ 289 nm | | | | Adenosine, $\lambda_{calcd}$ 282 nm | | | | Guanosine, $\lambda_{calcd}$ 291 nm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soln no. | CPD/ Cyd | $\Delta A$ | $(a-c)\times 10^4$ M | Log $K$ | CPD/ Ado | $\Delta A$ | $(a-c)\times 10^4$ M | Log $K$ | CPD/ Guo | $\Delta A$ | $(a-c)\times 10^5$ M | Log $K$ |
| 1 | 0.89 | 0.082 | 0.68 | 3.57 | 0.91 | 0.109 | 0.71 | 3.48 | 0.17 | 0.048 | 1.31 | 3.84 |
| 1′ | 0.89 | 0.083 | 0.68 | 3.58 | 0.91 | 0.108 | 0.71 | 3.47 | | | | |
| 2 | 1.77 | 0.146 | 1.40 | 3.61 | 1.81 | 0.195 | 1.46 | 3.50 | 0.22 | 0.057 | 1.94 | 3.76 |
| 2′ | 1.77 | 0.144 | 1.41 | 3.60 | 1.81 | 0.196 | 1.45 | 3.51 | 0.22 | 0.061 | 1.82 | 3.81 |
| 3 | 2.66 | 0.184 | 2.19 | 3.58 | 2.72 | 0.258 | 2.24 | 3.51 | 0.28 | 0.074 | 2.35 | 3.80 |
| 3′ | 2.66 | 0.185 | 2.19 | 3.59 | 2.72 | 0.258 | 2.24 | 3.51 | 0.28 | 0.077 | 2.27 | 3.83 |
| 4 | 3.54 | 0.213 | 3.00 | 3.57 | 4.09 | 0.318 | 3.47 | 3.49 | 0.41 | 0.111 | 3.52 | 3.83 |
| 4′ | 3.54 | 0.213 | 3.00 | 3.57 | 4.09 | 0.322 | 3.46 | 3.50 | 0.41 | 0.110 | 3.55 | 3.82 |
| 5 | 4.43 | 0.243 | 3.81 | 3.60 | 4.54 | 0.343 | 3.87 | 3.51 | 0.55 | 0.141 | 4.89 | 3.82 |
| 5′ | 4.43 | 0.238 | 3.82 | 3.58 | 4.54 | 0.336 | 3.88 | 3.49 | | | | |
| 6 | 5.32 | 0.256 | 4.66 | 3.57 | 5.00 | 0.352 | 4.29 | 3.49 | 0.69 | 0.169 | 6.31 | 3.82 |
| 6′ | 5.32 | 0.256 | 4.66 | 3.57 | 5.00 | 0.354 | 4.29 | 3.50 | | | | |
| 7 | 6.21 | 0.276 | 5.49 | 3.60 | 5.45 | 0.367 | 4.71 | 3.49 | 0.83 | 0.199 | 7.69 | 3.84 |
| 7′ | 6.21 | 0.277 | 5.49 | 3.60 | 5.45 | 0.364 | 4.71 | 3.48 | 0.83 | 0.197 | 7.74 | 3.83 |
| 8 | | | | | 6.81 | 0.401 | 5.98 | 3.49 | 0.96 | 0.219 | 9.33 | 3.82 |
| 8′ | | | | | 6.81 | 0.401 | 5.98 | 3.49 | | | | |
| 9 | | | | | | | | | 1.10 | 0.245 | 10.83 | 3.84 |
| 9′ | | | | | | | | | 1.10 | 0.244 | 10.86 | 3.83 |
| 10 | | | | | | | | | 1.38 | 0.268 | 14.58 | 3.78 |
| 10′ | | | | | | | | | | | | |
| | $[Cyd]_0 = 9.94\times 10^{-5}$ M<br>$\lambda_{iso}$ 268 nm<br>$\epsilon_{CPD\text{-}Cyd}$ $6.82\times 10^3$ $M^{-1}$ $cm^{-1}$ | | | | $[Ado]_0 = 9.70\times 10^{-5}$ M<br>$\lambda_{iso}$ 270 nm<br>$\epsilon_{CPD\text{-}Ado}$ $7.95\times 10^3$ $M^{-1}$ $cm^{-1}$ | | | | $[Guo]_0 = 1.72\times 10^{-4}$ M<br>$\lambda_{iso}$ 261, 270, 278 nm<br>$\epsilon_{CPD\text{-}Guo}$ $4.62\times 10^3$ $M^{-1}$ $cm^{-1}$ | | | |

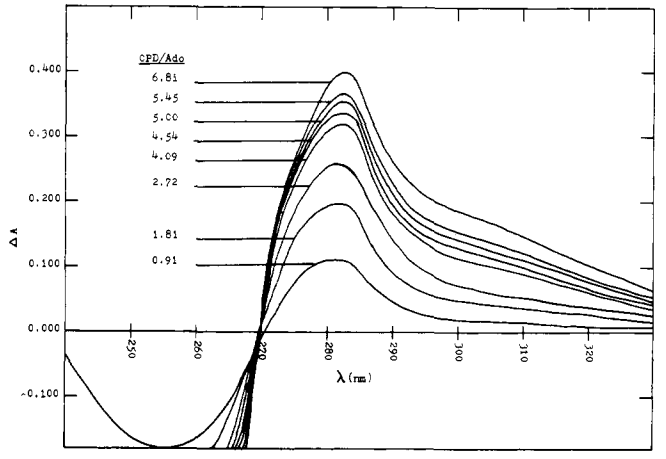


**Figure 4.** UV difference spectra resulting from aquated *cis*-$(NH_3)_2Pt^{II}$-adenosine interaction (pH 6.5 ± 0.2, $T$ = 25 °C, $t$ = 5 weeks). The *cis*-$(NH_3)_2Pt^{II}$/adenosine mole ratios are as indicated.

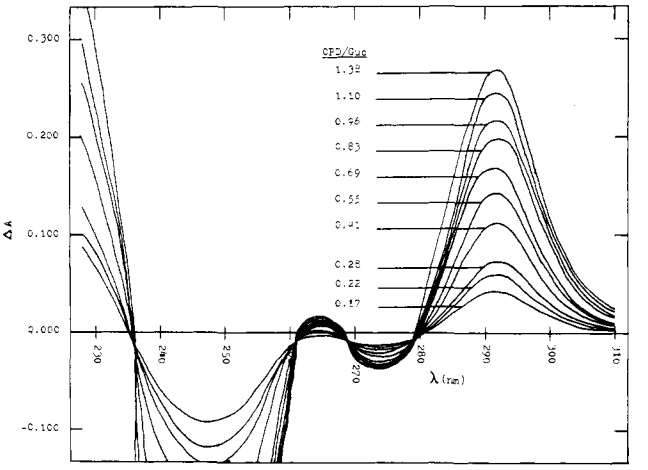


**Figure 5.** UV difference spectra resulting from aquated *cis*-$(NH_3)_2Pt^{II}$-guanosine interaction (pH 6.4–6.9, $T$ = 25 °C, $t$ = 5 weeks). The *cis*-$(NH_3)_2Pt^{II}$/guanosine mole ratios are as indicated.

of the reaction conditions in the study of the binding reaction and to the interpretation of the complexation (vide infra).

The linear plots for $b(a-c)/\Delta A$ vs. $(a-c)$ obtained for each complex are shown in Figure 6. The slope is indicative of $(1/\epsilon^*)$, while the intercept determines $[1/K\epsilon^*]$. The least-squares refinement of the data leads to $\epsilon$ values (at $\lambda_{max}$) of $6.82\times 10^3$, $7.95\times 10^3$, and $4.62\times 10^3$ $M^{-1}$ $cm^{-1}$ for the CPD-Cyd, CPD-Ado, and CPD-Guo complexes, respectively.

The respective $\lambda_{iso}$ and $\lambda_{max}$ for each reaction solution after 4 and 5 weeks incubation were virtually identical and therefore the calculation, which converged within a few interactions, yielded essentially invariant $K$ and $\epsilon$ values which were not strongly correlated. The $K$ and $\epsilon$ values calculated at different $\lambda$ values yielded comparable values. The difference spectra were again obtained after 8 weeks of incubation and in each study the $\Delta A$ values at $\lambda_{max}$ were greater; however, many of the individual spectra clearly did not pass through the isosbestic point. The magnitude of the calculated $K$ values for solutions having the isosbestic point increased by about a factor of 2. However, now the $K$ and $\epsilon$ values were more strongly correlated (i.e., did not exhibit a true minimum at the calculated best fit) and a comparison of the $K$ and $\epsilon$ values calculated at $\lambda_{max}$ and at other $\lambda$ values did not agree well. Incubation for 12 weeks further complicated the difference spectra. The calculations did not converge well and these data were not considered further.

## Discussion

The apparent formation constants, although not thermodynamic formation constants in the purest sense, do provide an excellent quantitative measure of the extent of binding of the aquated *cis*-$(NH_3)_2Pt^{II}$ species with the nucleosides. The $K$ values determined for the three nucleosides are listed in

**Table II.** Extinction Coefficients and $\lambda_{max}$ for Nucleoside Moieties[29,34]

| $\epsilon_{\lambda_{max}}$, $\times 10^3$ $M^{-1}$ $cm^{-1}$ | | $\epsilon_{\lambda_{max}}$, $\times 10^3$ $M^{-1}$ $cm^{-1}$ | | $\epsilon_{\lambda_{max}}$, $\times 10^3$ $M^{-1}$ $cm^{-1}$ | |
|---|---|---|---|---|---|
| Cyd | 8.9 (271) | Ado | 15.4 (259) | Guo | 13.7 (252) pH 7 |
| $CydH^+$ | 13.0 (280) | $AdoH^+$ | 15.1 (257) | $GuoH^+$ | 12.3 (256) pH 1 |
| 3-MeCyd | 11.8 (279) | 1-MeAdo | 13.8 (258) | $Guo^-$ | 11.3 (258) pH 11 |
| CPD-Cyd | 6.8 (289) | CPD-Ado | 8.0 (282) | 1-MeGuo | 10.9 (256) |
| | | | | 7-MeGuo | 8.5 (258); 7.4 (281) |
| | | | | CPD-Guo | 4.6 (291) |

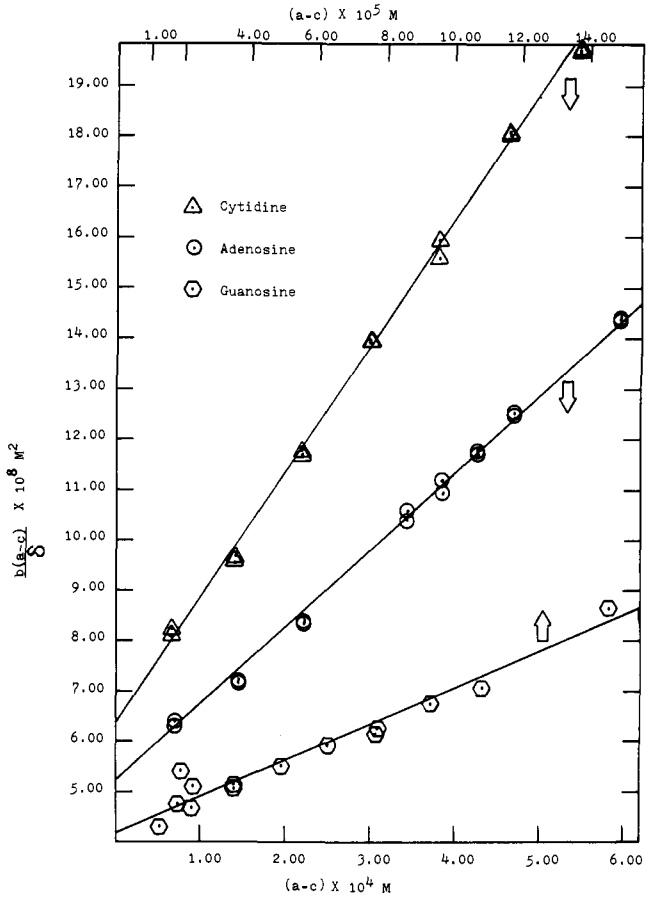


**Figure 6.** Least-squares fit of $b(a-c)/\delta$ vs. $(a-c)$ plot for complexation of: (a) *cis*-$(NH_3)_2Pt^{II}$-cytidine (▲), (b) *cis*-$(NH_3)_2Pt^{II}$-adenosine (⊙), (c) *cis*-$(NH_3)_2Pt^{II}$-guanosine (⊙).

Table III together with the reported observation that the *cis*-$(NH_3)_2Pt^{II}$ does not observably interact with thymidine under comparable conditions.[20] These results indicate that there is very little, if any, thermodynamic selectivity of aquated *cis*-$(NH_3)_2Pt^{II}$ for cytidine, adenosine, or guanosine under the conditions of this study; the extent of *cis*-$(NH_3)_2Pt^{II}$ binding to either of these three nucleosides differs only marginally with little or no observable interaction to thymidine. In addition, comparison of the CPD-Ado interaction with that for the CPD-[1-MeAdo]$^+$ interaction in acidic solution[35] indicates that the extent of interaction in both systems is comparable. Since the N-1 position in 1-$MeAdo^+$ is unavailable for coordination, the aquated *cis*-$(NH_3)_2Pt^{II}$ presumably binds at N-7.

As mentioned earlier, our results with the *cis*-$(NH_3)_2Pt^{II}$ interaction with adenosine and guanosine point out a number of items of concern in studies of this nature. First, from simply equilibrium considerations, the interaction or interactions occurring will be a function of the *cis*-$(NH_3)_2Pt^{II}$/nucleoside mole ratio. In this system or generally in any study in which

**Table III.** Log $K$ Values for CPD-Nucleoside Complexes

| Nucleoside | log $K$ (1:1 complex) |
|---|---|
| Cyd | 3.5 |
| Ado | 3.6 |
| Guo | 3.7 |
| Thd, Urd | |

a drug molecule interaction with a biomolecule is investigated, the biologically significant interaction is considered to be the binding that occurs at low drug/monomer mole ratios, i.e., the strong binding. Additional manifestations growing in as the drug/monomer mole ratio increases and, of course, which will complicate the simple equilibrium, are generally regarded, and in many cases shown, to indicate secondary interactions; these binding interactions have, of course, intrinsically lower binding constants and are therefore usually not biologically significant. For the adenosine and cytidine systems studied, *cis*-$(NH_3)_2Pt^{II}$/nucleoside mole ratios greater than 10 resulted in difference spectra which do not go through the respective $\lambda_{iso}$ indicated. For guanosine, *cis*-$(NH_3)_2Pt^{II}$/Guo mole ratios greater than 1 produced the same result. Also we have found that in studies at elevated temperatures with the same nucleoside reaction mixtures, further difficulties are encountered. Figure 7, for example, exhibits the UV difference spectra for the *cis*-$(NH_3)_2Pt^{II}$/adenosine interaction after incubation for 1 month at 37 °C with all other conditions comparable to those of the solutions shown in Figure 4. Notice that the spectra are quite different at the same mole ratios of *cis*-$(NH_3)_2Pt^{II}$/Ado and that the slight shoulder (to higher λ) observed in the 25 °C spectra is now the predominant broad band in all solutions except those at very low *cis*-$(NH_3)_2Pt^{II}$/Ado mole ratios. Notice also that the isosbestic point, which should theoretically occur at $\Delta A = 0$, is not observed for this series of solutions. A number of factors may contribute to this and possibly one of the most significant being secondary reactions occurring with *cis*-$(NH_3)_2Pt^{II}$ itself and also the *cis*-$(NH_3)_2Pt^{II}$ interacting at secondary positions on the nucleoside. Absorbance vs. concentration plots for *cis*-$(NH_3)_2Pt^{II}$ solutions in this concentration range and incubated at 37 °C (or higher) for 1 month do not follow Beer's law.[36]

The binding site can only be unequivocally determined after more thorough studies; however, if the information from x-ray crystallographic analyses, protonation studies, and other physical measurements obtained previously with platinum[20,23-27] and other metal-nucleoside complexes support any assignments,[37] it may be suggested that the binding site is at N-3 in cytidine, N-1 or N-7 in adenosine, and at N-7 in guanosine. The present data are consistent with only a 1:1 complex being formed and with no clear evidence for chelation, although the possibility exists. All three nucleosides studied have an amino group, but from studies in neutral solution, there is little or no evidence to indicate this position as the primary site of *cis*-$(NH_3)_2Pt^{II}$ interaction.[37]

Sigel has demonstrated generally that if the metal interaction occurs at the base in GTP, ITP (UTP and TTP), one can

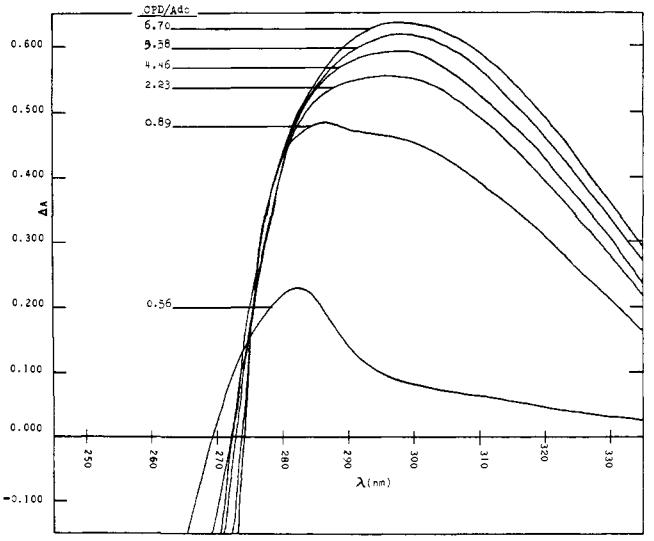


Figure 7. UV difference spectra resulting from aquated *cis*-$(NH_3)_2Pt^{II}$–adenosine interaction (pH 6.5, $T$ = 37 °C, $t$ = 4 weeks). The *cis*-$(NH_3)_2Pt(II)$/adenosine mole ratios are as indicated; $[Ado]_0 = 7.4 \times 10^{-5}$ M.

expect the proton at N-1 (or N-3) to ionize at a lower pH value than that of the free ligand.[38] Therefore complexation of *cis*-$(NH_3)_2Pt^{II}$ with guanosine should facilitate the ionization of the proton at N-1 also. Comparative data on Cu(II) interaction with guanosine and with GTP indicate that the decrease in p$K_a$ for deprotonation of the Cu(II)-guanosine (p$K$ = 7.05, $\Delta pK$ = 2.2) is even slightly greater than that for Cu(II)-GTP (p$K$ = 8.2, $\Delta pK$ = 1.8). Clarke and Taube likewise indicate that coordination of $(NH_3)_5Ru^{III}$ to guanosine enhances the acidity of the N-1 proton by two orders of magnitude.[39] With a similar enhancement of the guanosine p$K$ value due to *cis*-$(NH_3)_2Pt^{II}$ interaction, the result is not expected to be measurable under our conditions of reaction pH and concentrations. In a number of the solutions with guanosine, a noticeable *increase* in pH ($\Delta$pH as large as 0.4 from pH 6.5) is observed after completion of the reaction. This indicates a *net* liberation of $OH^-$ on complexation, if this is to be regarded as significant. The predominant platinum species, *cis*-$[(NH_3)_2Pt(OH)(H_2O)]^+$, could conceivably liberate a hydroxide ion from the coordination sphere on complexation, which would yield a net increase in pH. However, at the reaction conditions (pH 6.5, $[OH^-] \simeq 3 \times 10^{-8}$ M $OH^-$) studied, if one assumes at least 15% complexation at equilibrium, the final pH would be expected to be in the range of 9–10, which is not experimentally observed. Therefore, this process can also be eliminated. Alternatively, if *cis*-$(NH_3)_2Pt^{II}$ binds to N-1 with proton displacement or possibly binds at N-7 with resulting liberation of a proton at the N-1 position, as has been postulated in the analogous *cis*-$(NH_3)_2Pt$–inosine interaction,[40] the *cis*-$(NH_3)_2Pt^{II}$ species would therefore be required *to* liberate one hydroxide ion from the coordination sphere for each complex formed to completely negate any *decrease* in pH upon complexation. More complex schemes may also, of course, be presented;[50] we will, however, assume now that deprotonation of the guanosine does not occur and that in this complexation reaction monodentate guanosine coordination occurs with displacement of a water molecule from the coordination sphere. Recent x-ray crystallographic studies indicate *cis*-$(NH_3)_2Pt^{II}$ binding is at the N-7 position of 5′-IMP and similarly for $(en)Pt^{II}$ binding to 5′-GMP, which provides support for our suggestion of binding at N-7 in guanosine. However, Millard et al. have suggested that in the *cis*-$(NH_3)_2Pt^{II}$ interaction with DNA, guanosine acts as a chelate, binding through the N-7 and O-6 positions. The authors indicate that this interaction may manifest the antitumor activity observed in the *cis*-$(NH_3)_2PtCl_2$, in contrast to the *trans*-$(NH_3)_2PtCl_2$, which cannot bind in this manner and is not an effective antitumor drug.[41] The model reaction involved in formation of the complex of interest, *cis*-$[(NH_3)_2Pt(Guo)]Cl_2$, would involve liberation of a hydroxide ion, which, as previously indicated, is not consistent with our data.

In the binding process with cytidine and adenosine, protonation or deprotonation of the nucleoside is not expected to accompany *cis*-$(NH_3)_2Pt^{II}$ binding and we observe essentially no change in the pH. Consistent with an N-3 binding site in cytidine, Mansy has observed no significant interaction occurring between *cis*-$(NH_3)_2Pt^{II}$ and 3-methylcytidine after incubation at 37 °C for 1 month.[42]

In the reaction with adenosine (pH 6.5), the $\lambda_{max}$ in the difference spectra occurs at 282 nm ($\lambda_{iso}$ 270 nm) with a slight wing to higher $\lambda$, while for reaction with 1-methyladenosine at pH 3.3, $\lambda_{max}$ occurs at 290 nm with $\lambda_{iso}$ at 262 nm.[35] This may be interpreted as *cis*-$(NH_3)_2Pt^{II}$ binding at the N-7 position in 1-methyladenosine with binding possibly at a different site in adenosine, presumably the N-1 as has earlier been suggested for a proton and for $PtCl_4^{2-}$.[25]

In support of further work into this subject, the x-ray data recently presented by Collins et al. have demonstrated that the complex, $[Ni(5'\text{-AMP})(H_2O)_5]\cdot H_2O$, has a molecular structure in which the nickel coordinates to the N-7 position of the base.[26b] Clearly, the binding site on any nucleotide may be expected to be a sensitive function of the metal ion or metal complex and of the solution conditions.

From the formation constants for the *cis*-$(NH_3)_2Pt^{II}$–nucleoside interaction, it is evident that from a thermodynamic viewpoint, the *cis*-$(NH_3)_2Pt^{II}$ does discriminate against thymidine in its binding, but exhibits little or no thermodynamic selectivity with respect to cytidine, adenosine, or guanosine, the order of interaction being:

$$\text{Guo} \simeq \text{Cyd} \simeq \text{Ado} \gg \text{Thd}$$

In addition to obtaining the formation constants for the *cis*-$(NH_3)_2Pt^{II}$ interaction with the nucleosides, it was hoped that the constants might provide some guidance to the interpretation of data on the interactions at the polynucleotide level. This has proved very successful with $CH_3Hg^{II}$–nucleoside interactions,[43] and analogous extrapolations with Ag(I)[44,45] and Hg(II)[46] complexations conform well also. Making the following assumptions, thermodynamic selectivity of *cis*-$(NH_3)_2Pt^{II}$ binding may also be expected in the polynucleotides.

One may assume that in this transition, the extrapolation from the nucleoside to the polynucleotide level, the relative order of the $K$ values will not dramatically change and that the sites of interaction remain the same. That this is indeed reasonable, if not in fact usually observed, has been evidenced and is well documented.[37,43-46] Secondly, consider that only 1:1 interactions occur between the *cis*-$(NH_3)_2Pt^{II}$ and the bases with little cross-linking of bases (inter- or intrastrand). Experiments partially bear this out in that the degree of interstrand cross-linking in DNA with *cis*-$(NH_3)_2Pt^{II}$ has been observed to be less than 2%.[17,18] Unfortunately, it is not known whether there is any cooperativity or anticooperativity existing in the *cis*-$(NH_3)_2Pt^{II}$ binding process with polynucleotides. In the analogous Ag(I) and Hg(II) interactions with polynucleotides, cooperative interactions are indeed observed. However, this does not appear to alter the *relative* affinities for the particular bases.[43-46]

With these assumptions and considering the interaction with DNA in terms of the multiple interaction with the AT or the GC base pair, it follows that the probability of interactions at an AT base pair, as compared to the analogous interactions at a GC base pair, is proportional to the multiple of the formation

constants for the respective *cis*-$(NH_3)_2Pt$-nucleoside complexes. Therefore, from a *thermodynamic* standpoint, the probabilities, $P_{xy}$, are proportional to the multiple of the individual $K$ values, i.e.,

$$P_{A-T} \propto K_A K_T \simeq (10^4)(\sim 1) = 10^4$$

$$P_{G-C} \propto K_G K_C \simeq (10^4)(10^4) = 10^8$$

Therefore, one would predict the *cis*-$(NH_3)_2Pt^{II}$ to be *thermodynamically selective for GC base pairs or GC-rich regions of DNA as compared to the AT base pairs or AT-rich regions of DNA.*

On somewhat more idealized polynucleotide systems, the homopolynucleotides, one would expect poly G, poly A, and poly C to bind *cis*-$(NH_3)_2Pt^{II}$ strongly, while poly U or poly dT might be expected to interact to a far lesser degree.

This selective behavior is not unique to *cis*-$(NH_3)_2Pt^{II}$, but has been previously observed with other metal ions. In the case of Cu(II), evidence suggests binding primarily to the GC-rich regions of DNA.[37] This binding affinity is likewise true for Ag(I), whereas Hg(II) selectively binds to the AT-rich regions. In all three examples, however, it is of interest to note that the selectivity is predominantly the result of the metal ion having an especially high affinity for one base; Cu(II) and Ag(I) both bind very strongly to guanine, whereas Hg(II) binds tenaciously to thymine. In contrast, the binding selectivity of *cis*-$(NH_3)_2Pt^{II}$ is predicted because of the markedly lower affinity of *cis*-$(NH_3)_2Pt^{II}$ for one of the nucleosides, thymidine, when compared to the binding to cytidine, guanosine, or adenosine. The Hg(II), Ag(I), and $H_3CHg^{II}$ ions bind very strongly to the bases and the binding is greater than any of the first row transition metal ions. The *cis*-$(NH_3)_2Pt^{II}$ binds strongly also and can generally be considered in this group. It is also known that the reversible binding of Hg(II) and Ag(I) to DNA does not unwind the double helix, but in cross-linking the strands produces a very rigid metal-DNA complex.[43,44,46] This does not appear to be the case with *cis*-$(NH_3)_2Pt^{II}$. The selective binding affinity of Hg(II) and Ag(I) for AT-rich regions and GC-rich regions, respectively, led Davidson to use these metal ions to analytically separate two DNA components from crabs.[47] Possibly the *cis*-$(NH_3)_2Pt^{II}$ might also be of some utility in similar studies.

Recently Theophanides has studied the interaction of a number of Pt(II) complexes with salmon sperm DNA.[48] He suggests that the first binding site is located on the GC pairs, with preference being the N-7 on guanine. The $K$ values we obtained for the *cis*-$(NH_3)_2Pt^{II}$-nucleoside interactions would lead to just this prediction of a selective interaction with a GC pair in the DNA.

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(49) Inherent in the calculation is also the assumption that Beer's law is followed by the complex at $\lambda_{calcd}$ in this concentration range.
(50) The predominant reaction could be

$$cis\text{-}[(NH_3)_2Pt(OH)(H_2O)]^+ + Nu = cis\text{-}[(NH_3)_2Pt(Nu)]^+ + 2H_2O$$

in which the nucleoside (cytidine or adenosine) acts as a bidentate chelate at N-3 + 6-$NH_2$ and N-1 or N-7 + 6-$NH_2$, respectively, with proton displacement at the amino group to yield an equilibrium in which no *net* change of pH is observed. We cannot exclude this possibility, but cannot support it. In any event, the equilibrium expression remains the same ($K$ having units of $M^{-1}$). In addition, if the $\Delta$pH observed in the guanosine reaction can be assumed to be insignificant, the complexation reaction involving monodentate binding of guanosine (with proton release) to platinum, with hydroxide displacement, would also be consistent with our results.