spectra were run at the Catholic University of America Chemical Instrumentation Center, the FDA Office of Biologics, JEOL (U.S.A.), Inc., and the Southern California Regional NMR Center (the latter, an NSF-sponsored facility at Cal Tech). We thank Drs. William Egan, C. A. Evans, and Luciano Mueller for their assistance in obtaining spectra through the FDA, JEOL, and Cal Tech facilities, respectively. Financial support has been provided by the NIH, via Grant GM 26074.

Cytidylyl(3′–5′)guanosine Dinucleotides Give Two Platinum Chelates with *cis*-Diamminedichloroplatinum That Are Cytidine Syn–Anti Conformational Isomers

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Abstract: CpG and d(pCpG) react with *cis*-$[PtCl_2(NH_3)_2]$ (*cis*-DDP) or *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ (1 Pt per dinucleotide, 10^{-5}–(5×10^{-4}) M) in water at pH 5.5 to give as single adduct the CN3–GN7 chelate of the *cis*-$Pt(NH_3)_2^{2+}$ moiety. The nature of the ribo- and deoxy-(C-G)·*cis*-Pt chelates is established by atomic absorption spectroscopy, high-pressure gel permeation chromatography, and ^1H NMR. Reaction of CpG with [PtBr(dien)]Br and monitoring of its 10^{-2} M reaction with *cis*-DDP and its diaqua derivative show that the formation of the (CpG)·*cis*-Pt chelate is a two-step process starting with N7-platination of the guanine. The ribo- and deoxy-(G-C)·*cis*-Pt chelates exist as C(anti)-G(anti) and C(syn)-G(anti) isomers (respectively, ca. 20–80% at 20 °C) whose structures are established by ^1H NMR. When separated by HPLC, these two isomers slowly equilibrate at room temperature. The circular dichroism spectra of the two diastereoisomeric C(anti)-G(anti) and C(syn)-G(anti) chelates present a remarkable sign inversion, which can be related to the respective left- and right-handed pseudohelical arrangements of their sugar–phosphate backbones. For CpG the activation parameters of the C(anti)-G(anti) → C(syn)-G(anti) isomerization, determined from the evolution of the CD spectra at different temperatures between −9 and 18.5 °C, are $\Delta H^{\circ *} = 41$ (6) kJ mol^{-1} and $\Delta S^{\circ *} = -130$ (30) J mol^{-1} K^{-1}. From these values it is concluded that the equilibration process between the C(anti)-G(anti) and C(syn)-G(anti) platinum chelates is actually a conformational isomerization via the rotation of the cytosine about its glycosidic and N3–Pt bonds.

In the cell, DNA is considered as the primary target of the active aquated forms[2] of the antitumor drug *cis*-$[PtCl_2(NH_3)_2]$[3] (*cis*-DDP).[4] Studies with various DNAs have established that intrastrand cross-linking of two adjacent guanines is the major coordination fate of the *cis*-$Pt(NH_3)_2^{2+}$ moiety.[6-11] This is in agreement with model oligonucleotide studies which pointed to the facile GN7–GN7 platinum chelation by the GpG sequence to give only one complex with an anti–anti configuration.[12-16] In the case of DNA, there seems to be conflicting evidence about whether platinum first binds to all guanines with equal probability[17] or by a direct bifunctional attack at preferred sites.[11]

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(4) (a) Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); dien, diethylenetriamine; en, ethylenediamine; $TSPd_4$, sodium 3-(trimethylsilyl)-1-propionate-2,2,3,3-d_4; HPLC = high-pressure liquid chromatography; NOE = nuclear Overhauser effect; C and G represent cytidine and guanosine; d = deoxy; p to the left of a nucleoside symbol indicates a 5′-phosphate and to the right it indicates a 3′-phosphate; 5′-GMP = guanosine 5′-monophosphate. (b) Nomenclature: The common nomenclature for inorganic compounds is combined with that recommended by the IUPAC–IUB for nucleic acids,[5] i.e., the metal is listed first in formulas, followed by the ligands. The coordinating atom(s) of the ligand, in case of ambiguity, is (are) indicated in italics after the ligand, separated by a hyphen, according to ref 5, e.g., *cis*-$[Pt(NH_3)_2\{d(CpG)$-*N3,N7*$\}]^+$. Bridging ligands are indicated with the prefix μ, e.g., $[cis\text{-}Pt(NH_3)_2]_2\mu$-{CpG-*N3,N7*}$]_2^{2+}$. *cis*-Pt is used as an abbreviation for the *cis*-$Pt(NH_3)_2^{2+}$ moiety. Abbreviations used for the platinum dinucleotide complexes are CpG·*cis*-Pt, d(pCpG)·*cis*-Pt, and more generally (C-G)·*cis*-Pt for both of them.

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0002-7863/84/1506-7227$01.50/0

Actually other less frequent types of chelation have been demonstrated or proposed, from the analysis of enzymatic digests of platinated DNA. They involve either adenosine and guanosine or two guanosines of d(ApG),[11,18] d(ApXpG),[11] and d(GpXpG)[10,11] sequences with X = A, C, G, or T. The latter adducts, with X = C and A, lead to base-pair substitution in wild type *E. Coli* bacteria,[19] and model studies support a GN7–GN7 chelation by the d(GpCpG) sequence.[20] Dinucleotide model studies have shown that ApA,[12b,21] GpC and d(pGpC),[12b,22] CpC and d(pCpC),[22] and GpA.[23] all give platinum chelates upon reaction with *cis*-DDP, with formation of several isomers in the A–A, G–C, and G–A cases. For CpG, the formation of the *cis*-$[Pt(NH_3)_2$(CpG-*N3,N7*)$]^+$ and $[cis\text{-}Pt(NH_3)_2(\mu\text{-(CpG-}N3,N7))]_2^{2+}$ complexes, accounting for internal and external cross-linking, has been reported.[24] In this paper, following a preliminary report,[22] we show that CpG and d(pCpG) react stoichiometrically with *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. For both dinucleotides, the two-step reaction starts with GN7 platinum coordination and gives the C(anti)-G(anti) and the C(syn-G(anti) isomers of the CN3–GN7 platinum chelate *cis*-$[Pt(NH_3)_2\{$(C-G)-*N3,N7*$\}]^{+(-)}$ respectively **1** and **2** for CpG and **3** and **4** for d(pCpG). At room temperature, for both the ribo and deoxy complexes, a slow rotation of the cytosine, about the glycosidic and CN3–Pt bonds, leads to the equilibrium mixture of the two C(anti) and C(syn) conformers.

Experimental Section

CpG and d(pCpG) ammonium salts were from Sigma and Collaborative Research. The stoichiometric reactions (1 Pt per dinucleotide) were run at 10^{-5}–(5×10^{-4})M concentrations at pH ca. 5.5, in doubly distilled water, at 37 °C using *cis*-DDP, its diaqua derivative, or [PtBr(dien)]Br, under previously described conditions.[12b,14,22] The predominant reactive species were the aquachloro and aquahydroxo complexes for *cis*-DDP and the diaqua and aquahydroxo complexes for *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$.[2a] Concentrated stoichiometric reactions, 10^{-2} M in *cis*-Pt, have been run at pH 3.5 with *cis*-DDP and pH 5.5 and 3.5 with its diaqua derivative. At these pHs, the less reactive μ-hydroxo species can be neglected.[2]

Platinum content of the complexes was determined by atomic absorption using a A 560 Perkin-Elmer spectrometer coupled with a HGA 500 programmer.

HPLC analyses and preparative separations were performed on an Altex 420 liquid chromatograph, with 254-nm detection, on a Waters C18 μBondapak column used in reverse phase, with a 10^{-2} M aqueous $CH_3CO_2NH_4$ solution as eluant A and 10^{-2} M $CH_3CO_2NH_4$ in H_2O/CH_3OH (1:1) as eluant B, both solutions were at pH 4 (CH_3CO_2H added). Pure samples of the ribo- and deoxy-(C-G)·*cis*-Pt isomers were collected at liquid nitrogen temperature and lyophilized.

High-pressure gel-permeation chromatography analyses were performed on a Waters μ-Porasil GPC 60-A column using a 0.5 M $CH_3CO_2NH_4$ solution at pH 4 (CH_3CO_2H added) with 1% sodium dodecyl sulfate, as eluant.

The ^{1}H NMR spectra were recorded on Bruker WM 250 (250 MHz) and WM 400 (400 MHz) spectrometers, using standard Fourier transform techniques. The protons T_1 relaxation times were obtained by the inversion recovery method at ca. 20 °C under previously reported conditions.[14] The analyses of the spectra were obtained by homodecoupling techniques, NOE difference experiments, and two-dimensional homonuclear experiments (2D COSY 45 or 2D NOESY; the original data set consisted of 1024 points in the t_2 dimension and 256 points in the t_1 dimension, the resulting data matrix was processed with a sine-bell window in the two dimensions and a zero filling in the f_1 dimension, and the absolute value mode was used).

The CD spectra were recorded on a Jobin Yvon Mark III. The $\Delta\epsilon$ ($\epsilon_L - \epsilon_R$, M^{-1} cm^{-1}) are given per nucleotide residue. The molar extinction coefficients of the complexes were determined from the ratio of the optical densities of the dinucleotide solution before and after reaction with the platinum complex, assuming that no concentration change had occurred. For the mixtures of the (C-G)·*cis*-Pt isomers a mean extinction coefficient was obtained that was used for each isomer separated by HPLC. The variation of the equilibrium constant of the CpG·*cis*-Pt **1** and **2** isomers, with temperature, was determined from ^{1}H NMR spectra recorded between 2 and 87 °C. The rate constants of the CpG·*cis*-Pt **1** → **2** and **2** → **1** isomerizations were determined from the evolution vs. time of the CD spectra of the minor complex **1** (1.3×10^{-4} M) at four temperatures between -9 and 18.5 °C and the major complex **2** (2.2×10^{-4} M) at 1 and 18 °C, up to 30% conversion. The thermodynamic and kinetic parameters were determined from the $\ln K = f(1/T)$ and Eyring plots, using a classical regression analysis.

Results

Analysis of the Reactions. The reactions of 1 molar equiv of *cis*-$[PtCl_2(NH_3)_2]$ or *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ with CpG (2.8×10^{-4})–(1.8×10^{-5} M) or d(pCpG) (7.8×10^{-5})–(4.5×10^{-5} M) in water at pH 5.5 give a similar evolution of the UV characteristics of the solution (C-G, C-G λ_{max}, C-G·*cis*-Pt λ_{max} ratio of λ_{max} optical densities for C-G·*cis*-Pt and C-G: CpG 254 nm (sh 270), 261 nm, 0.9; d(pCpG) 254 (sh 270), 262, 1.0). A comparable evolution is observed for the stoichiometric reaction between [PtBr(dien)]Br and CpG (2.7×10^{-4} M) [(i.e., 254 (sh 270), 261, 0.87 nm).

The reactions between the dichloro or diaqua complex with CpG and d(pCpG) give, for each dinucleotide, the same two new HPLC peaks, with a shorter elution time than that of the starting C-G. With the bromodiethylenetriamine complex, only one new peak appears with a slightly longer elution time than that of CpG.

With the diaqua complex, CpG 2.8×10^{-4} M and d(pCpG) 7.8×10^{-5} M are completely converted into the final products after 17 and 21 h. The same reactions are about twice as long with the dichloro complex. For CpG 2.7×10^{-4} M and [PtBr(dien)]Br the reaction is over after 24 h.

For CpG 5×10^{-5} M reacting with the diaqua complex, C-G/Pt molar ratios of 0.5, 1, and 2 do not bring any change either in the products formed or in their proportions (in the latter case unreacted CpG is present). For the stoichiometric reaction run at the higher 10^{-2} M concentration at pH 5.5, between the diaqua complex and CpG, a monomeric intermediate complex is formed with an HPLC retention time close to that of the adduct obtained with [PtBr(dien)]Br. This intermediate is further converted into the two final products. Addition of 7 M aqueous KCl transforms the intermediate into another compound which, in these conditions, very slowly gives the final products. If one tries to isolate this new compound by HPLC, its transformation into the two final products is greatly accelerated.

After preparative HPLC separation, the two final adducts called **1** and **2** for CpG and **3** and **4** for d(pCpG), according to their elution order, slowly equilibrate at room temperature to give the same mixture as that of the reaction. At 20 °C this equilibrium mixture contains 20% **1**, 80% **2** or 17% **3**, 83% **4**, determined by ^{1}H NMR integration. Collection of the HPLC preparative fractions in the cold, followed by lyophilization, gave the pure isomers.

Analysis of the Complexes Formed. Atomic absorption spectroscopy allowed the determination of an ϵ_{max}/Pt of about 19 500 and 20 500 respectively for the adducts of CpG and d(pCpG), to be compared to 19 000 and 19 200 for the free dinucleotides.

The retention times of these adducts, in gel permeation chromatography, are respectively similar to those of the previously

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Table I. ^{1}H NMR Data for the (CpG)·*cis*-Pt **1** and **2** and for the [Pt(dien)(CpG-*N7*)]$^+$ (**5**) Complexes

	CpG (pH* 7.0)[25]		**1** (pH* 7.0)			**2** (pH* 7.0)			**5** (pH* 7.1)		
	δ	J	δ	J	Δδ[a]	δ	J	Δδ[a]	δ	J	Δδ[a]
H8	8.02 s		8.22 s		+0.2	8.44 s		+0.4	8.51 s		+0.5
H6	7.76 d	(5,6) = 7.9	8.10 d	(5,6) = 7.7	+0.35	7.62 d	(5,6) = 7.7	−0.15	7.83 d	(5,6) = 8	+0.05
H5	5.87 d		6.03 d		+0.15	5.99 d		+0.1	≈5.9 d		0
C-H1′	5.73 d	(1′,2′) = 3.2	5.78 s		+0.05	5.32 s		−0.4	5.9 d	(1′,2′) = 4	0, +0.15
G-H1′	5.90 d	(1′,2′) = 4.3	5.83 d	(1′,2′) = 6.0	−0.05	5.96 d	(1′,2′) = 7.7	+0.05	5.96 d	(1′,2′) = 4	0.05, +0.2
C-H2′	4.32	(2′,3′) = 5.1				4.37 d	(2′,3′) = 6.0	+0.05			
G-H2′	4.64	(2′3′) = 5.0				4.53	(2′,3′) = 5.8	−0.1			
C-H3′	4.44	(3′,4′) = 6.7 (3′,P) = 8.3				5.23	(3′,4′) = 9.8 (3′,P) = 9.8	+0.8			
G-H3′	4.52	(3′,4′) = 5.4				4.43	(3′,4′) = 2.5	−0.1			
C-H4′	4.21	(4′,5′) = 3.2				≈4.17		−0.04			
G-H4′	4.32	(4′,5′) = 2.4 (4′,5″) = 3.0				4.33	(4′,5′) ≈3–4 (4′,5″) ≈ 3–4	+0.01			
C-H5′	3.77	(5′,5″) = −13				4.05	(4′,5′) = 2.5 (5′,5″) = −12.5	+0.3			
C-H5″	3.82	(4′,5″) = 2.8				3.87	(4′,5″) = 7.2	+0.05			
G-H5′	4.29					≈4.17					
G-H5″	4.16										

[a] Chemical shift variation referred to CpG.

Table II. T_1 Relaxation Times[a] and Nuclear Overhauser Enhancements Observed for the Characteristic Protons of the (CpG)·*cis*-Pt Isomers **1** and **2**

	1 T_1, s	**2** T_1, s	**2** NOE
H8	0.6	0.5	
H6	0.6	0.2	+9%[b]
H5		0.6	
G-H1′	0.6	0.6	
C-H1′	0.6	0.2	+8%[b]
C-H3′		0.3	

[a] At 250 MHz and 17 °C. [b] Correlated NOEs.

described GpG·*cis*-Pt and d(pGpG)·*cis*-Pt complexes.[14] These results, together with the stoichiometry of the reaction, show that the complexes formed are monomeric and contain one platinum atom per dinucleotide.

Table I gives the ^{1}H NMR data for the two CpG·*cis*-Pt isomers, compared with those of CpG and of [Pt(dien)(CpG-*N7*)]$^+$ (**5**) (vide infra). Table II gives the T_1 relaxation times and the nuclear Overhauser enhancements measured for characteristic protons of the CpG·*cis*-Pt isomers, **1** and **2**. Table III gives the ^{1}H NMR data for the two d(pCpG)·*cis*-Pt isomers, **3** and **4**, compared with those of d(pCpG). The variation of the G-H8, C-H6, and C-H5 chemical shifts as a function of pH*[26] has been plotted between

Table III. ^{1}H NMR Data for the d(pCpG)·*cis*-Pt Complexes **3** and **4**

	d(pCpG) (pH* 5.1)		**3** (pH* 6.1)			**4** (pH* 6.1)		
	δ	J	δ	J	Δδ[a]	δ	J	Δδ[a]
H8	8.07[b]		8.19 s		+0.1	8.42 s		+0.3
H6	7.83[c]	(5,6) = 7.7	8.13 d	(5.6) = 7.5	+0.3	7.52 d	(5.6) = 7.5	−0.3
H5	6.08[c]		6.01 d			5.91 d		
C-H1′	6.13 d,d	(1′,2′) = 5.5, (1′,2″) = 7.7	6.06		−0.05, −0.2	5.65 d,d	(1′,2′) = 3.5 (1′2″) = 7	−0.5
G-H1′	6.26 t	(1′,2′) = (1′2″) = 6.7	6.09			6.29 d,d	(1′,2′) = 10 (1′2″) = 5	0
C-H2′	1.87	(2′,2″) = −14 (2′,3′) = 5.5				≈2.54		+0.7
C-H2″	2.41	(2″,3′) = 2.2						+0.15
G-H2′	2.85	(2′,2″) = −14 (2′,3′) = 6.5				2.5	(2′,2″) = −13.5 (2′,3′) = 6	−0.35
G-H2″	2.51	(2″,3′) = 3.5				2.43	(2″,3′) ≤ 1.5	−0.1
C-H3′	4.78	(3′,4′) = 2 (3′,P) = 6.5				5.25	(3′,4′) = 8 (3′,P) = 8	+0.5
G-H3′	4.72	(3′,4′) = 3.5				4.66	(3′,4′) = 2	−0.05
C-H4′	4.24					4.1		
G-H4′	4.20					4.1		
C-H5′ C-H5″	3.96							
G-H5′ G-H5″	4.09					4.26 4.00		

[a] Chemical shift variation referred to d(pCpG). [b] Broad. [c] Broad doublet.

Table IV. ^{1}H NMR Data for the Monocoordinated CpG **6** and [8] and bis(CpG) **7** Complexes

	cis-[PtCl(NH$_3$)$_2$(CpG-*N7*)] (**6**) (pH* 3.5)			*cis*-[Pt(NH$_3$)$_2$(CpG-*N7*)$_2$] (**7**) (pH* 3.5)			*cis*-[Pt(NH$_3$)$_2$(H$_2$O)(CpG-*N7*)]$^+$ (**8**) (pH* 3.5)		
	δ	J	$\Delta\delta^a$	δ	J	$\Delta\delta^a$	δ	J	$\Delta\delta^a$
H8	8.46 s		+0.45	8.51 s		+0.5	8.61 s		+0.6
H6	8.1 d	(5,6) = 7.7	+0.35	8.1 d	(5,6) = 7.7	+0.35	8.1 d	(5,6) = 7.7	+0.35
H5	6.19 d		+0.3	6.22 s		+0.3	6.24 d		+0.35
C-H1′	5.96 d			5.88 d	(1′,2′) = 4.8		6.02 d	(1′,2′) = 3.7	
G-H1′	5.88 d						5.97 d	(1′,2′) = 4.7	

[a] Chemical shift variation referred to CpG.

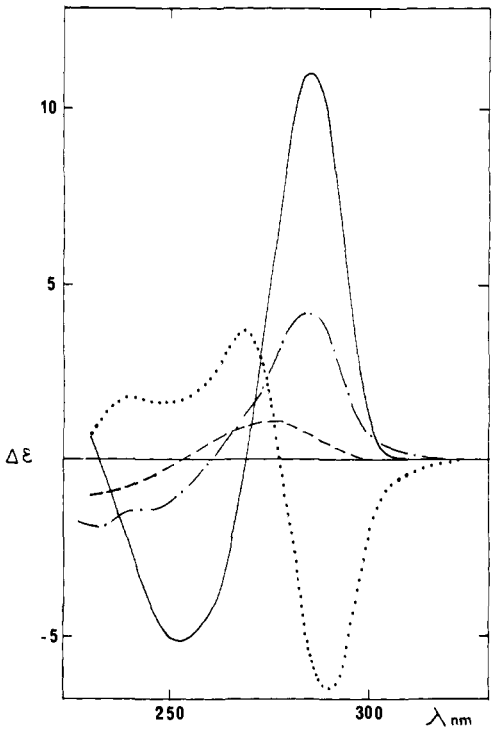

Figure 1. Circular dichroism spectra of CpG (---) (1.10^{-4} M, pH 6.4), (CpG)·*cis*-Pt **1** (···) (1.3×10^{-4} M, pH 5.5), (CpG)·*cis*-Pt **2** (—) (2.2×10^{-4} M, pH 5.5), and [Pt(dien)(CpG-*N7*)]$^+$ **5** (- - -) (1.6×10^{-4} M, pH 6.1) in NaCl 0.05 M for CpG and **5** at 20 °C and 1 M for **1** and **2** at 1 °C.

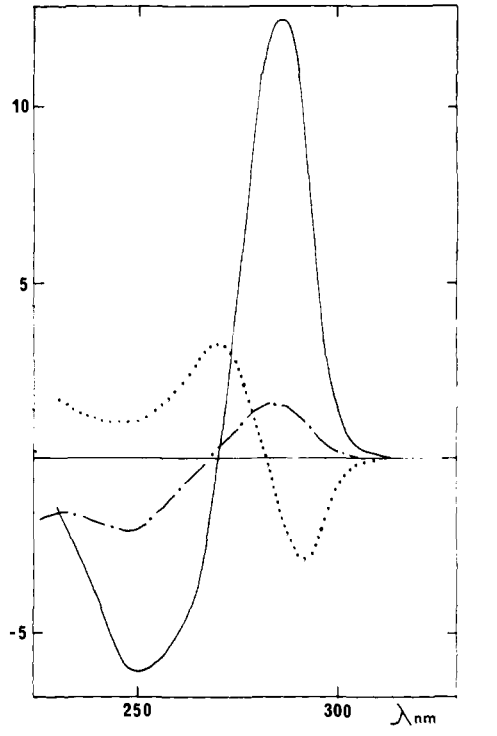

Figure 2. Circular dichroism spectra of d(pCpG) (---) (1.4×10^{-4} M, pH 6.9), d(pCpG)·*cis*-Pt **3** (···) (1.7×10^{-4} M, pH 5.5), and d-(pCpG)·*cis*-Pt **4** (—) (1.9×10^{-4} M, pH 5.5) in NaCl 0.05 M for d-(pCpG) at 20 °C and 1 M for **3** and **4** at 2.5 °C.

pH* 1.6 and 9.6 for the dinucleotides and the complexes **1–5**. For all these complexes, one notes the titration of a group with an apparent pK_a of ca. 8–8.5. Only for the Pt(dien) complex **5**, the C-H6 and C-H5 chemical shifts reflect the titration of a group with an apparent pK_a of ca. 4.5. The 8–8.5 pK_a can be assigned to the NH1 of an N7-platinated guanine (compared to ca. 10 in the free dinucleotide),[12-16,27,28] in agreement with the absence of GN7 titration (pK_a 2.3 in the free dinucleotide[29]). For complex **5** the 4.5 pK_a can be assigned to the free cytosine N3.[29] The absence of this titration for the **1** to **4** complexes shows that these complexes are CN3-GN7 chelates of the *cis*-Pt(NH$_3$)$_2^{2+}$ moiety.

It is noteworthy that the G-H8 downfield shifts for **1** and **3** are smaller than those usually reported for GN7 platination (0.3–0.7 ppm) and are also rather small for complexes **2** and **4**.[12-16,27,30] Moreover this chelation leads to C-H6 downfield shifts for complexes **1** and **3** and upfield shifts for their isomers **2** and **4** whereas CN3 platinum binding was reported to give 0.1–0.3 ppm downfield shifts of the H6 and H5 protons.[30] For the reaction of CpG with [PtCl$_2$(en)] a 0.11 ppm C-H6 upfield shift had been observed for one of the adducts.[24]

Whereas at pH* 9.6 the half-life of the free CpG G-H8 in D_2O is 98 h ($k = 7 \times 10^{-3}$ h^{-1}) and that of the complex **5** G-H8 at pH* 9.8 is 40.7 h ($k = 1.7 \times 10^{-2}$ h^{-1}), we have not detected any G-H8 exchange at pH* 9.6, after 1 week, for any of the complexes **1-4**. (Their stability in these conditions has been checked by returning to neutral pH*).

To identify the intermediate complex formed during the 10^{-2} M, pH 5.5, stoichiometric reaction, we have run two 10^{-2} M experiments with CpG and either *cis*-DDP or its diaqua derivative, at pH 3.5, in order to slow down the second step and monitored them by ^{1}H NMR. With *cis*-DDP, the *cis*-[PtCl(NH$_3$)$_2$(CpG-*N7*)] complex (**6**) (Table IV) appears after 1.5 h followed by the bis(CpG) complex *cis*-[Pt(NH$_3$)$_2$(CpG-*N7*)$_2$] (**7**) (Table IV), which becomes predominant (54% of the Pt). After 28 h a slow transformation of complex **6** into the **2** and **1** chelates is observed. Starting with *cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$, all CpG is converted within less than 5 min to give the *cis*-[Pt(NH$_3$)$_2$(H$_2$O)(CpG-*N7*)]$^+$ complex (**8**) (74% of the Pt) (Table IV), together with the bis-(CpG) adduct **7** (26%). After 10 min the transformation of **8** into **2** and **1** is detected and is nearly completed after about 14 h.

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The CD spectra of the ribo **1**, **2**, and **5** and deoxy **3** and **4** isomers, at 0 °C, are presented in Figures 1 and 2. The bisignated curves of the **1** and **3** isomers are both inverted when compared to those of their isomers **2** and **4** and of their parent dinucleotides. The spectrum presented in our preliminary report,[22] for d(pCpG)·*cis*-Pt isomer **3** at room temperature, actually corresponded to a partially isomerized complex. We have looked at the pH dependence of the CD spectra of the equilibrium mixtures of isomers **1** and **2**, and **3** and **4**. In both cases, the spectra at neutral and acidic pH are very similar with only a small increase of the amplitude of the couplet at pH 1.6. At pH 11 the amplitude of the bisignated curve is reduced to 50–60% of its value at pH 6.8, and the spectrum is shifted to shorter wavelength by about 10 nm.

Analysis of the CpG·*cis*-Pt 1 ⇄ 2 Interconversion. The constants of the **1** ⇄ **2** equilibrium, at five temperatures between 2 and 87 °C, have been determined by ^{1}H NMR analysis of the equilibrium mixtures (e.g., K_{275} = 5.9 (6) and K_{360} = 1.9 (2). From the ln $K = f(1/T)$ plot, we have determined the standard enthalpy variation $\Delta H°$ for the **1** → **2** transformation, assuming that $\Delta H°$ is constant within the temperature range used: $\Delta H°$ = -10.4 ± 0.6 kJ mol^{-1} (r^2 = 0.99). The other thermodynamic parameters of this reaction are $\Delta G°_{293}$ = -3.4 ± 0.3 kJ mol^{-1} and $\Delta S°$ = -23 ± 3 J mol^{-1} K^{-1}.

To find out whether the **1** → **2** isomerization occurred between two configurations, implying the dissociation of a nitrogen–platinum bond, or between two conformations of a single complex, we have tried to trap an intermediate complex bearing a monocoordinated CpG. ^{1}H NMR and CD have shown that there is no base protonation for the **1** and **2** isomers down to pH* 1.6. ^{1}H NMR has shown that 7 M KCl does not transform any of the isomers **1** and **2** into a monocoordinated-CpG species. HPLC has revealed no evolution of a 10^{-3} M **1** + **2** mixture in the presence of 10^{-2} M 5'-GMP. Taking advantage of the opposite signs of the CD signals of the isomers **1** and **2**, we have followed the evolution of the CD spectrum of the minor isomer **1** at four temperatures between -9 and 18.5 °C. The variation with time of $\Delta\epsilon$ at 288 nm, up to 30% conversion of **1**, gave a concentration variation that can be fit by a first-order approach to equilibrium. Although we could less accurately follow the reverse **2** → **1** isomerization, we checked that the forward (k_f) and reverse (k_r) rate constants were in reasonable agreement with the corresponding equilibrium constant; for example, at 18.5 °C k_f = 6.2 (3) × 10^{-4} s^{-1} and k_r = 1.8 (3) × 10^{-4} s^{-1}, for K_{293} = 4.0 (4) (from ^{1}H NMR) An Erying plot of the temperature dependence of the k_f rate constant gave the following activation parameters for the **1** → **2** isomerization: $\Delta H°^{\ddagger}$ = 41 ± 6 kJ mol^{-1}, $\Delta S°^{\ddagger}$ = -130 ± 30 J mol^{-1} K^{-1}, $\Delta G°^{\ddagger}$ = 80 ± 14 kJ mol^{-1}.

Discussion

The preceding results show that the stoichiometric reactions of CpG or d(pCpG) with either *cis*-[$PtCl_2(NH_3)_2$] or *cis*-[$Pt(NH_3)_2(H_2O)_2$]$^{2+}$ give in each case a ca. 20:80% equilibrium mixture of two isomeric CN3–GN7 chelates of the *cis*-$Pt(NH_3)_2^{2+}$ moiety (**1**, **2** and **3**, **4**). The ^{1}H NMR (Tables I and III) and CD (Figures 1, 2) data show that the ribo and deoxy complexes are completely similar. The only formation of [Pt(dien)(CpG-*N7*)]$^+$ (**5**), upon reaction of CpG with [PtBr(dien)]Br, together with the formation of *cis*-[$PtCl(NH_3)_2$(CpG-*N7*)] (**6**) and *cis*-[$Pt(NH_3)_2(H_2O)$(CpG-*N7*)]$^+$ (**8**) respectively during the high-concentration reactions of CpG with *cis*-DDP and its diaqua derivative, shows that platinum first binds to guanine N7 and that chelation by cytosine N3 occurs in a second step. At high concentrations of CpG, this second step is in competition with the GN7 binding of a second molecule of CpG to give *cis*-[$Pt(NH_3)_2$(CpG-*N7*)$_2$] (**7**), as observed in the GpC case.[28]

We assign the C(anti)-G(anti) structure to the CpG·*cis*-Pt **1** and d(pCpG)·*cis*-Pt **3** complexes and the C(syn)-G(anti) structure to their respective isomers, **2** and **4**, on the basis of the following ^{1}H NMR data: (a) For the CpG·*cis*-Pt isomer **2**, we observe correlated nuclear Overhauser enhancements of 9% for C-H6 and 8% for C-H1′ (Table II). These protons have the same T_1 relaxation time of 0.2 s, 3 times smaller than that of the C-H6 and C-H1′ of isomer **1**. This reveals a close proximity between the C-H6 and C-H1′ of CpG·*cis*-Pt **2**. (b) For the same isomer, **2**, the C-H1′ experiences a 0.4 ppm upfield shift, compared to its chemical shift in CpG, that is accompanied by a 0.8 ppm downfield shift of the C-H3′ (Table I). We assign these shifts to the deshielding effect of the carbonyl group of the syn cytosine in complex **2** compared to the flexible cytosine in CpG.[31] Comparable shifts of the C-H1′ and C-H3′ signals are observed for d(pCpG)·*cis*-Pt isomer **4** (Table III). (c) For d(pCpG)·*cis*-Pt, only the C-H6 chemical shift of isomer **3** is sensitive (+0.2 ppm) to the titration of the free 5′-phosphate group (apparent pK_a 6.9). This reflects a proximity between the C-H6 proton and the phosphate group.[32] All these data establish C(anti) and C(syn) structures respectively for the **1**,**3** and **2**,**4** isomers of the ribo and deoxy complexes.[33,34] (d) For both isomers of CpG·*cis*-Pt there is no detectable NOE between the G-H8 and G-H1′ protons. The T_1 relaxation times of these two protons are 0.6 s in isomer **1** and respectively 0.5 and 0.6 s in isomer **2**, reflecting no mutual relaxation (Table II). This is evidence for a G(anti) structure in **1** and **2**. The absence of G-H8/deuterium exchange for the two isomers (vide infra) precluded the confirmation of this result by the study of the T_1 relaxation times of the G-H1′ protons of the deuterated guanosines.[31]

The absence of deuterium exchange for the H8 of the platinated guanines of all the C-G chelated complexes, at basic pH*, must be due to the screening effects of the cytosine amino and carbonyl groups that are close to the G-H8 respectively in the C(anti)-G(anti) and C(syn)-G(anti) isomers. With these structure assignments, an examination of Corey–Pauling–Koltun (CPK) models shows that the G-H8 proton experiences the magnetic shielding of the cytosine ring in the C(anti)-G(anti) isomers (**1**, **3**) and of the cytosine carbonyl in the C(syn)-G(anti) isomers (**2**, **4**). This allows us to interpret the smaller than expected downfield shifts observed for this G-H8 proton upon CN3–GN7 platinum chelation by the C-G dinucleotides.

For the CpG·*cis*-Pt isomers **1** and **2** (Table I) the absence of C-H1′H2′ coupling shows that the cytidine ribose adopts an N-type conformation (C3′-endo) whereas the guanosine ribose has a predominantly S-type conformation (C2′-endo).[35] This is in agreement with the strong deshielding of the C-H3′ proton by the carbonyl group of the C(syn)-G(anti) isomer **2** (vide supra). For the d(pCpG)·*cis*-Pt isomer **4** (Table III) the ($J_{1'2'}$ + $J_{1'2''}$) values show that the 5′- and 3′-sugar conformations are respectively mainly of the N and S type. We have already shown that GN7–GN7 platinum chelation, by a G-G dinucleotide, leads to the adoption of an N-type conformation by the 5′-ribose or -deoxyribose.[14,36] This conformational change is also observed for platinum chelation by a GpG sequence within larger oligonucleotides.[16,37] It is noteworthy that it is also induced by CN3–GN7 platinum chelation by C-G dinucleotides and that it is present for both the C(anti)-G(anti) and C(syn)-G(anti) isomers.

The CD spectra of the two ribo- or deoxy-(C-G)·*cis*-Pt isomers present a remarkable sign inversion (Figures 1 and 2). The excitonic nature of the CD signals shows that the optical activity originates mainly from base–base interactions. The CPK models suggest that the larger couplet amplitude of the C(syn)-G(anti) isomers could result from the "face-to-face" situation of the bases that is not found in the C(anti)-G(anti) isomers. For the latter, the presence of the sugar–phosphate backbone diminishes the

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ring–ring dihedral angle compared to the 104° value reported for the *cis*-diammine(9-ethylguanine)(1-methylcytosine)platinum(II) model compound, having both G-O6 and C-O2 on the same side of the complex square plane.[38] Inspection of the CPK models reveals that the C(syn)-G(anti) chelation can be obtained with a slight modification of the right-handed helical conformation of the sugar–phosphate backbone of the free dinucleotide. In contrast, the C(anti)-G(anti) chelation implies an important perturbation leading to a zigzag left-handed pseudohelical conformation of the backbone.[39] It is noteworthy that in the C(anti)-G(anti) isomer, the G conformation is actually at the limit of the G(anti) and G(syn) assignments (χ = O(4′)C(1′)N(9)C(4) ≈ −90°).[40] In circular dichroism, for the C-G dinucleotides and their C(syn)-G(anti) platinum chelates, one observes a positive band for the first Cotton effect around 285 nm, followed by a negative band at shorter wavelength. For the C(anti)-G(anti) isomers there is a first negative band around 290 nm. If as a first approximation a positive chirality can be related to a rotation of the main electric transition moments[41] in the sense of a right-handed helix,[42-44] the sign of the first long-wavelength band appears to be in accordance with the chirality of the two types of isomers.

The energy difference between the C(anti)-G(anti) and C(syn)-G(anti) platinum chelates of the CpG and d(pCpG) dinucleotides is rather small. The -3.4 (3) kJ mol^{-1} standard free energy difference for the **1** → **2** conversion results from an exothermic transformation ($\Delta H°$ = -10.4 (6) kJ mol^{-1}) involving a rather unfavorable contribution of the entropy variation ($-T\Delta S°$ = +7.0 (9) kJ mol^{-1}). For the **1** ⇄ **2** isomerization, we have not been able to trap any "open intermediate" corresponding to the dissociation of the CN3–Pt bond. The activation parameters determined for the **1** → **2** conversion rule out a ligand exchange mechanism to account for the anti to syn isomerization of the cytosine.[45] On one hand, a dissociative process,[46] involving a rate-determining CN3–Pt bond-breaking step, can be excluded because of the rather large negative activation entropy of the **1** → **2** reaction ($\Delta S°^{\ddagger}$ = −130 (30) J mol^{-1} K^{-1}). Such a dissociative process with no charge separation, should present a positive $\Delta S°^{\ddagger}$.[47] Moreover the value of the enthalpy of activation ($\Delta H°^{\ddagger}$ = 41 (6) kJ mol^{-1}) is too small to account for a dissociative pathway.[47] On the other hand, this $\Delta H°^{\ddagger}$ value is also small when compared to those reported for ligand exchange reactions on platinum(II) and particularly for a water solvolytic associative process, e.g., 80 kJ mol^{-1}.[48] Actually the **1** → **2** $\Delta H°^{\ddagger}$ is of the order of magnitude of that of the chair ⇄ chair conformational inversion of cyclohexane (ca. 44 kJ mol^{-1}). Therefore we conclude that the **1** ⇄ **2** equilibration process is a conformational isomerization. It involves a rotation of the platinated cytosine about its glycosidic and N3–Pt bonds. Such a rotation seemed unlikely when considering the CPK models of the (C-G)·*cis*-Pt complexes. It is worth mentioning that other workers have observed a comparable equilibration of the two isomeric platinum chelates obtained from d(m^5-CpG) to give also an ca. 20–80% mixture at room temperature.[49] It is likely that a rotation of the platinated cytosine is also involved in this case, that is not influenced by the presence of the 5-methyl group which remains "outside" of the dinucleotide chelate.

In conclusion, at variance with a recent report concluding that no platinum cross-link occurred between cytosine and guanine for d(CpG),[50] we have shown that within a ribo- or deoxydinucleotide the CpG sequence is able to give a CN3–GN7 chelate of the *cis*-Pt(NH$_3$)$_2^{2+}$ moiety. This platinum chelate exists as two C(anti)-G(anti) and C(syn)-G(anti) conformational isomers, which equilibrate via a rotation of the cytosine about its glycosidic and N3–Pt bonds. These two isomers respectively present left- and right-handed pseudohelical arrangements of their sugar–phosphate backbones. It is likely that such a CpG chelation could occur after N7-platination of a guanine within a premelted region of kinked[51] or systematically bent B DNA[52] provided that more favorable G-G or A-G chelations are not available.[10,11,18] From the data of enzymatic digestions of platinated DNA,[10,11,18] CpG chelation does not appear among the detected cross-linking processes. Whether it could be a minor but significant event because of eventual implications of its conformational properties is an open question.

Acknowledgment. Support of this work by a grant from the "Association pour le développement de la Recherche sur le Cancer" (ARC) is gratefully acknowledged. We thank Dr. Gouyette, Institut Gustave Roussy, Villejuif, for the access to the AAS facility and Engelhardt Industries, France, for a loan of platinum salt.

Registry No. 1, 92344-06-4; **2**, 92344-07-5; **3**, 92269-77-7; **4**, 92269-78-8; **5**, 92269-79-9; **6**, 92269-80-2; **7**, 92269-81-3; **8**, 92269-82-4; *cis*-DDP, 15663-27-1; CpG ammonium salt, 27553-01-1; d(pCpG) ammonium salt, 92269-83-5; *cis*-[Pt(NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$, 52241-26-6; [PtBr(dien)]Br, 15633-95-1.

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