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Ground-State Stability and Rotational Activation Parameters for Individual Rotamers of (*R*,*S*,*S*,*R*)-(*N*,*N*′-Dimethyl-2,3-diaminobutane)PtG$_2$ Complexes (G = 9-EtG, 3′-GMP, and 5′-GMP)

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Rate constants for guanine rotation about the Pt–N7 bond in (*R*,*S*,*S*,*R*)-Me$_2$DABPt**G**$_2$ complexes (Me$_2$DAB = *N*,*N*′-dimethyl-2,3-diaminobutane; **G** = 9-EtG, 3′-GMP, and 5′-GMP) were evaluated from line-shape analysis of H8 resonances. Three diastereomers, two in head-to-tail (ΔHT and ΛHT) and one in head-to-head (HH) conformations, exist in equilibrium in solution. The two guanines are equivalent in ΔHT and ΛHT conformers and nonequivalent in the HH form; therefore, four rate constants ($k_{\Delta HT}$, $k_{\Lambda HT}$, k_{HH_s}, and k_{HH_d}; sub-subscripts s and d stand for H8-shielded and -deshielded guanine, respectively) were evaluated. Activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) were evaluated from the rate constant dependence on temperature. High values of $\Delta H^{\ddagger}$ (78–93 kJ mol^{-1}) and $\Delta S^{\ddagger}$ (51–71 J K^{-1} mol^{-1}) were found for **G** rotation in the preferred ΔHT rotamer having the six-membered ring of each guanine more canted toward the *cis*-**G** and a favorable dipole–dipole internucleotide interaction. Lower values of $\Delta H^{\ddagger}$ (64–76 kJ mol^{-1}) and very small values of $\Delta S^{\ddagger}$ (–7–11 J K^{-1} mol^{-1}) were found for **G** rotation in the less favorable ΛHT rotamer, indicating that the ground-state entropy of this rotamer is close to that of the activated complex and the ground-state enthalpy closer to that of the activated complex than for the ΔHT rotamer. For the two guanines in the HH rotamer there is no large difference in activation parameters. In general $\Delta H^{\ddagger}$ falls in the range 66–84 kJ mol^{-1} (rather close to the values for the ΛHT rotamer) and $\Delta S^{\ddagger}$ in the range 14–41 J K^{-1} mol^{-1}. The equilibrium constant between HT and HH rotamers was also evaluated together with the corresponding thermodynamic parameters (ΔH and ΔS). It is found that the low enthalpy is the major stabilizing factor for ΔHT as compared to HH, while the entropy factor would favor the latter rotamer. In contrast the greater entropy is the stabilizing factor for the ΛHT rotamer (the second most abundant conformer for 9-EtG and 3′-GMP) over the HH rotamer. In the latter case the enthalpy would favor the HH rotamer. In the case of the 5′-GMP derivative the greater entropy of the ΛHT rotamer is not such to compensate for the lower enthalpy of the HH rotamer, and the latter remains the second most abundant rotamer. This investigation has allowed for the first time the enthalpic and entropic contributions favoring different rotamers to be distinguished.

Introduction

Anticancer compounds such as *cis*-[PtCl$_2$(NH$_3$)$_2$] (cisplatin) and its less nephrotoxic derivative *cis*-[Pt{$\overline{CH_2CH_2CH_2C(COO)_2}$}(NH$_3$)$_2$] (carboplatin) are among the most effective anticancer agents currently in clinical use.[1]

The exact mechanism of action of these compounds is not fully understood, but the drugs are known to produce a critical lesion by releasing the two chlorides or the cyclobutanedicarboxylate anion and forming two Pt−N7 bonds[2,3] with adjacent purine bases of the same strand (either two guanines or an adenine and a guanine with the adenine in the 5′-position). These bifunctional lesions are referred to as "intrastrand cross-links". The platinum can also cross-

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link guanine bases of opposite strands to form "interstrand cross-links"; however, the latter constitutes only a minor lesion.[4]

Activity is nearly lost or remarkably diminished when the primary or secondary amines on platinum are replaced by tertiary amines.[5−7] The requirement that at least one hydrogen be attached to each amine has led to the hypothesis that an NH hydrogen bond to the O6 of guanine[8−11] or to a phosphate oxygen[12−20] may play an important role either in the formation or in the stabilization of the platinum adducts.

For intrastrand cross-links the adjacent purine residues generally assume a head-to-head (HH) conformation in which the H8 atoms of the purines are both on the same side of the platinum coordination plane.[21−24] In contrast, for interstrand cross-links the two purine residues have orientations in which the H8 atoms are on opposite sides of the platinum coordination plane. The latter orientation is referred to as head-to-tail (HT).

The simplest models for the bifunctional adducts described above are complexes of the type *cis*-PtA_2**G**$_2$, in which A_2 stands for two unidentate amine ligands or one bidentate amine ligand and **G** for a guanine derivative (boldface **G** denotes an unlinked guanine derivative).[25−35] These model compounds can form different rotamers having either an HH conformation (as found for intrastrand cross-links) or an HT conformation (as found for interstrand cross-links). Two HT conformations, designated Δ and Λ, are possible. A line connecting the O6 atoms of the two guanines and the line perpendicular to the coordination plane passing through the platinum atom will constitute a pair of right-handed skew lines in the former case and a pair of left-handed skew lines in the latter case. Addition of a ribose ring adds chirality to the **G** ligands and renders the HT rotamers diastereomeric.[28] Rotation about the Pt−N7 bonds leads to interconversion between rotamers.

In *cis*-PtA_2**G**$_2$ compounds in which A is ammonia or a primary amine, interconversion among possible rotamers is fast on the NMR time scale and a single signal is observed for each type of proton that is the average of all possible nucleotide orientations (fast rotation around the Pt−N7 bonds). However, the presence in the platinum compound of bulky and conformationally rigid A_2 ligands can diminish dynamic motion, and multiple rotamers can be observed through detection of several sets of NMR signals.[25−35]

In a few cases the activation energy for interconversion between rotamers has been determined. Cramer and Dahlstrom determined an energy barrier of ~86 kJ mol^{-1} for Me_4ENPt(Guo)$_2$ (Me_4EN = *N*,*N*,*N*′,*N*′-tetramethyl-1,2-diaminoethane; Guo = guanosine).[25] The same authors obtained energy barriers for Me_2ENPt(Guo)$_2$ (Me_2EN = *N*,*N*′-dimethyl-1,2-diaminoethane) in the range of 57−65 kJ mol^{-1}.[28] Finally, from line-shape analysis of ^{31}P and H8 resonances of *cis*-[Pt(5′-GMP)$_2$(NH_3)$_2$], which broaden to some extent on lowering the temperature below 0 °C, Li and Bose estimated an activation energy of 25 kJ mol^{-1}.[36] In all these studies the exact conformation of the rotamer undergoing guanosine rotation was not known.

The *cis*-PtA_2 moiety selected for the present study contains the bidentate amine ligand *N*,*N*′-dimethyl-2,3-diaminobutane (Me_2DAB). When this ligand is coordinated to platinum, four asymmetric centers (two at carbon and two at nitrogen chelate ring atoms) are present. The Me_2DABPt**G**$_2$ complexes in this study had *R*, *S*, *S*, and *R* configurations at the N, C, C, and N asymmetric centers, respectively. Me_2DABPt**G**$_2$ models are referred to as "retromodels" for reasons given in previous papers.[31,33,34] Three **G** ligands (9-EtG, 3′-GMP, and 5′-GMP) were used to assess the effect of the N9 substituents (ethyl, ribofuranosyl-3′-phosphate, and ribofuranosyl-5′-phosphate) upon the stabilities and the guanine rotation activation parameters of different conformers.

For (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ species, the two HT rotamers have C_2 symmetry; therefore, for each rotamer, only one H8

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signal for the two **G** ligands is expected. In contrast, the single HH rotamer has C_1 symmetry and nonequivalent guanines; therefore, two H8 signals are expected for this rotamer. Thus, four H8 signals will be observed when all three rotamers are present in solution and the rate of interconversion is slow on the NMR time scale.

The rotamer distribution depends on the Me_2DAB configuration. For (*R,S,S,R*)-$Me_2DABPt\mathbf{G}_2$ complexes (293 K) the ΔHT rotamer is the most abundant and accounts for over 60% of the total.[25,29,30,31,33,34] Each of the other two rotamers (ΛHT and HH) ranges from 7% to 20% depending upon the type of N9 substituent. This situation allowed, for the first time, the determination of individual rate constants for interconversion between HT and HH rotamers in *cis*-$PtA_2\mathbf{G}_2$ complexes. Moreover, from the Eyring equation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for rotation about the Pt−N7 bond of individual guanines in different rotamers were calculated.

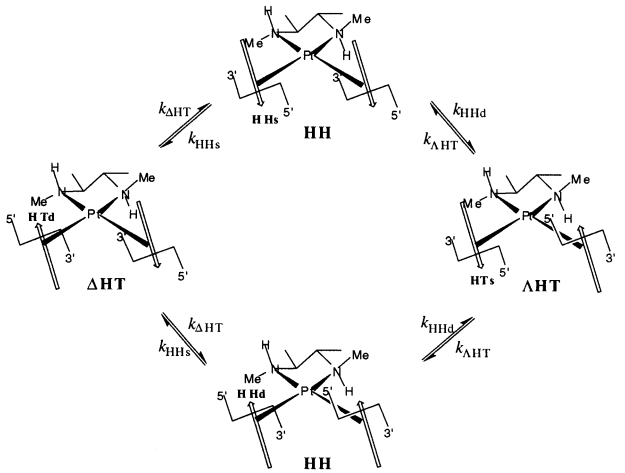
Experimental Section

Starting Materials. 9-EtG, 5′-GMP, and 3′-GMP were obtained from Sigma and used as received. The starting platinum substrate was of the form (*R,S,S,R*)-$Me_2DABPt(SO_4)(H_2O)$ and was prepared and characterized as described.[29] Briefly, the ligand was prepared by reduction of dimethylglyoxime with Raney nickel, followed by fractional crystallization of the HCl salts to separate the meso and racemic forms.[37] The racemic mixture was resolved with tartaric acid.[38] The diamine was converted to the bistrifluoromethylamide derivative and dimethylated with methyl iodide in KOH/DMSO (DMSO = dimethyl sulfoxide). The trifluoroacetyl groups were then removed by reaction with HCl in methanol to yield $Me_2DAB\cdot 2HCl$. $Me_2DABPtCl_2$ was prepared by addition of (*S,S*)-Me_2DAB to *cis*-$[PtCl_2(DMSO)_2]$ in methanol. The diastereoisomers differing in the configurations of the coordinated nitrogen atoms were then separated by fractional crystallization from dimethylformamide. Conversion of the dichloro to the aquasulfato species (*R,S,S,R*)-$Me_2DABPt(SO_4)(H_2O)$ was accomplished by reaction of (*R,S,S,R*)-$Me_2DABPtCl_2$ with a stoichiometric amount of silver sulfate in water.

Preparation of (*R,S,S,R*)-$Me_2DABPt(9\text{-}EtG)_2$. 9-EtG (4.0 mg, 0.022 mmol) was dissolved in D_2O (∼1 mL) and the pH adjusted to ∼1.5 (with DNO_3). The low pH was required to dissolve the 9-EtG. A stoichiometric amount of (*R,S,S,R*)-$Me_2DABPt(SO_4)(H_2O)$ (4.3 mg, 0.01 mmol) was added to the 9-EtG solution, and the progress of the reaction was monitored by ^1H NMR. The reaction could be considered complete after ∼4 h at ambient temperature, but usually we waited for 24 h. The use of a slight excess of 9-EtG ensures the complete transformation of the platinum complex into the bifunctional adduct. When the reaction was over, the sample was taken to dryness under reduced pressure and redissolved in 1 mL of D_2O (0.04 M phosphate buffer, pH 7.04). The neutral pH was sufficient to prevent hydroxide ion catalyzed isomerization of the asymmetric N centers of the diamine (no sign of isomerization was observed after 14 months at 24 °C). H8 signals (5 °C and pH 7.04) were at 8.30 (ΔHT), 8.03 (ΛHT), and 8.59 and 7.84 (HH) ppm.

Preparation of (*R,S,S,R*)-$Me_2DABPt(3'\text{-}GMP)_2$ and (*R,S,S,R*)-$Me_2DABPt(5'\text{-}GMP)_2$. 3′-GMP or 5′-GMP (0.02 mmol) was dissolved in D_2O (1 mL) and the pH adjusted to 3−4 (DNO_3). The nucleotide solution was treated with $Me_2DABPt(SO_4)(H_2O)$ (0.01 mmol) and the reaction monitored by NMR (the reaction was complete in 4−5 h). H8 signals for (*R,S,S,R*)-$Me_2DABPt(3'\text{-}GMP)_2$ (5 °C and pH 3.6) were at 8.61 (ΔHT), 8.31 (ΛHT), and 8.84 and 8.13 (HH) ppm. H8 signals for (*R,S,S,R*)-$Me_2DABPt(5'\text{-}GMP)_2$ (5 °C and pH 3.8) were at 8.85 (ΔHT), 8.37 (ΛHT), and 9.02 and 8.18 (HH) ppm.

Scheme 1



NMR Experiments. NMR spectra were recorded on a Bruker DPX 300 MHz spectrometer. All experiments were performed in D_2O to avoid baseline distortion consequent to H_2O suppression. The latter suppression is required if H_2O/D_2O is used as solvent. ^1H NMR spectra were recorded at 5 K intervals in the temperature range 273−353 K and were referenced against TSP. Temperatures were calibrated against MeOH and ethylene glycol in the appropriate ranges for these solvents. To avoid freezing of the solvent at low temperature, 5% deuterated methanol (v/v) was added. The ^1H NMR experiments at different temperatures were acquired with a total of 32K data points and 256 transients using a pulse width of 60°. For all spectra the spectral width was 4500 Hz, the acquisition time 3.64 s, and the relaxation delay 4 s.

Spectral Simulation. For (*R,S,S,R*)-$Me_2DABPt\mathbf{G}_2$ complexes (**G** = 9-EtG, 3′-GMP, and 5′-GMP), all three rotamers are present at equilibrium and interconversion is slow on the NMR time scale at temperatures close to 0 °C. Transitions between rotamers requiring simultaneous rotation of both **G** bases were considered to be very unlikely and were excluded. Consequently, only transitions between HT and HH rotamers that require rotation of only one **G** were considered.

Rate constants for the interconversion between rotamers were evaluated by using DNMR6, a dynamic NMR spectra simulation FORTRAN code.[39] For a given set of data, the program simulates the NMR spectrum. The input data that give the best fit with the experimental data are considered to be the real values. The set of input data is comprised of structural data (number of nuclei and conformations), NMR data (resonance frequencies and relaxation times), kinetic constants, and rotamer populations (see below). The conformations and the kinetic model are depicted in Scheme 1. The subscripts on the rate constants indicate which HT rotamer (ΔHT or ΛHT) and which guanine in the HH rotamer (HH_s and HH_d stand for H8-shielded and H8-deshielded guanine, respectively) is undergoing rotation. The nuclei selected for addressing the dynamic problem were the H8 atoms of the two guanines present in the three different conformers: HH (nonequivalent H8 nuclei), ΔHT (equivalent H8 nuclei), and ΛHT (equivalent H8 nuclei).

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Table 1. Percentages of Different Rotamers in (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ Complexes at 293 K

G	pH	ΔHT	HH	ΛHT
9-EtG	7.0	59.9	18.7	21.4
3′-GMP	3.6	77.7	9.9	12.4
	7.0	80.9	6.9	12.2
5′-GMP	3.8	73.4	20.0	6.6
	7.1	71.3	17.0	11.7

One guanine is in coordination position 1 (cp1, left-hand guanine in Scheme 1) and the other in coordination position 2 (cp2, right-hand guanine in Scheme 1). Each of them undergoes similar transformations; therefore, we can refer only to one of them. Let us consider the guanine in cp1. The H8 of this guanine can be found in four different magnetic environments along the overall cycle of transformations. Two of these magnetic environments involve the HH rotamer, in which the guanine in cp1 can be either shielded (high-frequency peak, upper HH rotamer of Scheme 1) or deshielded (low-frequency peak, lower HH rotamer of Scheme 1). These two situations have been labeled HH_s (shielded) and HH_d (deshielded), respectively. The other two magnetic environments are those of ΔHT (labeled HT_d because it is found at lower field) and ΛHT (labeled HT_s because it is found at higher field).

The four magnetic environments are linked by four kinetic parameters: the rate constants for the reactions ΔHT → HH ($k_{\Delta HT}$) and ΛHT → HH ($k_{\Lambda HT}$) and the rate constants for the reverse reactions (k_{HH_s} and k_{HH_d}, respectively). Moreover, the ratio $k_{\Delta HT}/k_{HH_s}$ is equal to [HH]/[ΔHT] = $K_{\Delta HT}$, and similarly, the ratio $k_{\Lambda HT}/k_{HH_d}$ is equal to [HH]/[ΛHT] = $K_{\Lambda HT}$. The values of the equilibrium constants $K_{\Delta HT}$ and $K_{\Lambda HT}$ can also be evaluated from the percentages of HH, ΔHT, and ΛHT rotamers (only two out of the three percentages are independent variables, as the three percentages must sum up to 100). In our case two rate constants ($k_{\Delta HT}$ and $k_{\Lambda HT}$) and the rotamer populations were used as input data; the values which give the best fit with the experimental spectrum at a given temperature were selected, and from them the equilibrium constants and the remaining two rate constants (k_{HH_s} and k_{HH_d}) were also evaluated.

For each compound the NMR spectrum was recorded at different temperatures starting from below 0 °C, where the rates of interconversion among rotamers are very slow compared to the NMR time scale [methanol-d_4 was added to D_2O (1:20 v/v) to avoid freezing of the solvent], and reaching temperatures above 70 °C, where the NMR signals coalesced.

The NMR spectrum at low temperature was used to determine the T_2 value for each H8 signal. At this temperature the interconversion between different conformations is slow on the NMR time scale, and the width of each signal depends only upon the relaxation time T_2. This situation is generally indicated as "absence of exchange".

Experimental (gray line) and simulated (dark line) H8 signals of (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ rotamers at the indicated temperatures are reported in Figures 1−3 for **G** = 9-EtG, 3′-GMP, and 5′-GMP, respectively. Estimated rate constants, equilibrium constants, and percentages are reported in Tables S1−S3 in the Supporting Information. Percentages at 293 K are reported in Table 1.

Dependence upon pH. Additional experiments were carried out in the cases of 3′-GMP and 5′-GMP, for which the pH is expected to influence the degree of deprotonation of the phosphate and hence the rate constants for interconversion. Therefore, solutions of (*R*,*S*,*S*,*R*)-Me_2DABPt(3′-GMP)$_2$ and (*R*,*S*,*S*,*R*)-Me_2DABPt(5′-GMP)$_2$ were adjusted to pH close to 7 by addition of dilute KOH. This value of pH ensures nearly complete ionization of the phosphate group (pK_a = 6.2) without significant isomerization of the Me_2-DAB ligand under normal temperature conditions. However, in the case of 5′-GMP the change of configuration at the nitrogen atoms of the carrier ligand became significant when the temperature was raised above 50 °C; consequently, the measured values of rate constants, equilibrium constants, and percentages are affected by larger errors.

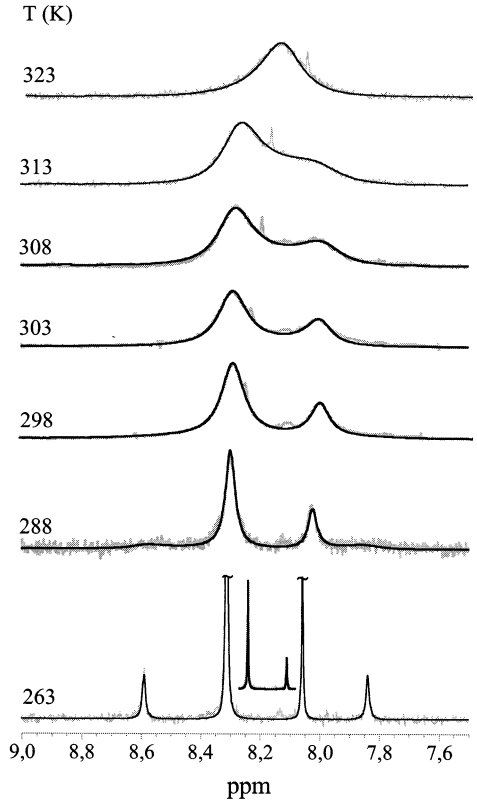

Figure 1. Experimental (gray line) and simulated (dark line) spectra in the region of H8 resonances for (*R*,*S*,*S*,*R*)-Me_2DABPt(9-EtG)$_2$ at different temperatures and pH 7.0.

Thermodynamic and Kinetic Parameters. From the values of rate constants at different temperatures it was possible to evaluate the enthalpy ($\Delta H^{\ddagger}$) and the entropy of activation ($\Delta S^{\ddagger}$) by applying the Eyring equation $\ln(k/T) = -\Delta H^{\ddagger}/RT + \ln(k_B/h) + \Delta S^{\ddagger}/R$ (k = rate constant, k_B = Boltzmann's constant, and h = Plank's constant).[40] The plots of $\ln(k/T)$ against $1/T$ are reported in Figures 4 and 5; the calculated activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are reported in Table 2.

The plots of $\ln K_{\Delta HT}$ and $\ln K_{\Lambda HT}$ as a function of $1/T$ were also linear within the experimental errors, and from their slopes and intercepts the values of ΔH and ΔS for the transformations were evaluated. The plots are reported in Figures S1 and S2, and the estimated thermodynamic parameters are listed in Table 3.

Results and Discussion

Populations of Different Rotamers. The stability of different rotamers in this type of complex has been investigated in previous papers.[29,31,33,34] For (*R*,*S*,*S*,*R*)-Me_2-DABPt**G2** complexes (**G** = 9-EtG, 3′-GMP, and 5′-GMP) the major rotamer was found to have the ΔHT conformation. It was deduced that for this rotamer a right-handed canting of the two coordinated guanine bases, favored by the *R*,*S*,*S*,*R* configuration of the Me_2DAB ligand resulting in the six-

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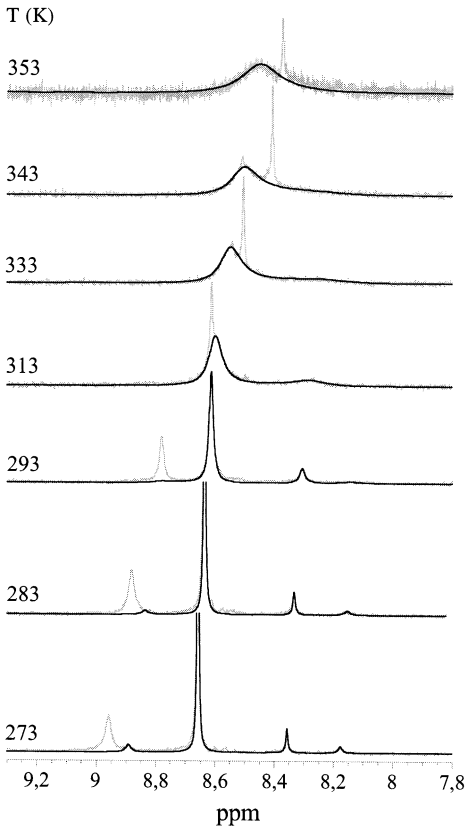

Figure 2. Experimental (gray line) and simulated (dark line) spectra in the region of H8 resonances for (*R*,*S*,*S*,*R*)-Me_2DABPt(3′-GMP)$_2$ at different temperatures and pH 3.6.

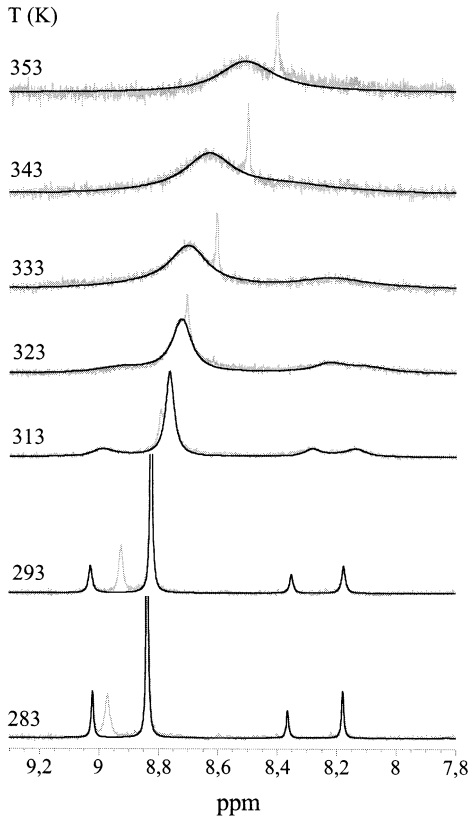

Figure 3. Experimental (gray line) and simulated (dark line) spectra in the region of H8 resonances for (*R*,*S*,*S*,*R*)-Me_2DABPt(5′-GMP)$_2$ at different temperatures and pH 3.8.

membered ring of each guanine to leaning towards the *cis*-**G** and in a better dipole−dipole (or stacking) interaction between the two bases, was also favored. The relative abundance of the ΔHT conformer was greater in the cases of 3′-GMP and 5′-GMP than in the case of 9-EtG. The explanation proposed in previous studies was that, assuming an *anti*-conformation for the nucleotides, the ΔHT conformer can be further stabilized by H-bond interaction between the phosphate and the N1H of the *cis*-**G** in the case of 3′-GMP and by H-bond interaction between the phosphate and the NH of the *cis*-amine in the case of 5′-GMP.[13,41,42]

The second most abundant rotamer at 293 K was ΛHT in the cases of 9-EtG and 3′-GMP, and HH in the case of 5′-GMP; the difference in abundance between ΛHT and HH rotamers, however, was rather small. It was proposed that in the case of 5′-GMP an extra stabilization of the HH rotamer could stem from H-bond interaction between one phosphate and the NH of the *cis*-amine.[29,31,33−35]

As far as H-bond interaction between the O6 of the guanine base and the NH of the *cis*-amine was concerned, the accumulated evidence indicated that this contribution was, at most, very weak.[43] Because such H-bonding interactions require the O6 and the NH of the *cis*-amine to be on the same side of the platinum coordination plane, such an interaction is not possible in the major ΔHT rotamer; two interactions would be possible in the minor ΛHT rotamer, and only one interaction would be possible in the HH rotamer.

For the 3′-GMP complex (in which the major ΔHT rotamer appears to be stabilized by H-bond interaction between the phosphate group and the N1H of the *cis*-**G**), the percentage of ΔHT increased slightly as the pH was raised from 3.6 to 7.0. Indeed, H-bond interactions are expected to be reinforced by deprotonation of the phosphate group. For the 5′-GMP complex, the ΔHT rotamer could be stabilized by H-bond interaction between the phosphate and the NH of the *cis*-amine while the ΛHT rotamer could be stabilized by H-bond interaction between the phosphate and the N1H of the *cis*-**G**. A slight decrease of ΔHT was observed on raising the pH from 3.8 to 7.1, while the ΛHT rotamer increased significantly.

Ground-State Stability of Different Rotamers. This investigation has allowed, for the first time, the enthalpy and the entropy contributions to the stability of different rotamers to be separated (Table 3).

The ΔHT rotamer is the most stable. The enthalpy of this rotamer is found to be lower than that of the HH rotamer by 13, 18−21, and 14−16 kJ mol^{-1} for the EtG, 3′-GMP, and 5′-GMP complexes, respectively (when two numbers are given, the first refers to acidic pH and the second to neutral pH). The ΔHT rotamer is also lower in entropy than the HH rotamer by 35, 45−50, and 37−42 J K^{-1} mol^{-1} for EtG,

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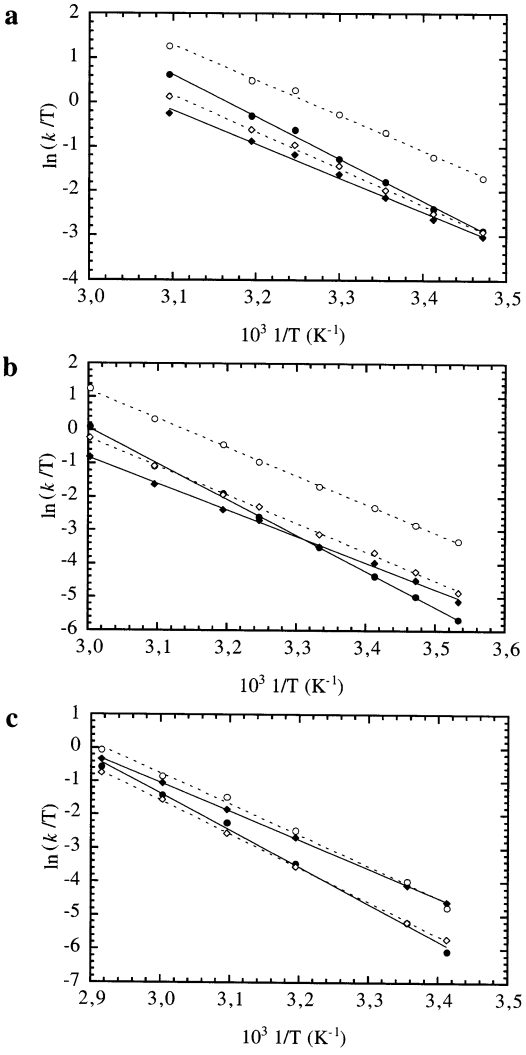

Figure 4. ln($k_{\Delta HT}/T$), ln($k_{\Lambda HT}/T$), ln(k_{HHd}/T), and ln(k_{HHs}/T) as a function of 1/T for (*R*,*S*,*S*,*R*)-Me_2DABPt$\mathbf{G}_2$ complexes at acidic pH (**G** = 9-EtG, a; 3′-GMP, b; 5′-GMP, c).

3′-GMP, and 5′-GMP complexes, respectively. Therefore, the ΔHT rotamer is stabilized by a lower enthalpy but destabilized by a lower entropy with respect to the HH rotamer. The negative contribution of the entropy, however, is not such to compensate for the positive contribution of the enthalpy, and the overall result is a lower free energy for ΔHT with respect to HH.

A completely different trend is observed for the ΛHT rotamer with respect to HH. In all cases examined, the ΛHT rotamer has higher enthalpy and higher entropy than the HH rotamer. In the cases of EtG and 3′-GMP complexes, the stabilizing contribution of the higher entropy compensates for the destabilizing contribution of the higher enthalpy and, as a consequence, the ΛHT rotamer becomes the second most abundant rotamer while the HH rotamer becomes the least abundant. In the case of 5′-GMP, the contribution to the stability of the entropic term is not sufficient to compensate for the instability determined by the higher enthalpy and the ΛHT rotamer remains the least abundant.

ΔHT lack of steric repulsion between **G** H8 and the *N*-Me of the *cis*-amine on opposite sides of the platinum coordination plane, favors leaning of the six-membered ring of each **G** toward the *cis*-**G** and a better dipole−dipole internucleotide interaction. The low enthalpy and entropy of the ΔHT rotamer are in accord with the major contribution to the stabilization of this rotamer coming from favorable dipole−dipole internucleotide interaction. Such an interaction not only lowers the energy but also reduces the degree of wagging of the nucleobases. Moreover, in the case of 3′-GMP and 5′-GMP, additional stabilization of the ΔHT rotamer was proposed to come from phosphate−N1H *cis*-**G** and phosphate−NH *cis*-amine H-bond interactions, respectively. Also these interactions are expected to lower the enthalpy and entropy of the species. Finally, the fact that the entropic and enthalpic terms give opposite contributions to the stability of this rotamer has the consequence that the stability of the ΔHT rotamer, with respect to that of HH, is less dramatic than expected on the basis of the enthalpic term alone.

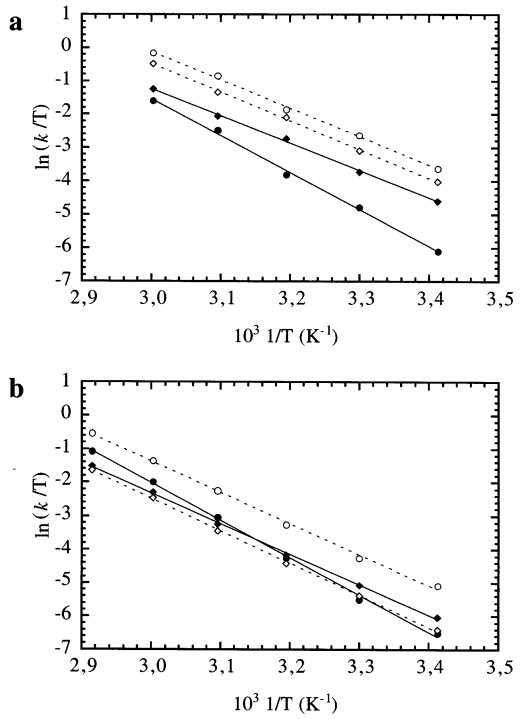

Figure 5. ln($k_{\Delta HT}/T$), ln($k_{\Lambda HT}/T$), ln(k_{HHd}/T), and ln(k_{HHs}/T) as a function of 1/T for (*R*,*S*,*S*,*R*)-Me_2DABPt$\mathbf{G}_2$ complexes at neutral pH (**G** = 3′-GMP, a; 5′-GMP, b).

Therefore, the difference in enthalpy and entropy between HH and ΔHT can be due, in part, to destacking between *cis*-**G** bases and, in part, to increasing wagging by one **G** base (having O6 on the same side as *cis*-NH) and, possibly, release of water molecules H-bonded to Me_2DAB NH *cis* to the latter **G**. The overall instability of the HH rotamer, with respect to the ΔHT rotamer, stems from an increase in enthalpy which is not balanced by an adequate increase in entropy.

The ΛHT rotamer has higher enthalpy and entropy than the HH rotamer. In the ΛHT rotamer both **G** base H8 atoms and *N*-Me the *cis*-amine are on the same side with respect to the coordination plane which reduces leadning of the six-membered ring of each **G** towards the *cis*-**G** and internucle-

Table 2. Activation Enthalpy (kJ mol^{-1}) and Entropy (J K^{-1} mol^{-1}) for Rotation of Nucleobases about the Pt−N7 Bond in Individual Rotamers[a] of (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ Complexes[b,c]

G	pH	$\Delta H^{\ddagger}_{\Delta HT}$	$\Delta H^{\ddagger}_{HH_s}$	$\Delta H^{\ddagger}_{HH_d}$	$\Delta H^{\ddagger}_{\Lambda HT}$	$\Delta S^{\ddagger}_{\Delta HT}$	$\Delta S^{\ddagger}_{HH_s}$	$\Delta S^{\ddagger}_{HH_d}$	$\Delta S^{\ddagger}_{\Lambda HT}$
9-EtG	7.0	78	66	69	64	51	18	18	−1
3′-GMP	3.6	89	71	71	66	71	26	14	−7
	7.0	91	71	72	68	64	14	14	−4
5′-GMP	3.8	91	77	84	71	65	28	41	8
	7.1	93	77	80	76	65	23	23	11

[a] Because in the HH rotamer the two guanines are not equivalent, two values of activation energies, one for the more shielded guanine (HH_s) and the other for the less shielded guanine (HH_d), are given. [b] For the 5′-GMP complex at neutral pH the standard deviations for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are estimated to be ±3 kJ mol^{-1} and ±4 J K^{-1} mol^{-1}, respectively. [c] Solvent D_2O + 5% CD_3OD (v/v). Standard deviations are estimated to be ±2 kJ mol^{-1} and ±3 J K^{-1} mol^{-1} for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, respectively.

Table 3. Enthalpy (ΔH, kJ mol^{-1}) and Entropy (ΔS, J K^{-1} mol^{-1}) for the Rotational Equilibria ΔHT ⇄ HH and ΛHT ⇄ HH ($K_{\Delta HT}$ = [HH]/[ΔHT]; $K_{\Lambda HT}$ = [HH]/[ΛHT]) in (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ Complexes[a]

G	pH	$\Delta H_{\Delta HT}$	$\Delta S_{\Delta HT}$	$\Delta H_{\Lambda HT}$	$\Delta S_{\Lambda HT}$
9-EtG	3	+13	+35	−5	−20
3′-GMP	3.6	+18	+45	−5	−21
	7.0	+21	+50	−4	−18
5′-GMP	3.8	+14	+37	−12	−33
	7.1	+16	+42	−4	−12

[a] Standard deviations are estimated to be ±2 kJ mol^{-1} for ΔH and ±3 J K^{-1} mol^{-1} for ΔS.

otide dipole−dipole interaction. Moreover increased wagging by **G** bases and, possibly, release of water H-bonded to Me_2-DAB NH (displaced by the wagging **G** bases) may account for the increase in entropy and enthalpy. For **G** = EtG and 3′-GMP, the increase in entropy is such to compensate for the increase in enthalpy and the ΛHT rotamer becomes favored with respect to the HH form. For **G** = 5′-GMP, the increase in entropy does not compensate for the increase in enthalpy and the ΛHT rotamer becomes the least abundant.

The thermodynamic parameters also account for the general observation that, by raising the temperature, the percentages of different rotamers tend to converge; in other words, the percentage of the most abundant rotamer decreases, while those of the less abundant rotamers increase. The major ΔHT rotamer is the lowest in enthalpy; therefore, by increasing the temperature, the enthalpic term will favor the HH and ΛHT rotamers over the ΔHT rotamer. Moreover, since the HH rotamer is lower in enthalpy than the ΛHT rotamer, by increasing the temperature, the ΛHT form increases with respect to the HH form.

Enthalpy and Entropy of Activation for Guanine Rotation about the Pt−N7 Bond. Another major objective of the present investigation was the determination of the enthalpy and entropy of activation for rotation of the **G** nucleobases about the Pt−N7 bonds in individual rotamers. Experimental values are reported in Table 2; the enthalpy profiles as a function of the reaction coordinate are reported in Figure 6.

Effect of the Carrier Ligand(s). Before rotational energy barriers for individual rotamers are discussed, it is helpful to consider data in the literature. In previous studies dealing with diamines such as Me_4EN[25] and Me_2EN,[28] the two HT rotamers had comparable stabilities and the HH rotamer was not detected; consequently, the kinetic parameters were evaluated for interconversion between HT rotamers. However, interconversion between HT rotamers undoubtedly requires the intermediacy of the HH rotamer; therefore, comparison can be made between the literature data and the average values of $\Delta H^{\ddagger}_{HT}$ found in the present investigation ($\Delta H^{\ddagger}_{HT}$ = $^1/_2$($\Delta H^{\ddagger}_{\Delta HT}$ + $\Delta H^{\ddagger}_{\Lambda HT}$)). The average value of $\Delta H^{\ddagger}_{HT}$ is 71 kJ mol^{-1} for Me_2DABPt(9-EtG)$_2$; the literature values of the activation energy (E^a) for Me_4ENPt(Guo)$_2$ and Me_2ENPt(Guo)$_2$ are 86 and 57−65 kJ mol^{-1}, respectively (the activation energy E^a is not exactly the activation enthalpy $\Delta H^{\ddagger}$ but is greater by a small amount equal to *RT*). Although Guo is slightly bulkier than 9-EtG, both lack H-bonding

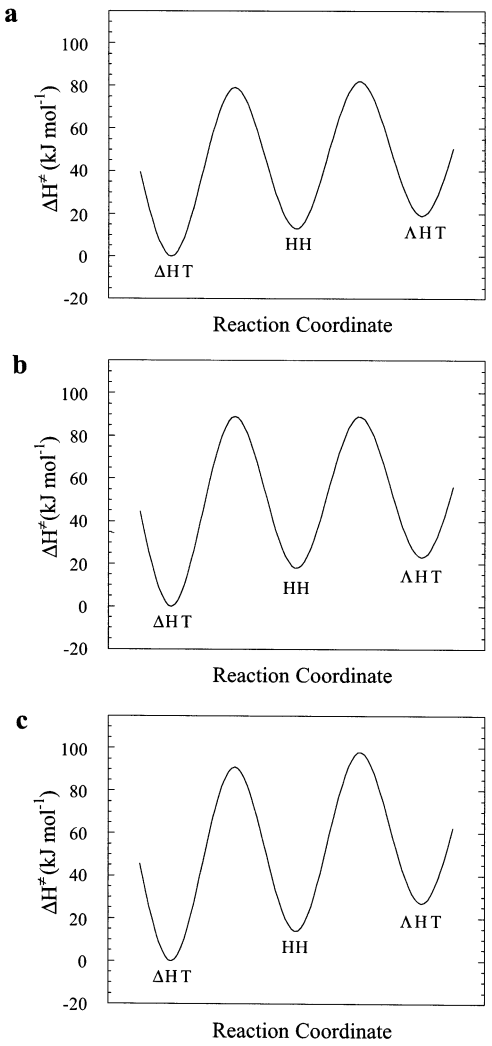

Figure 6. Enthalpy profiles as a function of the reaction coordinate for (*R*,*S*,*S*,*R*)-Me_2DABPt**G**$_2$ complexes at acidic pH (**G** = 9-EtG, a; 3′-GMP, b; 5′-GMP, c).

phosphate groups. Our value is exactly halfway between the literature data for Me_4EN and Me_2EN complexes, indicating that the Me_2DAB ligand exerts a steric impediment to rotation of the *cis*-guanines which is smaller than that of Me_4EN (having tertiary aminic groups), but greater than that of Me_2EN (having secondary aminic groups such as Me_2-DAB). It is most likely that the two Me substituents on the ring carbons render the chelate ring and the overall stereochemistry of Me_2DAB less flexible than those of Me_2EN. The average value for $\Delta H^{\ddagger}_{HT}$ for (*R,S,S,R*)-$Me_2DABPt(5'\text{-}GMP)_2$ is 81−85 kJ mol^{-1} (the former value refers to acidic pH and the latter to neutral pH). The estimated value of the activation energy for interconversion between HT rotamers in *cis*-$(NH_3)_2Pt(5'\text{-}GMP)_2$ was 25 kJ mol^{-1}.[35] The ∼60 kJ mol^{-1} increase in activation enthalpy on passing from *cis*-$(NH_3)_2$ to Me_2DAB carrier ligands is a direct consequence not only of the bulkiness of the alkyl substituents on the nitrogen atoms, but also of the decrease in rotational freedom of the aminic groups.

Effect of the Rotamer Conformation. In the case presently investigated the highest activation enthalpy and entropy were found for **G** rotation in the most stable ΔHT rotamer. On the average $\Delta H^{\ddagger}_{\Delta HT}$ is ca. 14 kJ mol^{-1} greater than $\Delta H^{\ddagger}_{HH}$ and 19 kJ mol^{-1} greater than $\Delta H^{\ddagger}_{\Lambda HT}$. Similarly $\Delta S^{\ddagger}_{\Delta HT}$ is ca. 40 J K^{-1} mol^{-1} greater than $\Delta S^{\ddagger}_{HH}$ and 60 J K^{-1} mol^{-1} greater than $\Delta S^{\ddagger}_{\Lambda HT}$. These results clearly indicate that the activation parameters are modulated by the enthalpy and entropy contents of the ground state. Therefore, the ΔHT rotamer, which has the lowest enthalpy and entropy, has the highest values for the enthalpy and entropy of activation, followed by the HH rotamer having enthalpy and entropy intermediate between those of the ΔHT and ΛHT rotamers, while the ΛHT rotamer having the highest values of enthalpy and entropy in its ground state has the lowest values for the enthalpy and entropy of activation.

It is worth noting the very small $\Delta S^{\ddagger}$ values for **G** rotation in the ΛHT rotamer. This is a clear indication that this rotamer, in its ground state, has an entropy content very close to that of the activated complex. In the previous discussion of the thermodynamic parameters it was suggested that the ΛHT rotamer (both **G** bases having the six-membered ring on the same side as the NH of the *cis*-amine with respect to the coordination plane) has the largest wagging by the **G** bases and, possibly, no water molecules H-bonded to the diamine NH groups.

The enthalpy and entropy of activation contribute to the reaction rate with opposite sign; therefore, the actual rate will depend on the balance between the two terms. For **G** rotation in the rotamer ΔHT, the negative contribution of the activation enthalpy is far greater than the positive contribution of the activation entropy and the rate of **G** rotation is the lowest. The ΛHT rotamer has the lowest activation enthalpy but also the lowest activation entropy (particularly in the cases of 9-EtG and 3′-GMP). As a consequence the rate of **G** rotation remains low (the second lowest rate after that of the ΔHT rotamer for the 9-EtG and 3′-GMP complexes). In contrast in the case of 5′-GMP, because of a slightly bigger activation entropy, the **G** rotation in ΛHT becomes faster than **G** rotation in the HH rotamer.

There are no remarkable differences between the activation parameters of the two unequivalent **G** bases in the HH rotamer. This is particularly true for the 9-EtG and 3′-GMP complexes. In the case of 5′-GMP, at acidic pH, the enthalpy and entropy for HH_s appear to be significantly greater than for HH_d. The shielded guanine has the 5′-phosphate in a favorable position (assuming an *anti*-conformation for the nucleotide) to interact with the NH of the *cis*-amine, and this could explain the greater values for the activation parameters.

Effect of the N9-Substituent. As a general trend, the activation enthalpy increases regularly on moving from 9-EtG to 3′-GMP and to 5′-GMP. The increase is particularly relevant for $\Delta H^{\ddagger}_{\Delta HT}$ on passing from 9-EtG to 3′-GMP and 5′-GMP, and for $\Delta H^{\ddagger}_{HH}$ on moving from 9-EtG and 3′-GMP to 5′-GMP. It has already been pointed out that, in the cases of 3′-GMP and 5′-GMP, the ΔHT rotamer can gain extra stabilization from phosphate−N(1)H *cis*-**G** and phosphate−NH *cis*-amine H-bond interactions, respectively. Moreover, it has also been pointed out that in the case of 5′-GMP the deshielded guanine of the HH rotamer has the phosphate in a suitable position to interact with the NH of the *cis*-amine.

Changes in $\Delta S^{\ddagger}$ as a function of the N9-substituents are difficult to rationalize. The reason is that several factors can contribute to this parameter, among others: wagging of the guanines, solvation/desolvation of Me_2DAB NH, guanine N1H, and phosphate, mobility of the sugar−phosphate moiety, etc.

Conclusions

Because all complexes investigated here have the three possible rotamers (ΔHT, ΛHT, and HH) present at equilibrium and in slow interconversion on the NMR time scale, we have been able to determine, for the first time, the equilibrium constants between conformers and to correlate the variations in enthalpy and entropy (ΔH and ΔS) with specific interactions.

The factors that in previous papers were proposed to give a major contribution to the ground-state stability of individual rotamers have been fully confirmed and quantified in terms of enthalpy and entropy. They are (a) dipole−dipole interaction between *cis*-guanines (particularly in HT rotamers having the six-membered ring of each guanine considerably leaning toward the *cis*-guanine), (b) nucleotide *cis*-amine H-bond interactions involving the 5′-phosphate, and (c) internucleotide H-bond interactions involving the 3′-phosphate and the N1H of the *cis*-nucleotide. In all cases the phosphate must be able to reach over the NH without altering the preferred *anti*-conformation of the nucleotide. Possible H-bond interactions between the O6 of the guanines and the NH of the *cis*-amines, at least in water solution, appear to be of much less importance.

The rate constants for rotation of a single **G** in individual rotamers as well as the corresponding activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) have also been determined for the first time. These activation parameters correlate with the ground-state

energy of the conformers, suggesting that the interactions that stabilize the ground state of a given conformer are much less effective in the stabilization of the activated complex for rotation about the Pt−N7 bonds.

An unexpected result of this investigation has been the highest value of enthalpy for the ΛHT rotamer and the crucial role of the entropy in modulating the relative stability of this conformer with respect to the HH rotamer. We hope that this investigation can give a significant contribution to the understanding of the energetics involved in the process of cross-link formation in cisplatin−DNA adducts.

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Supporting Information Available: Tables of rate constants, percentages of different rotamers, and equilibrium constants at different temperatures and plots of the logarithm of the equilibrium constants as a function of $1/T$. This material is available free of charge via the Internet at http://pubs.acs.org.

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