The Phosphodiester Groups of d(TpT)$^-$ and d(TpG)$^-$ Coordinate to Platinum(II) in *N*,*N*-Dimethylformamide

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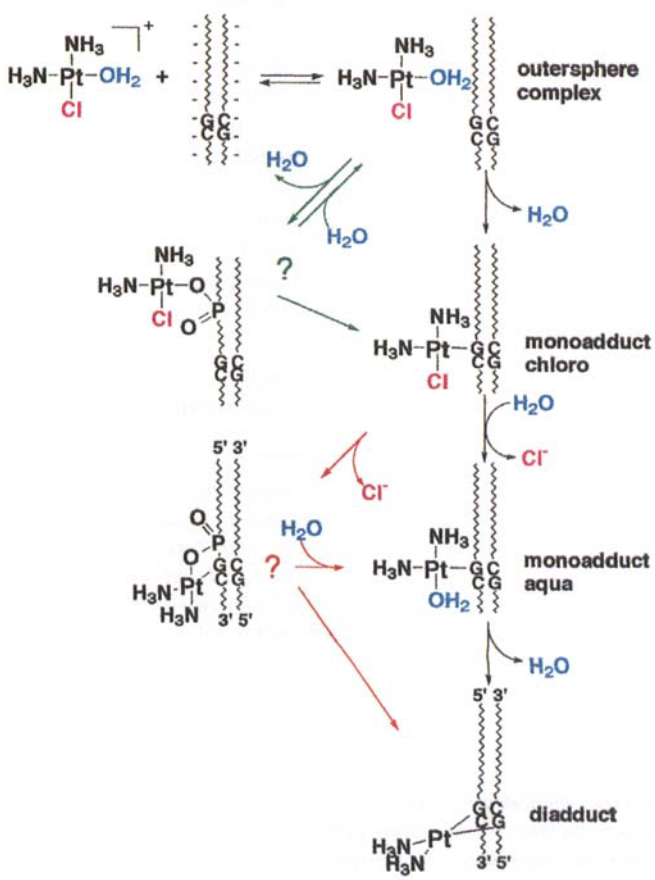
Abstract: The propensity of the phosphodiester group to bind platinum(II) was investigated by examining the reactions of the dinucleotide salts NH_4{d(TpT)} and NH_4{d(TpG)} with the complexes [Pt$(NO_3)(NH_3)_3]NO_3$ (**1**) and *cis*-[Pt$(NO_3)_2(NH_3)_2$] (**2**) in *N*,*N*-dimethylformamide. d(TpT)$^-$ coordinates to the platinum atom of both **1** and **2** through either of the two nonbridging phosphoryl oxygen atoms, as revealed by ^{31}P NMR spectroscopy. Conversely, d(TpG)$^-$ binds to **1** through the guanine N7 atom. With **2**, d(TpG)$^-$ forms monodentate adducts bound to N7(G), as well as two distinct macrochelates, in which N7(G) and one of the nonbridging phosphoryl oxygens are crosslinked by platinum. We suggest that guanine–phosphoryl macrochelate complexes play a role as intermediates in rearrangements of platinum monoadducts formed with DNA.

Keywords
antitumor agents · DNA · NMR spectroscopy · platinum

Introduction

The antitumor drug cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) reacts with DNA to form guanine–guanine and guanine–adenine crosslinks as major lesions in the molecule.[1] It is accepted that prior to reacting with a nucleobase, *cis*-[$PtCl_2(NH_3)_2$] has to be hydrolyzed to its monocationic {*cis*-[$PtCl(NH_3)_2(H_2O)]^+$} or dicationic {*cis*-[$Pt(NH_3)_2(H_2O)_2]^{2+}$} form. Accordingly, reactions between these cationic complexes and (oligo)nucleotides have been extensively studied as model systems for the more complicated interactions with DNA.[2] These studies have shown that platinum is coordinated almost exclusively by the N7 atoms of guanines and adenines. Phosphodiester binding has never been reported; this agrees with the general observation that the "soft" metal ion Pt^{II} has a greater affinity for polarizable nitrogen donors than for "hard" oxygen ligands.[3] However, this does not rule out the possibility of phosphodiester–platinum binding in transient reaction intermediates. It is, for instance, conceivable that the cationic complex associated with the polyanionic DNA molecule by electrostatic forces (outer-sphere complex in Scheme 1) would react with one of the phosphodiester groups located on the surface of the double helix, forming a transient phosphodiester-coordinated complex (green arrows in Scheme 1), and subsequently rearrange to a



Scheme 1. Reaction between *cis*-[$PtCl(NH_3)_2(H_2O)]^+$ (the monocationic form of the antitumor drug *cis*-[$PtCl_2(NH_3)_2$]) and DNA. Black arrows: generally accepted reaction steps [28–30]. Green arrows: hypothetical phosphodiester-mediated pathway for monoadduct formation. Red arrows: hypothetical phosphodiester-assisted path for mono-to-diadduct conversion via a guanine–phosphoryl macrochelate.

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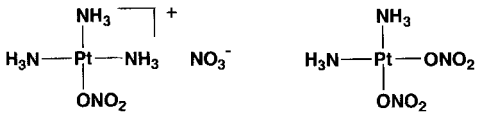
stable nucleobase-bound monoadduct. Alternatively, the conversion of the latter to a diadduct could be assisted by an adjacent phosphodiester group (red arrows in Scheme 1).

Whether or not phosphodiester-bound intermediates play a role in the reactions between platinum complexes and DNA depends primarily on the inherent propensity of the phosphodiester group to bind a Pt^{II} center. So far, no evidence for platinum–phosphodiester coordination has been demonstrated, although platinum complexes of dianionic phosphate monoesters are known.[4–7] In an attempt to detect platinum–phosphodiester binding, we have studied the reactions of two dinucleotide salts $NH_4\{d(TpT)\}$ and $NH_4\{d(TpG)\}$ with the monofunctional complex $[Pt(NO_3)(NH_3)_3](NO_3)$ (**1**) and the bifunctional complex *cis*-$[Pt(NO_3)_2(NH_3)_2]$ (**2**) in *N*,*N*-dimethylformamide (DMF). We find that in this low-dielectric solvent, platinum–phosphodiester coordination is readily observed by ^{31}P NMR.[8]



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Results

The phosphodiester group of d(TpT)$^-$ binds quantitatively to 1 and 2: d(TpT)$^-$ was chosen for this study since thymine is known not to react with platinum unless it is deprotonated.[11] Thus, in DMF the only potential sites available for platinum binding are the two nonbridging phosphoryl oxygen atoms, whose eventual coordination is expected to shift the ^{31}P NMR signal downfield.[7, 12, 13] The progress of the reaction between $NH_4\{d(TpT)\}$ and **1** can be conveniently followed by monitoring the change in the downfield region of the ^1H NMR spectrum (Figure 1). The resonance of the H3 imino protons of thymine at $\delta = 12.2$ is broad in $NH_4\{d(TpT)\}$ due to exchange with the ammonium protons (for atom numbering in thymine and guanine, see Scheme 2). Upon reaction with **1**, the signal sharpens, and after about 1 d at 25 °C, it splits into two signals at $\delta = 11.20$ and 11.25. This suggests that ion-pairing with NH_4^+ no longer takes place, which is in agreement with the formation of the cationic complex $[Pt(NH_3)_3\{d(TpT)\}]^+$. The ^{31}P NMR spectrum recorded at the end of the reaction (Figure 2) shows two singlets of roughly equal intensity at $\delta = 8.75$ and 8.79, that is, 8.6 ppm downfield from the signal of free $NH_4\{d(TpT)\}$ in DMF ($\delta = 0.13$). These observations indicate approximately equimolar formation of the two isomeric complexes $[Pt(NH_3)_3\{d(TpT)\text{-}OP\}]^+$, in which either terminal oxygen of the phosphodiester group is bound to platinum in a monodentate fashion. The complete disappearance of the peak due to unreacted

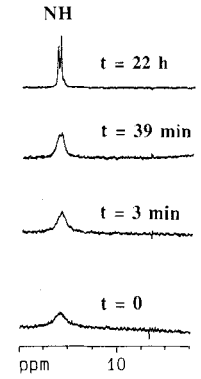
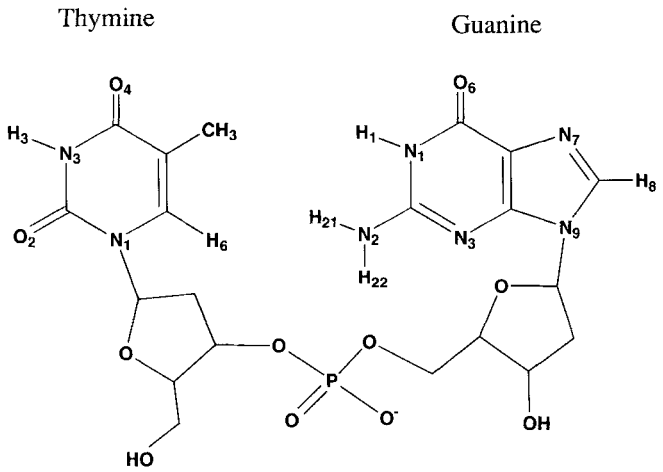


Figure 1. Downfield section of the ^1H NMR spectrum of 1.8×10^{-3} M $NH_4\{d(TpT)\}$ in $[D_7]$DMF, alone (bottom spectrum) and at the indicated time intervals after addition of an equimolar amount of **1** ($T = 25$ °C, scale unit = 0.5 ppm).



Scheme 2. Atom connectivity for d(TpG)$^-$ with atom numbering scheme for thymine and guanine.

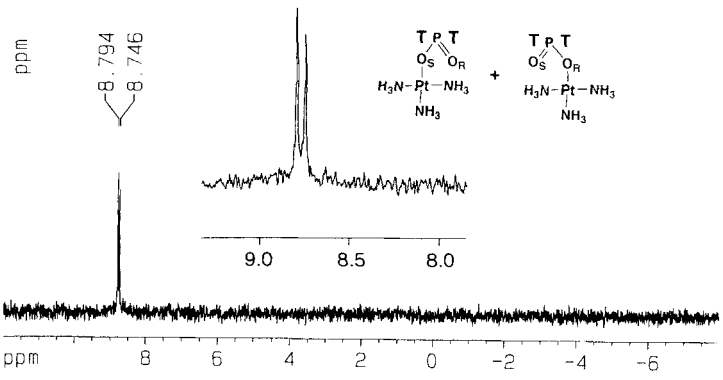


Figure 2. ^{31}P NMR spectrum of the reaction mixture of 1.8×10^{-3} M $NH_4\{d(TpT)\}$ with an equimolar amount of **1** after 1 d incubation at 25 °C. Insert: downfield region of the spectrum and proposed assignment of the two peaks.

Abstract in French: *L'aptitude du groupement phosphodiester à se fixer sur le platine(II) a été examinée en étudiant les réactions entre les sels d'ammonium de d(TpT)$^-$ et d(TpG)$^-$ et les complexes $[Pt(NO_3)(NH_3)_3]NO_3$ (**1**) et cis-$[Pt(NO_3)_2(NH_3)_2]$ (**2**) dans le N,N-diméthylformamide. La coordination du groupement phosphoryle de d(TpT)$^-$ au platine par l'un ou l'autre des atomes d'oxygène terminaux a été mise en évidence pour les deux complexes par spectroscopie RMN ^{31}P. Par contre, d(TpG)$^-$ se lie au platine de **1** par l'atome N7 de la guanine. Avec **2**, d(TpG)$^-$ forme, outre les complexes monodentes coordinés par N7(G), deux macrochélates où le platine ponte N7(G) et l'un des oxygènes terminaux du phosphoryl. Nous proposons un mécanisme pour les réarrangements des monoadduits de platine sur l'ADN, dans lequel des macrochélates guanine-phosphoryl joueraient un rôle d'intermédiaires.*

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d(TpT)$^-$ shows that the reaction is quantitative. A similar picture is obtained on reacting NH_4{d(TpT)} with a stoichiometric amount of **2** (Figure 3), except that the reaction is significantly faster, proceeding to completion within 2–3 h at 25 °C.

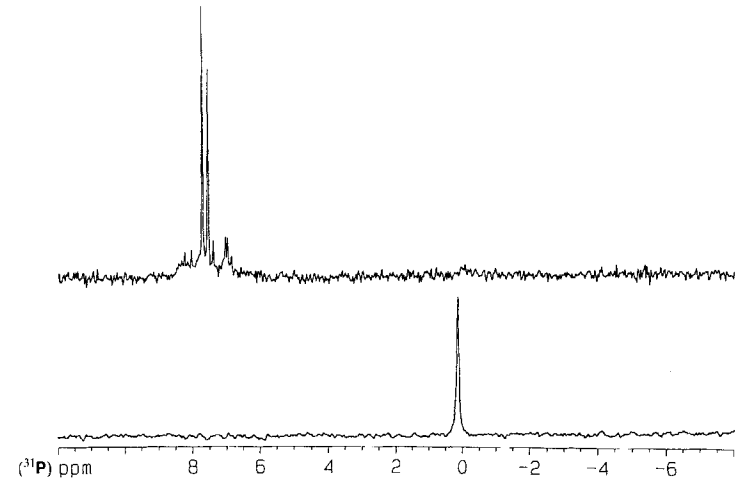

Figure 3. ^{31}P NMR spectrum of 1.7×10^{-3} M NH_4{d(TpT)}, alone (bottom) and after reaction with an equimolar amount of **2** in [D_7]DMF at 25 °C for 5 h (top).

d(TpG)$^-$ binds to 1 through guanine N7: In the ^{1}H NMR spectrum of NH_4{d(TpG)}, the imino proton resonances of the bases and the ammonium resonance appear as broad features between $\delta = 10$ and 12, and between $\delta = 7.5$ and 8, respectively (Figure 4a), indicating that NH_4^+ and d(TpG)$^-$ are largely undissociated in DMF, as was observed for NH_4{d(TpT)} (vide supra). The thymine H6 appears as a single peak at $\delta = 7.79$, whereas the guanine H8 resonance is masked by the DMF signal at $\delta = 8.0$. Mixing of approximately equimolar amounts of NH_4{d(TpG)} and **1** in [D_7]DMF resulted after a few minutes in the formation of a species characterized by a downfield H8 signal ($\delta = 9.1$). A subsequently recorded ^{31}P NMR spectrum revealed that NH_4{d(TpG)} ($\delta_P = +0.38$) had been quantitatively converted into a product showing a single peak at $\delta_P = -0.63$. The downfield shift of the H8 signal and the upfield shift of the phosphorus signal indicate that the product has the $Pt(NH_3)_3^{2+}$ group bound to the guanine N7 atom, the phosphodiester group remaining uncoordinated. Further addition of about 1 equiv of **1** shifted the H8 signal slightly downfield (probably a counterion effect) but did not lead to a downfield ^{31}P NMR signal. Thus, the phosphodiester group of the cationic complex $[Pt(NH_3)_3${d(TpG)-*N7*}]$^+$ does not readily coordinate a second equivalent of $Pt(NH_3)_3^{2+}$.[14]

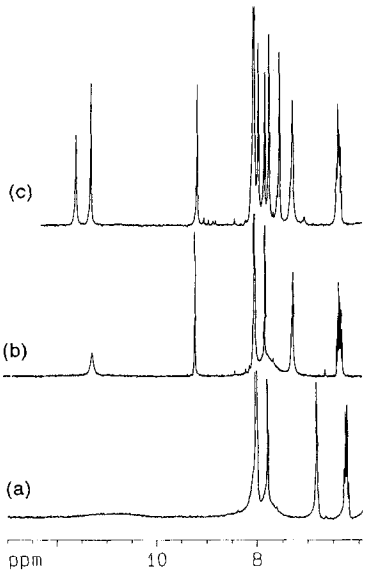

Figure 4. a) Downfield part of the ^{1}H NMR spectrum of 1.9×10^{-3} M NH_4{d(TpG)} in [D_7]DMF before reaction. b) Same sample after 30 min incubation at 25 °C with about 2 equiv of **1**. c) Same sample after an additional 1 d incubation at 60 °C.

The downfield region of the ^{1}H NMR spectrum of $[Pt(NH_3)_3${d(TpG)-*N7*}]$^+$ (Figure 4b) reveals an interesting detail: whereas the H8 resonance at $\delta = 9.17$ appears as a sharp peak, the thymine H3 and guanine H1 imino protons give a relatively broad and unresolved signal near $\delta = 11.2$. This, together with the observation of a broad feature near $\delta = 7.7$, where the ammonium resonance is expected, indicates that the anionic phosphodiester group is still ion-paired with ammonium, even though the total charge of the complex is +1. Heating at 60 °C for 24 h, however, transformed the broad signal at $\delta = 11.2$ into two sharp peaks at $\delta = 11.23$ and 11.52 (Figure 4c). The concomitant appearance of the well-resolved 1:1:1 triplet of NH_4^+ centered at $\delta = 7.70$ showed that ammonium was no longer associated with the dinucleotide. Our interpretation of these observations is as follows: the hydrogen-bonding/electrostatic association between NH_4^+ and the phosphodiester group of d(TpG)$^-$ is sufficiently tight in DMF to resist coordination of $Pt(NH_3)_3^{2+}$ by guanine N7. Only upon heating is this association disrupted, and NH_4^+ becomes solvated or pairs with NO_3^-. Conversely, when **1** reacts with NH_4{d(TpT)}, platinum binding to the phosphodiester group necessitates dissociation of NH_4^+ from d(TpT)$^-$.

Reaction between d(TpG)$^-$ and 2; evidence for a guanine-phosphoryl platinum macrochelate: As shown in Figure 4a, the signals due to the thymine H3 and guanine H1 imino protons are broad in the ^{1}H NMR spectrum of NH_4{d(TpG)}. Upon addition of about 1 equiv of **2**, considerable sharpening of both signals was observed, with concomitant appearance of a number of smaller peaks between $\delta = 8$ and 9 (Figure 5a) characteristic of H8 protons of N7(G)-coordinated species. Incubation at room temperature for 1 d led to increased intensity and further sharpening of the signal at $\delta = 11.2$, disappearance of that at $\delta = 10.7$ (thus, the T and G imino resonances overlap due to a downfield shift of the H1(G) signal,[15] which reflects guanine coordination to platinum), and, later, the appearance of two small peaks at $\delta = 11.46$ and 11.50 (Figure 5b,c). These changes were accompanied by an increase of the integrated area between $\delta = 8$ and 9, whereby two distinct peaks of approximately equal intensity appeared at $\delta = 8.51$ and 8.64.

In the ^{31}P NMR spectrum (Figure 6), the nearly complete disappearance of the initial peak and simultaneous appearance of a broad, composite signal centered at $\delta = -0.5$, together with two fine peaks at $\delta = 2.32$ and 3.88, were observed. These observations indicate that the reaction between NH_4{d(TpG)} and **2** yields a mixture of N7(G)-coordinated products. The broad ^{31}P resonance near $\delta = -0.5$ is assigned, in analogy to the monoadduct formed between **1** and d(TpG)$^-$, to monoden-

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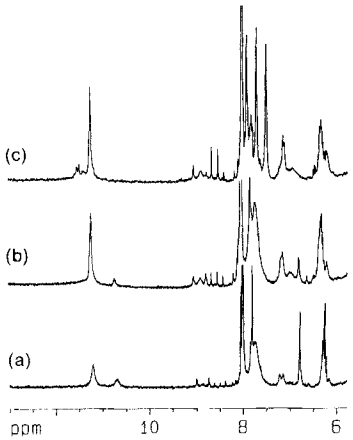


Figure 5. a) Downfield part of the ^{1}H NMR spectrum of 1.9×10^{-3} M NH_4{d(TpG)} in [D_7]DMF at 25 °C 10 min after mixing with about 1 equiv of **2**. b) Same sample after additional 20 min at 25 °C. c) Same sample after additional 14 h at 25 °C.

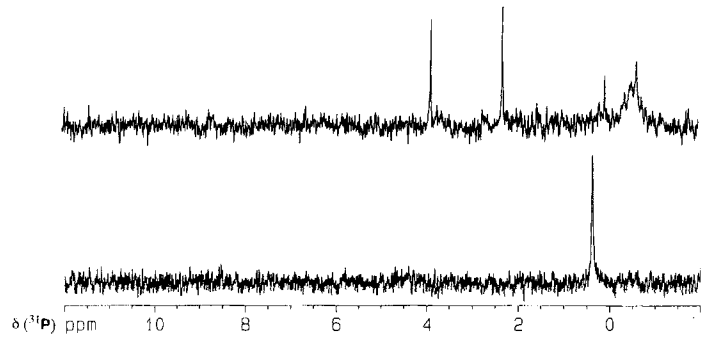
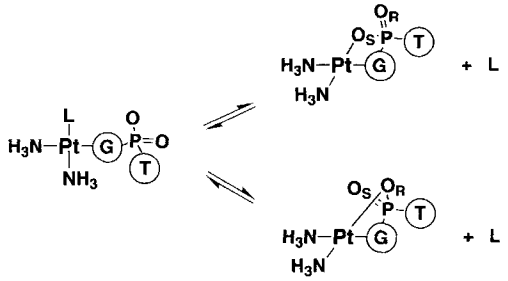


Figure 6. ^{31}P NMR spectrum of 1.9×10^{-3} M NH_4{d(TpG)} in [D_7]DMF at 25 °C, alone (bottom) and 30 h after mixing with about 1 equiv of **2** (top).

tate adducts bound to guanine but not to the phosphodiester group. These include the monoadducts bearing the N7(G)-bound *cis*-$[Pt(NO_3)(NH_3)_2]^+$ and *cis*-$[Pt(NH_3)_2(DMF)]^{2+}$ moieties,[16] and, possibly, the bis{d(TpG)} adduct *cis*-$[Pt(NH_3)_2\{d(TpG)\}_2]$, which may form even under stoichiometric conditions.[17] The two fine downfield ^{31}P signals are attributed to two different macrochelates in which platinum crosslinks N7(G) and either nonbridging phosphoryl oxygen atom.[18] This assignment is supported by the observation of a similar coordination shift ($\Delta\delta = 3.5$) for the analogous macrochelate formed between *cis*-$[Pt(CH_3NH_2)_2(H_2O)_2]^{2+}$ and 5′-IMP^{2-} in neutral aqueous solution.[5] The considerably smaller coordination shift ($\Delta\delta = 2.7$–4.3) of the phosphorus signals in the macrochelate species, as compared to that seen for the monofunctional adducts of **1** and **2** with $d(TpT)^-$ ($\Delta\delta = 7.4$–8.6), is consistent with a strained macrocyclic structure,[5, 6] which is expected to reduce the platinum–ligand orbital overlap and thus the ligand-to-platinum electron transfer. In addition, strain-induced variations in O-P-O bond angles could also considerably affect the ^{31}P chemical shift.[19]

Since the platinum–phosphodiester bond is expected to be labile, we interpret our observations in terms of an equilibrium between the guanine-bound monodentate complexes and the two isomeric guanine-phosphodiester macrochelates (Scheme 3).



Scheme 3. Equilibrium between a guanine-bound monodentate complex and two isomeric guanine–phosphodiester macrochelates (L = NO_3^-, DMF; charges omitted).

Discussion

Because of the "soft" character of the Pt^{II} ion, the typical DNA binding sites of platinum(II) are the heterocyclic nitrogen atoms of the bases, particularly the guanine N7 atoms. However, as noted previously by Sigel,[20] decreasing solvent polarity considerably enhances the affinity of metal ions for negatively charged moieties such as phosphodiester groups. It is therefore conceivable that in the densely packed cell nucleus, where the dielectric screening is expected to deviate from that in bulk water, interactions with the phosphodiester groups could play a significant role in the formation and/or processing of metal–DNA adducts (see Scheme 1).

On the basis of this idea and with the DNA-binding properties of platinum antitumor complexes in mind,[1, 21] we have examined in the present work the propensity of the phosphodiester group of $d(TpT)^-$ and $d(TpG)^-$ to bind platinum in the complexes **1** and **2** in DMF. As the first main result, we have found that in DMF, both **1** and **2** bind quantitatively to the nonbridging phosphoryl oxygen atoms of $d(TpT)^-$. This shows that under the conditions of moderately low dielectric screening ($\varepsilon_{DMF} = 36.7$), Pt^{II} has a non-negligible affinity for the phosphodiester group. Thus, although platinum binding occurs through guanine N7 in the complex formed between **1** and $d(TpG)^-$, we consider that transient binding to phosphodiester groups prior to base coordination (green arrows in Scheme 1) cannot be ruled out in reactions between cationic platinum complexes and DNA.

The second major finding of this study is that the *cis*-bifunctional platinum complex **2** (the dinitrato form of the antitumor drug cisplatin) forms a macrochelate with $d(TpG)^-$ in which the guanine N7 and either terminal phosphoryl oxygen are bound to platinum. Since in a platinum monoadduct bound to a guanine N7 within duplex DNA, the flanking phosphodiester group to the 5′-side is particularly well situated to carry out a nucleophilic attack on the platinum atom through one of its terminal oxygen atoms (Figure 3 in ref. [10]), such macrochelate complexes should be considered as possible intermediates in the monoadduct-to-diadduct conversion (red arrows in Scheme 1). The pathway via the macrocyclic complex would be entropically favored over bimolecular attack of a solvent molecule and would provide an explanation for the "catalytic effect" which double-stranded DNA has been reported to exert on reactions of various types of platinum monoadducts.[21] In particular, the macrochelate pathway plausibly explains the observed dependence of the monoadduct-to-diadduct conversion rate on se-

0947-6539/97/0309-1408 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 9

quence:[22] the stacking of the platinated guanine with its adjacent bases depends on the nature of these bases, as does the exact position of the 5′-phosphodiester group with respect to platinum. Thus, provided that nucleophilic attack of the phosphodiester group on platinum is the rate-determining step, the rate of the monoadduct-to-diadduct conversion should be sequence dependent, as is observed.

Based on the above arguments, we hypothesize that in reactions between platinum complexes and DNA, the phosphodiester groups of DNA could play the role of nucleophilic catalysts.

Experimental Section

The platinum complexes **1**[23] and **2**[24] were prepared according to published methods and predissolved in a small volume of [D_7]DMF before addition to the dinucleotide solutions. The dinucleotides (Sigma) were used without further purification. The NMR spectra were recorded on Bruker ARX250 and AC100 spectrometers. Typically, a 45° pulse and a 2–3 s repetition delay were used for the acquisition of the ^{31}P NMR spectra. Chemical shifts are referenced to external 85% aqueous H_3PO_4 (^{31}P) and to the downfield DMF signal set to $\delta = 8.000$ (^{1}H). The spectra reproduced in the figures were recorded with the ARX250 spectrometer, except for Figure 3 (AC100).

The low-field instrument (AC100) was employed in a search for ^{31}P–^{195}Pt coupling, since ^{195}Pt chemical shift anisotropy relaxation, whose contribution to the relaxation rate increases with B_0^2, can lead to the disappearance of doublets due to ^2J(Pt,X) coupling.[25] However, no splitting of the ^{31}P NMR signals was observed for any of the adducts. It is possible that additional broadening of the satellites due to ^{31}P–^{195}Pt coupling arises from a dependence of ^2J(Pt,P) on the dihedral angle Pt-O-P=O, in analogy to the stereospecificity of ^{31}P-C-^{1}H and ^{31}P-C-^{19}F spin–spin coupling constants.[26] Undetectable ^{31}P–^{195}Pt coupling, even with low-field spectrometers, has been previously reported for a number of phosphato complexes of platinum.[5, 6, 27]

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[1] M. J. Bloemink, J. Reedijk, *Met. Ions Biol. Syst.* **1996,** *32*, 641–685.
[2] J.-C. Chottard, in *Handbook of Metal–Ligand Interactions in Biological Fluids, Vol. 2* (Ed.: G. Berthon), Marcel Dekker, New York, **1995**, pp. 990–1009.
[3] R. G. Pearson, *Science* **1966,** *151*, 172–177.
[4] S. Louie, R. Bau, *J. Am. Chem. Soc.* **1977,** *99*, 3874–3876.
[5] M. D. Reily, L. G. Marzilli, *J. Am. Chem. Soc.* **1986,** *108*, 8299–8300.
[6] M. D. Reily, T. W. Hambley, L. G. Marzilli, *J. Am. Chem. Soc.* **1988,** *110*, 2999–3007.
[7] R. N. Bose, L. L. Slavin, J. W. Cameron, D. L. Luellen, R. E. Viola, *Inorg. Chem.* **1993,** *32*, 1795–1802.
[8] Preliminary accounts of this work have been presented at the EUROBIC-3 conference [9] and in a review article [10].
[9] J. Kozelka, G. Barre, *Proceedings of the 3rd European Conference on Bio-Inorganic Chemistry*, **1996,** Noordwijkerhout (The Netherlands), B10.
[10] P. Augé, J. Kozelka, *Transition Met. Chem.* **1997,** *22*, 91–96.
[11] B. Lippert, *Prog. Inorg. Chem.* **1989,** *37*, 1–97.
[12] R. N. Bose, R. E. Viola, R. D. Cornelius, *J. Am. Chem. Soc.* **1984,** *106*, 3336–3343.
[13] L. L. Slavin, R. N. Bose, *J. Chem. Soc. Chem. Commun.* **1990,** *18*, 1256–1258.
[14] No attempt was made to use more than a twofold molar excess of **1**.
[15] The chemical shifts of the imino protons of 2′-deoxyguanosine and thymidine in DMF are, according to our measurements, $\delta = 10.63$ and 11.19, respectively.
[16] Only partial solvolysis of the NO_3^- ligands occurs in DMF. For instance, we have found that equilibrated solutions of **1** contain 36% of [Pt(DMF)$(NH_3)_3$]$^{2+}$ [$\delta(^{195}Pt) = -2063$] and 64% of [Pt$(NO_3)(NH_3)_3$]$^+$ [$\delta(^{195}Pt) = -2094$].
[17] J.-P. Girault, G. Chottard, J.-Y. Lallemand, F. Huguenin, J.-C. Chottard, *J. Am. Chem. Soc.* **1984,** *106*, 7227–7232.
[18] We cannot, however, rule out the possibility that in both phosphodiester-bound species, the same phosphoryl oxygen is bound to platinum, in which case the two downfield ^{31}P NMR signals would correspond to slowly interconverting conformers.
[19] D. G. Gorenstein in *Phosphorus-31 NMR* (Ed.: D. G. Gorenstein), Academic Press, Orlando, **1984,** p. 11.
[20] H. Sigel, *Chem. Soc. Rev.* **1993,** 255–267.
[21] M. Boudvillain, R. Dalbiès, M. Leng, *Met. Ions Biol. Syst.* **1996,** *33*, 87–104.
[22] D. Payet, F. Gaucheron, M. Sip, M. Leng, *Nucleic Acids Res.* **1993,** *25*, 5846–5851.
[23] F. Reeder, J. Kozelka, J.-C. Chottard, *Inorg. Chem.* **1996,** *35*, 1413–1415.
[24] H. J. S. King, *J. Chem. Soc.* **1938,** 1338–1346.
[25] J.-Y. Lallemand, J. Soulié, J.-C. Chottard, *J. Chem. Soc. Chem. Commun.* **1980,** 436–437.
[26] G. Mavel, *Annu. Rep. NMR Spectrosc.* **1973,** *5b*, 1–350, pp. 26–37.
[27] R. N. Bose, N. Goswami, S. Moghaddas, *Inorg. Chem.* **1990,** *29*, 3461–3467.
[28] D. P. Bancroft, C. A. Lepre, S. J. Lippard, *J. Am. Chem. Soc.* **1990,** *112*, 6860–6871.
[29] S. K. C. Elmroth, S. J. Lippard, *Inorg.Chem.* **1995,** *34*, 5234–5243.
[30] F. Bernges, E. Holler, *Eur. J. Biochem.* **1992,** *208*, 573–579.

  0947-6539/97/0309-1409 $ 17.50+.50/0