9.7: Structure of Platinum-DNA Complexes

a. NMR Studies of Platinated Oligonucleotides

Once the major spectrum of adducts formed by *cis*- and *trans*-DDP with DNA began to emerge, it was of immediate interest to learn to what positions on the nucleobases the platinum atom was coordinated. Proton NMR spectroscopy soon proved to be an invaluable tool for obtaining this information.[71,125,126] Several ribo- and deoxyribooligonucleotides containing GG, AG, or GNG sequences were synthesized, and allowed to react with *cis*-DDP or its hydrolysis products, and the resulting complexes were purified by chromatography. All GG-containing oligomers formed intrastrand crosslinks with the $\{Pt(NH_3)_2\}^{2+}$ moiety coordinated to the N7 atoms. This structure was deduced from several criteria. Most frequently studied were the nonexchangeable base protons H8 and H2 of adenine, H6 of thymine, H8 of guanine, and H5 and H6 of cytosine (Figure 9.9). Coordination of platinum to N7 of guanine causes a downfield shift of the H8 proton resonance. More importantly, however, it also lowers the pK_a of the N1 proton by ~ 2 units, because platination adds positive charge to the base. Thus, titration of the platinated oligonucleotide over a pH range, and comparison of the results to those obtained for the unplatinated oligomer, reveals a difference in the midpoint of the transition in chemical shift of the H8 proton by 2 pH units if coordination occurs at N7. This effect is illustrated in Figure 9. 19 for the adduct *cis*- $[Pt(NH_3)_2\{d(ApGpGpCpCpT)\}N7\text{-}G(2),N7\text{-}G(3)]$, where the pK_a of N1 is seen to shift from ~ 10 to ~ 8 upon platination.[71] The pH titration in this example also reveals the pH-dependent chemical shift of the cytosine ^1H resonances at a pH of ~ 4.5, corresponding to protonation of the N3 atoms. The protonation of adenine N7 (pK_a ~ 4) is also frequently observed in these studies. These results conclusively demonstrate platinum coordination at N7 of the two guanosine nucleosides.

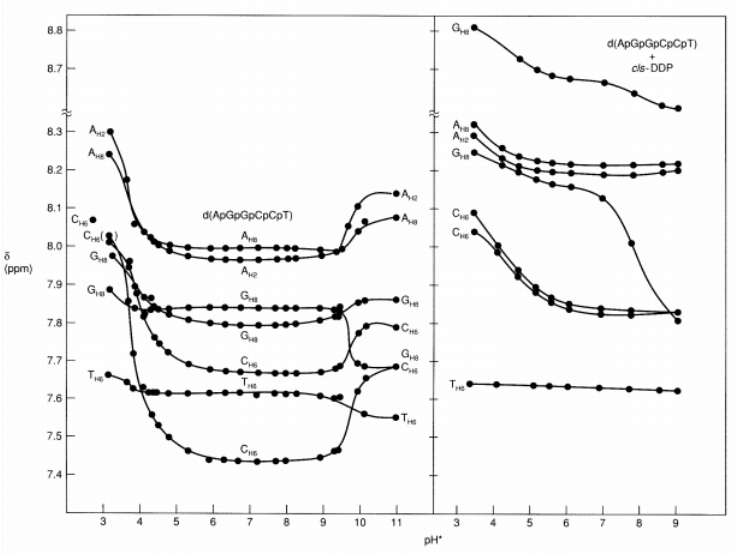


Figure 9.19 - Chemical shift (δ) vs. pH* of the nonexchangeable base protons of D_2O solutions of $[d(ApGpGpCpCpT)]_2$ (3.5 mM, 35 °C) and its cis-DDP adduct (2.5 mM, 70 °C). The pyrimidine resonances of the latter sample show no chemical shift changes with temperature over the range 35 < T < 70 °C while the purine resonances show a slight temperature-dependent chemical shift change of up to 0.1 ppm. Tetramethylammonium chloride was used as the internal standard (δ 3.180). Reproduced by permission from J. C. Caradonna, S. J. Lippard, M. J. Gait, and M. Singh, J. Am. Chem. Soc. **104** (1982), 5793.

Although several of the oligonucleotides studied have self-complementary sequences, such that they can form a double helix when unplatinated, in no such case was a duplex observed for their platinated forms. The presence of the platinum-induced crosslink presumably decreases the stability of the doublestranded form of the oligonucleotide. Another interesting result is that all intrastrand $\{Pt(NH_3)_2\}^{2+}$ adducts of d(GpG) or d(ApG) have an altered deoxyribosesugar ring conformation. In normal, unplatinated form, these single-stranded or duplex oligonucleotides have a C2'-endo sugar pucker (Figure 9.9). Upon platination, the 5'-nucleotide switches to C3'-endo. This change is readily monitored by the ring proton-coupling constants $J_{H1'\text{-}H2'}$ and $J_{H1'\text{-}H2''}$. These protons constitute an ABX spin system such that the sum, $\Sigma^3J = {}^3J_{1'2'} + {}^3J_{1'2'}$, is most easily measured as the separation between the outermost peaks in the multiplet. For the C2'-endo conformation, a pseudotriplet occurs with Σ^3J = 13.6 Hz, and for C3'-endo, Σ^3J = 7.5 Hz. The 3'-guanosines in the adducts show greater conformational flexibility, having ~ 70 to 80 percent C2'-endo sugar puckers, depending upon the temperature.

Another conformational feature that could be deduced from ^1H NMR studies of all *cis*-DDP-platinated oligonucleotides containing an embedded d(GpG) sequence is that both guanosine nucleosides retain the anti orientation of the base around the C1'-N9

glycosidic linkage (Figure 9.9). This result was deduced from the lack of a pronounced nuclear Overhauser effect (NOE) between H8 and the H1' protons, such as would occur in the syn conformation. An NOE between H8 resonances on the two coordinated nucleosides was observed for adducts of d(ApTpGpG) and d(CpGpG), indicating that the two bases are in a head-to-head orientation with respect to the platinum coordination plane. In other words, both O6 atoms lie on the same side of that plane. Two oligonucleotides containing *cis*-[$Pt(NH_3)_2$(ApG)] adducts have been examined; their structural properties closely resemble those of the (GpG) adducts, with platinum coordinated to N7 of both purine bases.

In order to study double-stranded DNAs platinated on one strand, it was necessary to adopt a special strategy. First, the desired oligonucleotide is synthesized. It is preferable that the DNA strands not be self-complementary, since the affinity of such an oligomer for itself is so much greater than that for its platinated form that the desired, singly platinated duplex will not form. After the platinated single strand is synthesized and purified, the complementary strand is added. Several duplex oligonucleotide-containing *cis*-[$Pt(NH_3)_2${d(pGpG)}]-embedded adducts prepared in this manner have been studied by ^1H NMR spectroscopy. With the use of two-dimensional and temperature-dependent techniques, both the nonexchangeable base and sugar protons as well as the exchangeable (guanine N1 and thymine N3) N-H (imino) proton resonances were examined. The last are useful, since they give some measure of the extent to which the double helix remains intact. When not base-paired to their complements in the other strand, these protons exchange more rapidly with solvent (water) protons, leading at moderate exchange rates to broadening of the resonances and, at high exchange rates ($>10^7$ s^{-1}), disappearance of the signals. Several interesting results were obtained in these studies. In all of them, platination of the d(GpG) sequence brought about the same C2'-endo → C3'-endo sugar-ring pucker switch for the 5'-guanosine as seen in the single-stranded adducts. Head-to-head, anti conformations were also observed. At low temperatures, below the melting transition temperature, above which the duplex becomes single-stranded, the imino proton resonances were observed. This result was interpreted to mean that normal, Watson-Crick base pairs can still exist between the *cis*-DDP d(GpG) adduct and the d(CpC) sequence on the complementary strand. In the case of [d(TpCpTpCpG*pG*pTpCpTpC)]•[d(GpApGpApCpCpGpApGpA)], where the asterisks refer to the sites of platination, the imino proton resonances were assigned with the assistance of NOE experiments.[125] Temperature-dependent studies showed that, in the range - 4° < T < 46 °C, the imino resonances of the coordinated guanosine nucleosides broadened first with increasing temperature. Apparently the base pairs of the intrastrand crosslinked, platinated duplex DNA are disrupted, or "melted," outward from the point of platination as well as from the ends. Since the amino hydrogen atoms involved in base pairing were not observed in this study, a completely definitive structural analysis was not possible. Nevertheless, the authors proposed that the duplex would be kinked by an angle of ~ 60° at the *cis*-DDP binding site in order to preserve full duplex character.

Another useful NMR nucleus for monitoring *cis*-DDP-DNA interactions is ^{195}Pt, which is 34 percent abundant with I = $\frac{1}{2}$. When used in conjunction with ^{15}N (I = $\frac{1}{2}$) enriched NH_3 ligands, ^{195}Pt NMR resonances provide a powerful means for characterizing complexes in solution. The ^{195}Pt and ^{15}N chemical shifts are both sensitive to the ligand trans to the NH_3 group, as is the ^{195}Pt-^{15}N coupling constant.[127] ^{195}Pt NMR studies of *cis*-DDP binding have been carried out using nucleobases, small oligonucleotides, and even double-stranded fragments of 20 to 40 bp in length, as previously described (Section V.D.l). The major contribution of this method is to show whether platinum coordinates to a nitrogen or an oxygen donor atom on the DNA, since the ^{195}Pt chemical shift is sensitive to this difference in ligation.

b. X-ray Structural Studies

In recent years several oligonucleotide duplexes have been crystallized and characterized by x-ray diffraction methods. The probability of forming suitable single crystals of DNA fragments is disappointingly low, however, with only 1 in 10 such attempts being successful. Correspondingly, it has been difficult to crystallize platinated oligonucleotides. An alternative approach has been to soak nucleic-acid crystals of known structure with the platinum reagent in the hope of forming an isomorphous derivative, the structure of which could be obtained by using the changes in phases from the native material. In attempts to characterize a *cis*-DDP nucleic acid adduct, crystals of $tRNA^{Phe}$ and the self-complementary dodecamer d(CpGpCpGpApApTpTpCpGpCpG) were soaked with cisplatin solutions in the hope of obtaining useful metric information.[123,128,129] These efforts have thus far failed to produce a high-resolution structure, although they confirm the predilection for platinum to coordinate to the N7 position of purine rings. Addition of *cis*-DDP tends to disorder the crystal, with platinum going to several sites of partial occupancy.

A more fruitful approach has been to crystallize a purified oligonucleotide containing the coordinated *cis*-$\{Pt(NH_3)_2\}^{2+}$ moiety. The first x-ray structure to be deciphered through such a strategy was that of *cis*-[$Pt(NH_3)_2${d(pGpG)}].[130] This compound crystallizes with water solvent and glycine buffer molecules in the lattice. The crystals were grown at pH 3.8, where the terminal phosphate is monoprotonated in order to provide a neutral complex of diminished solubility. Two crystalline forms have been obtained, and both structures solved, one to 0.94 Å resolution. The latter contains four crystallographically independent molecules,

which, although complicating the structure solution, afforded four independent views of the major adduct formed by *cis*-DDP with DNA. The four molecules form an aggregate, held together by hydrogen bonding and intermolecular base-base stacking interactions (Figure 9.20). There are two conformationally distinct classes that comprise molecules 1 and 2, and molecules 3 and 4; within each class, the molecules are related by an approximate C_2 symmetry axis.

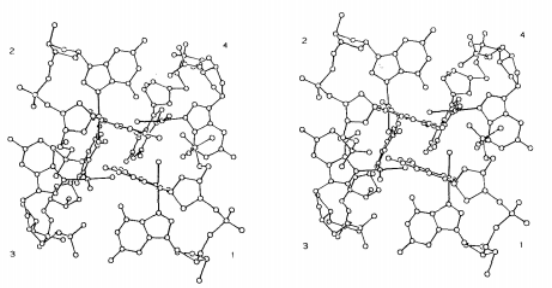


Figure 9.20 - Stereoview of aggregate of four cis-[Pt(NH_3)$_2${d(pGpG)}] molecules (reproduced by permission from Reference 130).

The molecular structure of molecule 1 is displayed in Figure 9.21; geometric information about all four molecules is contained in Table 9.4. As expected from the NMR studies, platinum coordinates to N7 atoms of the guanine bases, which are completely destacked (dihedral angles range from 76.2 to 86.7°), to form a square-planar geometry. The bases have a head-to-head configuration and conformational angles χ (Table 9.4 and Figure 9.9) that fall in the anti range. The sugar puckers of the 5'-deoxyribose rings for all four molecules have a C3'-endo conformation, and some of the 3'-sugar carbon atoms exhibit large thermal parameters suggestive of a less well-ordered structure. These results further demonstrate the similarity of the structure as detected in the solid state by x-ray diffraction and in the solution state by NMR spectroscopy.

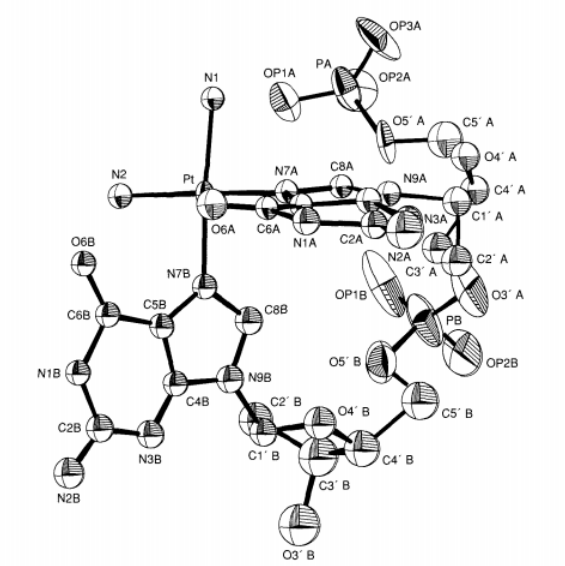


Figure 9.21 Molecular structure of cis-[Pt(NH_3)$_2$d(pGpG)}].

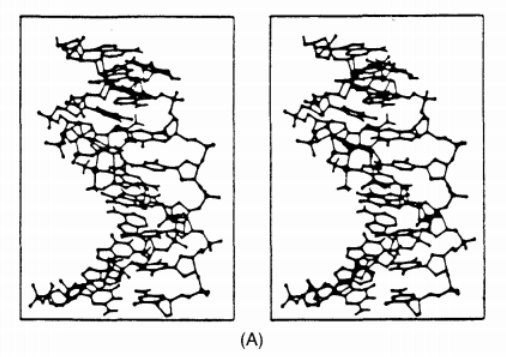
An interesting additional feature of the *cis*-[Pt(NH_3)$_2${d(pGpG)}] crystal structure is a hydrogen bonding interaction between an ammine ligand and the oxygen atom of the terminal phosphate group (OP1A•••N1, Figure 9.21). This intramolecular hydrogen bond is prominent in three of the four molecules in the asymmetric unit. Although the relevance of this hydrogen bonding interaction to the solution structure and molecular mechanism of cisplatin is presently unknown, it is interesting to note that the antitumor activity of platinum amine halide complexes is reduced when protons on coordinated NH_3 are replaced by alkyl groups.[34]

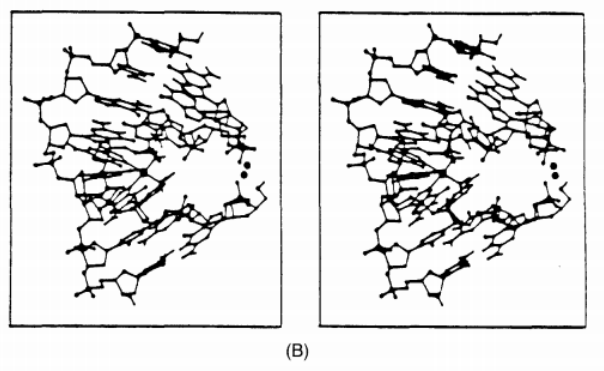
A second *cis*-DDP-oligonucleotide adduct characterized by x-ray crystallography is the neutral molecule *cis*-[Pt(NH_3)$_2${d(CpGpG)}].[131] Here again, there are several (three) molecules in the asymmetric unit. Although determined at lower resolution, the structure is similar in most respects to that of *cis*-[Pt(NH_3)$_2${d(pGpG)}] except for the presence of some weak NH_3•••O6(guanosine) intramolecular hydrogen bonding interactions and a few unusual sugar-phosphate backbone torsional angles. Also, no NH_3(H)••• phosphate(O) hydrogen bonds were observed.

From the foregoing discussion, it is apparent that adequate x-ray structure information is available for the *cis*-$\{Pt(NH_3)_2\}^{2+}$/d(pGpG) intrastrand crosslink. What is needed now are structures of the minor adducts and, most importantly, of adducts in double-stranded DNA. Very recently, dodecanucleotide duplexes containing *cis*-$\{Pt(NH_3)_2\}^{2+}$/d(pGpG) adducts have been crystallized, the structures of which are currently being investigated.[132]

c. Molecular Mechanics Calculations on Platinated Duplexes

As a supplement to x-ray structural information on double-stranded oligonucleotides containing an embedded *cis*-[$Pt(NH_3)_2${d(pGpG)}] adduct, several models have been constructed by using a molecular mechanics approach.[133] In this work, a set of coordinates was first obtained by amalgamation of structural information about standard double-helical DNAs and the platinated d(pGpG) fragment. Various starting structures were assumed, both linear and bent. The models were then refined according to various charge and stereochemical constraints built into the calculation. The results, which can reveal only what is feasible and not necessarily what actually happens, for both linear and bent structures are depicted in Figure 9.22 for two of the duplex sequences studied. In the linear model, the 5'-coordinated guanosine is rotated out of the stack, and its hydrogen bonding to the cytosine on the complementary strand is seriously disrupted. The imino N-H group is still involved in H-bonding, however; so this structure is not inconsistent with the NMR results. Two classes of kinked platinated duplex structures were encountered, with bending angles of 61 and 50°. In one of these, all Watson-Crick hydrogen bonds remain intact. These kinked structures are supported most strongly by the gel-electrophoresis experiments discussed in Section V.D.3.b.v.





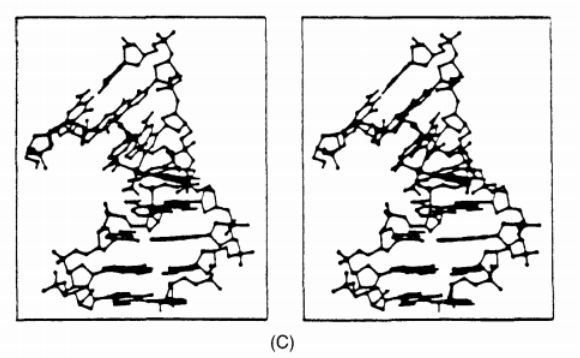


Figure 9.22 - (A) Stereoscopic view of the unkinked, platinated model of duplex d(TpCpTpCpG*pG*pTpCpTpC) from molecular-mechanics calculations. Counter ions used to stabilize the negative charge of the phosphates are not shown. (B) Stereoscopic view of the "high-salt" kinked, platinated model of duplex d(GpGpCpCpG*pG*pCpC) from molecular-mechanics calculations. Counter-ions are not depicted, with the exception of the bridging ion. (C) Stereoscopic view of the "low-salt" kinked, platinated model of duplex d(GpGpCpCpG*pG*pCpC). Counter-ions are not depicted. Reproduced with permission from Reference 133.

Molecular mechanics and the related molecular dynamics calculations are a potentially valuable tool for the bioinorganic chemist interested in how metal complexes might perturb the structures of biopolymers. Analysis of the results for cisplatin-DNA binding reveals that, compared with the sum of all contributions from the biopolymer, the Pt-DNA interactions constitute a small part of the overall energy. For the most accurate results, it is important to know the charge distributions on the metal and its ligands as well as the effects of solvent interactions. Much work needs to be done in these areas before the results of molecular mechanics and dynamics calculations can be used reliably to predict or analyze structures. At present, however, they are far superior to examination of space-filling molecular models, for example, and produce quantitatively revealing structural diagrams.

d. Platinum-Nucleobase Model Complexes

Several studies have been carried out of the *cis*-diammineplatinum(II) moiety coordinated to nucleobases in which the N9 (purine) or N3 (pyrimidine) positions either have been alkylated, to simulate the glycosidic linkages, or in which the actual nucleotide (AMP, dGMP, etc.) is employed.[134] These investigations are in many respects analogous to the synthesis and characterization by bioinorganic chemists of model complexes for the active site of a metalloenzyme. Their purpose is to simplify the problem, revealing kinetic, thennodynamic, and structural preferences of the primary building blocks involved in the metallodrug-biopolymer interaction, without the profound stereochemical constraints of the latter. Early studies of *cis*- and *trans*-DDP adducts with nucleobases (i) revealed the kinetic preferences for platinum binding to GMP and AMP, (ii) mapped out the preferred sites of platination (N7 of A and G; N1 of A; N3 of C; no N7-O6 chelate; no ribose or deoxyribose binding; only rare binding to phosphate oxygen atoms), (iii) demonstrated that Pt-N7 binding to G lowered the pK_a of N1-H by ~ 2 units, and (iv) led to the discovery of interesting new classes of coordination complexes such as the *cis*-diammineplatinum pyrimidine blues and metal-metal bonded diplatinum(III) complexes.

Attempts to model the intrastrand d(GpG) crosslink with nucleobases have met with only moderate success. Usually the O6 atoms of the two guanosine rings are on opposite sides of the platinum coordination plane ("head-to-tail" isomer). Only for *cis*-$[Pt(NH_3)_2(9\text{-EtG})_2]^{2+}$ was the correct isomer obtained. Nucleobase complexes of the *cis*-diammineplatinum(II) moiety have been valuable for testing the controversial proposal of N7,O6 chelate formation, which to date has not been observed. Several interesting discoveries of metal-nucleobase chemistry are that metal binding can stabilize rare tautomers, for example, the 4-imino, 2-oxo form of cytosine, through N4 binding, that coordination of platinum often produces unusual base pairing, and that metal migration from one donor site to another on an isolated nucleobase can occur. These model studies will continue to provide valuable insights into the possible chemistry of platinum antitumor drugs with DNA.

e. trans-DDP-DNA Adducts

Because *trans*-DDP is biologically inactive, it has received less attention than the *cis* isomer. Nevertheless, knowledge of its binding to DNA is important to have as a reference point for mechanistic comparison with the active compounds. Shortly after replication mapping experiments established that *trans*-DDP binds preferentially to d(GpNpG) and d(ApNpG) sequences,[135] several synthetic oligonucleotides containing such sequences were prepared and used to investigate reactions with the *trans* isomer.[136-138] Kinetic studies of *trans*-DDP with d(GpCpG) and d(ApGpGpCpCpT) revealed the presence of, presumably monofunctional, intermediates that closed to form both intra- and interstrand products. In the reaction with d(GpCpG), the 1,3-intrastrand G-G chelate accounted for 70 percent of the product, and 21 percent of the remaining material was unreacted

oligonucleotide. Proton NMR studies of purified *trans*-[$Pt(NH_3)_2${d(GpCpG)}] as well as the d(GpTpG) analog established platinum binding to N7 positions of the two trans guanosine nucleosides. As with the *cis*-[$Pt(NH_3)_2${d(pGpG)}] adducts, the 5'-guanosine residue no longer retained the normal B-DNA type conformation; instead, the sugar ring pucker switched to C3'-endo. A fairly detailed ^1H NMR characterization of *trans*- [$Pt(NH_3)_2${d(ApGpGpCpCpT)-N7-A(1),N7-G(3)}] revealed very similar features. This example nicely illustrates the different stereoselectivity of *cis*- and *trans*-DDP binding to DNA. The *cis* isomer forms exclusively an intrastrand d(GpG) crosslink, whereas the *trans* isomer makes a 1,3-d(A*pGpG*) adduct. A schematic depiction of the *trans*-$\{Pt(NH_3)_2\}^{2+}$ adduct is shown in Figure 9.23. As can be seen, the two purine rings enclose a large, 23-membered ring, the central guanosine residue is "bulged out," and the 5'-residue has a C3'-endo sugar pucker. This structure may be compared with that of *cis*-[$Pt(NH_3)_2${d(pGpG)}] (Figure 9.21), where the platinum is part of a smaller, 17-membered ring. Both space-filling model building studies and molecular mechanics calculations reveal that it would be stereochemically very unfavorable for the *trans*-$\{Pt(NH_3)_2\}^{2+}$ fragment to replace the *cis* analogue in an intrastrand crosslinked d(GpG) structure of the kind shown in Figure 9.21. Thus, for bidentate adducts, it seems clear that the important difference between *cis*- and *trans*-DDP binding to single-stranded DNA is revealed by the structures shown in Figures 9.21 and 9.23, respectively.

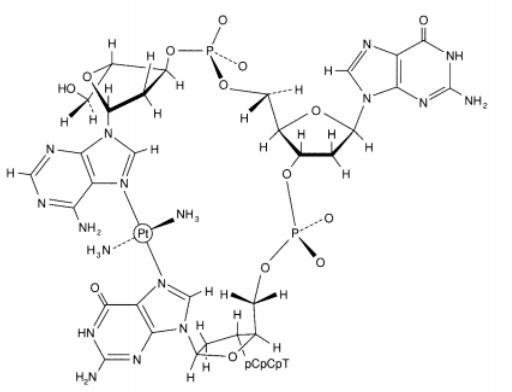


Figure 9.23 - Structure of the intrastrand 1,3-d(A*pGpG*) crosslink formed in the reaction of trans-DDP with d(ApGpGpCpCpT). Reproduced by permission from Reference 136.

Information about *trans*-DDP binding to double-stranded DNA is scanty, but very recent studies indicate that the *trans*-[$Pt(NH_3)_2${d(GpApG)-N7-G(1),N7-G(3)}] intrastrand crosslinked fragment can be embedded in duplex dodecamers.[139] Interestingly, for one sequence the melting temperature (T_M) of this duplex is not reduced over that of the unplatinated DNA fragment, in contrast to results for *cis*-DDP intrastrand d(GpG) adducts. This intriguing result, which agrees with earlier T_M studies of DNA platinated by *trans*-DDP, does not yet have a structural rationale. It is possibly relevant to the processing of bifunctional *trans*-DDP-DNA adducts *in vivo*.

f. Effects of Platination on DNA Structure

It is valuable to summarize at this stage all that has been learned concerning the changes in DNA structure that occur upon *cis*- or *trans*-DDP binding. *cis*-DDP intrastrand crosslinks result in unstacking of neighboring bases and a switch in the sugar pucker of the 5'-nucleoside from C2'-endo, the standard B-DNA conformation, to C3'-endo, a conformation encountered in A-DNA. These various forms of DNA have already been introduced in the previous chapter. Watson-Crick base pairing, although weakened, is probably maintained. Evidence that base pairing is altered comes from studies with antinucleoside antibodies that bind appreciably better to DNA platinated with *cis*-DDP than to unmodified DNA. These antibodies recognize the nucleobases much better in platinated than in unplatinated DNA, presumably because platination disrupts the double helix. Additional support for base-pair disruption comes from gradient gel-denaturation experiments using sitespecifically platinated DNA (see Section V.D.8.b). Intrastrand crosslinking by *cis*-DDP also bends the helix by about 34° and unwinds the duplex by 13°. When *trans*-DDP forms 1,3-intrastrand crosslinks, the nucleotides situated between the platinated residues may be bulged out; consistent with this picture is the fact that they present an especially good target for antinucleoside antibodies. In 1,3-intrastrand d(GpNpG) or d(ApNpG) adducts, the 5'-nucleoside sugar pucker is altered to C3 I-endo. Intrastrand crosslink formation by *trans*-DDP also leads to DNA bending, but the platinum serves as the locus for a hinge joint and not for cooperative bending. These different effects of platination on DNA structure brought about by the two isomers are likely to be related to their different biological activities.