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]

Acknowledgment. This study was supported in part by the Netherlands Organization of Chemical Research (SON), with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO), through Grant 11-28-17, and from the Netherlands Organization for Fight against Cancer (KWF). F. J. Dijt and C. J. van Garderen are thanked for critical reading of the manuscript. Stimulating discussions with the group of Prof. Dr. J. C. Chottard (Paris), made possible by the sponsorship of the French-Dutch cultural agreement, are gratefully acknowledged. We are indebted to Johnson-Matthey Chemicals Ltd. (Reading, England) for a generous loan of K_2PtCl_4.

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.

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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

Dan Gibson and Stephen J. Lippard*

Received December 5, 1986

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.

0020-1669/87/1326-2275$01.50/0

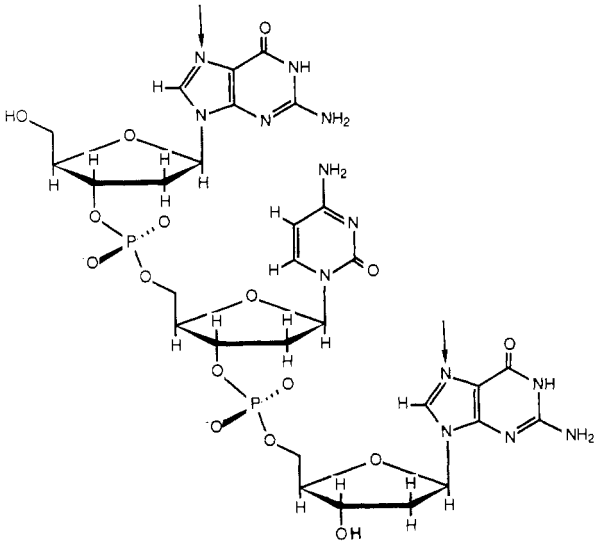

Figure 1. Trinucleoside diphosphate d(GpCpG) with the *trans*-DDP binding sites indicated by arrows.

by *cis*-DDP.[10] Immunochemical studies with antinucleoside antibodies have revealed that a greater disruption of the DNA double helix occurs for low levels of bound *trans*-DDP vs. *cis*-DDP.[11]

Most of the work aimed at elucidating the structures of platinated DNA has focused on adducts of *cis*-DDP with various oligonucleotides containing two adjacent guanosine or d(GpNpG) moieties.[12-19] Only very recently, during the course of the present investigation, has work been communicated[20] showing that d(GpTpG) can form a chelate with *trans*-DDP via both guanine bases in a fashion similar to that of the cis isomer.[18] Here we report the results of HPLC chromatographic and NMR spectroscopic investigations of the reaction of *trans*-DDP with d(GpCpG), which establish the nature of the binding sites and distortions of the DNA that occur upon platination. These structural alterations may be responsible for the differential ability of cells to process DNA that has reacted with *cis*- vs. *trans*-DDP and, consequently, the differential cytotoxicity of the two isomers.

Experimental Section

Synthesis of *trans*-[Pt(NH$_3$)$_2$\{d(GpCpG)-*N7-G(1)*, *N7-G(3)*\}]. The trinucleoside diphosphate d(GpCpG) (Figure 1) was synthesized by the phosphotriester method.[21] It was purified by anion-exchange chromatography using a DE-52 cellulose resin and eluting with triethylammonium bicarbonate over a gradient ranging from 0.03 to 0.3 M, monitoring the absorbance of the eluate at 254 nm. After lyophilization, the triethylammonium salt was passed through a cation-exchange resin to yield the sodium salt of d(GpCpG).

In a typical procedure, 5.7 mg of the sodium salt of d(GpCpG) was allowed to react with 1.94 mg of *trans*-[Pt(NH$_3$)$_2$Cl$_2$] in the presence of 2 μL of 250 mM Na$_2$EDTA in a total volume of 20 mL (0.3 mM of reactants) at 37 °C in the dark. The progress of the reaction was monitored by reversed-phase high-performance liquid chromatography (HPLC).

After the reaction appeared to go to completion (45 h), the mixture was concentrated in a rotary evaporator and purified by reversed-phase HPLC using an isocratic elution of 90% 0.1 M NH$_4$OAc and 10% 1:1 0.1 M NH$_4$OAc–CH$_3$CN over a reversed-phase C$_{18}$ column while monitoring at 260 nm. The purified product was lyophilized several times from H$_2$O and then desalted by using a reversed-phase SEP-PAK column and eluting with a solution of 60% methanol in water.

NMR Spectroscopy. The NMR samples were lyophilized three times from 99.6% D$_2$O (Aldrich) and then dissolved in 99.98% D$_2$O (Stohler Isotopes) containing a trace amount of Me$_4$NCl added as an internal reference. The low solubility of the neutral platinated oligonucleotide resulted in NMR sample concentrations in the 3.6 mM range.

Proton NMR spectra were recorded at both 250 MHz (for the pH titration) and 500 MHz. The former were acquired on a Bruker WM-250 spectrometer, where the free induction decay signals (128 accumulations, quadrature detection) were averaged on the Bruker Aspect 2000 data system. Resolution enhancement was achieved by applying a Gaussian function. All 500-MHz proton spectra were obtained on a home-built spectrometer at the Francis Bitter National Magnet Laboratory (NML).

COSY experiments were carried out at 500 MHz, using the pulse sequence $[\pi/2-t_1-\pi/2-\text{acq}]_n$. Typically, 1024 data points per free induction decay were collected, with 512 t_1 values. The remaining blocks were zero-filled prior to Fourier transformation. Usually 32 scans per FID were sufficient to give a good signal-to-noise ratio, with 4 Hz line broadening applied in each dimension. Processing was done on a VAX

Table I. Time Course of the Reaction between *trans*-DDP and d(GpCpG) As Monitored by HPLC[a,b]

species[b]	percent UV-absorbing species[a]			
	t = 0	t = 40 min	t = 84 min	t = 45 h
d(GpCpG)	100	63	49	21
A		19	24	
B		11	14	5
trans-[Pt(NH$_3$)$_2$-{d(GpCpG)}] (C)		2	6	70

[a] Numbers refer to percent total UV-absorbing species in the HPLC chromatograms. [b] See Figure 2.

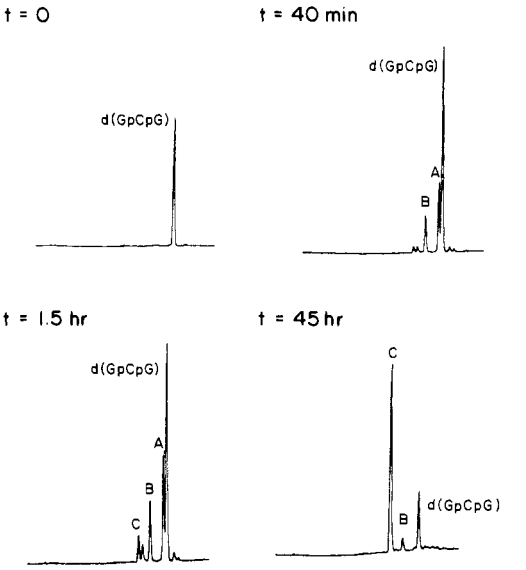

Figure 2. HPLC traces showing the time course of the appearance and disappearance of species formed in the reaction of *trans*-DDP with d(GpCpG) at 37 °C. The first fractions off the column appear at the left-hand sides of the traces.

(10) Ciccarelli, R. B.; Solomon, M. J.; Varshavsky, A.; Lippard, S. J. *Biochemistry* **1985**, *24*, 7533–7540.
(11) Sundquist, W. I.; Lippard, S. J.; Stollar, B. D. *Biochemistry* **1986**, *25*, 1520–1524.
(12) 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 Acids Res.* **1982**, *10*, 4715–4730.
(13) (a) Caradonna, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793–5795. (b) Caradonna, J. P.; Lippard, S. J., submitted for publication.
(14) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528–1530.
(15) van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J.-P.; Huynh-Dinh, T.; Lallemand, J.-Y.; Igolen, J.; Chottard, J.-C. *J. Am. Chem. Soc.* **1984**, *106*, 3037–3039.
(16) Marcelis, A. T. M.; den Hartog, J. H. J.; van der Marel, G. A.; Wille, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *135*, 343–349.
(17) den Hartog, J. H. J.; Altona, C.; van der Marel, G. A.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371–379.
(18) den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Marcelis, A. T. M. van der Marel, G. A.; Rinkle, L. J.; Wille-Hazeleger, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *134*, 485–495.
(19) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science (Washington, D.C.)* **1985**, *230*, 412–417.
(20) van der Veer, J. L.; Ligtvoet, C. J. van der Elst, H.; Reedijk, J. *J. Am. Chem. Soc.* **1986**, *108*, 3860–3862.
(21) (a) Efimov, V. A.; Reverdato, S. V.; Chakhmakhcheva, O. G. *Nucleic Acids Rev.* **1982**, *10*, 6675–6694. (b) Gait, M. J. In *Oligonucleotide Synthesis. A Practical Approach*; Gait, M. J., Ed.; IRL: Washington, DC, 1984; Chapter 1.

Table II. Comparison of the Proton Chemical Shifts of d(GpCpG), *cis*-[$Pt(NH_3)_2${d(GpCpC)}], and *trans*-[$Pt(NH_3)_2${d(GpCpG)}]

species	nucleotide	chem shift[a] H1′	H3′	H8	H5	H6	ref
d(GpCpG)	Gp–	6.136	4.848	7.865			18
	–pCp–	6.123	4.789		5.788	7.554	
	–pG	6.190	4.714	7.978			
cis-[$Pt(NH_3)_2${d(GpCpG)}]	Gp–	6.222	4.861	8.216			18
	–pCp–	6.322	4.823		6.024	7.820	
	–pG	6.244	4.598	8.320			
trans-[$Pt(NH_3)_2${d(GpCpG)}]	Gp–	6.407	4.598	8.586			this work
	–pCp	6.477	5.177		5.850	7.985	
	–pG	6.311	4.768	8.713			

[a] Chemical shifts are given in ppm vs. Me_4Si.

750 computer with software developed by the NML.

The difference 1D-NOE experiments were performed at 5 °C on the 500-MHz spectrometer on degassed samples. The sequence employed was [Irr–PW–AQ–RD]$_n$ (PW = pulse width; RD = relaxation delay) with irradiation times varying from 250 to 600 ms. In a typical experiment, the sample was irradiated off-resonance, close to the nonexchangeable proton peaks (to minimize the Bloch–Siegert shifts) and then, in turn, at each of the three nonexchangeable base protons. Thirty-two scans were accumulated for each FID, and the four FID's were stored separately. This cycle was repeated approximately 80 times to afford FID's consisting of approximately 3,000 scans. The FID's were subtracted from each other and the resultant was Fourier transformed to give the difference NOE spectrum.

Other Measurements. HPLC work was done on a Perkin-Elmer Series 4 liquid chromatograph, equipped with an LCI-100 laboratory computing integrator. UV spectra were recorded on a Perkin-Elmer Lambda 7 spectrometer. The pH measurements were made with an Orion Research digital pH meter, with the use of a 5-mm electrode. The pH was adjusted with DCl and NaOD and was not corrected for isotope effects.

Results

The platination of d(GpCpG) was followed by reversed-phase HPLC (see Figure 2 and Table I), monitored at 260 nm. After 40 min of reaction time, two new products (A and B) appear, accounting for approximately 30% of the UV-absorbing material, while the starting material, d(GpCpG), integrates to approximately 63% of the total. After 84 min, products A and B comprise approximately 38%, the starting material comprises 49%, and a new product, C, comprises 6%. After 45 h, peak C integrates to ~70% of the total UV-absorbing material, while the starting material is 21%, and the only other species (B) is approximately 5%. The reaction is essentially complete after 45 h, but was allowed to proceed for an additional 48 h, after which time the profile of the reaction mixture had not changed significantly. Addition of another portion (10% of the initial amount) of *trans*-DDP, followed by reaction for an additional 24 h, enhances the yield of the final adduct.

Inspection of the ^1H NMR spectrum of the final purified adduct (C) of *trans*-DDP with d(GpCpG) (Figure 3) reveals a single set of resonances for the nonexchangeable base and H1′ deoxyribose protons, as expected from the HPLC traces which showed only a single product. Almost all of the protons of this adduct resonate at lower fields (less shielded environments) than their counterparts in the *cis*-DDP adduct of d(GpCpG).[18] This difference is especially conspicuous for the H2′ proton of the –pCp– fragment.

Spectral assignments were obtained by applying the following strategy. The protons belonging to the same sugar residue were identified by two-dimensional correlation spectroscopy (COSY), which connects protons that are scalar coupled to one another. This procedure was sufficient to classify the 21 deoxyribose protons into three groups of seven, corresponding to each of the three sugar residues. Since the H1′ protons of these moieties are well resolved, they are readily identified and assigned.

By the use of one-dimensional nuclear Overhauser effect studies, we assigned the nonexchangeable base protons to their respective deoxyribose sugar rings. Since the H6 resonance of the deoxycytidine residue appears as a doublet, the assignment of all resonances of the pCp nucleotide was straightforward. The remaining problem of distinguishing the two guanosine residues was accomplished by identifying either the H3′ proton of G(3) and/or

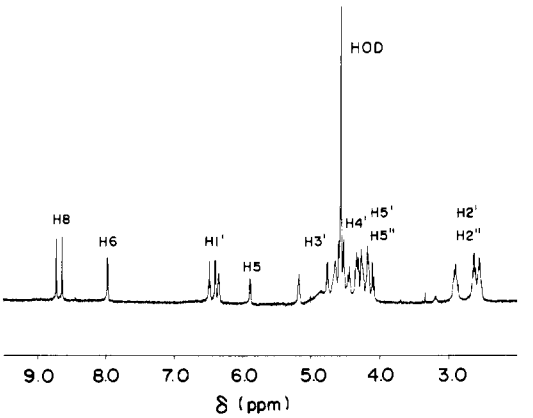

Figure 3. 500-MHz proton NMR spectrum of *trans*-[$Pt(NH_3)_2${d(GpCpG)-*N7-G(1)*, *N7-G(3)*}]. Labels refer to the various classes of proton resonances (see text).

the H5′, H5″ protons of G(1), neither of which is coupled to a ^{31}P nucleus.

The two-dimensional COSY experiment was carried out at 35 °C on a nonspinning sample, which afforded the best resolution of the resonances of interest, from the H1′ region to the H2′ H2″ region. Owing to the proximity of two of the H3′ resonances to the HOD peak, no attempt was made to use any form of water suppression. The H1′, H2′, H2″, H3′, and H4′ protons could be identified from this study (Table II), but extensive overlap of the H4′ and H5′, H5″ resonances (see Figure 3) made assignment of these protons extremely difficult. The H2′ and H2″ protons could not be individually distinguished, owing to extensive spectral overlap.

As was the case for both d(GpCpG) and *cis*-[$Pt(NH_3)_2${d(GpCpG)}],[18] the rotational correlation times of *trans*-[$Pt(NH_3)_2${d(GpCpG)}] are such that no NOE effects were observed at ambient temperatures. In order to obtain any reasonable signal, the temperature had to be decreased to 5 °C, which led to broadening of most of the resonances. The difference NOE experiments were conducted with varying NOE buildup times (250–600 ms) in order to find the best compromise between optimal signal/noise ratios and minimal spin diffusion effects.

The NOE experiments proved useful in assigning the nonexchangeable base protons but not for determining molecular conformations. The nonexchangeable protons of all three bases exhibit clear NOE effects with their respective H1′ sugar protons and, in almost all cases, to the H2′, H2″, H3′, and sometimes even H4′ protons as well. The results of a typical experiment are shown in Figure 4. Variation of the irradiation time between 200 and 600 ms made little difference in the observed NOE's.

Since the NOE data were obtained at 5 °C and the COSY study was carried out at 35 °C, the temperature-dependence of the ^1H NMR spectrum was investigated to correlate the results at these two different temperatures.

The distinction between the 5′- and the 3′-guanosine residues could be made by observing that one of the three H3′ resonances at 45 °C does not exhibit any line broadening due to ^{31}P coupling (Figure 5). Accordingly, this resonance was assigned to H3′ of

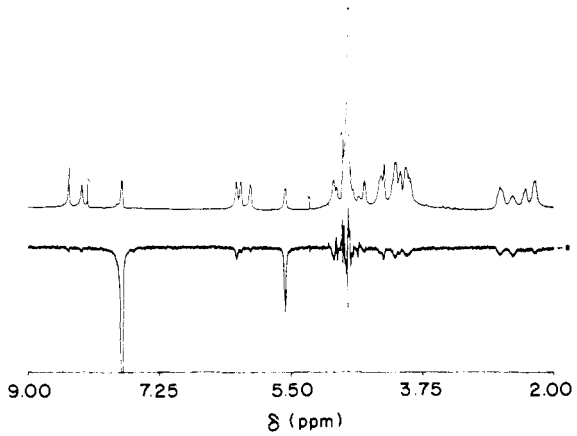

Figure 4. One-dimensional NOE difference spectrum of *trans*-[Pt-$(NH_3)_2${d(GpCpG)}] obtained upon irradiation of the H6 resonance of C(2). Peaks marked by an × are impurities in the commercial deuterated solvents.

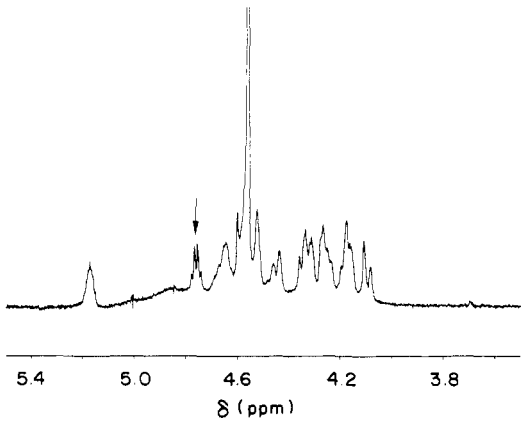

Figure 5. 500-MHz ^1H NMR spectrum of the H3′ region depicting the lack of ^{31}P coupling to the H3′ resonance of G(3), designated by an arrow.

G(3), which allows a unique assignment of the spectrum.

The binding sites of the *trans*-$\{Pt(NH_3)_2\}^{2+}$ moiety on d-(GpCpG) were determined by performing a pH titration (Figure 6). The unplatinated d(GpCpG) oligonucleotide exhibits an end point at about pH 2, which is due to the protonation of the guanosine N7 atoms, and an additional midpoint corresponding to a pK_a of 4.5, due to the protonation of the cytosine N3 atom.[18] For the platinated adduct oligonucleotide, the two guanine bases are no longer protonated at N7 at low pH, while the cytosine base retains its pK_a of 4.5. These results indicate that platinum binds to both guanosine N7 atoms, and that the cytosine residue is clearly not platinated at its only likely metal binding site. The pK_a for N1 of the two guanine bases, normally about 10, is reduced upon metal binding at their N7 positions. This reduction in pK_a of both guanosine nucleosides is clearly evident in the pH titration of *trans*-[$Pt(NH_3)_2${d(GpCpG)}], further corroborating the coordination of platinum to the N7 atoms of both guanosines (Figure 6).

In the unplatinated oligonucleotide d(GpCpG), the H1′ resonances of the deoxyribose moieties appear as triplets in the spectrum and the sum of coupling constants $J_{H1',H2'} + J_{H1',H2''}$ is on the order of 14 Hz. This value indicates that all sugar puckers are in the S (or C2′-endo) conformation, in agreement with the detailed conformational study.[18] Upon platination by *trans*-DDP, one of the H1′ resonances appears as a doublet (Figure 7) with a coupling constant of 6.7 Hz. This value corresponds to a change in sugar pucker[22] to the N (or C3′-endo) conformation. Specifically, for a C3′-endo sugar pucker the coupling between H1′

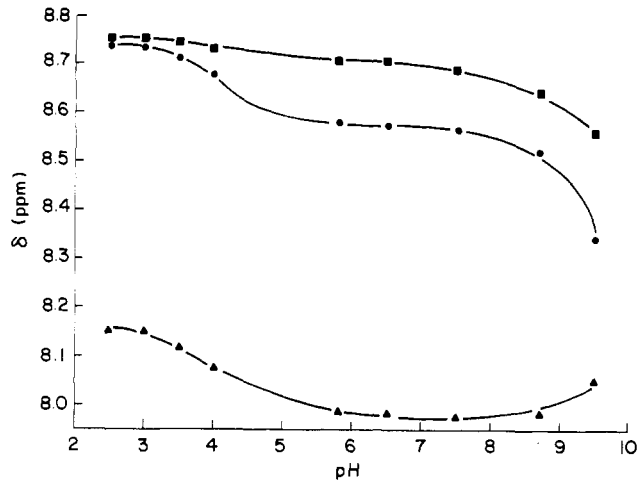

Figure 6. pH titration of the nonexchangeable base protons H8 of G(1) (●), H8 of G(3) (■) and H6 of C(2) (▲).

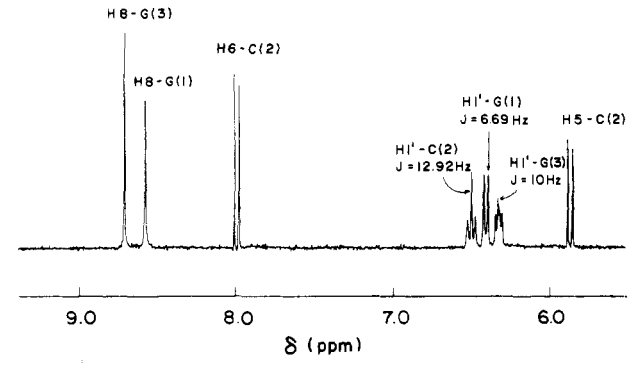

Figure 7. 250-MHz proton NMR spectrum of *trans*-[$Pt(NH_3)_2$-{d(GpCpG)}] displaying the downfield region of the spectrum, the H1′ coupling constants, and the resonance assignments.

and H2′ vanishes, leaving only that between H1′ and H2″, which appears as a doublet with a coupling constant of approximately 7 Hz. Thus, it appears that the sugar pucker of the 5′-guanosine has changed its conformation from S to N upon platination with *trans*-DDP.

Discussion

Comparison of the HPLC chromatogram of the initial reaction mixture with that obtained after 45 h would appear to indicate an uncomplicated reaction between d(GpCpG) and *trans*-[Pt-$(NH_3)_2Cl_2$] at 37 °C, pH 6, to form one major adduct in relatively high yield. Closer scrutiny of the time course of the reaction reveals, however, that two intermediate adducts initially form. As the peaks corresponding to these two intermediates grow in, that due to the starting material diminishes before any of the final adduct is observed.

Although the extinction coefficients of the two intermediates, the starting material, and the final adduct are not identical, they are expected to be within 10% of each other and the integrations of the HPLC chromatograms thus provide useful information. Specifically, after 84 min of reaction time, the two intermediates account for approximately 40% of the UV-absorbing material, and after 45 h, the final adduct and starting material account for 70% and 21%, respectively, of the total material. This high yield of 70% could not have been obtained without the intermediates having been converted to the final products.

The kinetic preference of platinum(II) complexes for the N7 atom of guanine is well established.[23] The formation of bifunctional adducts of *cis*- and *trans*-DDP with DNA may be considered to occur in a two-step reaction.[24] Initially, platinum

(22) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer: New York, 1984; pp 17–21.

(23) Mansy, S.; Chu, G. Y. H.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 607–616.

Table III. Differential Shielding[a] of the -pCp- Fragment of d(GpCpG), *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}], and *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}]

species	shielding H1′	H5	H6	ref
d(GpCpG)	−0.23	−0.37	−0.50	18
cis-[Pt(NH$_3$)$_2$-{d(GpCpG)}]	−0.03	−0.14	−0.24	18
trans-[Pt(NH$_3$)$_2$-{d(GpCpG)}]	+0.13	−0.31	−0.06	this work

[a] Relative to 8–10 mM d(pCp) at 23 °C, pH 5.0.[18]

will bind to the N7 atom of either the 5′- or the 3′-guanosine in d(GpCpG), and then to the other available N7 position to close a large, 23-membered ring (Figure 1). This scenario is consistent with the observation of two intermediates that later convert to final product. Although we have not isolated and characterized the intermediates, we believe the rationale to be self-consistent and intuitively appealing. Similar behavior has been observed in the reaction of *cis*-DDP with the trinucleotide d(GpApG).[25] Studies of the reaction of this oligonucleotide with [Pt(dien)Cl]Cl revealed no platinum binding to the adenosine. Rather, 65% of the adducts had platinum coordinated to the 3′-guanosine and 35% had platinum coordinated to the 5′-guanosine base.

We considered the possibility that *trans*-DDP might form an interstrand cross-link between two d(GpCpG) dinuclear species in parallel or antiparallel orientation. Previous studies of *trans*-DDP binding to d(GpTpG) demonstrated that only monomeric adducts form.[20] When we carried out the platinum reaction at one-third of the previous concentration (i.e. 0.1 mM vs. 0.3 mM), no change was observed in the HPLC chromatograms. A single set of resonances in the ^{1}H NMR spectrum, coupled with a change in the sugar pucker of the 5′-guanosine (it is not obvious why a sugar pucker change would occur in a dinuclear compound), provides further support for the conclusion that the major product is a mononuclear adduct.

The difference NOE spectra enabled the three nonexchangeable base protons to be assigned to their respective nucleosides, and the sugar ring protons were identified by the two-dimensional correlation study and lack of phosphorus coupling to the H3′ proton of G(3). Since no NOE's could be observed at 35 °C, a temperature-dependent study was conducted to correlate the COSY with the NOE results. No peak crossover was observed for either the H1′ or the base protons, demonstrating that the assignments made at 5 °C are valid at 35 °C. No information on the glycosyl bond orientations could be discerned from the 5 °C NOE data, and in any case, attempting to extrapolate these results to 35 °C would be questionable. In the study of the *cis*-DDP adduct of d(GpCpG)[18] it was concluded from NOE results that the cytosine residue adopts the anti conformation, while both the guanosine residues show syn ↔ anti equilibria.

The most striking feature of the spectrum of *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] is the downfield shift of almost all the resonances relative to those of both d(GpCpG) and *cis*-[Pt(NH$_3$)$_2${d(GpCpG)}] (see Table II). This difference is especially pronounced for the deoxycytidine protons, as shown in Table III, where the chemical shifts of this fragment in the three species are reported relative to the values of d(pCp). The H1′ proton of the -pCp- moiety in *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] is less shielded than its counterpart in d(pCp), while the H6 proton shows only very slight shielding. By comparison, the -pCp- nucleotide in both d(GpCpG) and its *cis*-DDP adduct exhibits substantially more shielding than in the *trans*-DDP adduct. The H5 resonance of the *trans*-DDP adduct seems to be more shielded than its *cis*-DDP counterpart; the H2′ resonance is strongly deshielded in comparison with those in the two other trinucleotides (cf. ref 18). These results reveal that, as concluded from the analysis of the *cis*-DDP complex of d(GpCpG),[18] the deoxycytidine residue of *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] does not spend much time stacked between the two guanosine residues.

The platinum binding sites were determined by the pH titration study. The chemical shifts of the nonexchangeable base protons are influenced by the extent of protonation at purine N7 and N1 positions and at N3 for pyrimidines. Protonation of N7 of guanosine causes a large change in the chemical shift of the H8 resonances in d(GpNpG).[25] Platination of these sites blocks protonation such that no sharp titration end point is observed at pH ~2. From the pH titration, it is clear that the cytosine N3 atom is protonated at its normal pK_a, while the two N7 atoms are not. In addition, the pK_a values of the two N1 atoms decrease upon platination, indicative of platinum binding.[21,25] Large downfield shifts of the H8 protons are typically observed for platination of mononucleotides[8] and trinucleotides[20] by *trans*-DDP. The larger downfield shifts of the H8 resonances in the *trans*- vs. the *cis*-DDP adduct are attributed to the lack of mutual shielding by two cis-oriented guanosine bases.

The sugar pucker conformations of d(GpCpG) were shown to be of the conventional S (C2′-endo) variety, as expected for B DNA.[18] In the *trans*-DDP adduct, the sugar pucker of the 5′-guanosine changes to the N (C3′-endo) conformation. This phenomenon is most clearly seen in Figure 7, where the pseudotriplet or quartet of the G(1) H1′ resonance, typical of S conformation, has been converted to a doublet with a coupling constant of approximately 7 Hz. The lack of H1′-H2′ coupling is typical of pure N conformations.[26] This result is very similar to the change in sugar pucker that is observed upon platination by *cis*-DDP of d(GpG) units.[12-15] The *cis*-DDP adduct of d(GpCpG) apparently does not exhibit such a sugar pucker alteration.[18] The other two H1′ coupling constants for *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}] (Figure 7) reveal a C2′-endo sugar pucker for the cytosine nucleoside and an indeterminate value for the 3′-guanosine conformation.

In summary, the NMR spectral studies of the major adduct, *trans*-[Pt(NH$_3$)$_2${d(GpCpG)}], reveal the structure to be comprised of an intrastrand cross-link in which two guanosine residues bind to platinum through their N7 atoms. This result is in good agreement with the predictions of the replication mapping of the adducts of *trans*-DDP on DNA.[9] The cytosine base is destacked compared to unplatinated d(GpCpG), as predicted by studies of platinated DNAs using antinucleoside antibodies.[11] Finally, the 5′-guanosine nucleoside switches its sugar pucker to C3′-endo upon platination with *trans*-DDP but apparently not *cis*-DDP. This same sugar pucker alteration is also a characteristic feature of *cis*-[Pt(NH$_3$)$_2${d(GpG)}] adducts on DNA.

Acknowledgment. This work was supported by U.S. Public Health Service Grant CA 34992 awarded by the National Cancer Institute, Department of Health and Human Services. D.G. was a Chaim Weizmann Postdoctoral Fellow and also thanks the National Institute of General Medical Services for a postdoctoral National Research Service Award, GM 09253. We are grateful to Engelhard Corp. for a loan of K_2PtCl_4, from which the complexes were prepared. The 500-MHz NMR spectroscopic studies were performed at the Francis Bitter National Magnet Laboratory, which is supported by the National Science Foundation.

(24) Reedijk, J.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Marcelis, A. T. M. In *Platinum Coordination Complexes in Cancer Chemotherapy*; Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds.; Martinus Nijhoff: Boston, MA, 1984; p 39.

(25) van der Veer, J. L.; van der Elst, H.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Inorg. Chem.* 1986, *25*, 4657–4663.

(26) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413–433.