X-ray Structure of Cytotoxic *trans*-[PtCl₂(dimethylamine)(isopropylamine)]: Interstrand Cross-Link Efficiency, DNA Sequence Specificity, and Inhibition of the B−Z Transition

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We report here the X-ray structure of cytotoxic *trans*-[PtCl₂(dimethylamine)(isopropylamine)]. This *trans*-platinum compound crystallizes in the monoclinic system, with $Z = 8$, in the spatial group $C2/c$ with unit cell parameters $a = 19.862(17)$ Å, $b = 6.581(3)$ Å, $c = 18.563(3)$ Å, $\alpha = 90°$, $\beta = 119.16(3)°$, $\gamma = 90°$, $V = 2119(2)$ Å³, $\rho = 2.321$ Mg/m³, $R = 0.0505$, and $R_w = 0.1166$ on the basis of 2339 independent reflections. To our knowledge this is the first report of the crystal structure of a biologically active *trans*-platinum compound containing different aliphatic amines. The DNA binding mode of *trans*-[PtCl₂(dimethylamine)(isopropylamine)] may be a consequence of the spatial disposition of the dimethylamine and isopropylamine ligands around the *trans*-Pt(II) center. We have found that *trans*-[PtCl₂(dimethylamine)(isopropylamine)] readily forms DNA interstrand cross-links. In addition, the compound shows binding affinity toward alternating purine−pyrimidine sequences and inhibits the B−Z transition. These particular DNA binding properties might be related to the capacity of *trans*-[PtCl₂(dimethylamine)-(isopropylamine)] for inducing some selective killing in a H-*ras* overexpresssing cell line.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)], commonly known as *cis*-DDP, is an antineoplastic drug effective against ovarian, testicular, and head and neck malignancies. However, this antitumor agent exhibits some important dose-limiting toxicities, mainly nephrotoxicity, neurotoxicity, and ototoxicity.[1] In the last 30 years, many investigations have been directed toward the synthesis of *cis*-Pt(II) compounds endowed with higher anticancer activity and lower toxic effects than *cis*-DDP. Of the thousand *cis*-Pt(II) analogues synthesized, none of them have surpassed the parent drug in efficacy, although some of them display reduced toxicity and alternative modes of clinical delivery.[2]

It is now well-established that nuclear DNA is the main cellular target for *cis*-DDP and related compounds and that the formation of kinetically stable Pt:DNA adducts is responsible for the biological activity of *cis*-platinum drugs.[3a,b] On the other hand, it has been generally accepted as the central paradigm of the biochemical pharmacology of platinum antitumor drugs that a *cis* configuration of the two leaving groups is a prerequisite to obtain therapeutically active Pt(II) complexes. However, some years ago it was reported that certain analogues of clinically ineffective transplatin show antitumor properties[4a−e] (for a review see ref 4e). Because, from a mechanistic point of view, the cytotoxic activity of *cis*-DDP should be associated with its binding
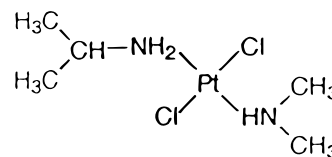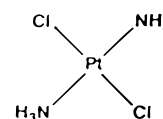

trans-[PtCl₂(**dimethylamine**)(**isopropylamine**)]

M.W. = 370.18
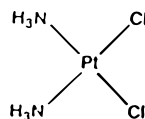
CISPLATIN   TRANSPLATIN

Figure 1. Structures of *trans*-[PtCl₂(dimethylamine)(isopropylamine)], cisplatin, and transplatin.

mode to nuclear DNA, biologically active *trans*-platinum complexes must interact with nuclear DNA by a molecular mechanism that differs from that of *cis*-DDP.[5]

To identify new *trans*-platinum complexes endowed with biological activity, we have recently synthesized and characterized several *trans*-platinum complexes with different aliphatic amines as non-leaving groups.[6] The first representative of this novel class of *trans*-platinum complexes is *trans*-[PtCl₂(dimethylamine)-(isopropylamine)], which was initially characterized by elemental analysis, mass spectrum, and IR and NMR spectroscopies (Figure 1). The compound is able to circumvent *cis*-DDP resistance in Pam212-*ras* tumor

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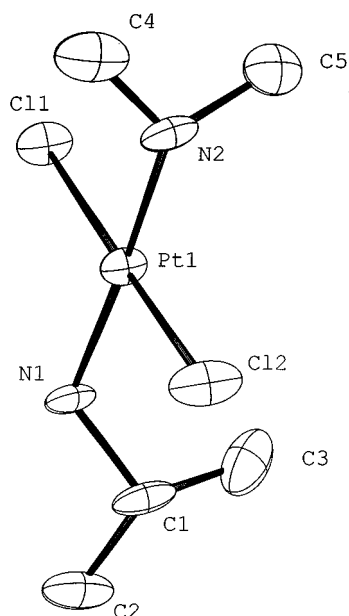
Figure 2. ORTEP diagram of *trans*-[PtCl₂(dimethylamine)-(isopropylamine)].

cells and induces cell death through apoptosis.[6] Moreover, inhibition of H-*ras* overexpression is a prerequisite for apoptosis induction in this H-*ras*-transformed cell line.[7]

We report here the crystal structure of *trans*-[PtCl₂-(dimethylamine)(isopropylamine)]. As far as we know, this is the first reported X-ray structure of a *trans*-platinum complex with different aliphatic amines as non-leaving groups. So far, only the X-ray structure of *trans*-[PtCl₂(NH₃)(1-methylcytosine-N3)] has been previously reported.[8] To shed light on the biochemical mechanism of cytotoxic activity of this nonclassical *trans*-platinum compound, it is necessary to examine its DNA interaction and to compare the major features with those previously established for *cis*- and *trans*-DDP. The biophysical and biochemical data reported in this paper indicate that *trans*-[PtCl₂(dimethylamine)-(isopropylamine)] readily forms DNA interstrand cross-links (≈6% of total Pt:DNA adducts). Moreover, the compound shows binding affinity toward alternating purine−pyrimidine sequences. Because DNA is the main cellular target of platinum antitumor drugs,[3b] it is likely that the specific DNA binding behavior of *trans*-[PtCl₂(dimethylamine)(isopropylamine)] might be responsible for its biological activity.

Results

X-ray Structure of *trans*-[PtCl₂(dimethylamine)-(isopropylamine)]. The ORTEP diagram for *trans*-[PtCl₂(dimethylamine)(isopropylamine)] is shown in Figure 2 together with the atomic numbering scheme. Bond distances and bond angles are given in Tables 1 and 2, and crystal data and structure refinement are listed in Table 3. The enviroment of the Pt atom is distorted square planar with the following bond length values: Pt−N1 = 2.065(9) Å, Pt−N2 = 2.048(9) Å, Pt−Cl1 = 2.309(3) Å, and Pt−Cl2 = 2.287(3) Å. These values are comparable with those found in other *trans*-Pt complexes with planar amines.[9]

Table 1. Bond Lengths for *trans*-[PtCl₂(dimethylamine)-(isopropylamine)]

bond	length (Å)	bond	length (Å)
Pt1−N2	2.048(9)	N2−C4	1.355(18)
Pt1−N1	2.065(9)	N2−C5	1.546(17)
Pt1−Cl2	2.287(3)	C1−C3	1.489(19)
Pt1−Cl1	2.309(3)	C1−C2	1.510(17)
N1−C1	1.466(13)		

Table 2. Angles for *trans*-[PtCl₂(dimethylamine)-(isopropylamine)]

angle	deg	angle	deg
N2−Pt1−N1	177.6(4)	C4−N2−C5	108.4(11)
N2−Pt1−Cl2	87.2(3)	C4−N2−Pt1	121.5(10)
N1−Pt1−Cl2	93.7(3)	C5−N2−Pt1	111.2(7)
N2−Pt1−Cl1	91.1(3)	N1−C1−C3	108.2(10)
N1−Pt1−Cl1	88.1(3)	N1−C1−C2	109.7(11)
Cl2−Pt1−Cl1	177.98(12)	C3−C1−C2	113.2(12)
C1−N1−Pt1	120.4(8)		

Table 3. Crystal Data and Structure Refinement for *trans*-[PtCl₂(dimethylamine)(isopropylamine)]

empirical formula	$C_5H_{16}Cl_2N_2Pt$
formula weight	370.18
temperature	293(2) K
wavelength	0.71069 Å
crystal system, space group	monoclinic, $C2/c$
unit cell dimensions	$a = 19.862(17)$ Å, $\alpha = 90°$
	$b = 6.581(3)$ Å, $\beta = 119.16(3)°$
	$c = 18.563(3)$ Å, $\gamma = 90°$
volume	2119(2) Å³
Z	8
calculated density	2.321 mg/m³
absorption coefficient	13.7 mm⁻¹
$F(000)$	1376
crystal size	0.1 × 0.1 × 0.2 mm³
θ range for data collection	3.31−29.97°
index ranges	$-27 <= h <= 24$
	$0 <= k <= 9$
	$0 <= l <= 25$
reflections collected/unique	2478/2339 [R(int) = 0.0611]
refinement method	full-matrix least-squares on F^2
data/restraints/parameters	2339/0/92
goodness-of-fit on F^2	1.008
final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0505$, $wR_2 = 0.1166$
R indices (all data)	$R_1 = 0.0582$, $wR_2 = 0.1191$
largest difference peak and hole	0.949 and −0.985 e.Å⁻³

Looking at the plane formed by the N2, Pt1, Cl1, and Cl2 atoms (see Figure 2) it may be observed that N1 is 0.107(10) Å out of this plane. This distortion is due to the fact that N1 and N2 are bound to Cl1 by means of weak hydrogen bonds. The hydrogen bonds are: N1−H1···Cl1 (symmetry code for Cl1 = −*x*, *y*, 1/2−*z*) with bond lengths D−H = 0.86 Å, H···A = 2.65 Å, and D···A = 3.454(11) Å and with D−H···A angle = 155.9°; and N2−H2N···Cl1 (symmetry code for Cl1 = *x*, 1+*y*, *z*) with bond lengths of 0.91, 2.72, and 3.524(11) Å, respectively, and with D−H···A angle = 147.3°. Taking into account these hydrogen bonds, the structure consits of a chain of hydrogen-bonded molecules along the [010] direction. The hydrogen bond of N1 provokes that the N1−C1 bond forms an angle of −38.3(9)° relative to the Pt1, Cl1, Cl2, and N1 plane. This hydrogen bond also produces a *cis* conformation between C1 and Cl2 in the Pt−N1 bond. The steric hindrance between the C1 and Cl2 atoms produces an increase of the Pt−N1−C1 angle. Analogously, the hydrogen bond from N2 suggests that the N2−C4 bond is at 54.58(11) Å from the plane formed by Pt1, Cl1, Cl2, and N2, that the N2−C5 bond is at
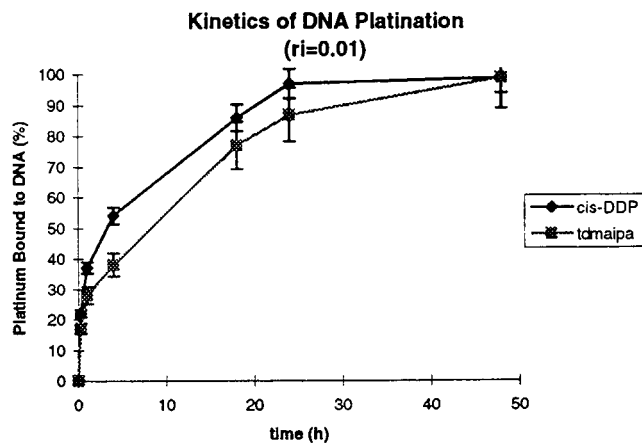
Figure 3. Kinetics of DNA platination by *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] (tdmaipa) and *cis*-[PtCl₂-(NH₃)₂] *(cis*-DDP) at $r_i = 0.01$.

Table 4. r_b Values Obtained by TXRF in Linear pBR322 DNA after Incubation with Various r_i Values of *cis*-DDP or *trans*-[PtCl₂(dimethylamine)(isopropylamine)] for 24 h at 37 °C in 10 mM NaClO₄[a]

compd	r_i	$r_b \pm$ SD	r_b/r_i (%)
cis-DDP	0.01	0.0097 ± 0.0002	97
	0.05	0.047 ± 0.003	95
	0.10	0.09 ± 0.005	90
	0.15	0.12 ± 0.007	80
	0.20	0.15 ± 0.006	75
tdmaipa	0.01	0.0085 ± 0.0002	85
	0.05	0.041 ± 0.002	82
	0.10	0.076 ± 0.003	76
	0.15	0.105 ± 0.005	70
	0.20	0.136 ± 0.007	68

[a] SD, standard deviation; *cis*-DDP, *cis*-[PtCl₂(NH₃)₂]; tdmaipa, *trans*-[PtCl₂(dimethylamine)(isopropylamine)].

−74.89(11)° from the same plane, and that H2N is in *cis* conformation with respect to Cl2. The steric hindrance between the C2H7N ligand and the chlorine atoms suggests that the C4−N2−Pt1 angle becomes longer and that the N2 atom is −0.1048(10) Å out of the plane defined by N1, Pt1, Cl1, and Cl2 atoms (see Figure 2).

Kinetics of DNA Platination by *trans*-[PtCl₂-(dimethylamine)(isopropylamine)]. Figure 3 shows that at $r_i = 0.01$ the kinetics of platination of linear pBR322 DNA due to *trans*-[PtCl₂(dimethylamine)-(isopropylamine)] is slightly slower than that due to *cis*-DDP. After 1 h of incubation at 37 °C the percentage of platinum binding to pBR322 DNA was 29% for *trans*-[PtCl₂(dimethylamine)(isopropylamine)] and 38% for *cis*-DDP. After 5 h it was 38% and 55%, respectively. After 24 h of drug:DNA complex formation, 97% of the *cis*-Pt(II) centers of *cis*-DDP are bound to pBR322 DNA in contrast with 85% of the *trans*-Pt(II) centers of *trans*-[PtCl₂(dimethylamine)(isopropylamine)]. After 48 h of incubation, platinum binding to DNA has been totally accomplished reaching a plateau for both compounds.

Table 4 summarizes the r_b values obtained after 24 h of incubation of linear pBR322 DNA with *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] and *cis*-DDP at several r_i. The data show that at increasing r_i the amount of Pt bound per nucleotide (r_b) decreases for both platinum compounds. This decrease is higher for the *trans*-Pt(II) compound than for *cis*-DDP since it goes
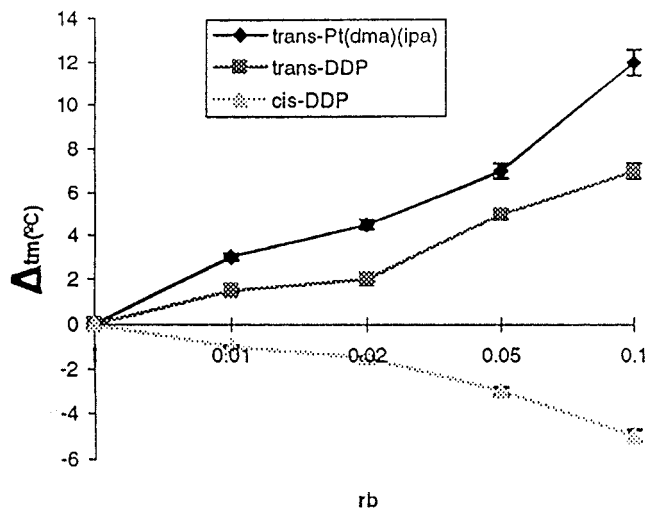

Figure 4. Plots of Δt_m values of CT DNA modified by *trans*-[PtCl₂(dimethylamine)(isopropylamine)], *cis*-DDP, and *trans*-DDP at r_b from 0.01 to 0.1 (Δt_m is defined as the difference between the t_m values of unmodified and platinated DNAs).

from 85% ($r_i = 0.01$) to 68% ($r_i = 0.2$) in *trans*-Pt:DNA complexes and from 97% ($r_i = 0.01$) to 75% ($r_i = 0.2$) in *cis*-Pt:DNA complexes. These data suggest that the isopropylamine and dimethylamine ligands impose some steric hindrance to the DNA binding of the *trans*-Pt(II) center and/or that the rate of hydrolisis of *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] is slower than that of *cis*-DDP.

Melting Temperature (t_m) of CT DNA Modified by *trans*-[PtCl₂(dimethylamine)(isopropylamine)]. The effect on melting temperature (t_m) of modification of CT DNA with *trans*-[PtCl₂(dimethylamine)(iso-propylamine)] at r_b from 0.01 to 0.1 was measured at 260 nm (Figure 4). The results shows that increasing r_b *trans*-[PtCl₂(dimethylamine)(isopropylamine)] gradually increases the t_m of CT DNA therefore increasing the thermal stability of the double helix. At $r_b = 0.1$ the increase in t_m in *trans*-[PtCl₂(dimethylamine)(iso-propylamine)]:CT DNA complexes is 12 °C relative to control unmodified CT DNA. *trans*-DDP also induces an increase in t_m in CT DNA but much lower than that of *trans*-[PtCl₂(dimethylamine)(isopropylamine)] ($\Delta t_m = 6$ °C at $r_b = 0.1$). In contrast, *cis*-DDP decreases the thermal stability of DNA under identical conditions ($\Delta t_m = -5$ °C at $r_b = 0.1$). The finding that the t_m values of DNA modified by *trans*-[PtCl₂(dimethylamine)(iso-propylamine)] strongly increase at increasing r_b is consistent with conformational alterations on the double helix leading to DNA stabilization.[10] Moreover, we observed that at 95 °C the percentage of hyperchromicity of *trans*-[PtCl₂(dimethylamine)(isopropylamine)]:CT DNA complexes (25%) was lower than that of control CT DNA (32%) indicating that most likely the DNA in the drug:DNA complexes was not totally melted due to interstrand adduct formation (data not shown).

***trans*-[PtCl₂(dimethylamine)(isopropylamine)] Binding to Supercoiled pBR322 DNA.** The effect of binding of *trans*-[PtCl₂(dimethylamine)(isopropylamine)] on supercoiled DNA was determined by the ability of this compound to alter the electrophoretic mobility of the ccc and oc forms of pBR322 plasmid. Figure 5 shows the electrophoretic mobility of native pBR322 DNA and pBR322 DNA incubated with *trans*-[PtCl₂(dimethyl-
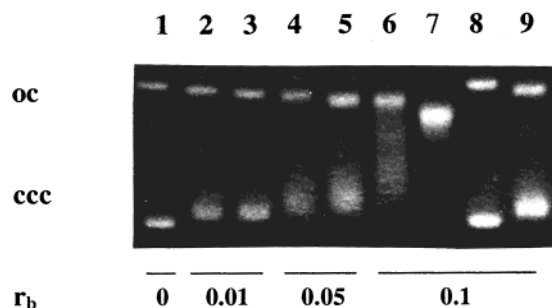
Figure 5. Unwinding of supercoiled pBR322 DNA by platinum drugs. Lanes 3, 5, and 7: pBR322 modified by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] at r_b of 0.01, 0.05, and 0.10, respectively. Lanes 2, 4, and 6: pBR322 modified by *cis*-DDP at r_b of 0.01, 0.05, and 0.10, respectively. Lanes 8 and 9: pBR322 modified by isopropylamine and *trans*-DDP at r_b = 0.10, respectively. Lane 1: control unmodified pBR322 DNA. oc, open circular DNA form; ccc, covalently closed circular DNA form.

Figure 6. Kinetics of DNA interstrand cross-link formation in linear pBR322 DNA by *cis*-DDP (lanes 3–6) and *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] (lanes 7–10) at r_i = 0.01. Cn, control native linear pBR322 (dsDNA); Cd, control denatured linear pBR322 (ssDNA); top bands, dsDNA; botom bands, ssDNA.

amine)(isopropylamine)] and *cis*-DDP at r_b = 0.01, 0.05, and 0.1 (lanes 2–7) and with isopropylamine and *trans*-DDP at r_b = 0.1 (lanes 8 and 9). It has been reported that at increasing r_b the rate of migration of the ccc DNA band decreases until it comigrates with the oc DNA band so that the r_b at the coalescence point, $r_b(c)$, corresponds to the amount of platinum needed for complete removal of all supercoils from DNA. The DNA unwinding angle, ϕ, can be calculated as previously reported.[11] It may be seen in Figure 5 that the $r_b(c)$ value for *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] is 0.100 (lane 7). Under our experimental conditions (density of supercoiling, $\sigma = -0.086$) this $r_b(c)$ gives a ϕ value of 15°. For *cis*- and *trans*-DDP the calculated r_b-(c) values are 0.11 and 0.22 (data not shown) which render ϕ values of 13° and 7°, respectively. The ϕ values of 12° and 7° obtained for *cis*- and *trans*-DDP, respectively, are in agreement with those reported by other authors.[11-13] Because the ϕ value of *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] is close to the ϕ value of *cis*-DDP but more than 2 times higher than the ϕ value of *trans*-DDP, it is most likely that *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] forms bifunctional DNA adducts.[14]

Interstrand Cross-Link Formation by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] in Linear pBR322 DNA. The DNA melting data reported above support the idea that the DNA in *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)]:CT DNA complexes is thermally stabilized and not totally melted at 95 °C. Because these two facts may indicate that the *trans*-Pt(II) drug is forming DNA interstrand cross-links,[15] we analyzed the ability of *trans*-[PtCl$_2$(dimethylamine)-(isopropylamine)] to form interstrand cross-links in linear pBR322 plasmid DNA. Figure 6 shows the kinetics of interstrand cross-link formation in linear pBR322 DNA due to *trans*-[PtCl$_2$(dimethylamine)-(isopropylamine)] after several times of incubation. It may be seen that all pBR322 DNA is already in double-stranded form (dsDNA) only after 15 min of incubation of DNA with the *trans*-Pt(II) compound at r_i = 0.01 (lane 7). Interestingly, the electrophoretic mobility of dsDNA in *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)]:pBR322 complexes decreases relative to unmodified pBR322 DNA when the period of incubation increases (lanes
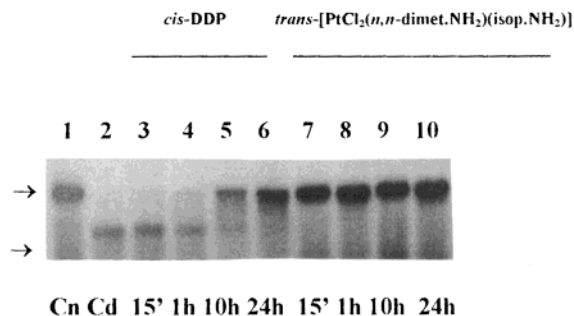
7–10 versus lane 1) as an indication that the number of DNA interstrand adducts increases.[16] After 15 min of incubation at r_i = 0.01 of *trans*-[PtCl$_2$(dimethylamine)-(isopropylamine)] the r_b is 0.0018 (see Figure 3) which means that there is 1 trans-Pt(II) center per 550 nucleotides. Therefore, there are about 16 *trans*-Pt(II) centers per plasmid molecule considering that pBR322 has 4363 base pairs.[17] From the ratio of the intensities of dsDNA versus ssDNA (single-stranded DNA) bands, the percentage of interstrand adducts may be calculated assuming that at low r_b there is one interstrand adduct per dsDNA fragment.[18] Thus, at r_b = 0.0018 (15 min of incubation at r_i = 0.01) it may be calculated that the number of interstrand cross-links is at least 6% of the total number of adducts produced by *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] in linear pBR322 DNA. In contrast, *cis*-DDP has a much slower kinetics of DNA interstrand cross-links formation. Thus, Figure 6 also shows that *cis*-DDP does not form DNA interstrand cross-links after 15 min of incubation at r_i = 0.01 since the band corresponding to dsDNA is not detected (lane 3). After 1 and 10 h of incubation at r_i = 0.01 of *cis*-DDP the amount of dsDNA increases from 10% to 45%, respectively (lanes 4 and 5) as revealed by the appearance of the corresponding dsDNA bands. After 24 h of incubation at r_i = 0.01 of *cis*-DDP all DNA molecules are in double-stranded form. Under these conditions the r_b value for *cis*-DDP is 0.0097 (see Table 4) which means that there is 1 cis-Pt(II) center per 103 nucleotides and, therefore, there are about 85 *cis*-Pt(II) centers per pBR322 molecule. So, the percentage of interstrand cross-links would be 1.2% of the whole amount of *cis*-Pt:DNA adducts in linear pBR322 plasmid. These data indicate that *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] has an enhanced capacity of DNA interstrand cross-linking relative to *cis*-DDP.

DNA Sequence Specificity of *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] Adducts. The $3' \rightarrow 5'$ exonuclease activity of T4-DNA polymerase has been extensively used to determine the sites of reaction with DNA of platinum agents.[19,20] Inhibition of the $3' \rightarrow 5'$ exonuclease activity of T4 polymerase by the various Pt:DNA adducts renders a pool of DNA fragments of different lengths that may be resolved by 12% polyacrylamide gel electrophoresis under denaturing conditions. Figure 7 shows that *cis*-DDP (lane 2) produced much fewer stop sites of T4 DNA polymerase than

1 G A T C 2 3
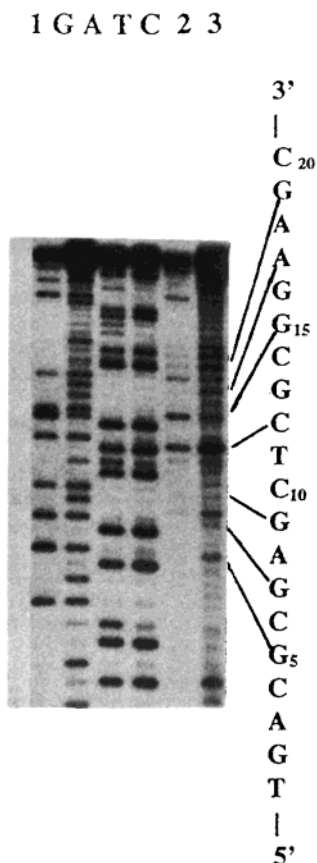
Figure 7. Autoradiogram of 12% polyacrylamide/8 M urea gel showing inhibition of 3′→5′ exonuclease activity of T4 DNA polymerase on a 5′-^{32}P-labeled DNA fragment of 50 base pairs (from the binding domain of Epstein−Barr virus nuclear antigen) modified by platinum compounds. Lanes: 1, control unmodified DNA; 2, DNA modified by *cis*-DDP at $r_b = 0.01$; 3, DNA modified by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] at $r_b = 0.01$; G, A, T, and C, Maxam and Gilbert sequencing bands.

trans-[PtCl$_2$(dimethylamine)(isopropylamine)] (lane 3). The major stop sites for *cis*-DDP were observed at C$_{12}$, G$_{15}$-G$_{16}$, and A$_{17}$ in agreement with previously reported data about the DNA binding specificity of *cis*-DDP.[3a,19] In contrast, *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] induced strong stop sites throughout the DNA. Of interest was the production of unique stretches of stop sites corresponding to platination within C$_4$-G$_7$ and C$_{12}$-G$_{15}$. It should be pointed out that alternating purine−pyrimidine sequences have been reported as a good source of interstrand cross-links in the reaction of platinum compounds with DNA.[16,18] So, these data suggest that *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] forms DNA interstrand cross-links in purine−pyrimidine stretches.

Effect of *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] on the NaCl-Induced B−Z DNA Transition of Poly(dG-dC)·Poly(dG-dC). Because *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] forms DNA interstrand cross-links and it binds efficiently to alternating purine−pyrimidine sequences, we have analyzed the salt-induced B−Z transition of double-stranded poly(dG-dC) modified by the drug. We previously reported that the salt-induced B−Z DNA transition of double-stranded poly(dG-dC) is a time-dependent process than can be described by a two-state kinetic model characterized by
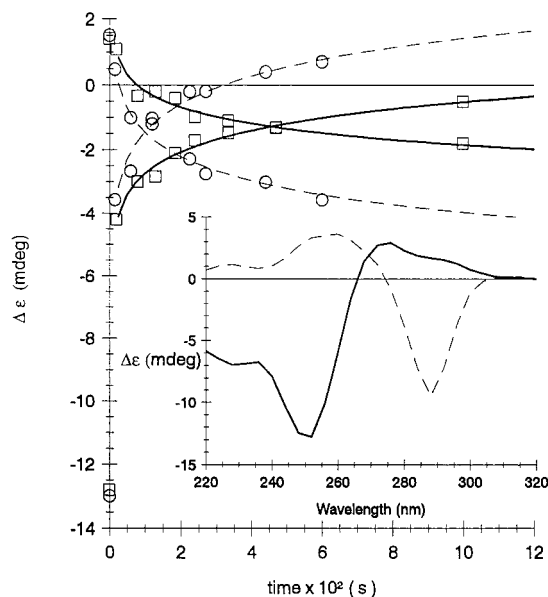
Figure 8. Plots of the variation of the ellipticities ($\Delta\epsilon$ = mdeg) at 250 and 290 nm (see insert) as a function of time for the NaCl-induced B−Z transition of poly(dG-dC)·poly(dG-dC) (○) and poly(dG-dC)·poly(dG-dC) modified at $r_b = 0.01$ by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] (□). The B−Z transition of poly(dG-dC)·poly(dG-dC) was induced with 2.5 M NaCl. The insert shows the CD spectra of unmodified poly(dG-dC)·poly(dG-dC) in the B-conformation in 10 mM NaClO$_4$ (continuous line) and in the Z-conformation (dashed line) after addition of 2.5 M NaCl and subsequent incubation for 2 h.

a transition midpoint time ($t_{1/2}$) that is the time at which the amounts of B-DNA and Z-DNA are equivalent.[21,22] At $t_{1/2}$, the ellipticity values at 250 and 290 nm of the polynucleotide are the same.[21] Figure 8 shows the variation of the ellipticity at 250 and 290 nm versus time for poly(dG-dC)·poly(dG-dC) incubated with 2.5 M NaCl and poly(dG-dC)·poly(dG-dC) previously modified with *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] at $r_b = 0.01$ and subsequently incubated with 2.5 M NaCl. It may be observed that *trans*-[PtCl$_2$(dimethylamine)-(isopropylamine)] delays the midpoint time of the B−Z transition relative to the native polynucleotide ($t_{1/2}$ of 450 s versus $t_{1/2}$ of 105 s). Thus, we may calculate from the data of Figure 8 that after 105 s of incubation with 2.5 M NaCl the amount of DNA in Z-conformation in control unmodified poly(dG-dC)·poly(dG-dC) is around 50% versus 25% in the polynucleotide modified by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)]. *trans*-DDP also induces a decrease in the midpoint time of the B−Z transition of the polynucleotide but to a much higher extent ($t_{1/2} = 840$ s, data not shown). In contrast, *cis*-DDP accelerates the midpoint time of the B−Z transition of the polynucleotide ($t_{1/2} = 20$ s, data not shown). These data suggest that the DNA interstrand adducts formed by *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] hinder the B−Z transition decreasing, therefore, the cooperativity of the process. In fact, it has been reported previously that the interstrand adducts formed by platinum drugs prevent the flipping from B-DNA to Z-DNA.[15]

Discussion

The crystal structure of *trans*-[PtCl$_2$(dimethylamine)-(isopropylamine)] reveals that the square-planar geom-

etry around the *trans*-Pt(II) center is distorted. In view of this fact, we hypothesized that in *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] the *trans*-Pt(II) center might efficiently form DNA interstrand cross-links in order to avoid a close contact between the dimethylamine and isopropylamine bulky ligands.[23] We tested this hypothesis via analyzing, by means of various biophysical and biochemical methods, the DNA interstrand cross-linking efficiency of the compound.

The high increase in t_m found in *trans*-[PtCl₂(dimethylamine)(isopropylamine)]:CT DNA complexes relative to unmodified CT DNA together with the fact that the DNA in *trans*-[PtCl₂(dimethylamine)(isopropylamine)]:CT DNA complexes is not totally melted at 95 °C suggests that formation of interstrand adducts might prevent separation of DNA complementary strands. Formation of DNA interstrand adducts by *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] was confirmed by the presence of dsDNA formation after denaturation of linear pBR322 plasmid treated with the drug. At a r_b value as low as 0.0018 the amount of DNA interstrand cross-links produced by *trans*-[PtCl₂(dimethylamine)-(isopropylamine)] is at least 6% of the total number of *trans*-Pt:DNA adducts. In contrast, *cis*-DDP forms 1.2% of DNA interstrand cross-links relative to the total amount of *cis*-Pt:DNA adducts and at a higher molar ratio of Pt to nucleotides ($r_b = 0.0097$). It should be pointed out that interstrand cross-linking efficiency by cytotoxic *trans*-Pt(II) complexes varies considerably from one compound to another. For instance, *trans*-[PtCl₂(*E*-iminoether)₂] has a greatly reduced ability to form DNA interstrand cross-links,[4d,11] while *trans*-[PtCl₂(NH₃)-(quinoline)] forms a high percentage of DNA interstrand cross-links.[13]

The ability of *trans*-[PtCl₂(dimethylamine)(isopropylamine)] to form DNA interstrand cross-links is in agreement with the binding affinity of this compound toward alternating purine–pyrimidine sequences. In fact, pBR322 plasmid DNA contains an alternating purine–pyrimidine stretch between nucleotides 1440 and 1465 that might be attacked by *trans*-[PtCl₂-(dimethylamine)(isopropylamine)] through interstrand cross-linking. Interestingly, this purine–pyrimidine sequence may adopt the Z-DNA conformation in the supercoiled form of the plasmid.[24]

The B–Z transition of DNA modified by platinum(II) complexes has attracted considerable attention because it might be involved in the biochemical mechanism of antitumor activity of these drugs.[15,25] The results reported here show that *trans*-[PtCl₂(dimethylamine)-(isopropylamine)] delays the midpoint time ($t_{1/2}$) of the salt-induced B–Z transition of poly(dG-dC)·poly(dG-dC). Interestingly, clinically active *cis*-DDP accelerates the B–Z transition. It is also interesting to note that antitumor *trans*-[PtCl₂(*E*-iminoether)₂] and *trans*-DDP delays the B–Z transition.[26] Because the DNA in poly-(dG-dC)·poly(dG-dC) modified by *trans*-[PtCl₂(dimethylamine)(isopropylamine)] is in double-stranded form after heating at 95 °C, cooling on ice, and subsequent agarose electrophoresis in denaturing conditions while the unmodified polynucleotide is in single-stranded form (laboratory data), we think that the interstrand adducts formed by *trans*-[PtCl₂(dimethylamine)(isopropylamine)] block the B–Z transition of poly(dG-dC)·poly(dG-dC).

Although the exact biological consequences of Z-DNA are still obscure, it has been recently reported that the B–Z transition may be involved in DNA transcription through the Z-DNA binding activity of double-stranded RNA adenosine deaminase (DRADA) proteins.[27]

Because H-*ras* oncogene contains several GC tracts in its sequence, including a GC-rich strong promoter,[28,29] it is attractive to think about the possibility that the inhibition of H-*ras* overexpression in Pam212-*ras* cells treated with *trans*-[PtCl₂(dimethylamine)(isopropylamine)][7] might be related to the ability of the drug to form interstrand cross-links in guanine–cytosine stretches. Further research is warranted to test this hypothesis.

It is well-established that DNA is the main pharmacological target of Pt(II) complexes[30] and, on the other hand, DNA interstrand adducts have been often implicated with cytotoxicity of platinum drugs.[31,32] So, it is likely that this kind of adduct may play an important role in the mechanism of cytotoxic activity of *trans*-[PtCl₂(dimethylamine)(isopropylamine)].

Experimental Section

Structural Determination and Refinement of the Complex. A prismatic crystal (0.1 × 0.1 × 0.2 mm³) was selected and mounted on a Enraf-Nonius Cad4 four-circle diffractometer. Unit-cell parameters were determined from automatic centering of 25 reflections (12 < θ < 21°) and refined by least-squares method. Intensities were collected with graphite monochromatized Mo Kα radiation, using ω/2θ scan technique; 2478 reflections were measured in the range 3.31 ≤ θ ≤ 29.97, 2339 of which were nonequivalent by symmetry (R_{int} (on *I*) = 0061); 2127 reflections were assumed as observed applying the condition I ≥ 2σ(*I*). Three reflections were measured every 2 h as orientation and intensity control; significant intensity decay was not observed. Lorentz polarization and absorption corrections were made.

The structure was solved by direct methods, using the SHELXS computer program,[33] and refined by full-matrix least-squares method with the ShelX93 computer program[34] using 2339 reflections (very negative intensities were not assumed). The function minimized was $\sum w ||F_o|^2 - |F_c|^2|^2$, where $w = [\sigma^2-(I) + (0.0782P)^2]^{-1}$, $P = (|F_o|^2 + 2|F_c|^2)/3$, and *f*, *f'*, and *f''* were taken from *International Tables of X-ray Crystallography*.[35] All H atoms were computed and refined with an overall isotropic temperature factor using a riding model. The final *R* (on *F*) factor was 0.050, R_w (on $|F|^2$) = 0.116 and goodness of fit = 1.008 for all observed reflections. Number of refined parameters was 92, maximum shift/esd = 0.0, mean shift/esd = 0.0. Maximum and minimum peaks in final difference synthesis were 0.949 and −0.985 eÅ⁻³, respectively. The details of the data collected and the structural analyses are summarized in Table 3.

Reagents and Compounds. Stock solutions of the Pt(II) compounds were prepared in 10 mM NaClO₄ to a final concentration of 1 mg/mL. The drug solutions were freshly prepared before use. *trans*-[PtCl₂(dimethylamine)(isopropylamine)] was synthesized as described previously.[6] *cis*- and *trans*-DDP were purchased from Sigma Co. Sonicated calf thymus DNA (CT DNA) (average length of 1000 base pairs) and pBR322 plasmid DNA (4363 base pairs in length, density of supercoiling σ = −0.086) were supplied from Sigma Co. The *Bam*HI restriction enzyme and the Klenow fragment of *E. coli* DNA polymerase I were obtained from Boehringer Mannheim. [α-³²P]dCTP (10 mCi/mL) was purchased from Amershan International.

Formation of Drug:DNA Complexes. Formation of drug: DNA complexes was carried out by addition to CT DNA or pBR322 DNA of aliquots of each compound at different concentrations in 10 mM NaClO₄. The amount of compound added to the DNA solution was expressed as r_i (input molar

ratio of Pt/nucleotide). The mixture was incubated at 37 °C for various periods of time.

Quantitation of Pt Binding to DNA. 20 μg/mL solutions of CT DNA and linear or supercoiled pBR322 DNA in 10 mM NaClO$_4$ were incubated at 37 °C with the platinum drugs at several r_i. Aliquots of 250 μL were withdrawn at various times. The fraction of unreacted compounds was separated by precipitation of the DNA with 2.5 volumes of ethanol and 0.3 M sodium acetate, pH 4.8. The amount of platinum bound per nucleotide (r_b) was determined at every reaction time by total X-ray fluorescence (TXRF) using a Seifert EXTRA-II apparatus.[36]

DNA Melting. CT DNA was modified with *trans*-[PtCl$_2$-(dimethylamine)(isopropylamine)] and *cis*- or *trans*-DDP in 10 mM NaClO$_4$ at r_b values from 0.01 to 0.1. Subsequently, the drug:DNA complexes were precipitated with ethanol/sodium acetate dried in a speed vac concentrator and resuspended in a buffer containing 4 mM NaCl plus 1 mM Tris·HCl with 0.1 mM EDTA, pH 7.4. Melting curves were recorded at 260 nm by differential spectrophotometry and at increase rate of 1 °C/min from 37 to 95 °C. The maximum value of hyperchromicity in control DNA at 95 °C was 32%. The value of the melting temperature (t_m) was calculated as the temperature corresponding to the maximum of the first-derivation profile of the melting curves. The t_m values had an accuracy of ±0.2 °C.

Interstrand Cross-Link Assays. To linearize pBR322 plasmid the DNA was digested in 150 mM NaCl with 10 units/mg DNA of *Bam*HI (unique restriction site in pBR322) at 37 °C for 4 h. The linear double-stranded plasmid DNA was 3′-end labeled by incubation with 2.5 mCi/mg DNA of [α-^{32}P]-dCTP and 1.25 units/mg DNA of the Klenow fragment of *E. coli* DNA polymerase I for 30 min at room temperature. The reaction was stopped by heating at 70 °C for 5 min. The unincorporated radioactivity was removed by passing the labeling reaction through a Sephadex G-50 column. The labeled DNA was precipitated with 0.1 volumes of sodium acetate and 2 volumes of cold ethanol. Sonicated CT DNA was added to the eluted solution of the labeled pBR322 DNA to a final DNA concentration of 180 μg/mL. Afterward, the DNA, at a concentration of 90 ng/mL, was incubated with the platinum drugs in 10 mM NaClO$_4$ at r_i = 0.01 for several periods of time. Then, aliquots of 10 μL were removed and the reactions were ended by addition of an equal volume of the loading dye (90% formamide, 10 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue). Monitoring of Pt bound to DNA (r_b) was carried out by TXRF. The DNA was melted for 10 min at 90 °C and chilled on ice. 1.5% agarose gel electrophoresis in denaturing conditions was carried out at 20 V for 16 h.[15] The gels were dried and autoradiographed. Band quantification was made using a Molecular Dynamics model 300A densitometer.

Interaction of *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] with Supercoiled pBR322 Plasmid DNA. pBR322 DNA aliquots (25 μg/mL, σ = −0.086) were incubated with the platinum compounds at 37 °C in 10 mM NaClO$_4$ until achieving r_b values of 0.01, 0.05, 0.10, 0.11, 0.12, 0.13, 0.14, and 0.15. Aliquots of 20 μL of compound:DNA complexes containing 0.5 μg of DNA were subjected to 1.5% agarose gel electrophoresis for 16 h at 25 V in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0) as previously reported.[37]

Sequence Specificity of Pt:DNA Adducts. A 5′-^{32}P-labeled DNA fragment of 50 base pairs (5 ng) from the binding domain of the Epstein−Barr virus nuclear antigen was platinated with *trans*-[PtCl$_2$(dimethylamine)(isopropylamine)] or *cis*-DDP at r_b = 0.01 in 10 mM NaClO$_4$ and then used as substrate for the 3′→5′ exonuclease activity of T4 DNA polymerase (10 units) in a reaction buffer containing Tris-HCl (50 mM), KCl (50 mM), and MgCl$_2$ (10 mM) for 10 min at 37 °C. The rections were terminated by the addition of EDTA to 25 mM. T4-polymerase digestion yielded a pool of DNA fragments of different lengths due to inhibition of the 3′→5′ exonuclease activity of the enzyme by the various Pt:DNA

adducts. the DNA fragments were resolved after running in a 12% polyacrylamide gel under denaturing conditions (8 M urea).

CD Spectroscopy. CD spectra were recorded in a JASCO J-600 spectropolarimeter interfaced to a computer. Measurements were performed at 37 °C using a 1-cm path length cuvettes. CD spectra were run in a range of wavelength from 220 to 320 nm and at a speed of 200 nm/min. Scans were recorded at 0.4-nm intervals. Measurements of the NaCl-induced B−Z transition of aliquots of 15 μg/mL of native poly-(dG-dC)·poly(dG-dC) and of poly(dG- dC)·poly(dG-dC) modified by the platinum drugs at r_b = 0.01 in 10 mM NaClO$_4$ were carried out adding NaCl to reach a final concentration of 2.5 M. Inmediately after, the CD spectrum of the polynucleotide was recorded at several times. Plots of the ellipticity values at 250 and 290 nm versus time were constructed and the midpoint times ($t_{1/2}$) of the salt-induced B−Z DNA transition of the polynucleotide were obtained from the crossing point of both ellipticities.[21] All the experiments were made in triplicate.

Abbreviations: A, acceptor atom in the hydrogen bond; ccc, covalently closed circular DNA; D, donor atom in the hydrogen bond; CT DNA, calf thymus DNA; dsDNA, double-strand DNA; ssDNA, single-strand DNA; *cis*-DDP, cisplatin, *cis*-diamminedichloroplatinum(II); *trans*-DDP, transplatin, *trans*-diamminedichloroplatinum(II); EDTA, ethylenediamine-tetraacetate; oc, open circular DNA; r_b, molar ratio of platinum bound per nucleotide; r_i, input molar ratio of platinum per nucleotide; SD, standard deviation; Tris, tris(hydroxymethy-lamino)methane; TXRF, total X-ray fluorescence.

Supporting Information Available: Crystal data and structure refinement, listings of isotropic parameters, atomic coordinates, bond distances and angles, and anisotropic parameters for non-hydrogen atoms in *trans*-[PtCl$_2$(dimethyl-amine)(isopropylamine)]. This material is available free of charge via the Internet at http://pubs.acs.org.

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