Rotational Energy Barrier in Pt^{II}–N^7 (Purine) Bonds by One- and Two-dimensional Nuclear Magnetic Resonance Spectroscopy

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Rotational energy barriers about Pt^{II}–N^7 purine bonds for *cis*-$[Pt(NH_3)_2L_2]$ [L = 1-methyladenosine, adenosine 5'-mono-, -di- or -tri-phosphate, or guanosine 5'-monophosphate) were evaluated from one- and two-dimensional phosphorus-31 exchange spectroscopy. Two diastereomers in a head-to-tail conformation exist for all the aminopurine complexes in the temperature range 25–60 °C due to slow rotation about Pt–N^7 bonds. The rotational activation energies for all the 6-aminopurine complexes lie in the range 46–95 kJ mol^{-1} while the 6-oxopurine complex exhibits the lowest energy barrier, 25 kJ mol^{-1}. The two exocyclic amines of the aminopurine complexes in a head-to-tail configuration are positioned above and below the platinum square plane. The increased rotational energy barrier in the AMP compared to that of GMP complexes is attributed to the absence of hydrogen bonding with amines, a direct interaction of the lone-pair electrons of the 6-NH_2 group with the σ-bonding orbitals of the platinum atom, and a decrease in the crystal-field stabilization energy due to the interaction with the filled d_{z^2} orbital. The rotational energy barrier can be taken as the ground-state energy difference between the head-to-tail and head-to-head configurations since the latter is encountered near to the highest potential-energy surface during the conversion of one head-to-tail rotamer into the other. An energy difference >45 kJ mol^{-1} may be estimated between the bis(GMP) complex and the AMP analogue in a head-to-head configuration.

It is generally believed that the antitumour activity of *cis*-diamminedichloroplatinum(II) is due to an intrastrand binding to DNA predominantly through the two adjacent guanine bases.[1] It is established that reactions of this complex with guanosine 5'-monophosphate (GMP)[2] and DNA[3] take place in a stepwise manner mainly through rate-limiting aquation processes. The rate of the initial step to form the Pt–DNA monofunctional adduct (bound to one base) is faster than that of the final step which leads to a bifunctional adduct (bound to two bases). Reactions of platinum complexes with DNA are usually viewed as kinetically controlled processes.[1] Theoretical calculations point to an enhanced basicity of atom N^7 of guanine compared to the same donor site of the adenine base.[4] An intrastrand DNA binding by platinum gives rise to a head-to-head conformation in which two six-membered ring moieties of the purine base are on the same side of the square plane.[5,6] However, in addition to such conformations, slow rotations about Pt–N^7 purine bonds in bis(purine)platinum complexes of mononucleotides and nucleosides give rise to a head-to-tail conformation in which two six-membered ring moieties are on the opposite sides of the plane.[7] Structures of head-to-head and head-to-tail rotamers for the *cis*-$[Pt(NH_3)_2(AMP)_2]$ (AMP = adenosine 5'-monophosphate)† complex are shown in **I** and **II**. The corresponding bis(GMP) complexes do not exhibit such rotamers at room temperature due to rapid rotations about Pt–N^7 bonds.[8] However, rotamers in several platinum complexes containing bulky amine ligands were observed due to restricted rotations about the Pt–N^7(guanine) bonds.[7,9]

In this article we report rotational energy barriers in the Pt–N^7 bonds determined from one- and two-dimensional NMR spectroscopy, compare the energetics of GMP and AMP complexes in head-to-head conformations, and pinpoint the specific interactions responsible for the variation of the energy barrier within closely related platinum(II)–purine complexes. Although rotational energy barriers have been assessed from molecular mechanics calculation,[10] experimental results to compare the energetics of various nucleotide complexes are scarce. To the best of our knowledge, this is the first report which utilizes ^{31}P two-dimensional exchange spectroscopy (EXSY) to evaluate quantitatively the activation energy associated with the rotations about Pt–N^7 purine bonds and directly compares the energies of the AMP and GMP systems. Phosphorus-31 has an advantage over ^1H primarily because the nuclear Overhauser effect (NOE) is solely due to the exchange phenomena resulting from the presence of only one ^{31}P resonance per nucleotide. An initial communication of this work has appeared.[11]

† At neutral pH AMP, ADP, ATP and GMP exist predominantly as their 2−, 3−, 4− and 2− ions. Assuming there is no substantial change in the pK_a values of the phosphate moieties upon co-ordination through the N^7 sites of the nucleotides, the charges of the platinum complexes at neutral pH would be $[Pt(NH_3)_2L_2]^{-(2n+2)}$ where L represents the nucleotide and n its charge. At low pH the N^1 site of 1-methyladenosine becomes quaternary.

Experimental

Reagents.—Adenosine, 1-methyladenosine, adenosine 5'-mono-, -di- and -tri-phosphate (sodium salts), guanosine 5'-monophosphate (sodium salt), and D_2O (99.9% atom) (Sigma) were used without further purification. *cis*-Diamminedichloroplatinum(II) was prepared following the method of Dhara.[12]

Platinum nucleoside and nucleotide complexes were prepared *in situ* by mixing *cis*-$[Pt(NH_3)_2Cl_2]$ and the desired base at pH 7.0, with the base : metal mole ratio ranging from 2 : 1 to 3 : 1 and keeping [complex] at 5.0 mmol dm^{-3}. The reaction mixture was thermostatted at 40 °C for 24 h. No significant amounts of unreacted bases were detected by proton NMR spectroscopy when the complex was treated with exactly 2 equivalents of base.

Physical Measurements.—The NMR experiments were carried out using a 300 MHz (GN 300) instrument equipped with a variable-temperature probe. The chemical shifts for the ^{31}P and ^1H signals are with respect to 85% phosphoric acid and the HOD signal (at δ 4.67) respectively. For a typical ^{31}P spectrum, a 2000 Hz frequency window, 25 μs pulse width, 2–3 s pulse delay time, and 4–8 K data points were selected. A line-broadening factor of 3 Hz was introduced before Fourier transformation. The HOD signal was presaturated for 500 ms

in order to suppress the water peak in the proton spectra. Solutions containing platinum complexes were adjusted to pH 6.0–7.0 by adding dilute NaOD or DNO_3. Solid $NaNO_3$ was added to the aqueous solutions of the platinum complexes for the NMR experiments below 0 °C. The lowest temperature reported here was about 5° higher than the freezing point of the solution. The NMR spectra were recorded in the temperature range −13 to 85 °C.

Longitudinal relaxations for ^{31}P were measured by a conventional inversion-recovery method[13] following the pulse sequence π–t–$\frac{\pi}{2}$–A_t where t and A_t are the delay time between 180 and 90° pulses and the acquisition time, respectively. In these experiments the solutions were purged with N_2 to remove dissolved oxygen. At least fifteen time intervals between 180 and 90° pulses were selected. Delay times between acquisitions were chosen to ensure larger than 95% relaxations. The values of T_1 were evaluated from an exponential fit of intensity *vs.* time data assuming perfect π and $\frac{\pi}{2}$ pulses by utilizing the GE software supplied with the instrument.

Two-dimensional exchange experiments were performed following the conventional phase-sensitive nuclear Overhauser effect spectroscopy (NOESY) pulse sequence[14] $\frac{\pi}{2}$–t_1–$\frac{\pi}{2}$–τ_m–$\frac{\pi}{2}$–A_t in which t_1 and τ_m are the evolution and mixing times. For the rate measurements the mixing times were varied between 200 and 1200 ms. The intensity of the cross-peaks reaches its maximum near 600 ms and then steadily declines.*[15] The rate constants were found to be invariant with mixing times in the range 500–900 ms. Rate constants compiled in Table 1 were based on a mixing time of 600 ms. The intensities of the diagonal and cross-peaks in the two-dimensional contour plots were evaluated utilizing the GE software. For two-site exchange processes (1) the normalized peak intensities in the contours are

$$A \underset{k_{BA}}{\overset{k_{AB}}{\rightleftharpoons}} B \qquad (1)$$

related to the rate process[16] by equation (2) where τ_m is the

$$A = e^{-R\tau_m} \qquad (2)$$

mixing time for the EXSY experiment, and A and R are the intensity and rate matrices (3). In these matrices, a_{AA}, a_{BB}, a_{AB}

$$A = \begin{vmatrix} a_{AA/A_0} & a_{AB/B_0} \\ a_{BA/A_0} & a_{BB/B_0} \end{vmatrix} \quad R = \begin{vmatrix} \rho_A & -k_{BA} \\ -k_{AB} & \rho_B \end{vmatrix} \qquad (3)$$

and a_{BA} are the intensities of the diagonal (AA,BB) and cross-(AB,BA) peaks; a_{A_0} and a_{B_0} are the intensities of A and B in the one-dimensional spectrum which was recorded with sufficient delays to ensure the establishment of >90% equilibrium magnetization before irradiating with the next pulse. In the R matrix ρ_A and ρ_B are longitudinal relaxation rates and k_{AB} and k_{BA} are the rate constants for the exchange process as indicated in equation (1). This matrix can be obtained by diagonalizing A and then calculating the eigenvector matrix X and its inverse X^{-1} according to the established method.[16c] The values of the rate constants were read from the R matrix directly.

When the signal intensities of two species which are undergoing exchange processes were approximately equal a simpler method was used to evaluate the rate constant. It has been shown that for a symmetrical two-site exchange process

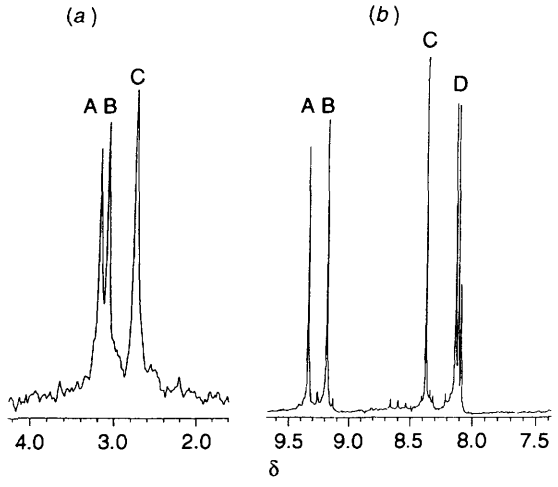


Fig. 1 (*a*) Phosphorus-31 NMR spectrum of *cis*-$[Pt(NH_3)_2Cl_2]$ (5.0 mmol dm^{-3}) and AMP (13.0 mmol dm^{-3}) at 25 °C. Peaks A and B are for the Pt–AMP complexes and C is the residual unreacted AMP. (*b*) Proton NMR of the same reaction mixture (purine part only). Two downfield signals, A and B, are for the AMP complex, and C and D are for the H^8 and H^2 resonances of free AMP

the normalized intensities of the diagonal (I_{diag}) and off-diagonal ($I_{off\text{-}diag}$) matrix elements in the A matrix are related as in equation (4).[16d] The value of k was calculated from the ratio of the normalized intensities using this equation.

$$I_{diag}/I_{off\text{-}diag} = (1 - k\tau_m)/k\tau_m \qquad (4)$$

Rate constants were also obtained by solution of the conventional equation for a two-site exchange process.[17] Selected signals which manifest the exchange phenomenon were simulated and superimposed over the experimental signals. In this computer simulation the natural linewidth was determined from non-exchangeable resonances and the correlation time was varied until an optimum agreement between observed and simulated spectra was achieved. First-order rate constants were then obtained from the correlation times.

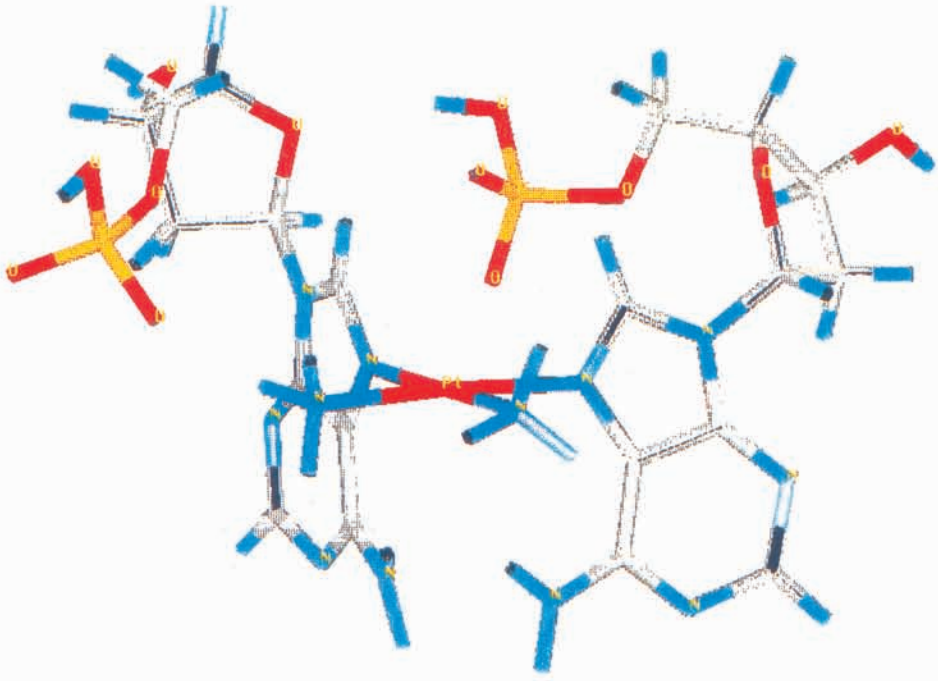
The activation energies for the exchange processes were calculated from the slopes of plots of ln k *vs.* $1/T$. These energies can be reproduced to within 10%.

Results

Fig. 1 shows the ^{31}P and proton NMR spectra† of a solution containing *cis*-$[Pt(NH_3)_2Cl_2]$ (5.0 mmol dm^{-3}) and AMP (13.0 mmol dm^{-3}) after reaction for 24 h. The two downfield ^{31}P signals at δ 3.18 and 3.03 are for the $[Pt(NH_3)_2(AMP)_2]$ products and the peak at δ 2.76 for the unreacted nucleotide. Similarly, the two signals furthest downfield at δ 9.33 and 9.18 in the proton NMR spectrum are for the complexes and that at δ 8.36 is of unreacted AMP. These two ^{31}P and ^1H signals of products are for the two diastereomers in a head-to-tail conformation due to the slow rotations about the Pt–N^7 purine bonds (see below). Fig. 2 shows the EXSY contours for the ^{31}P resonances. The existence of cross-peaks in these contours establishes the exchange processes between the two rotamers. In this EXSY experiment free AMP was kept intentionally in order to demonstrate that there are no cross-peaks between the unreacted materials and the products. The rate constants

* A long mixing time does not create a spin-diffusion problem in our EXSY experiments. Spin-diffusion rates depend on the internuclear dipolar interaction and are proportional to r^{-6} for the interacting spins. For small molecules with correlation time $\ll\omega_0^{-1}$, $\alpha\alpha = \beta\beta$ relaxation pathways dominate. A mutual enhancement of relaxation but not an exchange of magnetization is expected. In any event, since we did not observe any systematic variation of rate constant with τ_m in the time domain 600–900 ms, we conclude that spin diffusion did not contribute significantly to the magnetization transfer. The magnetization-transfer processes observed here are purely due to the exchange phenomenon.

† One reviewer noted that the ratio of the peak intensity for the bound to free nucleotide in Fig. 1 should be 1.0:0.3. However, the spectra presented exhibit a slightly higher intensity for the unreacted nucleotide. This is primarily due to the fact that near neutral pH a small amount of hydroxo-bridged diplatinum complex is formed through a parallel reaction. The formation of the dimer did not affect the rotational energy barrier since AMP did not react with the dimer under our experimental conditions.



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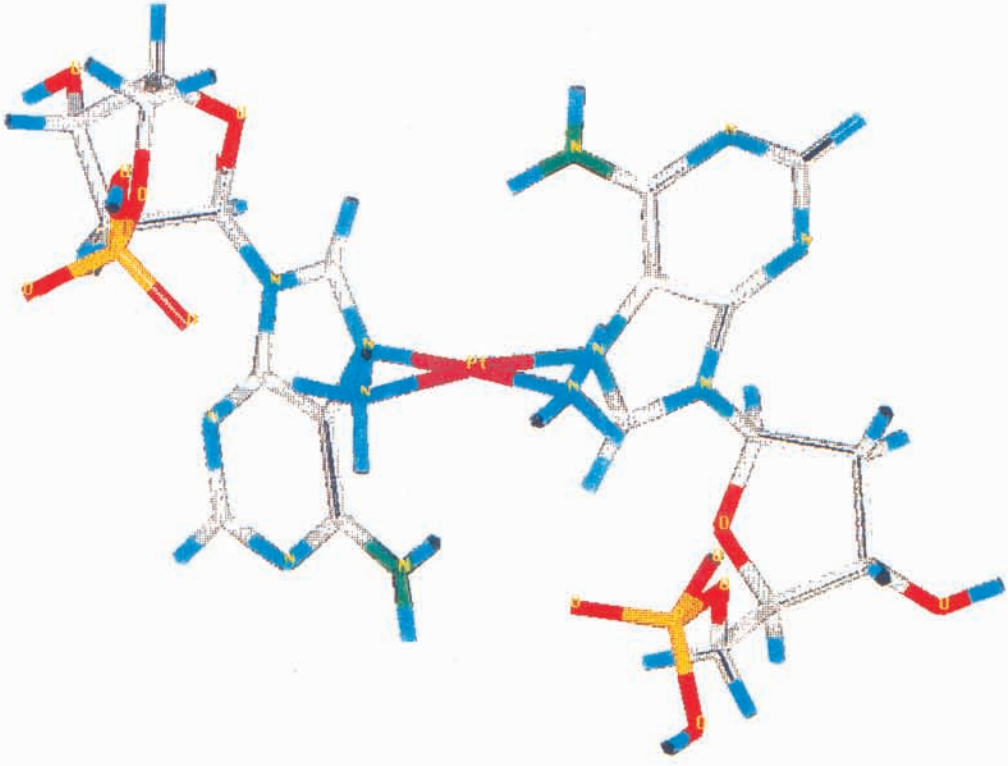

II

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obtained from the intensity contours of the ^{31}P NMR spectra following the procedure outlined in the Experimental section are listed in Table 1. They were compiled from experiments with a selected mixing time of 600 ms. Proton two-dimensional NOE experiments also exhibited NOE connectivity between two H^8 signals of the diastereomers. However, the intensities of the diagonal and cross-peaks were not utilized to calculate the rate constants since the NOE enhancements were not purely of exchange origins. Nearby and distant protons also contributed towards the intensities of the cross-peaks in the two-dimensional contours.

Both the proton and phosphorus-31 NMR signals broaden significantly with increasing temperature initially and then merge at 85 °C. Fig. 3 compares the observed and simulated ^{31}P NMR spectra based on a two-site exchange process. As can be seen in Table 1 there is a small difference in magnitude between k_{AB} and k_{BA}. The intensities of the ^{31}P peaks are also unequal in the one-dimensional spectra in keeping with the fact that the rate constants must be unequal. However, $k_{AB}(\text{intensity})_A = k_{BA}(\text{intensity})_B$, since we are dealing with equilibrium kinetics. Table 1 also shows an approximate rate constant, k_{ap}, calculated by assuming a two-site exchange process according to equation (4). This rate constant was then compared with the average value, $k_{av} = (k_{AB} + k_{BA})/2$, and also with the rate constants obtained from one-dimensional proton spectra by solving the two-site exchange equation. A good agreement between the rate constants obtained from the one- and two-dimensional NMR methods is evident.

Reactions of ADP and ATP also resulted in a pair of diasteromers as is evident from the appearance of two sets of signals and their NOE enhancements due to the exchange phenomenon. Rate constants associated with these exchange processes are listed in Table 2. For the ATP and ADP products coalescence points were not observed even at 85 °C. Two H^8 resonances at δ 8.43 and 8.41 were also observed for bis(1-methyladenosine) products. The resonances coalesced at 65 °C; exchange rate constants are also listed in Table 2.

The complex *cis*-$[Pt(NH_3)_2(GMP)_2]$ exhibited single ^{31}P and H^8 resonances indicating rapid rotation about the Pt–N^7 bond on the NMR time-scale. These resonances were broadened to some extent in the temperature range −13 to 0 °C (Fig. 4). An activation energy of 25 kJ mol^{-1} can be estimated for the 6-oxopurine complexes from the line-shape analysis.

Table 1 Rate constants for *cis*-$[Pt(NH_3)_2(AMP)_2]$ rotamers at different temperatures obtained from ^{31}P NMR two-dimensional EXSY and solutions of one-dimensional spectra based on symmetrical two-site exchange processes

T/°C	k_{AB}[a]/s^{-1}	k_{BA}[a]/s^{-1}	k_{av}[b]/s^{-1}	k_{ap}[c]/s^{-1}	k_{1D}[d]/s^{-1}
26.5	0.24	0.25	0.25	0.21	0.25
31.5	0.34	0.40	0.37	0.37	0.38
39.3	0.81	1.7	0.94	0.89	0.77
48.8	1.8	1.8	1.8	1.3	1.7

[a] k_{AB} and k_{BA} refer to the rate of exchange of two rotamers represented by peaks A and B in Fig. 1. [b] $k_{av} = (k_{AB} + k_{BA})/2$. [c] Calculated based on the symmetrical exchange two-dimensional method [equation (4)]. [d] Obtained from solutions of the two-site exchange equation using an iterative computer program.

Table 2 Rate constants* for AMP, ADP, ATP, and 1-methyladenosine (made) rotamers in $[Pt(NH_3)_2L_2]$ at different temperatures

	Rate constant/s^{-1}			
T/°C	L = AMP	ADP	ATP	made
40.0	0.80	0.033	0.01	0.72
60.0	4.0	0.20	0.03	1.5
80.0	17	0.31	0.09	3.8

* Obtained by solving the symmetrical two-site exchange equation using an iterative computer program.

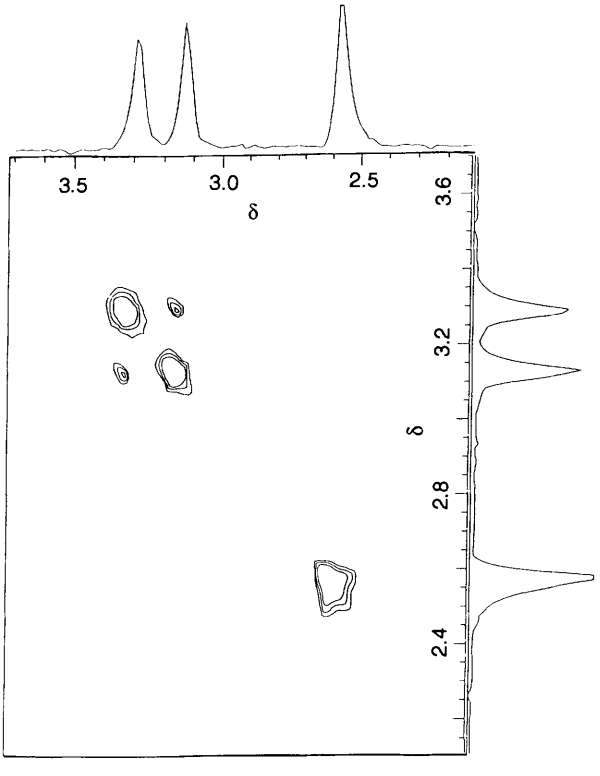

Fig. 2 121.5 MHz Phosphorus-31 two-dimensional EXSY contours of *cis*-$[Pt(NH_3)_2Cl_2]$ (5.0 mmol dm^{-3}) and AMP (15.0 mmol dm^{-3}) mixed in D_2O at 25 °C and pD 5.3. The two downfield ^{31}P signals at δ 3.28 and 3.13 are for the *cis*-$[Pt(NH_3)_2(AMP)_2]$ products and the peak at δ 2.55 for the unreacted nucleotide

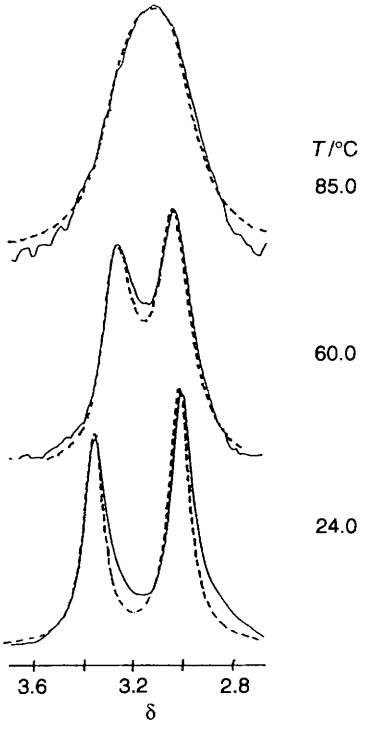

Fig. 3 Experimental (solid lines) and simulated (dashed lines) phosphorus-31 resonances of bis(AMP) rotamers at the indicated temperatures

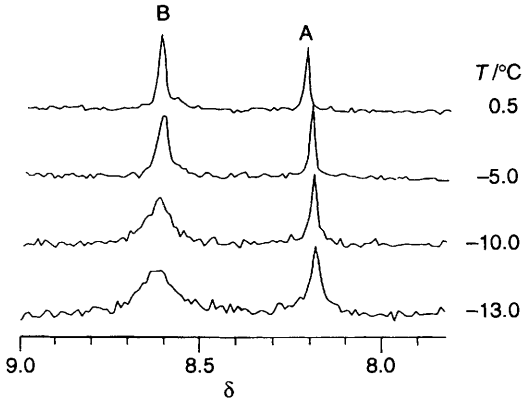

Fig. 4 The H^8 proton signals of free GMP (A) and *cis*-$[Pt(NH_3)_2(GMP)_2]$ (B) recorded in the temperature range 0.5 to −13 °C

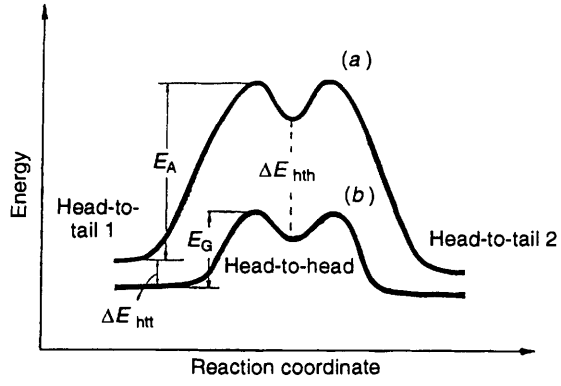

Fig. 5 A schematic representation of the rotational energy barrier for the conversion of one head-to-tail rotamer to the other for (*a*) bis(AMP)- and (*b*) bis(GMP)-platinum(II) complexes; E_A and E_G represent the activation energies for head-to-tail ⟶ head-to-head interconversions, ΔE_{htt} is the ground-state energy difference between the head-to-tail rotamers. The energy difference between the head-to-head configurations of AMP and GMP can be estimated as $\Delta E_A + \Delta E_{htt}$

Table 3 Activation energies related to Pt–N^7 purine bonds in various nucleotide and nucleoside complexes

Complex	Activation energy/kJ mol^{-1}
$[Pt(NH_3)(AMP)_2]$	70 ± 5[a]
	73 ± 5[b]
$[Pt(NH_3)_2(GMP)_2]$	25 ± 5[a]
$[Pt(NH_3)_2(made)_2]$	46 ± 3[a]
$[Pt(NH_3)_2(ADP)_2]$	89 ± 5[a]
$[Pt(NH_3)_2(ATP)_2]$	95 ± 10[a]

[a] From a one-dimensional experiment. [b] From two-dimensional EXSY methods.

The activation energies calculated from plots of ln *k* *vs.* $1/T$ are listed in Table 3. The ATP complexes exhibit the highest rotational energy barrier.

Discussion

The reaction of adenosine mononucleotides with *cis*-$[Pt(NH_3)_2Cl_2]$ proceed with N^7-co-ordinated intermediates (1 : 1) which upon further reaction formed two products.[18] Based on proton, ^{31}P, and ^{13}C NMR measurements we proposed several structures for the products all of which are N^7-bound platinum complexes. Further, it was suggested that the phosphate moiety may be involved in the co-ordination to Pt^{II} as well. Based on the reactions of deoxyadenine nucleotides with $[PtCl_2(en)]$ (en = ethane-1,2-diamine), Reily and Marzilli[19] pointed out that the co-ordination chemical shifts of ^{31}P NMR signals are not large enough to conclude any direct phosphate complexation in their and our systems. In the light of our recent work related to the complexation of platinum(II) with uridine[20] and cytidine nucleotides,[21] and with inorganic phosphates[22] where we have unequivocally established phosphate co-ordination, we concur with their assessment[19] that a large co-ordination chemical shift is warranted for direct phosphate binding. According to Marzilli's assignments, the two products observed in the *cis*-$[Pt(NH_3)_2Cl_2]$–AMP reaction are two diastereomers of the head-to-tail rotamer.

To convert one head-to-tail rotamer to the other through rotation about the Pt–N^7 bonds, head-to-head configurations must be encountered. A schematic potential-energy diagram is depicted in Fig. 5. Since no head-to-head diastereomer was detected, this conformation should lie in close proximity to the highest potential energy along the reaction coordinate. The observed activation energies then approximately represent the difference in ground-state energies between the head-to-head and head-to-tail rotamers. There is a small but reproducible intensity difference between the two AMP rotamers in the one-dimensional ^{31}P NMR spectra although the longitudinal relaxation times are about the same, which points to an unequal distribution of the two diastereomers. Two-dimensional EXSY experiments permit us to estimate two rate constants for the conversion of one head-to-tail rotamer to the other and *vice versa*. Therefore, the difference in activation energies associated with k_{AB} and k_{BA} would provide an estimate of the difference in ground-state energies of the two head-to-tail rotamers. An energy difference of ≈ 5 kJ mol^{-1} may be estimated between the ground states of these two rotamers.

The difference in activation energy associated with the interconversions of rotamers of AMP and GMP complexes can be related to the energy difference in their head-to-head configurations. As shown in Fig. 5 this energy difference should be equal to ΔE plus the ground-state energy difference between the head-to-tail configurations of the two nucleotides assuming that the head-to-head conformations are near the highest potential-energy surface. Basch *et al.*[23] estimated that the binding energy for the Pt–N^7 bond for the GMP complex is higher than that of the AMP complex. We, therefore, conclude that the energy difference between the two head-to-head configurations must be greater than 45 kJ mol^{-1} with the GMP complex at lower energy.

An analysis of activation energies for the diastereomers should aid us to understand specific interactions between platinum(II) and various nucleotides and nucleosides. First, the activation energy difference between AMP and 1-methyladenosine, 24 kJ mol^{-1}, must be due to the presence of the phosphate group. The phosphate groups of nucleotides have been shown to form hydrogen bonds with the hydrogen of the co-ordinated amine.[5,24] Hydrogen bonding along with the increased bulkiness should account for the additional activation energy of the AMP complex. Secondly, a systematic increase in activation energy from AMP to ADP and then to ATP should be attributed to the bulkiness of the phosphate groups. Finally, reasons for an increased rotational energy barrier, 45 kJ mol^{-1}, for the AMP compared to the GMP complexes should be addressed. This additional rotational energy barrier in the 6-aminopurine system cannot be of purely steric origin since both the 6-oxo- and 6-amino-purine nucleotides are of about the same size. Moreover, restricted rotation in a Pt^{II}–CMP complex containing only the pyrimidine base has been observed.[19] Further, the hydrogen bonding between the phosphate group and hydrogen atoms of co-ordinated amine must be present in both cases. This additional energy barrier in the AMP complexes must originate from the specific interactions between the platinum and exocyclic amine group. Hambley[10] in his molecular mechanics calculation stressed the importance of hydrogen bonding between the exocyclic oxygen and the co-

ordinated amine ligand in the head-to-head configuration. Such hydrogen bonding between the exocyclic amine and co-ordinated amine is not possible. However, this large energy difference cannot be attributed to the lack of hydrogen bonding only in the head-to-head configuration of the AMP complex. A 'pseudo-octahedral' environment positioning two NH_2 groups for two AMP molecules above and below the platinum plane may establish a weak interaction with the σ-bonding platinum orbitals. This weak interaction between the lone pair of the nitrogen and filled d_{z^2} orbital should lower the crystal-field stabilization energy of the aminopurine complexes as compared to the oxopurine complexes. Although a weak interaction may also exist in oxopurine complexes with two exocyclic oxygen atoms,[25] its magnitude must be substantially less than in the 6-aminopurine complexes.

An intrastrand binding by two adjacent guanine bases in DNA to *cis*-$[Pt(NH_3)_2Cl_2]$ is expected to result in a head-to-head conformation. Indeed, X-ray crystallographic data[5,6] from Lippard's and Reedijk's groups for short oligonucleotide complexes of *cis*-$[Pt(NH_3)_2Cl_2]$ establish such a configuration. The results presented here support the view that intrastrand binding through two adjacent adenine bases would be unfavourable by more than 45 kJ mol^{-1}.

Acknowledgements

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