Contribution from the Department of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Conformational Changes in a Single- and Double-Stranded Nonanucleotide upon Complexation of a Monofunctional Platinum Compound As Studied by ^{1}H NMR, ^{31}P NMR, and CD Methods

Carla J. van Garderen, Cornelis Altona, and Jan Reedijk*

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The structure of the nonanucleotide d(T-C-T-C-G-T-C-T-C), after binding of a monofunctional platinum compound $[PtCl(NH_3)_3]Cl$ to the N7 of the central guanine residue, is described. The observed conformational changes are limited to the central residues; the intramolecular interactions between dC(4) and dG(5) are reduced, and the sugar conformation of the platinated guanosine, dG(5), is changed from the S-type conformer toward an N-type conformer upon platination. When the complementary strand d(G-A-G-A-C-G-A-G-A) is added to the platinated nonanucleotide, a duplex is formed. The imino proton of the platinated G·C base pair is clearly observed in the ^{1}H NMR spectrum at low temperatures. The melting temperature of the double-stranded nonanucleotide is reduced by 15–20 °C as a result of monofunctional platinum binding to dG(5). The conformation of this $Pt(NH_3)_3$–DNA adduct is compared with the structure of a DNA fragment to which the antitumor agent *cis*-$PtCl_2(NH_3)_2$ is bound bifunctionally. The "kinked" structure, which is proposed for *cis*-$Pt(NH_3)_2$–DNA, does not occur in the monofunctional-platinated nonanucleotide, although the double-helical structure as such is destabilized.

Introduction

In 1969, Rosenberg reported the antitumor activity of *cis*-$PtCl_2(NH_3)_2$ (abbreviated as cDDP[1]).[2] Thereafter, many studies have been published concerning the working mechanism of cDDP. After hydrolysis of cDDP inside the cell, which results in the reactive species, binding to DNA appeared to be the most important interaction for the antitumor activity.[3,4] Binding to DNA occurs preferentially at the N7 of a guanine base.[5,6] Because of the two active sites available after hydrolysis of cDDP, several bifunctional adducts can be formed. Two major adducts arise from an intrastrand chelate between two neighboring guanine bases and a chelate between an adjacent adenine and a guanine base.[7] Minor adducts arise from interstrand cross-links between two guanine bases and from intrastrand cross-links between two guanines separated by one or more bases. Also DNA–Pt–protein interactions occur.[7]

The main adduct found after enzymatic digestion of DNA treated with cDDP is the intrastrand chelate of two neighboring guanines (the –GpG-*N7*,*N7* chelate).[8] Therefore, the conformations of *cis*-$Pt(NH_3)_2$[d(GpG)-*N7*(*1*),*N7*(*2*)] and *cis*-$Pt(NH_3)_2$[d(CpGpG)-*N7*(*2*),*N7*(*3*)] were studied in detail with high-resolution NMR spectroscopy by den Hartog et al.[9,10] These structures are characterized by a significant distortion of the DNA backbone and a change of the conformation of the 5′-guanosine sugar ring from the S-type conformer, commonly found in DNA fragments,[11] into an N-type conformation. In the crystal structures of *cis*-$Pt(NH_3)_2$[d(pGpG)-*N7*(*1*),*N7*(*2*)] and *cis*-$Pt(NH_3)_2$[d(CpGpG)-*N7*(*2*),*N7*(*3*)] the same conformation for the GpG part was found.[12,13]

The distortion, induced by cDDP binding to a GpG sequences in larger double-stranded DNA fragments, appeared to be relatively small. Destabilization of the duplex results in a reduction of the melting temperature by 9–27 °C, dependent on the sequence of the fragment, the number of base pairs involved in duplex formation, and the location of GpG in the sequence.[14–16] The changes in the DNA conformation can be described best by a "kinked" structure of the double helix.[17,18]

The interaction of cDDP to DNA is a two-step reaction. The first reaction step, binding to a guanine N7, appears to be slow. This step is followed by a relatively fast chelation step to a neighboring guanine. To obtain more information about the binding mechanism, the first reaction step and the structure of the intermediate is of particular interest. Therefore, the monofunctional platinum compounds (which are non antitumor active) are used to simulate the first reaction step. The structure of the products of the reaction of monofunctional platinum compounds, like [PtCl(dien)]Cl (dien denotes diethylenetriamine) and $[PtCl(NH_3)_3]Cl$, with DNA fragments can be used as model for the conformation of the intermediate.

The changes in the conformation of Pt(dien)[d(CpGpT)-*N7*(*2*)] with respect to the noncomplexed trinucleotide, d(CpGpT), are only located in the guanine part of the fragment. The sugar conformation of the guanosine is changed into an almost 50% N/S conformational mixture, and the stacking interactions between the cytidine and guanine base are reduced.[19] From these results it is concluded that the first reaction step already induces a change in the sugar conformation of the guanine residue. On the other

(1) Abbreviations: cDDP, *cis*-$PtCl_2(NH_3)_2$; dien, diethylenetriamine; pH*, uncorrected meter reading of solutions in D_2O; TMA, tetramethylammonium chloride; TMP, trimethyl phosphate. Abbreviations for nonanucleotides: I, d(T-C-T-C-G-T-C-T-C), (numbering T(1), C(2) to C(9)); I–Pt, $Pt(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7*(*5*)]; II, d(G-A-G-A-C-G-A-G-A) (numbering: G(10), A(11) to A(18)); III, I + II; III–Pt, I–Pt + II.

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hand, the CD spectrum of the platinated trinucleotide suggests more conformational changes, as deduced from earlier investigations on Pt(dien)–DNA complexes,[20,21] probably because this small trinucleotide has a larger degree of conformational freedom than the DNA. Therefore, it was decided to study the structure of a larger oligonucleotide, coordinated by a monofunctional platinum compound.

This paper deals with the results of an NMR and CD study of the –CGT-containing single-stranded nonanucleotides d(T-C-T-C-G-T-C-T-C) (I) and $Pt(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7(5)*] (I-Pt), together with the double-stranded molecules d(T-C-T-C-G-T-C-T-C)·d(G-A-G-A-C-G-A-G-A) (III) and Pt-$(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7(5)*]·d(G-A-G-A-C-G-A-G-A) (III–Pt) (the phosphate groups are omitted for clarity). The conformation of I–Pt is compared with that of Pt(dien)[d-(CGT)-*N7(2)*][19] and the structure of the double-stranded oligonucleotide, III–Pt, with that of the analogous double-helical decanucleotide d(T-C-T-C-G-G-T-C-T-C)·d(G-A-G-A-C-C-G-A-G-A), modified with cDDP on G(5) and G(6), studied by den Hartog et al.[14,17,22]

A preliminary optical study on the melting behavior of the double-stranded nonanucleotides showed that platination of the nonanucleotide with $[Pt(dien)]^{2+}$ or $[Pt(NH_3)_3]^{2+}$ decreases the melting temperature with 16 °C.[23] This decrease is of the same order of magnitude as the decrease in melting temperature induced by cDDP.[14–16]

Materials and Methods

$[PtCl(NH_3)_3]Cl$ was prepared according to Morita and Bailar.[24] The purity was checked by infrared spectroscopy and elemental analysis (Microanalytical Laboratory, University College, Dublin, Ireland). The nonanucleotide d(T-C-T-C-G-T-C-T-C) (I) and its complementary strand d(G-A-G-A-C-G-A-G-A) (II) were synthesized by using an improved phosphotriester method.[25] After purification, the fragments were treated with a Dowex AG ion-exchange resin (Na^+ form), type 50W-X2 (Serva, Heidelberg, FRG) to yield the sodium salt.

Strand I was allowed to react with $[PtCl(NH_3)_3]Cl$ (1:1) for 6 days at room temperature in the dark (2×10^{-5} M, pH 6). The reaction product, $Pt(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7(5)*] (I–Pt), was purified on a DEAE Sephadex A-25 column eluted with a NaCl gradient (0.0–0.8 M). Desalting was performed by Sephadex G-25 gel filtration using water as eluent. An equimolar amount of strand II was added to strands I and I–Pt to obtain the double-stranded nonanucleotides III and III–Pt, respectively.

NMR samples were lyophilized three times from 99.7% D_2O after adjustment of pH* to 7.0 (pH* denotes the uncorrected meter reading of solutions in D_2O) and finally taken up in 99.95% D_2O. Tetramethylammonium chloride (TMA) was used as internal reference (chemical shift relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) is approximately 3.18 ppm at 25 °C).

^1H NMR spectra were recorded on a Bruker WM-300 spectrometer equipped with an Aspect-2000 computer and on Bruker AM-500 and AM-600 spectrometers, both interfaced with an Aspect-3000 computer. In the case of D_2O samples, the residual HDO peak was suppressed by selective irradiation of the HDO resonance. The H_2O signal in the imino proton spectra was minimized by means of a time-shared long pulse in combination with a data shift accumulation routine.[26,27] In order to study the pH dependence of the chemical shift of the nonexchangeable proton resonances, spectra were recorded over the pH* range 1.5–12. The pH* was adjusted with small quantities of 0.1 M NaOD or DCl solutions. ^1H NMR spectra were recorded at several temperatures between 275 and 360 K in order to obtain chemical shift/temperature profiles. Exact temperatures were determined from the chemical shift of the HDO peak relative to TMA as described earlier.[28]

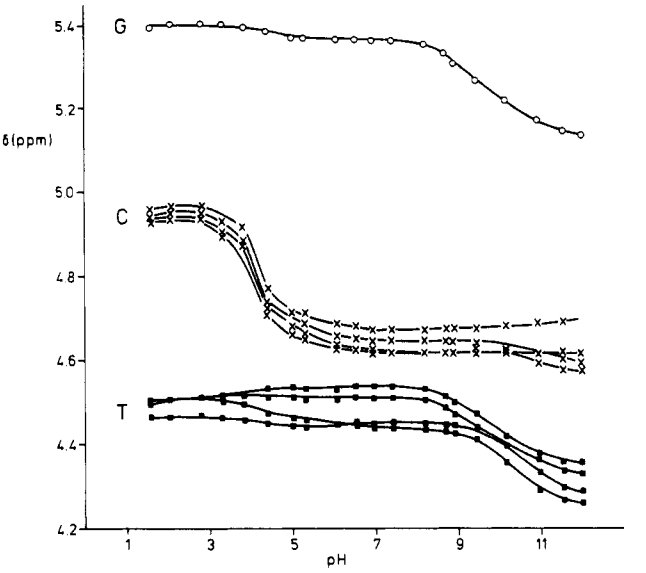

Figure 1. pH dependence of the chemical shift (δ) of the nonexchangeable base protons of I-Pt: (○) guanine H8; (×) cytosine H6; (■) thymine H6. Chemical shifts are reported relative to TMA; T = 293 K.

Phase-sensitive 2D-NOE experiments (mixing time 0.5 s) were performed at 285 K on carefully degassed samples as previously described.[29]

^{31}P NMR spectra were recorded on a Bruker WM-300 spectrometer, operating at 121.5 MHz. Heteronuclear proton-noise decoupling was used throughout. Trimethyl phosphate (TMP) was used as an internal reference.

CD spectra were recorded at room temperature for I and I–Pt and at 9 °C for III and III–Pt on a CNRS Roussel-Jouan III dichrograph (Jobin Yvon, France). CD samples were dissolved in redistilled water (pH 7.0–7.2). UV absorbance of the samples varies from 0.7 for I and I–Pt to 1.4 for III and III–Pt, corresponding to molar concentrations of 11.5 and 8.7 μM, respectively. The absorption coefficients at 254 nm determined as previously described[19] were 61 600 M^{-1} cm^{-1} for the single-stranded fragments (23 °C) and 159 000 M^{-1} cm^{-1} for the duplexes (9 °C).

Nomenclature. The conformational notation and numbering of the residues accords with the recommendations by the IUPAC-IUB Joint Commission on Biochemical Nomenclature.[30]

Results and Discussion

Identification and Analysis of the Single-Stranded Nonanucleotides I and I–Pt. The reaction of strand I with the monofunctional platinum compound $[PtCl(NH_3)_3]Cl$ yields a single product (I–Pt) comprising 70–80% of the UV-absorbing material. To ascertain the platinum binding site in the single-stranded adduct, the pH dependence of the nonexchangeable base protons was studied (Figure 1). The chemical shift changes at pH 4 (four protons) and at pH 10 (four protons) are ascribed to the protonation of the N3 of non-platinated cytosine and thymine, respectively.[31,32] The N1 deprotonation of guanine is shifted from pH 9.5[32] to pH 8.5 upon platinum binding and the N7 protonation (before platination at pH 2.3[33]) is no longer observed. These effects prove that the platinum complex is bound to the N7 of guanine.[34–37] Hence, the reaction product I–Pt is assigned to $Pt(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7(5)*].

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Table I. ^{1}H NMR Chemical Shifts (ppm) of the Nonexchangeable Base, 1′, 2′, 2″ and 3′ Protons of the Single-Stranded Nonanucleotides I and I–Pt Reported Relative to That of TMA

	H8/H6		H5/5CH$_3$		H1′		H2′		H2″		H3′	
residue	I	I–Pt	I	I–Pt	I	I–Pt	I	I–Pt	I	I–Pt	I	I–Pt
T(1)[a]	4.440	4.447	−1.337	−1.340	3.050	3.015	−0.880	−0.826	−0.723	−0.660	1.566	1.583
C(2)	4.593	4.640	2.758	2.785	3.002	3.020	−0.964	−0.925	−0.666	*c*	1.635	1.736
T(3)	4.461	4.564	−1.312	−1.282	*c*	3.122	−0.989	−0.980	−0.766	−0.718	1.571	1.662
C(4)	4.381	4.590	2.688	2.729	2.860	2.920	−1.300	−1.063	−0.868	−0.726	1.561	1.641
G(5)	4.772	5.375	...	...	2.900	2.984	−0.336	−0.319	−0.474	−0.432	1.800	1.880
T(6)	4.275	4.516	−1.495	−1.373	2.975	3.081	−0.973	−0.900	−0.779	−0.668	1.654	1.690
C(7)	4.634	4.605	2.770	2.778	3.033	2.968	−0.932	−0.965	*b*	*b*	*c*	1.660
T(8)[a]	4.440	4.447	−1.337	−1.340	*c*	*c*	*c*	−0.868	*c*	−0.676	*c*	1.658
C(9)	4.668	4.660	2.849	2.842	3.084	3.069	−0.895	−0.882	−0.805	−0.811	1.385	1.367

[a] H6 and 5CH$_3$ resonances of T(1) and T(8) are isochronous in both compounds. [b] H2″ resonances are isochronous with H2′ resonances. [c] Not assigned due to severe overlap.

The ^{1}H NMR spectra of the single-stranded nonanucleotides were assigned with the aid of 2D-NOE experiments by following established procedures.[38-40] In a 2D-NOE experiment, cross peaks are observed between a base proton and its H1′ proton and also between the same base proton and the H1′ proton of the residue at its 5′ side. These intra- and interresidual connectivities allow us to assign the base and H1′ resonances in a sequential manner. An example of this procedure is given for the platinated double-stranded nonanucleotide in the next section of this paper. The assignments of the H2′ and H2″ protons are based on intensity differences observed for the NOE's between the base and H2′/H2″ protons resonances[38] and on the chemical shift rule $\delta(2') < \delta(2'')$ for pyrimidines, except for the 3′ residue.[41]

Table I gives the chemical shift values of the base, H1′, H2′, H2″, and H3′ protons of both fragments (I and I–Pt) at 285 K. The chemical shift of the following resonances are shifted more than 0.1 ppm downfield upon platination: 6(3), 6(4), 8(5), 6(6), 5CH$_3$(6), 1′(6), 2′(4), 2″(4), 2″(6). All these protons belong to the central residues T(3)–T(6), i.e. the two residues on the 5′ side of the platinated guanine and the one on the 3′ side. Just as was found in the case of Pt(dien)[d(CGT)-*N7*(*5*)],[19] the influence of the platination on the chemical shift is more pronounced at the 5′ side. The downfield parts of the ^{1}H NMR spectra of the two single-stranded nonanucleotides recorded at 285 K are displayed in Figure 2A,B, together with the chemical shift changes of the above-mentioned H8, H6, and H1′ resonances. The larger downfield shift of the 8(5) signal is a direct result of platinum binding to the N7 and is commonly found for platinated guanine residues.[14,34-36]

The chemical shift difference between the two nonanucleotides can be explained by conformational changes of the backbone and/or sugar ring at the central part of the fragments. Scheek et al.[38] reported the influence of the negative charge of the 3′ P on the chemical shift of the H2′/H2″ protons. In particular, the chemical shift of the H2′/H2″ protons of the C(4) residue are affected by monofunctional platinum binding, suggesting a change in the environment of P(5), the phosphorus nucleus located between the C(4) and G(5) residues.

Any change in the sugar ring can be determined from the equilibrium of the two common sugar conformations; the S-type and the N-type conformation.[11] Binding of [Pt(dien)]$^{2+}$ to the guanine N7 of the deoxytrinucleotide d(CGT) results in a change of the guanine sugar conformation from 80% S in the "free" trinucleotide to 55% S in the platinated one,[19] while the sugar conformation of the dC and dT residues remains the same as in the non-platinated trimer. To find out whether this change also occurs in our nonanucleotide fragments, the sum of coupling constants of the H1′ and H3′ proton can be used.[41] If the total width of the H3′ resonance appears to be smaller than 22 Hz, the sugar ring adopts the S conformation. In case of an N conformation, the total width is expected to be larger than 30 Hz.[17] The total width of the 3′(5) peak in fragment I appeared to be 17 Hz; thus, this sugar ring prefers the S conformation. The total width of the 3′(5) peak in I–Pt is significantly larger, 22 Hz, indicating a definite change in the sugar conformation upon platinum binding. Detailed investigation of the shape and the width of the 1′(5) resonance of I–Pt points to a 50–60% S conformation for the guanosine sugar ring; this resonance appears to be a triplet, while the H2′/H2″ protons are not isochronous and the total width is about 13 Hz.[41] Also the total width of the 1′(4) resonance in I–Pt could be determined. This cytidine sugar ring does not seem to be affected by binding of the platinum complex. The total width is about 14 Hz, indicating that the sugar ring adopts an S conformation for more than 60%.[41]

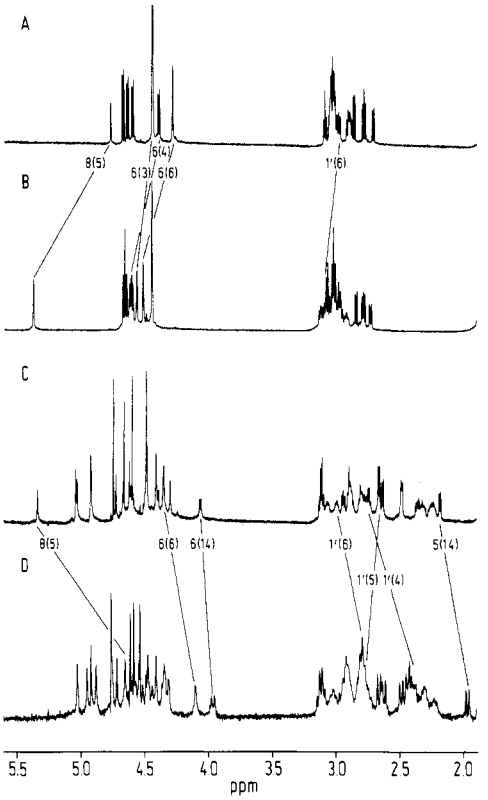

Figure 2. Downfield part (6–2 ppm) of the ^{1}H NMR spectra of (A) I (10 mM), (B) I–Pt (6 mM), (C) III–Pt (2 mM), and (D) III (2 mM). Chemical shift changes of more than 0.1 ppm upon platination are indicated. Spectra are recorded relative to TMA at pH 7; T = 285 K.

Also the influence of the inter- and intramolecular interactions on the chemical shift have to be taken into account. The intermolecular interactions are concentration dependent. Therefore, spectra of diluted samples of the two compounds (I, 2 mM, 5 times diluted; I–Pt, 1 mM, 6 times diluted) were recorded at several temperatures. The chemical shifts of all nonexchangeable protons appear to be independent of the concentration (data not shown).

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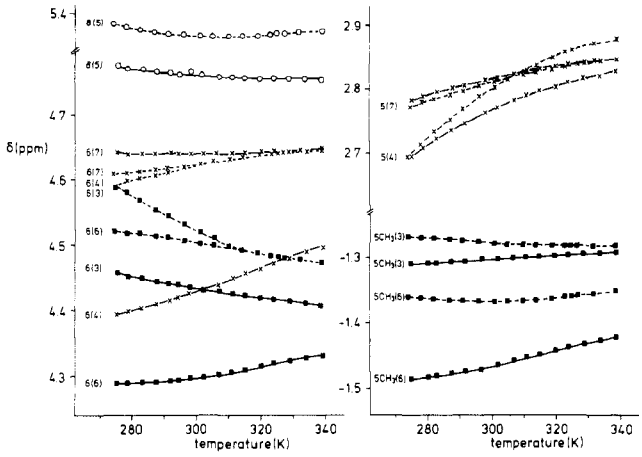

Figure 3. Chemical shift/temperature profiles of the nonexchangeable base protons of the central five residues of the fragments I (—) and I-Pt (- -). Concentrations are 10 and 6 mM, respectively at pH 7. Chemical shifts are reported relative to TMA.

Chemical shift/temperature profiles are frequently used to obtain information about intramolecular or stacking interactions. Chemical shifts are highly sensitive toward the ring current exerted by the aromatic planes of the bases. In particular, protons lying above or below the plane of a purine base are strongly shielded in a stacked structure with respect to their position in a random-coil conformation. Pyrimidine (Y)–Y interactions are less strong than purine (R)–Y, Y–R, and R–R interactions and have a smaller effect on the chemical shift.[42] Nonanucleotide I consists mainly of pyrimidine bases; a purine base occurs only in the center of the fragment. The chemical shift/temperature profiles of the nonexchangeable protons of the five central residues of the two single-stranded nonanucleotides are drawn in Figure 3. The profiles of the protons of the unmodified nonanucleotide show that the three core residues, from C(4) to T(6), are in a (partially) stacked structure at low temperatures; the protons of these residues show shielding changes larger than 0.05 ppm when the temperature decreases from 339 to 274 K. The Y–Y interactions are much weaker, and the outer parts of the molecule appear to be relatively more unstacked even at low temperatures.

Also, the Y–Y part of the platinated nonanucleotide appears to be present in a stacked/unstacked equilibrium. However, the stacking interactions of the three core residues are changed compared to that of nonanucleotide I. The opposite shielding effect on the chemical shift of the 6(6) and $5CH_3(6)$ protons, with respect to these protons in the unmodified nonanucleotide, and the different behavior of other protons must be due to a change in the conformation of the platinated guanine residue. Perhaps, the alteration of the guanine deoxyribose ring changes the environment of the protons of the bases on the 5′ and 3′ sides of the guanine and a shielding of the protons of these residues is the result. As in Pt(dien)[d(CGT)-*N7(2)*],[19] the chemical shift difference with temperature of the 6(4) proton, comparable with that of the 6(1) proton and d(CGT), is reduced after platinum binding. The chemical shift of the 6(4) proton in I changes 0.1 ppm by increasing the temperature from 274 to 340 K, whereas the 6(4) proton of I–Pt only shifts 0.05 ppm over the same temperature range. This implies a reduction of the stacking interaction between dC and dG. The chemical shift/temperatures profiles of the 8(5) and 6(6) protons of I–Pt are exactly the same as those for the analogous protons 8(2) and 6(3) in Pt(dien)[d(CGT)-*N7(2)*]. This suggests that the stacking interactions between dG and dT of I–Pt are not affected upon platination, just as reported for Pt(dien)[d(CGT)-*N7(2)*].[19]

The structure of the –CGT– part of the platinated nonanucleotide indeed resembles that of Pt(dien)[d(CGT)-*N7(2)*]: an N preference of the guanine sugar ring, which is only found in

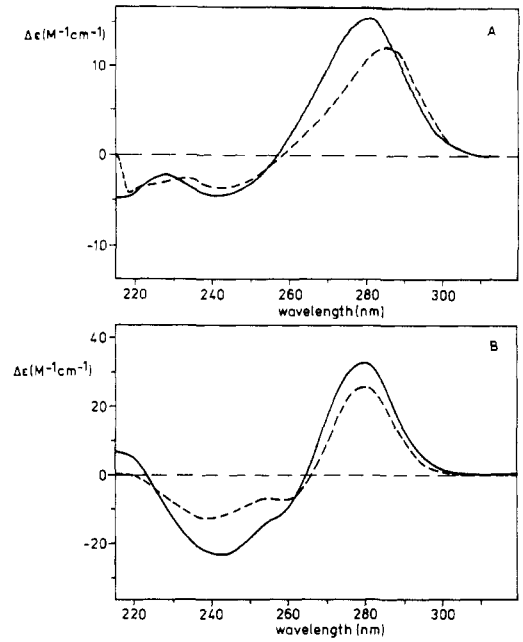

Figure 4. (A) CD spectra of I (—) and I–Pt (- -) at room temperature, pH 7, and 11.5 μM. (B) CD spectra of III (—) and III–Pt (- -) at 281 K, pH 7, and 8.7 μM in 0.5 M NaCl.

Chart I

d(5′T - C - T - C - G - T - C - T - C3′)
1 2 3 4 5 6 7 8 9
d(3′A - G - A - G - C - A - G - A - G5′)
18 17 16 15 14 13 12 11 10

platinated DNA fragments,[9,43] and the effect of platination upon the 5′ side of the guanine base, resulting in a reduction of the stacking interactions of dC-dG, while that of dG-dT remains intact.

The CD spectra of Pt(dien)[d(CGT)-*N7(2)*] were not comparable with those of Pt(dien)–DNA complexes.[19-21] Therefore, the CD spectra of the nonanucleotides, the extended fragment of d(CGT), have also been recorded. Figure 4A shows the CD spectra at room temperature of the platinated and "free" nonanucleotide. Upon platination of fragment I, the positive band in the CD spectra is red-shifted (λ_{max} goes from 279 to 283 nm) and the intensity is decreased, just as found for d(CGT).[19] The second positive band at 250 nm in the CD spectrum of Pt(dien)[d(CGT)-*N7(2)*] does not occur in I–Pt and also is not present in the spectra of the Pt(dien)–DNA complexes. Thus, the CD spectra of larger single-stranded fragments compare better with those of the Pt(dien)–DNA complexes than the CD spectra of the small trinucleotide. The structural change of the central bases is masked by the structure of the other bases. The effect of platination on the long-wavelength positive band is still significantly larger in I–Pt than that for the Pt(dien)–DNA complexes, perhaps because these latter complexes are double-stranded. In the next section of this paper, the CD spectra of the double-stranded nonanucleotides are reported.

Analysis of the Two Double-Stranded Nonanucleotides III and III–Pt. Equimolar amounts of strand II were added to strand I and to I–Pt, yielding the double-stranded nonanucleotides d(T-C-T-C-G-T-C-T-C)·d(G-A-G-A-C-G-A-G-A) (III) and Pt-$(NH_3)_3$[d(T-C-T-C-G-T-C-T-C)-*N7(5)*]·d(G-A-G-A-C-G-A-G-A) (III–Pt), respectively. To check the ratios of I and II (in III) and I–Pt and II (in III–Pt) the NMR intensities of the H5′/H5″ resonances of residues 1 and 10 were integrated. The strands were found to be present in equimolar amounts (error less than 5%).

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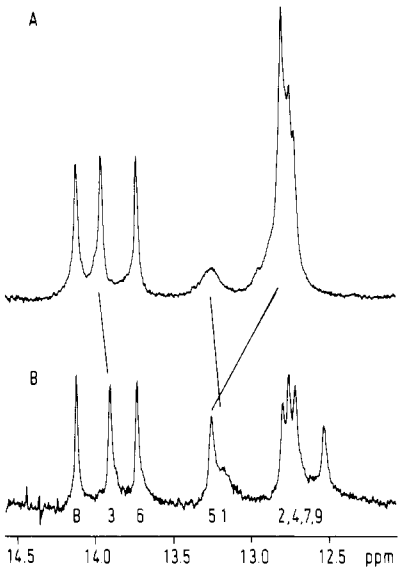

Figure 5. ^{1}H NMR spectra of the imino protons of III (A) and III–Pt (B). The numbering of the base pairs given in the lower trace spectrum is according to Chart I. Chemical shift changes of more than 0.05 ppm are alos indicated. Concentrations are 4 (III) and 2 mM (III–Pt) in 90% H_2O/10% D_2O, respectively at pH 6.8 and 270 K. Spectra are recorded relative to DSS.

The ability to form a duplex is studied by recording the ^{1}H NMR spectra of the imino protons of both fragments (Figure 5). The numbering of the base pairs is indicated in Chart I. The four A·T base pairs could be assigned on the basis of their melting behavior and by analogy with the imino protons of the undecanucleotide d(T-C-T-C-G-T-G-T-C-T-C)·d(G-A-G-A-C-A-C-G-A-G-A) studied by den Hartog et al.[44] The chemical shift of the G·C(5) base pair, to which $[Pt(NH_3)_3]^{2+}$ is bound, follows from the positions of the cDDP-platinated G·C base pairs in the analogous decanucleotide *cis*-$Pt(NH_3)_2$[d(T-C-T-C-G-G-T-C-T-C)-*N7*(5),*N7*(6)]·d(G-A-G-A-C-C-G-A-G-A),[17] but the remaining C·G base pairs could not be assigned unambiguously. Chemical shift changes of more than 0.05 ppm upon platinum binding are also indicated in Figure 5. It is striking that the A·T(3) base pair is more affected upon platination than the A·T(6) base pair. Apparently, it is also seen in the double-stranded nonanucleotides that the influence of platinum binding is larger at the 5′ side of the platinated guanine than at its 3′ side. This is probably a result of the steric hindrance of the platinum moiety. From a model study, it was seen that the platinum group is more directed to the cytosine residue (not shown). The imino proton signals of the central residues broaden upon raising the temperature and disappear at 315 K for III and at 298 K for III–Pt (not shown). This finding indicates that the lifetime of the base pairs in the platinated nonanucleotide is significantly shorter than that in the unmodified one. It is noted that the signal of the G·C base pair to which platinum is bound remains visible up to 298 K, which means that there is no local denaturation of the duplex.

As described for the single-stranded nonanucleotides, the ^{1}H NMR spectra of the nonexchangeable protons of III and III–Pt at low temperatures were assigned. This is demonstrated in Figure 6A for the platinated duplex. A part of the contour plot of the 2D-NOE experiment, i.e. the area with the cross peaks between the base protons and the H1′, H5, and $5CH_3$ resonances, is drawn. For reasons of clarity, only the assignment of the platinated strand is shown. The solid line, starting with the {6(1)–1′(1)} connectivity, outlines the procedure followed for the sequential assignment of the base and 1′ resonances. Additional information is obtained from the fact that the cytosine H5 and the thymine $5CH_3$ are sufficiently close to both the base proton of their own residue and to the base proton of the residue at the 5′ side to permit NOEs to build up.[39] These intra- and interresidual NOE cross peaks are connected with dashed lines (Figure 6A). In the same way the base protons and H1′ resonances of the complementary strand

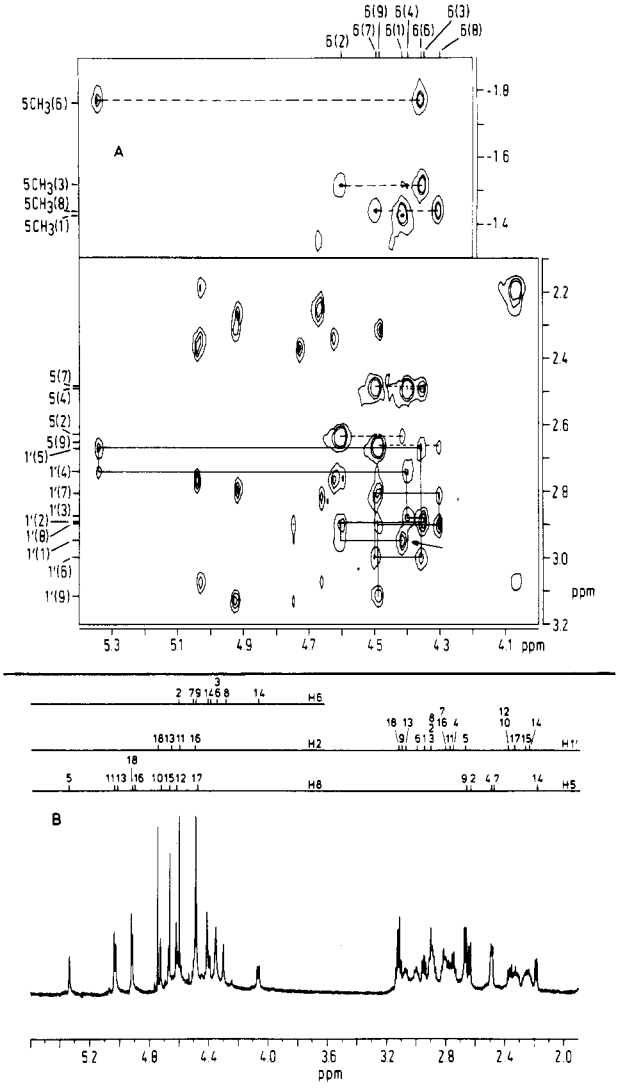

Figure 6. (A) Part of the 2D-NOE contour plot of III–Pt. The solid lines show assignment of the base and H1′ resonances. The arrow indicates the {6(1)–1′(1)} connectivity, starting point of the sequential procedure. The dashed lines show connections of the H5 and $5CH_3$ resonances between the inter- and intraresidual NOE cross peaks. (B) Downfield part of the 600-MHz ^{1}H NMR spectrum of III–Pt at 285 K and pH 7. Upper traces show residue numbers for the assignment of the H6, H2, and H8 resonances (5.6–3.8 ppm) and of the H1′ and H5 resonances (3.4–2.0 ppm), respectively.

could be assigned. All {base(n)–1′(n),1′($n-1$)} connectivities were observed in both strands, indicating that the overall B-DNA structure is indeed maintained after platination. The H2 protons of the adenine residues could not be assigned in the same way because there are no nonexchangeable protons in their vicinity. However, these protons were assigned by comparison of the chemical shift of the analogous protons of the double-stranded decanucleotide.[17] Complete assignment of the H2′/H2″ protons of the unmodified duplex (III) appeared not to be possible. In particular, connectivities within the repeating d(-T-C-T-C-) fragment were not observed clearly. The assignment of the base and 1′ protons of the platinated duplex is given in Figure 6B. Parts C and D of Figure 2 exhibit the downfield part of the ^{1}H NMR spectra of both double-stranded fragments. The proton resonances that are shifted more than 0.1 ppm upon platination are also indicated. Again, these are only protons of the core residues C(4)–T(6) and C(14); the conformation of the three central bases of the platinated strand and only the central cytosine residue of the complementary strand are altered.

The chemical shifts of the base and 1′ protons are listed in Table II. Comparison of the chemical shift values of the protons in I–Pt with those of III–Pt shows that all resonances are shifted

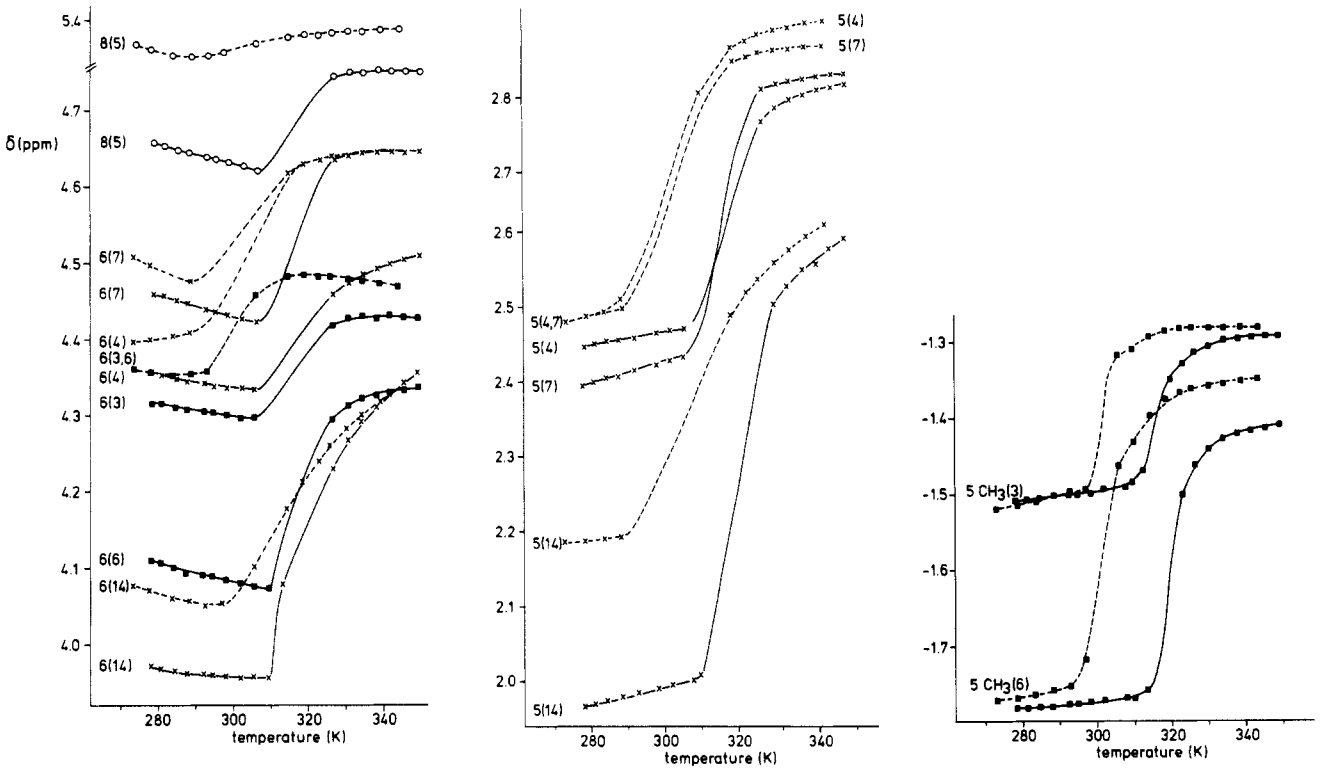

Figure 7. Chemical shift/temperature profiles of the nonexchangeable base protons of the five central residues of the d(T-C-T-C-G-T-C-T-C) strand of the fragments III (—) and III–Pt (--). Concentrations are 2 mM at pH 7. Chemical shifts are reported relative to TMA. Around the midpoint temperature of the duplex/single-strand transition, some protons exhibit a large chemical shift change; therefore, the spectra become too broad to assign all protons unambiguously.

Table II. ^{1}H NMR Chemical Shifts (ppm) of the Nonexchangeable Base and 1′ Protons of the Double-Stranded Nonanucleotides III and III–Pt Reported Relative to That of TMA

	H8/H6		H2/H5/5CH$_3$		H1′	
residue	III	III–Pt	III	III–Pt	III	III–Pt
T(1)	4.408	4.424	−1.441	−1.432	2.952	2.945
C(2)	4.578	4.605	2.606	2.634	2.902	2.896
T(3)	4.308	4.350	−1.529	−1.520	2.899	2.884
C(4)	4.347	4.408	2.476	2.488	2.421	2.748
G(5)	4.603	5.345	...	...	2.784	2.675
T(6)	4.101	4.360	−1.788	−1.773	2.796	2.996
C(7)	4.453	4.504	2.421	2.480	2.799	2.808
T(8)	4.340	4.314	−1.470	−1.440	2.902	2.897
C(9)	4.486	4.488	2.649	2.664	3.102	3.117
G(10)	4.716	4.732	...	...	2.388	2.368
A(11)	5.035	5.040	4.572	4.600	2.800	2.765
G(12)	4.546	4.622	...	...	2.382	2.342
A(13)	4.953	5.029	4.603	4.663	2.990	3.073
C(14)	3.961	4.066	1.960	2.187	2.288	2.240
G(15)	4.753	4.676	...	...	2.218	2.265
A(16)	4.887	4.914	4.521	4.489	2.736	2.799
G(17)	4.476	4.488	...	...	2.295	2.312
A(18)	4.920	4.928	4.761	4.749	3.120	3.127

upfield in going from the single- to the double-stranded nonanucleotide, due to shielding of the protons by the aromatic bases of the added strand. The exceptional chemical shift changes as found in the analogous decanucleotide upon binding of cDDP[17] are not observed in this nonanucleotide. This is an indication of a conformational difference between monofunctional and bifunctional platinum–DNA fragments. The structure of the monodentate Pt–DNA adduct is more flexible than that of the GpG chelate. The monodentate Pt–DNA adduct probably still approximates the original DNA structure, and therefore, the effect of monofunctional platination on the chemical shift values is less pronounced compared with that of bifunctional platinum binding.

At high temperatures, the two fragments III and III–Pt are single stranded. The chemical shifts of the protons of the d(T-C-T-C-G-T-C-T-C) strand under this condition are the same as for the single-stranded nonanucleotides I and I–Pt, respectively. The chemical shift/temperature profiles of the nonexchangeable base pairs of III and III–Pt are drawn in Figure 7. In particular, the chemical shift changes of the 5CH$_3$ resonances (in the right-handed side of Figure 7) clearly demonstrate the difference in the melting temperature of both fragments. The melting-transition midpoint of the nonanucleotide is shifted by 15–20 K to lower temperature after binding of the monofunctional platinum complex. This difference in melting temperature agrees with the results of the UV melting experiments reported earlier.[23]

There is an opposite shielding effect on the 6(4) proton in comparison with the other H6 resonances in the double-stranded part of the chemical shift/temperature profiles. This suggesta a change in the intramolecular interactions of the cytosine residue after platinum binding. Probably, just as in the single-stranded fragment, the stacking between dC and dG is reduced upon platination.

The ^{31}P NMR spectra of both duplexes (III and III–Pt) at 285 K are depicted in Figure 8. The resonances of the unplatinated nonanucleotide are found between −3.8 and −4.5 ppm relative to TMP (Figure 8B). This is in the range commonly observed for double-stranded DNA fragments.[45,46] All signals of the platinated nonanucleotides, except the resonance at −4.7 ppm, fall also within this range. Comparing the spectrum of III–Pt (Figure 8A) with that of the platinated decanucleotide[22] shows by analogy that the resonance at −4.7 ppm corresponds with the ^{31}P resonance of P(5); the phosphorus nucleus between dC(4) and dG(5). As reported in the single-stranded nonanucleotide (see above), the environment of P(5) is changed upon platination. The upfield-shifted resonance observed in the platinated decanucleotide[22] and in salmon sperm DNA treated with cDDP[48,49] belongs to the P(6) resonance and

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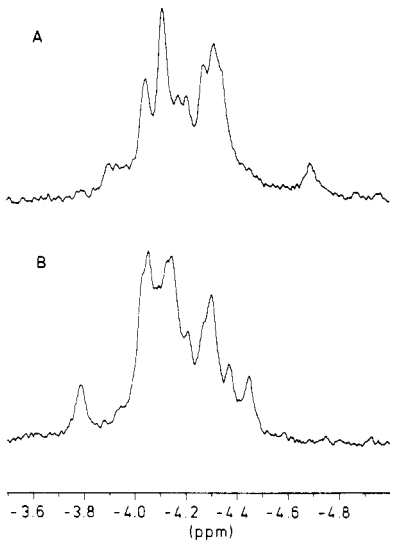

Figure 8. ^{31}P NMR spectra (121.5 MHz) of III–Pt (A) and III (B) at 285 K. Concentrations are 2 mM in the presence of 0.1 mM EDTA at pH 7. Spectra are recorded relative to TMP.

is not present in the nonanucleotide. The ^{31}P NMR spectra again indicate that the conformational changes after binding of $[Pt(NH_3)_3]^{2+}$ is restricted to the 5′ side of the guanine residue, since only the P(5) resonance is shifted upon platination.

The change in structure induced by monofunctional platinum binding is clearly demonstrated by the CD spectra of the two nonanucleotide duplexes in Figure 4B. The experimental conditions are the same as reported for the UV melting experiments.[23] In order to make certain that the platinated nonanucleotide is double-stranded at 10 μM, NaCl was added to a concentration of 0.5 M and the sample temperature was decreased below 10 °C.[23] Upon platination, the positive band at 280 nm is slightly red-shifted. The decrease in intensity has been reported earlier for the single-stranded monofunctional platinum–DNA fragments Pt(dien)–d(CGT)[19], Pt(dien)–d(CCGG)[49] and the single-stranded nonanucleotide (see above) and is likely due to destacking of the bases in the fragments. The CD spectra of the double-stranded nonanucleotides are more similar to the CD spectra of Pt(dien)–DNA complexes reported by Macquet and Butour[20,21] than to those of the single-stranded oligonucleotides. The positive band at 210–220 nm indicates that the nonanucleotide after binding of a monofunctional platinum compound retains its B-type conformation in solution.[34]

Conclusions

All experiments described above, i.e. ^1H NMR, ^{31}P NMR, and CD spectroscopy, clearly indicate that the conformation of the single- and double-stranded nonanucleotide changes after binding of the monofunctional platinum compound $[PtCl(NH_3)_3]Cl$. Only the central residues of the fragments are involved in this distortion. The outer parts remain of the B-DNA type. The features of this conformational alteration appears to be a change in the N/S equilibrium of the sugar ring of the guanine residue and a reduction of the stacking interaction between dC(4) and dG(5). In particular, the protons of the residue at the 5′ side of the guanine, to which the platinum complex is bound, are affected by platination. The ^1H NMR spectrum of the imino protons of the double-stranded fragment clearly demonstrates that there is no local denaturation of the duplex. The distortion is more pronounced in the platinated strand; the conformation of the three core residues is changed upon platinum binding. In the complementary strand, the distortion is limited to the central cytosine base. Due to these conformational changes and increased flexibility, the DNA backbone will be directed to a better position to form a chelate (in the case of cDDP binding) more easily. The conformation of the central –CGT– part of the nonanucleotides I–Pt and III–Pt resembles very much the structure of the single-stranded fragment Pt(dien)[d(CGT)-*N7(2)*] reported earlier.[19]

The nonanucleotide duplex is destabilized upon binding of $[Pt(NH_3)_3]^{2+}$ as shown by a reduction of the melting temperature of 15–20 K. This result was also obtained from UV melting experiments of the same nonanucleotide.[23]

The distortion of the structure of DNA fragments induced by bifunctional binding of cDDP differs from that reported in this paper, although some resemblances exist. For the analogous decanucleotide modified with cDDP, *cis*-$Pt(NH_3)_2$[d(T-C-T-C-G-G-T-C-T-C)-*N7(5)*,*N7(6)*]·d(G-A-G-A-C-C-G-A-G-A), a change in the intramolecular interactions of dC(4)–dG(5) was also reported.[17] However, the sugar ring of the dG(5) residue of the decanucleotide is completely turned into an N conformer after bifunctional binding of cDDP, whereas after monofunctional platinum binding to the nonanucleotide the N/S equilibrium of dG(5) is shifted only about halfway toward an N conformation. In both cases, the distortion is limited to the central residues, and base pairing, even that of the platinated guanines, remains possible at low temperatures. The reduction of the melting temperature as a result of mono- and bifunctional platinum binding is of the same order of magnitude.

In addition, the ^{31}P NMR and CD spectra indicate a clear difference in the structures of the mono- and bifunctional platinum–oligonucleotide adducts. The downfield shift of a ^{31}P signal (at ca. –3 ppm) in the platinated adduct is not observed in the nonanucleotide. This resonance is assigned to the phosphorus nucleus in the –GpG– chelate and is only observed for DNA fragments complexed with antitumor active platinum compounds. In the CD spectra of cDDP–DNA adducts, the intensity of the positive 280-nm band is increased, while monofunctional platinum compounds causes a decrease of this band.

Presuming that the downfield shifted resonance in the ^{31}P NMR and the increase of the intensity of the 280-nm band in the CD spectra are clues for a "kinked" structure of the DNA fragments, it is not likely that the monofunctional platinated nonanucleotide appears in such a "kinked" conformation. This "kinked" structure might be important for the antitumor activity. Binding of a platinum compound to a guanine N7 distorts the DNA structure in such a way that chelation can occur more easily, due to the larger conformational freedom. Furthermore, Pinto and Lippard[50] demonstrated that cDDP forms an adduct with DNA, which inhibits the DNA synthesis in vitro. In contrast, the monofunctional adducts are easily bypassed by the polymerase I enzyme. This agrees with the observation of Alazard et al.[51] that $[PtCl(dien)]^+$ does not inhibit replication in bacteria. This distinction between cDDP and Pt(dien) adducts can be explained with the larger molecular flexibility of the monofunctional Pt–DNA fragment in contrast to the more rigid structure of the –GpG–*N7,N7* chelate. Moreover, the conformation of mono- and bifunctional Pt–DNA fragments differs: i.e., the "kinked" versus the "unkinked" structure. These differences in conformation and conformational behavior between the mono- and bifunctional DNA adducts may be part of the reason why monofunctional platinum compounds are not antitumor active.

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