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Rate of Displacement of Guanosine by Cyanide from *cis*-Diamminebis(guanosine)platinum(II) Chloride Determined by Using ^{13}C NMR Spectroscopy

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The rate of displacement of guanosine by cyanide from *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ has been determined by using the peak heights of ^{13}C NMR resonances, which are linearly related to concentrations. Cyanide displaces guanosine from platinum in *cis*-$[(NH_3)_2Pt(guanosine)_2]Cl_2 \cdot 2H_2O$ over the temperature range 298-313 K in a second-order process having an activation energy of 79 ± 2.8 kJ/mol. For this reaction, an estimation of the enthalpy of activation and entropy of activation gives 76 kJ/mol and −75 J/(K mol), respectively. The relatively slow rate of the reaction suggests that removal of platinum from inter- and intrastrand cross-links with DNA does not play a significant role in the various processes that utilize nucleophiles for controlling the adverse effects of *cis*-platinum.

Introduction

A variety of adverse effects ascribed to reactions with thiol groups[1] or to the accumulation of platinated nucleotides following strand repair[2] accompanies the clinical use of the common antineoplastic agent *cis*-diamminedichloroplatinum(II) (**1**). Extensive studies regarding the control of these adverse effects via treatments with other agents indicate a common use of sulfur-based nucleophiles. Thus, thiosulfate,[3] dithiocarbamates,[4] glutathione,[5] WR-2721,[6] and a variety of other thiols and thioethers are used,[7] especially to suppress the nephrotoxicity of **1**. Limitations exist for the time frame within which such agents must be administered in order to attain renal protection—if administered too early they will sometimes,[8] but not always,[9] destroy the antineoplastic activity of the *cis*-platinum; if administered too late, irreversible renal damage may occur.[10] The destruction of the antineoplastic activity may involve reaction with *cis*-platinum itself or with the nucleotide complexes that it forms with DNA. *cis*-Platinum binds preferentially to the N7 position of the guanine nucleobase, both mono- and bifunctionally,[11] as supported by the X-ray crystal structures of *cis*-$[Pt(NH_3)_2\{d(pGpG)\}]$[12a] and *cis*-$[Pt(NH_3)_2\{d(CpGpG)\}]$.[12b] A previous report using high-resolution NMR techniques indicated that the most likely structure in solution consisted of the two guanine bases coordinated through N7 and oriented in a head-to-head, anti conformation (as found in B-DNA) with a dihedral angle of about 60°.[13] Moreover, the head-to-head arrangement has been reported for complexes of the type *cis*-$[(NH_3)_2Pt(9\text{-}EtGH)_2]X_2$, for which the purine dihedral angles range from 68 to 78°.[14] Recent studies have explored further the conformational changes in nucleotides upon binding of platinum(II) complexes.[15] The formation of a bis(guanosine) complex as an intrastrand cross-link between two adjacent guanine bases in DNA is especially important, since it is proposed to play a vital part in the biological activity of such compounds. Dissociation of nucleosides from platinum complexes requires the presence of a competing ligand with a very strong trans effect, such as cyanide.[16] The present study determined the rate of removal of guanosine from *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ (**2**) by cyanide, the most effective of the few compounds previously reported capable of removing *cis*-platinum from DNA[17] and from oligonucleotides.[18] Marzilli and co-workers have examined the use of model compounds such as **2**, as well as related compounds, and have found them to mirror many of the most significant reaction patterns of platinated DNA.[19]

Experimental Section

cis-Diamminedichloroplatinum(II) was obtained from AESAR, and the guanosine, from Sigma Chemical Co. as the dihydrate; the guanosine was recrystallized from water before use. The *cis*-platinum guanosine adduct, *cis*-diamminebis(guanosine)platinum(II) chloride dihydrate (**2**) was prepared via a modification in previously reported procedures.[20] To 6 mmol of guanosine stirring in 100 mL of deionized water at 50–60 °C was added 3 mmol of **1**. The reaction vessel was covered with aluminum

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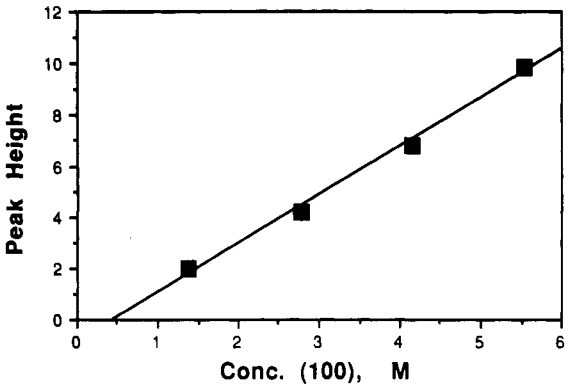

Figure 1. Graph of peak height (as signal to noise ratio) versus concentration for the ^{13}C NMR resonance corresponding to the C1′ carbon of ribose over the concentration range 1.38×10^{-2} to 5.54×10^{-2} at 298 K.

foil, to keep out light, and the solution stirred at 60 °C until a clear colorless solution was attained, signifying complete reaction. The solution was evaporated under reduced pressure to about half-volume; the resulting solution was placed in a refrigerator, and the white precipitate that formed was filtered off. This procedure was followed until no more precipitate formed upon refrigeration. The solution was then evaporated to approximately 20–30 mL at room temperature and subsequently lyophilized to give a white styrofoam-like solid, **2**. The UV and IR spectra of **2** are consistent with previously reported spectra and differ distinctly from those of free guanosine.[20a,21] Likewise, the ^1H NMR spectrum of **2** (including peak assignments) is consistent with proton NMR spectra reported elsewhere for this compound.[22] Anal. Calcd for **2**: C, 26.6; H, 3.99; N, 18.6. Found: C, 26.15; H, 4.16; N, 18.16. Platinum analyses using furnace atomic absorption were accurate within experimental error for this method.[23] A proton-decoupled ^{13}C NMR spectrum of **2** showed complete accordance with a previously published ^{13}C NMR spectrum of *cis*-$[(NH_3)_2Pt(Guo)_2]Cl_2$, for which numerical values of the resonances were not listed.[24] We obtained the following resonances (referenced to an external standard of DMSO at 39.5 ppm): 157.84 (C6), 155.57 (C2), 151.62 (C4), 140.95 (C8), 115.37 (C5), 89.72 (C1′), 86.58 (C4′), 75.01 (C2′), 71.04 (C3′), 62.04 (C5′) ppm. Assignments are based on a proton-coupled ^{13}C spectrum and are comparable to spectra reported for analogous complexes.[25]

^{13}C NMR Studies. The rate of displacement of guanosine by cyanide in 1:5, 1:7.5, 1:10, 1:11.25, 1:12.5, and 1:15 molar ratios of complex to cyanide was studied in unbuffered solutions (pH ≈ 10) at 298, 303, 308, and 313 K. Samples were prepared in 10-mm NMR tubes and dissolved in a mixture of 90% H_2O and 10% D_2O with DMSO as an external reference at 39.5 ppm. The spectra were obtained at 50.3 MHz on a Bruker 200-MHz instrument employing WALTZ ^1H decoupling[26] to prevent the sample from overheating. Use of a variable-temperature unit kept the temperature constant throughout the experiment. The reaction was followed by measuring the change in the ribose ^{13}C peak heights for the disappearance of **2** as a function of time, measured as a signal to noise ratio. Concentrations used for the 1:10 ratio were 2.77×10^{-2} M in complex and 2.77×10^{-1} M in KCN. Acquisition of spectra began immediately after mixing the above solutions. Each spectrum took 10 min to acquire, with a 30–60-min interval (depending on ratio and temperature) between acquisitions.

^1H NMR Studies. The samples were prepared by dissolving the complex, **2**, in D_2O and lyophilizing several times. In a glovebag under nitrogen, the "deuterated" complex and KCN were combined in a 1:1 and 1:10 mole ratio in D_2O (minimum isotopic purity of 99.996 atom % D) from Aldrich Chemical Co. The spectra were obtained at 300.13 MHz on a Bruker 300-MHz spectrometer and referenced to the HDO peak at 4.6 ppm. Presaturation was utilized to reduce the intensity of the residual HDO resonance.

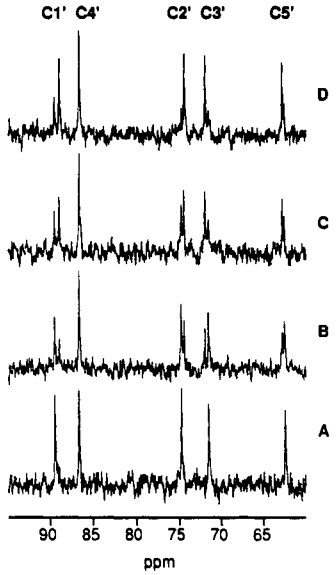

Figure 2. Representative ^{13}C NMR spectra with peak assignments showing the decrease in reactant and increase in product with time at 303 K for a 1:10 ratio of **2** to cyanide: (A) t = 15 min; (B) t = 135 min; (C) t = 255 min; (D) t = 435 min.

Calculations using the integrated form of the first-order rate equation

$$\ln [C]_t = -kt + \ln [C]_0 \quad (1)$$

where $[C]_t$ is the concentration of complex at time t and $[C]_0$ is the initial concentration of complex, provided pseudo-first-order rate constants for the disappearance of **2**. Determination of the activation energy followed from a plot of $\ln k$ vs T^{-1}. Both enthalpies and entropies of activation were calculated from standard equations.[27]

Results

The peak heights (at constant peak width) of the ^{13}C resonances for each of the ribose carbons in *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ were measured as a function of concentration over the range 1.38×10^{-2} to 5.54×10^{-2} M at 298, 303, 308, and 313 K. Figure 1 shows a representative plot of peak height vs concentration for the ^{13}C resonance of the C1′ carbon on ribose at 298 K. When fit to a linear regression, the concentration to peak height correlation coefficients for the ribose carbon atoms ranged from $r^2 = 0.947$ to $r^2 = 1.000$, indicating that the relaxation times of the carbons were not changing over this concentration range. As the substitution of cyanide for guanosine proceeds and the ^{13}C NMR resonances of ribose in **2** decrease steadily, a white solid ultimately begins to precipitate from solution.[28] At the same time, ^{13}C rsonances characteristic of the reaction product, *cis*-$[(NH_3)_2Pt(Guo)(CN)]^+$ (**3**), formed in eq **2** appear and increase steadily

$$cis\text{-}[(NH_3)_2Pt(Guo)_2]^{2+} + CN^- \rightarrow cis\text{-}[(NH_3)_2Pt(Guo)(CN)]^+ + Guo \quad (2)$$

(see Figure 2). Characterization by elemental analysis, melting point, and UV, IR, and ^{13}C NMR spectroscopy found the white solid that precipitated from solution to be free guanosine. It is important to note that the measurement of the reaction rate is stopped when guanosine begins to precipitate, thereby making the reaction shown in eq 2 the sole reaction occurring over the time of the study. Moreover, no other species are observed either in the ^{13}C NMR or in the ^1H NMR spectra during this time. A ^{13}C NMR spectrum was obtained (*several days after initiation of reaction*) on the freeze-dried filtrate resulting from the removal

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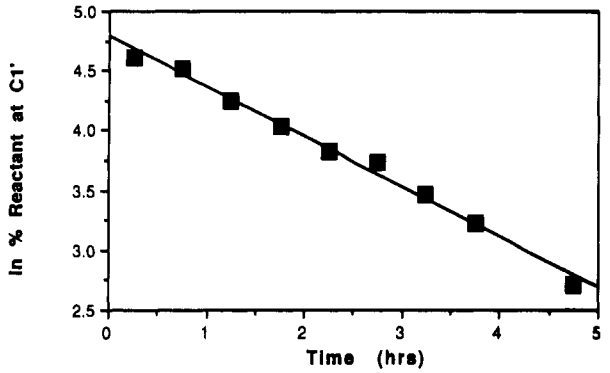

Figure 3. Plot of ln (% reactant) for the C1′ ribose carbon versus reaction time for a 1:10 ratio of **2** to cyanide, using ^{13}C NMR data at 308 K.

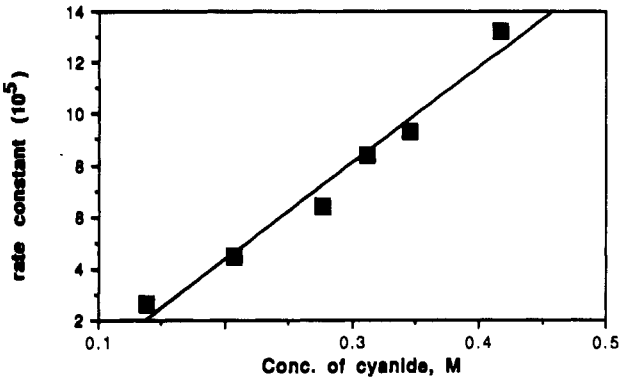

Figure 4. Graph of pseudo-first-order rate constant versus initial cyanide concentration for 1:5, 1:7.5, 1:10, 1:11.25, 1:12.5, and 1:15 mole ratios at 303 K.

of the guanosine precipitate from the 1:10 ratio reaction mixture; it showed resonances similar in chemical shift to those already assigned as the ribose carbons of the starting complex, **2** (see Experimental Section), and a peak at 126 ppm, characteristic of the resonance expected for $Pt(CN)_4^{2-}$. Furthermore, the assignment of this peak as the tetracyanoplatinate ion is supported by two other facts: platinum satellites are observed for this resonance and a proton-coupled ^{13}C NMR spectrum shows that this is a quaternary carbon. A peak at 167 ppm corresponding to the value for KCN in water was present in the ^{13}C NMR spectrum of the filtrate, also. An elemental analysis of the solid material resulting from lyophilization of this filtrate indicates a mixture of species may be present.

In order to verify the presence of **3**, a 1:1 mole ratio of **2** and KCN was mixed and allowed to react only until a white precipitate (guanosine) formed. After removal of the guanosine precipitate, a ^{13}C NMR spectrum of the lyophilized filtrate showed resonances at 158.88, 156.33, 151.39, 140.52, 115.39, 89.65, 86.39, 74.85, 70.94, and 61.96 ppm, which are different from those of the starting complex, **2**, and of free guanosine. Similarly in the ^1H NMR spectrum, peaks are observed that are characteristic of guanosine but are at different chemical shifts than the guanosine resonances either in the starting complex or in free guanosine. In addition, neither the ^{13}C NMR nor the ^1H NMR spectra show evidence of other species being present at this stage of reaction—as expected, since there is not an excess of cyanide. The freeze-dried filtrate from the equimolar reaction mixture had carbon, hydrogen, and nitrogen analyses of the sort expected for a mixture of **2** and **3**.

The order of the reaction with respect to both *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ and CN^- was determined. Figure 3 shows a typical pseudo-first-order plot for the ^{13}C resonances corresponding to C1′ of the ribose of **2** in the presence of a 10-fold excess of cyanide. In addition, the rate constants for this reaction at 303 K were determined in solutions with 5-fold, 7.5-fold, 11.25-fold, 12.5-fold, and 15-fold excesses of cyanide and found to be 2.6 (±0.2) × 10^{-5}, 4.5 (±0.5) × 10^{-5}, 8.4 (±1.1) × 10^{-5}, 9.3 (±0.5) × 10^{-5}, and 1.3 (±0.2) × 10^{-4} s^{-1}, respectively. The graph of pseudo-first-order rate constants for various mole ratios of complex to initial cyanide concentration, shown in Figure 4, indicates a second-order rate constant of 2.5 (±0.4) × 10^{-4} L mol^{-1} s^{-1} for this process at 303 K.

Table I. Rate Constants and Activation Parameters[a]

T, K	k, s^{-1}	ΔH^*, kJ/mol	ΔS^*, J/(K mol)
298	3.1 (±0.2) × 10^{-5}	76.2 ± 2.8	−75.6 ± 9.2
303	5.5 (±0.5) × 10^{-5}	76.2 ± 2.8	−75.2 ± 9.0
308	9.6 (±1.7) × 10^{-5}	76.2 ± 2.8	−75.0 ± 10.0
313	1.5 (±0.4) × 10^{-4}	76.1 ± 2.8	−76.1 ± 8.9

[a] Averages of the rate constants and activation parameters (with standard deviations) over at least three trials at each temperature, for the ribose carbons in the reaction of 2.77 × 10^{-2} M complex with 2.77 × 10^{-1} M cyanide.

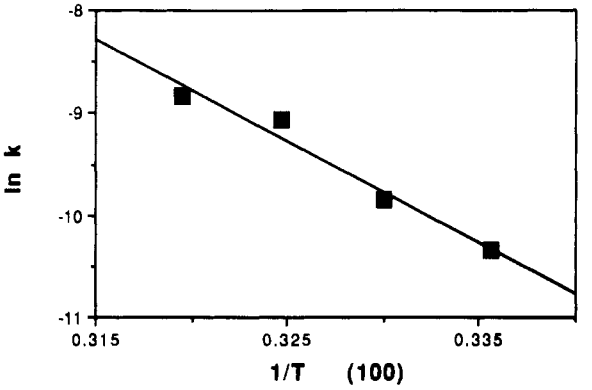

Figure 5. ln k versus T^{-1} for the ^{13}C NMR resonances corresponding to the C1′ carbon on ribose following reaction of **2** with a 10-fold excess of cyanide.

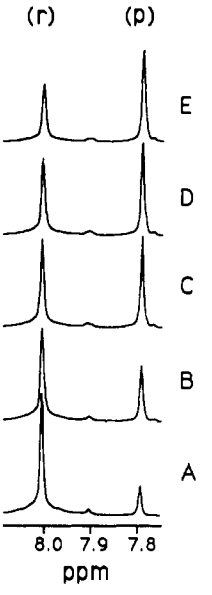

Figure 6. ^1H NMR spectra showing the changes in the H8 proton as the reactant peak (r) decreases and the product peak (p) increases over time, for a 1:10 ratio of **2** to cyanide at 303 K: (A) t = 15 min; (B) t = 75 min; (C) t = 140 min; (D) t = 170 min; (E) t = 230 min.

Our ^{13}C NMR data, obtained on the initial stages of the first step of the reaction (e.g. eq 2) of a 1:10 ratio of **2** to cyanide, provided the pseudo-first-order rate constants shown in Table I. The activation parameters listed in Table I were obtained from a plot of the natural logarithm of the rate vs T^{-1} (see Figure 5). In addition, the ^1H NMR spectra for a 1:10 mole ratio of **2** to KCN at 303 K were collected over the same time frame as the ^{13}C NMR spectra to see if the rate data were comparable. The rate was determined by studying the C8 proton, since it was the proton most affected by a change in the environment around the

platinum, as expected (see Figure 6). No significant changes in the ribose protons were evident. As the reaction proceeds, the C8 proton peak of **2** decreases as a new (product) peak forms; the observed rate of 4.88×10^{-5} s^{-1} is within the standard deviation of the rate reported in Table I for the ribose carbons at 303 K by using ^{13}C NMR spectroscopy.

Discussion

Before discussing the results obtained by using ^{13}C NMR spectroscopy, it is important to address why this technique was preferred over UV spectrometry. UV studies proved unsatisfactory for following these reactions because the shift in wavelength was not significant enough to distinguish between the area under the complex, **2**, and that under the complex plus cyanide, **3**, in that the latter was simply a shoulder on the former.

The present study uses ^{13}C NMR spectroscopy to study the kinetics of the reaction of **2** with cyanide in H_2O. Quantitative integration of proton-decoupled ^{13}C resonances is complicated because carbon atoms with long spin–lattice relaxation times (T_1) may not completely return to a Boltzmann distribution between pulses, thus resulting in signals that are considerably weaker than expected from the number of carbons responsible for those signals. Therefore, only the ribose carbons, which relax most readily, were used in obtaining the data for the present study, thus minimizing the spectral acquisition time—a crucial requirement for kinetic studies. Since only the relative integral of a single peak is considered, it is essential only that the relaxation time for the peak being measured remain constant. Also, it is essential that T_1 and T_2 (the spin–spin relaxation time) stay constant during the course of the reaction. The collection of data ended prior to the formation of a precipitate, such that T_2 would not change due to a change in the homogeneity of the sample.

The ^{13}C NMR spectra of the filtrate from the reaction mixtures containing an *excess* of cyanide ions were examined after longer reaction times than those used for the collection of kinetic data. These suggest the formation of several products, including $[(NH_3)_2Pt(Guo)(CN)]^+$ (**3**). The reaction was allowed to proceed for this extended period of time so that subsequent reaction products might appear in the NMR spectrum and thus enable them to be identified. Knowing the chemical shifts of these subsequent products allowed a means by which to ensure that the collection of data was halted prior to their formation. Similarly, the formation of the guanosine precipitate, which caused a decrease in the lock signal, indicated that the first step of the reaction was proceeding as expected. Once **3** is formed, the ligands cis and trans to the cyanide become strongly labilized, thereby facilitating further reaction. Lippert and Raudaschl-Sieber report that using 2 equiv of KCN for every 1 equiv of *trans*-$[(NH_3)_2PtG(Cl)]^+$ yields *trans*-$(NH_3)_2Pt(CN)_2$ as well as several mixed cyanide–nucleobase–ammine complexes and eventually $Pt(CN)_4^{2-}$ when all of the original ligands are replaced.[17] Thus, in our reaction mixtures that contained a large excess of cyanide, resonances corresponding to $Pt(CN)_4^{2-}$ as well as to CN^- should have been observed in the ^{13}C spectrum, as they were.

A ^{13}C and a ^1H NMR spectrum of the filtrate from an *equimolar* mixture of **2** and KCN were taken immediately after precipitation of the guanosine to determine the product formed by the reaction over the course of time of the kinetic data collection. Since a 1:1 ratio can only substitute one of the guanosines with a cyanide and the guanosine precipitates, the species remaining in solution should be *cis*-$[(NH_3)_2Pt(Guo)(CN)]^+$, thereby enabling this species to be isolated and identified. The reaction in eq 2 is not likely to proceed in the reverse direction due to the low concentration of guanosine in solution and the high thermodynamic stability of the platinum–ammine linkage.[29] In addition, the elemental analysis of the lyophilized filtrate from the equimolar mixture corresponds to complexes that contain at least one guanosine; this supports the point that the rate data were obtained under conditions where the second guanosine had not been replaced yet. Studies by Leng et al. indicate that a similar adduct, *cis*-$[Pt(NH_3)_2(dGuo\text{-}N7)(CN)]^+$, is the likely intermediate species in the reaction between cyanide and a platinated double-strand oligonucleotide, for which the cyanide ions react selectively with the 5′-guanosine residues, at a rate k_1, to give the intermediate before subsequent reaction, at rate k_2, to give the unplatinated double-strand oligonucleotide.[30]

The activation parameters given in Table I for $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ compare well to values found for displacement of similar platinum(II) complexes.[31] The entropy of activation for this reaction is −75 J/(K mol), a value which suggests that a considerable degree of steric rearrangement is necessary to attain the transition state. In view of the structure of **2**, considerable movement of the guanosine is expected to occur during substitution. Entropies of activation for substitution process in square-planar complexes tend to be negative because of the associative mechanism by which they proceed.[32] Few previous studies pertaining to the determination of the activation parameters for the displacement of a large ligand, such as guanosine, are available.

Our results suggest that ^{13}C NMR peak heights may provide an opportunity for the estimation of reaction rates that are otherwise difficult to obtain. This technique could prove useful when rate constants are needed in aqueous solutions for reactants with exchangeable hydrogen atoms. The cyanide ion is one of the best entering groups for platinum(II) substitution reactions[33] and can be expected to displace a guanosine at least as rapidly as the nucleophiles typically used to control the nephrotoxicity of *cis*-platinum. Also, the cyanide ion has an extremely large trans effect and is a rather poor leaving group. The reaction of cyanide with *cis*-$[(NH_3)_2Pt(Guo)_2]^{2+}$ examined here was distinctly slower than the reaction of *cis*-$[(NH_3)_2PtCl_2]$ with thiosulfate,[34] glutathione,[35] or diethyldithiocarbamate.[35] These differences in rates indicate that the principal route by which such nucleophiles can decrease the antitumor effect of *cis*-platinum is via direct reaction with the parent compound, rather than by reactions in which they displace platinum from DNA. Moreover, the fate of platinated nucleic acids subsequent to DNA repair processes may be affected by the continuing presence of platinum. One can expect that metabolism of platined nucleotides will be subject to considerable interference, as previously described by Muller and Holler for several enzymatic processes.[2]

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