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# The Antitumor Drug *cis*-Diamminedichloroplatinum(II) Preferentially Chelating Neighboring Guanines in the Trinucleotide d(pGpGpG)

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The products from the reaction of *cis*-$PtCl_2(NH_3)_2$ with the trinucleotide d(pGpGpG) have been investigated by NMR spectroscopy. The main product appeared to be *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(1),N7(2)*], whereas a minor product could be assigned to *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(2),N7(3)*]. Although NOE and homodecoupling techniques did not allow straightforward assignment, the characterization of the main adduct was possible by pH-dependent chemical shift data in combination with comparison of the ribose H1′ splitting patterns. Phosphorus-31 NMR data of this product were in agreement with a cis-Pt chelate of neighboring guanines. The minor product was established by comparing chemical shift data. No indications for the so-called GNG adduct with this trinucleotide—i.e. *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(1),N7(3)*]—have been found.

## Introduction

It is generally accepted that platinum–DNA interactions play an important role in the antineoplastic activity of the well-known antitumor drug *cis*-$PtCl_2(NH_3)_2$ (cis-Pt).[1-4] These interactions with this bifunctional platinum compound result in various platinum–DNA adducts, which have been found in different quantities. At the moment, it is not known whether there is a specific "key lesion" that causes the cell killing.

Recent studies have made clear that, both in vitro[5,6] and in tissue culture,[7] cis-Pt treatment of naked DNA or cells, respectively, yield mainly intrastrand chelates with neighboring purines, in which the platinum moiety is bound to the N7 sites. It has been proposed[8] that, in addition to neighboring purines, also next-neighboring guanines should be able to coordinate to cis-Pt. Indications for this binding mode were obtained from a bacterial mutation study.[9] More recently, quantitation results obtained from in vitro experiments[6] gave also indirect evidence for this binding fashion.

This latter binding mode, in which cis-Pt is bound to two guanines separated by a noncoordinated nucleobase N (the GNG chelate), has afterwards been established for short oligonucleotides such as d(GpCpG),[10,11] d(GpTpG),[12] d(GpApG),[13] d(GpCpGpC),[14] and the undecamer d(TpCpTpCpGpTpGpTpCpTpC).[15]

Interactions of cis-Pt with trinucleotides that contain three purines are interesting from a kinetic point of view, since both neighboring and next-neighboring chelates can be formed. These two binding fashions have indeed been demonstrated for the cis-Pt interaction with d(GpApG),[13] which resulted both in the GNG chelate (80%) and in the AG chelate (20%).

It has been shown that cis-Pt binds preferently to d(GpG) sequences in the DNA.[5-7] This makes the reaction of the platinum compound with d(pGpGpG) especially interesting, since in principle the formation of both GG and GNG chelates is possible with this trinucleotide. In the present paper, the results of an investigation focused on the interaction of cis-Pt with this trinucleotide are described.

## Experimental Section

**Starting Materials.** The trideoxynucleoside triphosphate d(pGpGpG) ($Na^+$ salt) was synthesized via an improved phosphotriester method.[16] *cis*-$PtCl_2(NH_3)_2$ was prepared[17] and purified[18] by literature procedures.

**Reactions and Purification of the Products.** Stoichiometric amounts of cis-Pt and d(pGpGpG) ($\sim 10^{-5}$ M; based on a molar absorption of 32.000 for the trinucleotide at 254 nm and pH 7) were incubated for 5 days at room temperature, at both pH 5.0 and pH 7.5. The reactions were monitored by UV spectroscopy (Perkin-Elmer EPS-3T). After concentration, the products were separated by gel filtration chromatography (Sephadex G25; Pharmacia) with a 0.02 M triethylammonium hydrogen carbonate (TEAB) solution as eluent. Detection was performed by UV spectroscopy (254 nm). The peak fractions were pooled and desalted by gel filtration chromatography, using doubly distilled water as eluent.

**NMR.** The desalted peak fractions were lyophilized twice from $D_2O$ (99.8%; Merck), pH adjusted (pH 7.0, uncorrected for isotope effect), and eventually dissolved in 0.4 mL of $D_2O$ (99.95%; Merck). A trace amount of tetramethylammonium nitrate (TMA) was added for internal $^1H$ reference (3.18 ppm downfield from DSS). The proton NMR spectra were recorded on a Bruker WM 300 NMR spectrometer, interfaced with an Aspect 2000 computer. Resolution enhancement was obtained by applying a Gaussian window, according to Ernst.[19] The pH dependence of the chemical shift of the nonexchangeable base protons was monitored by adding trace amounts of DCl or NaOD. If necessary, the HDO signal was reduced in these experiments by selective saturation. Phosphorus-31 NMR spectra were recorded at 121.5 MHz on a Bruker WM 300 NMR spectrometer after adding a trace amount of TMPB (tetramethylphosphanium bromide), which was used as an internal reference, and EDTA (Merck) for removing traces of metals. The $^{31}P$ chemical shifts are reported relative to 3′5′-cAMP.[20]

**Nomenclature.** The notation of the chain direction is according to the recommendations of ref 21; i.e., the oligonucleotides are notated sequentially from the left to the right from the 5′- to the 3′-terminus.[21]

## Results and Discussion

**General Observations and Purification of the Adducts.** The reaction of cis-Pt and the trinucleotide d(pGpGpG) proved to be

(1) Marcelis, A. T. M.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 121.
(2) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616.
(3) Roberts, J. J.; Thomson, A. J. *Prog. Nucleic Acid Res. Mol. Biol.* **1979**, *22*, 71.
(4) Reedijk, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding (Berlin)*, in press.
(5) Fichtinger-Schepman, A. M. J.; Lohman, P. H. M.; Reedijk, J. *Nucleic Acid Res.* **1982**, *10*, 5345.
(6) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707.
(7) Plooy, A. C. M.; van Dijk, .; Lohman, P. H. M. *Cancer Res.* **1984**, *44*, 2043.
(8) Macquet, J. P.; Butour, J. L. *Biochimie* **1978**, *60*, 901.
(9) Brouwer, J.; van de Putte, P.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7010.
(10) Marcelis, A. T. M.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 2664.
(11) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 322.
(12) van der Veer, J. L.; Ligtvoet, G. J.; van den Elst, H.; Reedijk, J. *J. Am. Chem. Soc.* **1986**, *108*, 3860.
(13) van der Veer, J. L.; den Hartog, J. H. J.; van den Elst, H.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Inorg. Chem.* **1986**, *25*, 4657.
(14) Marcelis, A. T. M.; den Hartog, J. H. J.; van der Marel, G. A.; Wille, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *135*, 343.
(15) den Hartog, J. H. J.; Altona, C.; van den Elst, H.; van der Marel, G. A.; Reedijk, J. *Inorg. Chem.* **1985**, *24*, 986.
(16) van der Marel, G. A.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Nucleic Acid Res.* **1982**, *10*, 2337.
(17) Dhara, S. *Indian J. Chem.* **1970**, *8*, 193.
(18) Raudaschl, G.; Lippert, B.; Hoeschele, J. D. *Inorg. Chim. Acta* **1983**, *78*, L43.
(19) Ernst, R. R. *Adv. Magn. Reson.* **1966**, *2*, 1.
(20) Haasnoot, C. A. G.; Altona, C. *Nucleic Acid Res.* **1979**, *6*, 1135.
(21) IUPAC-IUB Joint Commission on Biochemical Nomenclature Recommendations. *Eur. J. Biochem.* **1982**, *131*, 9.

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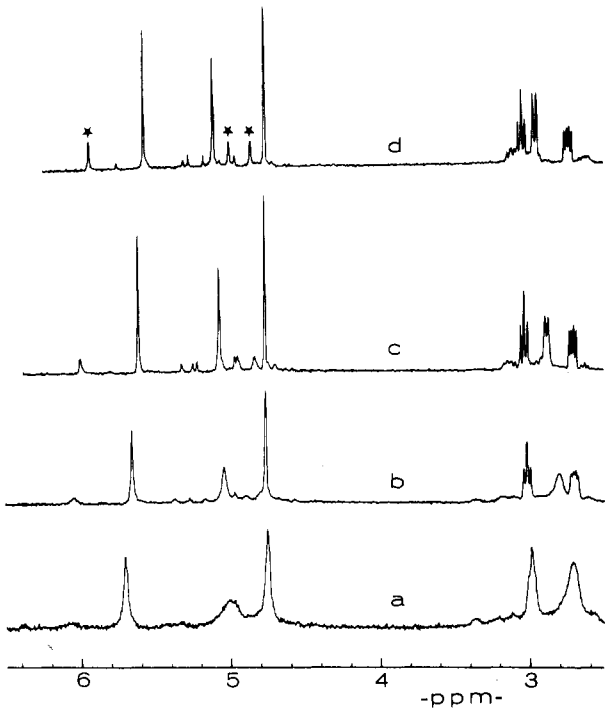


**Figure 1.** Downfield region of the 300-MHz proton NMR spectrum of the products eluted under peak II, recorded at 280 (a), 295 (b), 310 (c), and 325 K (d). In spectrum d, the discussed minor adduct has been indicated.

complete within 5 days, since no changes were observed after that time interval in the UV spectrum of the reaction mixtures. During this period, a bathochromic shift had occurred, which is also described for other platinum–oligonucleotide interactions.[13,22,23] The $UV_{max}$ and the $UV_{min}$ increased from 252 and 224 nm to 258 and 229 nm, respectively.

It was noted that the reaction at pH 5.0 proceeded somewhat faster than that at pH 7.5. This was deduced from the $UV_{max}/UV_{min}$ ratio, which decreased more rapidly in the former reaction mixture. The decrease of this ratio was considered to be proportional to the reaction rate, since it was at the onset (3.85) and the end (1.45) of the reaction virtually the same for both reactions. Moreover, the same products had been formed in equal quantities (vide infra). The somewhat faster reaction at the low pH value can be related to the presence of different amounts of the various cis-Pt hydrolysis products under both conditions,[26] which might have different reactivities. Phosphate groups are also known to influence the reaction rate of platinum binding.[24,25] However, since virtually the same amounts of products were found (vide infra), the effect of the 5′-phosphate protonation on the reaction rate is expected to be negligible.

The gel filtration chromatography of both reaction mixtures resulted in the separation of three UV-absorbing products, comprising successively 15% (peak I), 80% (peak II), and 5% (peak III) of the total peak area, as monitored by UV detection ($A_{254}$). No differences between both profiles were observed. Proton NMR spectra of the desalted peak I and peak II were recorded. The amount of the products eluted under peak III, however, was too small to investigate by NMR spectroscopy.

**Proton NMR Analysis.** Elution peak I appeared to contain mainly unreacted d(pGpGpG), together with some—probably platinated—product(s). The proton signals of the latter species

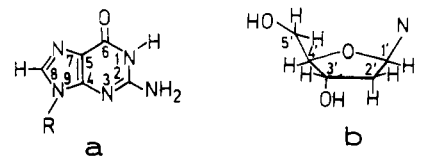


**Figure 2.** Depiction of the nucleobase guanine (a) and the 2′-deoxyribose (b), with recommended notations.

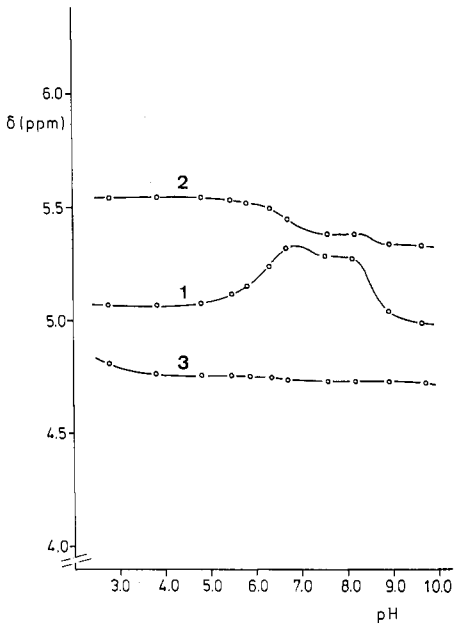


**Figure 3.** Plot of the chemical shift of the H8 proton resonances vs. the pH of the main product that is eluted under peak II. The spectra were recorded at 330 K.

were poorly resolved. Neither the addition of EDTA nor recording the spectra at higher temperature improved the resolution. Since the latter product(s) represent(s) only a minor fraction, i.e. less than 10% of the total product yield, it was not investigated further.

The downfield part of the proton NMR spectrum of elution peak II, recorded at 280, 295, 310, and 325 K is redrawn in Figure 1. Since only one nonexchangeable nucleobase proton signal is expected for guanine residues (the H8 proton; see Figure 2), three proton resonances can be expected for one trinucleotide adduct in the aromatic region of the NMR spectrum. From Figure 1, it is clear that one major and one minor product are eluted under peak II. The proton signals of the major product appear to be broadened at low temperature. This is possibly due to intermolecular interactions, since the NMR solution has become viscous at this temperature. When using the trinucleotide d(GpGpG), the line broadening due to intermolecular interactions appeared to be even larger than that for d(pGpGpG) (J. L. van der Veer, unpublished results). This in fact led us to use the present trinucleotide. The minor product (indicated in the NMR spectrum at 325 K) becomes only well resolved at higher temperature. The amount of this adduct, however, does not exceed 15% of the main product. Also H8 resonances of some other minor products are present, which are not fully reproducible and possibly originate from degradation products.

To characterize the main product, a pH-dependent chemical shift study of the guanine H8 resonances was performed. Guanine contains two nitrogen donor sites, which can be (de)protonated[27] between pH 1 and pH 13, i.e. the N1 ($pK_a \approx 10$) and the N7 ($pK_a \approx 2.4$). These (de)protonations can induce changes in the chemical shift of the H8 proton signals. Since platination at the preferred N7 site excludes N7 protonation and also causes a decrease of the $pK_a$ of the N1 deprotonation[1,14,23,28] to about 8.5, recording

(22) Chottard, J. C.; Girault, J. P.; Chottard, G.; Lallemand, J. Y.; Mansuy, D. *J. Am. Chem. Soc.* **1980**, *102*, 5565.
(23) Girault, J. P.; Chottard, G.; Lallemand, J. Y.; Chottard, J. C. *Biochemistry* **1982**, *21*, 1352.
(24) Marcelis, A. T. M.; Erkelens, C.; Reedijk, J. *Inorg. Chim. Acta* **1984**, *91*, 129.
(25) Eapen, S.; Green, M.; Ismail, I. M. *J. Inorg. Biochem.* **1985**, *24*, 233.
(26) Martin, R. B. *ACS Symp. Ser.* **1983**, *209*, 231.
(27) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32.

of the spectra at different pH values can yield valuable information about the number of platinated guanines in the trinucleotide.

In Figure 3, a plot showing the chemical shift of the H8 signals of the main product as a function of the pH is depicted. At pH 2, the onset of an N7 protonation is seen, only at the H8(3) signal. At pH 8.5, N1 deprotonations can be observed only at the H8(1) and H8(2) signals. A clear 5′-phosphate protonation ($pK_a \approx 6.3$) is present at the H8(1) signal, indicating that this proton signal belongs to the 5′-guanine. It should be noted that the curvature of the two most downfield H8 signals (i.e. the H8(1) and the H8(2)) is essentially similar to that derived from the cis-Pt adduct of d(pGpG).[23] In summary, from the pH-dependent chemical shift data it can be concluded that in the main product two guanines are platinated at the N7, including the one at the 5′-end.

After ascertaining the binding of platinum to two guanines in d(pGpGpG), homodecoupling experiments in combination with NOE techniques can in principle lead to a straightforward assignment of the three H8 signals to the proper nucleotide residue. This can immediately reveal whether the main product is a GG chelate or a GNG chelate. Unfortunately, at the temperature needed for NOE measurements at 500 MHz (275 K; to obtain a convenient rotational correlation time), the proton signals of the product appeared to be broadened severely. Moreover, under these conditions, the H4′, H5′, and H5″ signals of the adduct strongly overlap, making assignment of the deoxyribose proton signals to the proper residue very difficult.

Careful inspection of the H1′ splitting patterns of the main product, however, gave evidence for the GG chelate. The H1′ resonances can be observed in the range 2.6–3.1 ppm. In Figure 1, it can be seen that an H1′ with a clear doublet splitting pattern is present. With decreasing temperature, this signal strongly broadens and shifts to higher field. This doublet splitting pattern has also been observed in oligonucleotides containing a cis-Pt chelate on neighboring guanines such as d(GpG),[23] d(pGpG),[23] d(ApTpGpG),[29] and d(TpGpGpCpCpA)[28a] and also in the AG chelate of d(GpApG).[13] From a conformational analysis[31] of the cis-Pt adduct of d(GpG), it has been concluded that the H1′ doublet splitting pattern is caused by an almost pure N-type ribose conformation of the 5′-purine. Cis-Pt chelation of next-neighboring purines, however, does not induce N-type conformations in any ribose of the resulting GNG chelate.[13,30,32] So, the observation of an H1′ doublet in the main product indicates that cis-Pt is bound here to two neighboring guanines and not via a GNG binding fashion. Combining these results with the pH dependence chemical shift data (vide supra) results in the conclusion that the main product is *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(1),N7(2)*].

**Phosphorus-31 NMR Analysis.** It has been shown that phosphorus-31 NMR spectral data can supply useful information about the local distortion of the structure of platinated oligonucleotides[33] or DNA.[34,35] Therefore, $^{31}P$ NMR spectra of unreacted d(pGpGpG) were recorded, and the product was eluted under peak II. The $^{31}P$ chemical shift vs. temperature profiles are depicted in Figure 4. In Figure 4a, signals 2 and 3 are present in the range 0–1 ppm (relative to 3′5′-cAMP), where the $^{31}P$ resonances of

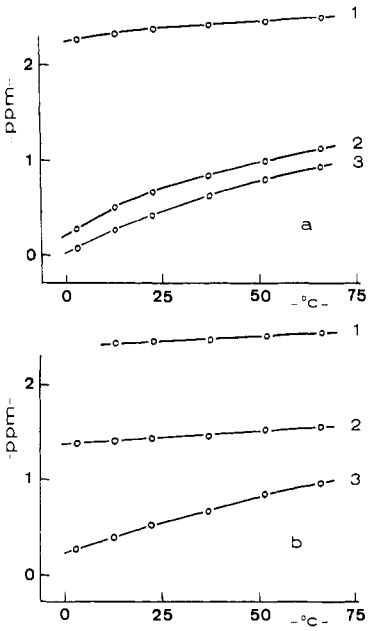


**Figure 4.** Plots of the phosphorus-31 chemical shifts vs. the temperature of unbound d(pGpGpG) (a) and of the main product eluted under peak II (b).

single- and double-stranded oligonucleotides are usually found.[33,36] Signal 1 is therefore expected to belong to the 5′-terminal phosphate group, which is known to resonate at lower field.[37] This resonance could only be observed as a sharp singlet after the addition of a trace amount of EDTA. It is known that terminal phosphate signals can readily broaden in the presence of traces of metals. This observation therefore supported the assignment of this signal. Resonance 1 in Figure 4b was also only observed after addition of EDTA. Since the profile of this signal almost coincides with that from Figure 4a, this resonance belongs most likely to the terminal phosphate group. Curves 3 in both figures also seem to coincide, at least at higher temperature. The remaining signal 2 in Figure 4b has been shifted downfield upon platination, from 0.3 ppm (65 °C) to 1.1 ppm (2 °C). According to other data, this signal must originate from the phosphate group that connects the two chelated guanines.[33,38] The downfield shift may even be larger, in case that resonances 2 and 3 would have crossed. Then also, a small upfield shift must have occurred for signal 2 of the unreacted trinucleotide (Figure 4a). Similar small upfield shifts for nearby phosphate resonances after cis-Pt chelation of a GG sequence in a single-stranded oligonucleotide have been reported.[33]

In conclusion, the $^{31}P$ NMR data of the platinated main product of d(pGpGpG), showing one clear downfield shift of a phosphate resonance, is in agreement with similar reported[33,38] shifts in single-stranded GG-platinated oligonucleotides. Although no $^{31}P$ NMR data are available for GNG-containing single-stranded oligonucleotides, the results of the platinated double-stranded alternating DNA polynucleotide poly(dG–dC)$_2$ at least indicate that no strong $^{31}P$ shifts resulting from platination will occur.[35]

**Characterization of the Minor Adduct.** Finally, some comments can be made on the nature of the minor adduct indicated in Figure 1d. For this adduct, no separated phosphorus-31 resonances could be observed in the above-mentioned $^{31}P$ NMR spectra of elution peak II, possibly due to the low signal-to-noise ratio. Since in the proton NMR spectra no N7 protonation is seen on the two most downfield H8 resonances at pH 2 (not shown), two guanines must be chelated by cis-Pt. It can be excluded that this minor product represents the GNG adduct for the following reason. In our experience, the chemical shift of the guanine H8 signals in

(28) (a) Girault, J. P.; Chottard, J. C.; Guittet, E. R.; Lallemand, J. Y.; Huyhn-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157. (b) Caradonna, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793.
(29) Neumann, J. M.; Tran-Dinh, S.; Girault, J. P.; Chottard, J. C.; Huyhn-Dinh, T.; Igolen, J. *Eur. J. Biochem.* **1984**, *141*, 465.
(30) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Marcelis, A. T. M.; van der Marel, G. A.; Rinkel, L. J.; Wille-Hazeleger, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *134*, 458.
(31) den Hartog, J. H. J.; Altona, C.; Chottard, J. C.; Girault, J. P.; Lallemand, J. Y.; de Leeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucleic Acid Res.* **1982**, *10*, 4715.
(32) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Reedijk, J. *Recl. Chim. Trav. Pays-Bas* **1984**, *103*, 322.
(33) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Reedijk, J. *FEBS Lett.* **1984**, *176*, 393.
(34) Wilson, W. D.; Heyl, B. L.; Reddy, R.; Marzilli, L. G. *Inorg. Chem.* **1982**, *21*, 2527.
(35) Marzilli, L. G.; Reily, M. D.; Heyl, B. L.; Mc Murray, C. T.; Wilson, W. D. *FEBS Lett.* **1984**, *176*, 389.
(36) Gorenstein, D. G.; Findlay, J. B.; Momii, R. K.; Luxon, B. A.; Kar, D. *Biochemistry* **1976**, *15*, 796.
(37) Corzone, P. J.; Jardeztky, O. *Biochemistry* **1976**, *15*, 4853.
(38) Marcelis, A. T. M. Ph.D. Thesis, Leiden University, 1982.

all GNG chelates with cis-Pt, when recorded at ambient temperature and neutral pH, ranges from 5.0 to 5.3 ppm, as can be seen in the adducts of d(GpCpG),[14,30] d(CpGpCpG),[14] d(GpTpG),[12] and d(GpApG).[23] On the other hand, at least one purine H8 signal of a cis-Pt chelated d(GpG) sequence is always found above 5.4 ppm, as has been found for d(GpG),[31] d(CpGpG),[39] d(CpCpGpG),[14] d(CpGpGpT),[40] d(ApTpGpG),[29] d(TpGpGpCpCpA),[28a] and d(ApGpGpCpCpT).[28b] On the basis of this criterion, the minor adduct clearly belongs to the latter class of chelates. Since the main product has been proven to be *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(1),N7(2)*] (vide supra), the minor one must be *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(2),N7(3)*].

**Final Remarks.** Summarizing the described results, it can be concluded that the reaction of cis-Pt with the trinucleotide d(pGpGpG) yields mainly—and perhaps exclusively—adducts in which cis-Pt is bound to neighboring guanine residues. No indication for the presence of a so-called GNG chelate has been found. It should be noted that deprotonation of the 5'-terminal phosphate group did not cause a different product yield. The fact that the same proportions of the two adducts were obtained at both pH's suggests that in this case both $OPO_3H^-$ and $OPO_3^{2-}$ have similar directing effects for the reactive platinum species. The high preference of cis-Pt for neighboring guanines over next-neighboring guanines is in agreement with other studies in which also a high preference for GG sequences has been reported.[6,41]

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**Registry No.** *cis*-$PtCl_2(NH_3)_2$, 15663-27-1; d(pGpGpG), 51246-89-0; *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(1),N7(2)*], 108563-01-5; *cis*-$Pt(NH_3)_2$[d(pGpGpG)-*N7(2),N7(3)*], 108594-53-2.

(39) den Hartog, J. H. J.; Altona, C.; van der Marel, G. A.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371.
(40) Peters, A. R.; van der Veer, J. L., unpublished results.
(41) Stone, P. J.; Kelman, A. D.; Sinex, F. M. *Nature (London)* **1974**, *251*, 736.

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# Synthesis and NMR Structural Studies of the Adduct of *trans*-Diamminedichloroplatinum(II) with the DNA Fragment d(GpCpG)

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The reaction of *trans*-$[Pt(NH_3)_2Cl_2]$ with the sodium salt of the deoxytrinucleoside diphosphate d(GpCpG) at 37 °C in water at pH 6 leads to *trans*-$[Pt(NH_3)_2$[d(GpCpG)]] as the one major product formed after 45 h. At earlier time periods (1-2 h) two intermediates, tentatively assigned as monofunctional adducts containing platinum bound to the N7 position of G(1) or G(3), were observed by high-performance liquid chromatography (HPLC). Experiments carried out include pH-dependent titrations of the nonexchangeable base protons, two-dimensional COSY, and one-dimensional nuclear Overhauser effect proton NMR studies on 250- and 500-MHz instruments. The results indicate that the *trans*-diammineplatinum(II) fragment forms an intrastrand cross-link between the N7 atoms of guanosine nucleosides G(1) and G(3). In this adduct the intervening cytidine nucleotide is destacked and the G(1) deoxyribose sugar ring switches its puckering from an S (C2'-endo) to an N (C3'-endo) conformation. This change in sugar pucker is similar to that observed for the 5'-nucleotide in *cis*-$[Pt(NH_3)_2$[d(GpG)]] intrastrand adducts on DNA. This structural information is likely to be relevant to the fact that, unlike its cis isomer, *trans*-DDP is inactive as an anticancer drug.

## Introduction

The platinum complex *cis*-diamminedichloroplatinum(II), *cis*-DDP, is an important anticancer drug.[1] The trans isomer, however, is clinically ineffective and less cytotoxic and mutagenic than the cis stereoisomer.[2] Although the structural changes that occur when *cis*-DDP binds to DNA, its likely biological target, have been thoroughly studied, the DNA binding properties of the trans isomer have been less well investigated. For stereochemical reasons, *trans*-DDP prefers not to form a 1,2-intrastrand cross-link between two guanosine nucleosides, the major adduct formed by *cis*-DDP with DNA, and its failure to do so may be the reason for the difference in biological activity between the two stereoisomers.[3,4]

Previous spectroscopic work has shown that the bifunctional adducts made by *cis*- and *trans*-DDP with DNA disrupt the double helix to a much greater extent than monofunctional adducts, such as those made by [Pt(dien)Cl]Cl.[5-8] Replication mapping studies revealed that both *cis*- and *trans*-DDP inhibit DNA synthesis by DNA polymerase I, whereas [Pt(dien)Cl]Cl does not.[9] These studies have also shown that *trans*-DDP binding to DNA is less regio- and stereoselective than the binding of *cis*-DDP, although 1,3-intrastrand cross-links of guanine bases to form platinated d(GpNpG), where N is an intervening nucleotide, appear to be the most prevalent adducts formed when single-stranded DNA is treated with the trans isomer. Recent in vivo experiments suggest that adducts formed between *trans*-DDP and DNA may be recognized and repaired more efficiently than those formed

(1) Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds. *Platinum Coordination Complexes in Cancer Chemotherapy*; Martinus Nijhoff: Boston, MA, 1984.
(2) Roberts, J. J.; Thomson, A. J. *Prog. Nucleic Acid Res. Mol. Biol.* **1979**, *22*, 71-133.
(3) Plooy, A. C. M. van Dijk, M.; Lohman, P. H. M. *Cancer Res.* **1984**, *44*, 2043-2051.
(4) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167-180.
(5) Macquet, J.-P.; Butour, J.-L. *Biochimie* **1978**, *60*, 901-914.
(6) Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1980**, *46*, 35-39.
(7) Houssier, C.; Maquet, M. N.; Fredericq, E. *Biochim. Biophys. Acta* **1983**, *739*, 312-316.
(8) Polissiou, M.; Viet, M. T. P.; St.-Jacques, M.; Theophanides, T. *Inorg. Chim. Acta* **1985**, *107*, 203-210.
(9) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad Sci. U.S.A.* **1985**, *82*, 4616-4619.

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