dominated coordination spheres. These results may contribute to answer the question why nature uses in enzymes like nitrogenases or hydrogenases transition metals and sulfur ligands for the active centers in order to generate catalysts that render possible the most difficult reactions even under the mildest conditions.

Acknowledgment. We thank W. Preetz, Institut für Anorganische Chemie, Universität Kiel, for Raman measurements. These investigations were supported by the Deutsche Forschungsgemeinschaft, by the Fonds der Chemischen Industrie, and by a donation of $RuCl_3 \cdot xH_2O$ by Degussa AG, Hanau. We gratefully acknowledge this support.

Registry No. 1·THF, 132699-25-3; **2**, 127139-84-8; $2 \cdot CH_2Cl_2$, 138052-72-9; $2 \cdot CS_2$, 138052-73-0; **3**, 138052-71-8; $3 \cdot CS_2$, 138128-09-3; $[Ru(PPh_3)'S_4']_x$, 124401-90-7; $H_2'S_4'$, 83209-89-6; CS_2, 75-15-0.

Supplementary Material Available: Listings of isotropic thermal parameters, anisotropic displacement parameters, bond distances as well as bond angles of 1·THF, $2 \cdot CS_2$, and $3 \cdot CS_2$, and coordinates of hydrogen atoms of 1·THF and $2 \cdot CS_2$ (35 pages); listings of F_o and F_c values (24 pages). Ordering information is given on any current masthead page. Further details of the X-ray crystal structure analyses have been deposited and can be obtained from the Fachinformationszentrum Energie, Physik, Mathematik, D-7514 Eggenstein-Leopoldshafen 2 by citing the deposition numbers CSD 320193 ($[Ru(SH_2)(PPh_3)'S_4'] \cdot$THF (1·THF)), CSD 320250 ($[(\mu\text{-}S_2)\{Ru(PPh_3)'S_4'\}_2] \cdot CS_2$ ($2 \cdot CS_2$)), CSD 320249 ($[\{Ru(PPh_3)\}(\mu\text{-}'S_4'CS_2)\{Ru(PPh_3)'S_4'\}] \cdot CS_2$ ($3 \cdot CS_2$)), the authors, and the reference.

Conformation of Pt(dien)[d(ApGpA)-*N*7(2)] in the Solid State and in Aqueous Solution, As Determined with Single-Crystal X-ray Diffraction and High-Resolution NMR Spectroscopy in Solution

Gert Admiraal, Maarten Alink, Cornelis Altona, Fransje J. Dijt, Carla J. van Garderen, Rudolf A. G. de Graaff, and Jan Reedijk*

Contribution from the Department of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands. *Received June 26, 1991*

Abstract: The structure in the solid state and in solution of the adduct between $Pt(dien)^{2+}$ and the trinucleotide d(ApGpA), Pt(dien)[d(ApGpA)-*N*7(2)], has been studied with high-resolution NMR techniques and X-ray diffraction. This adduct is a model for the intermediate in the binding of the antitumor drug *cis*-$PtCl_2(NH_3)_2$, cisplatin, to DNA. In aqueous solution the intramolecular interactions between the guanine residue and the 3′ adenine are reduced upon platination, while the stacking between the 5′ adenine and guanine remains intact. Furthermore, binding of the monofunctional platinum compound to the N7 of guanine results in a change in the sugar conformation of all three residues. Instead of the usual 80–100% *S* conformation, all three Pt(dien)[d(ApGpA)-*N*7(2)] deoxyribose rings are in an almost 50% N/S equilibrium. The change of the sugar conformation causes a change in the DNA backbone; this change seems to be the reason for the more easy Pt binding at the 5′ side of the molecule. The conformational changes were found to be sequence dependent as seen from comparison with Pt(dien)[d(CpGpT)-*N*7(2)]. In that case the conformation of only the guanine residue is changed upon platinum binding. Crystallographic data of Pt(dien)[d(ApGpA)-*N*7(2)] are as follows: space group $P2_12_12_1$, $Z = 8$, $a = 20.094$ (5) Å, $b = 21.418$ (5) Å, $c = 29.631$ (6) Å, $V = 12752$ Å^3, resolution = 1.15 Å, $R_w = 0.087$ for 4855 reflections and 776 variables. Apart from two Pt(dien)[d(ApGpA)-*N*7(2)] molecules 18 well-ordered water molecules are present in the asymmetric unit. The two independent molecules which have a similar conformation are held together in the unit cell by stacking interactions and hydrogen bonding of the bases. Characteristic features are unusual G·G base pairs (using N3 and N2, N···N = 2.84–2.99 Å) and A·A base pairs (using N6 and N1 of A(1), i.e. the 5′ adenine, N···N distances are 2.80 and 3.04 Å; and also using N6 and N7 of A(3), N···N = 2.96–2.99 Å). These G·G and A·A base pairs are stacked in the *b* direction of the crystal. The backbone of each d(ApGpA) molecule has an extended conformation. The only intramolecular interactions are relatively weak H bridges between an amine ligand and a phosphate oxygen and between another amine ligand and a guanine O6 (N···O = 3.10–3.15 Å). Such an intramolecular interaction has been observed earlier in the crystal structure of *cis*-$Pt(NH_3)_2$[d(CpGpG-N7(2),N7(3)]. The intermolecular interactions (i.e. the crystal packing effects) are clearly stronger than the stacking forces within a molecule, resulting in different molecular structures and conformations in solution and in the crystal.

Introduction

It is well-known that the antitumor drug cisplatin (*cis*-$PtCl_2(NH_3)_2$, cDDP[1]) forms a didentate complex with DNA.[2-4] Studies on the enzymatic digestion of platinated salmon sperm DNA[2] show a perference of cDDP for two neighboring guanines (about 65% of the DNA-bound platinum), while binding occurs at the N7 position of the bases (a -GpG-N7,N7 chelate). A smaller amount (up to 20%) of an -ApG-N7,N7 chelate is formed. In contrast, the -GpA-N7,N7 chelate has not yet been found in DNA. Also two minor platinum-containing compounds were found, i.e. monofunctionally bound $Pt(NH_3)_3$dGMP (2%) and *cis*-$Pt(NH_3)_2(dGMP)_2$ (13%). This last adduct originates from both interstrand cross-links and enzymatically degraded d(Gp-{Np}$_n$G), $n \geq 1$, in which platinum is bound to two guanines with one or more nonbound bases in between. The monofunctional adduct at a guanine-N7 is thought to be an intermediate in the

(1) Abbreviations: cisplatin, cDDP, *cis*-$PtCl_2(NH_3)_2$; d(ApGpA), deoxyadenylyl(3′-5′)deoxyguanylyl(3′-5′)deoxyadenosine; dien, diethylenetriamine; NMR, nuclear magnetic resonance; pH*, uncorrected meter reading of solutions of D_2O; TMA, tetramethylammonium chloride; TMP, trimethylphosphate.

(2) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707–713.

(3) Inagaki, K.; Kidani, Y. *Inorg. Chim. Acta* **1985**, *106*, 187–191.

(4) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167–180.

0002-7863/92/1514-930$03.00/0 © 1992 American Chemical Society

formation of the bifunctional adducts.[3,5,6]

Many studies have been reported dealing with the binding mechanism of cDDP to DNA. The kinetics of the two-step reaction have been studied by several groups.[7-9] The first binding step at the N7 of a guanine appears to be slow followed by a very fast, almost simultaneous, chelation step to a guanine or adenine base. Experiments with the hydrolyzed forms of cDDP, i.e. *cis*-diamminediaquaplatinum(II), show that the chelation step favors the 5′ base.[10]

The structure of the d((p)GpG)-N7,N7 chelate has been extensively studied by NMR spectroscopy[10] and X-ray diffraction.[11,12] The most important change in the conformation of the dinucleotide as a result of platination is a reduction in the intramolecular interaction between the two guanines. Due to the square-planar coordination of the platinum atom, the two guanines become almost perpendicular to one another, resulting in a significant change in the sugar conformation of the 5′ residue.

To obtain more insight in the mechanism of action of cDDP, the monofunctional platinum adduct, the assumed intermediate in the bifunctional reaction of cDDP with DNA, is studied. In a previous paper the structure in solution of Pt(diene)[d(CpGpT)-*N*7(2)] (diene denotes diethylenetriamine) was described.[13] That monofunctional adduct was used as a model for the intermediate structure of the two-step binding reaction of cDDP. In that case the sugar conformation of the guanosine residue only is changed to 55–60% *S*, compared to 80% *S* conformer in the unmodified oligonucleotide.[13] This change causes a distortion of the DNA backbone, possibly facilitating the chelation step. However, in a d(GG) or d(AG) chelate only purine–purine interactions exist, while in the case of d(CpGpT) pyrimidine–purine and purine–pyrimidine interactions occur. Therefore, in this paper the structure of a trinucleotide with only purine–purine interactions, namely Pt(dien)[d(ApGpA)-*N*7(2)], is studied. A special feature of this molecule is its poor solubility under acid and neutral conditions, probably as a result of the purine–purine interactions, since this feature is not found in Pt(dien)[d(CpGpT)-*N*7(2)].[13]

The present paper describes a detailed study of the structure of Pt(dien)[d(ApGpA)-*N*7(2)] both in solution, as determined by using ^{1}H NMR and ^{31}P NMR spectroscopy, and in the crystalline state determined by single-crystal X-ray diffraction. The schematic structure of d(ApGpA) together with the numbering system is shown in Figure 1A.

Materials and Methods

Synthesis and Starting Materials. [PtCl(dien)]Cl was prepared according to Watt and Cude.[14] Purity was checked by infrared spectroscopy and elemental analysis (Microanalytical Laboratory, University College, Dublin, Ireland). The trinucleotide d(ApGpA) was synthesized via an improved phosphotriester method[15] and used as its disodium salt.

The reaction between [PtCl(dien)]Cl and d(ApGpA) (1:1, ca. 10^{-5} M) was carried out at pH 6 and 37 °C in the dark. The reaction was followed by UV spectroscopy and seemed to be complete after 3 days (estimated yield 60%). Separation of the reaction products was achieved by gel permeation chromatography (Sephadex G25, Pharmacia), using 0.02 M triethylammonium hydrogencarbonate (TEAB) as eluent. TEAB, a volatile salt, was removed from the products by repeated evaporation with a few drops of NH_4OH, whereafter a Chelex 100 cation-exchange resin (Bio-rad) was used to obtain the sodium salt.

NMR Spectroscopy. NMR samples (5.5 mM) were lyophilized three times from 99.7% D_2O (Merck) after pH* adjustment (pH* denotes the uncorrected meter reading of solutions in D_2O) and finally dissolved in 0.4 mL of 99.95% D_2O (Merck). A trace of tetramethylammonium chloride (TMA) was added to the samples as an internal reference (chemical shift relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate is 3.18 ppm at 25 °C). For NOE and T_1 measurements the samples were first carefully degassed.

^{1}H-NMR spectra at 300 and 600 MHz were recorded on a Bruker WM-300 on a Bruker AM-600 spectrometer, respectively. When necessary the residual HDO peak was suppressed by selective irradiation of the HDO resonance. Resolution enhancement was achieved according to Ernst by Gaussian multiplication.[16]

^{1}H-NMR spectra were recorded at several pH values between pH* 1.5 and 12. The pH* was adjusted with small quantities of 0.1 M NaOD or DCl solutions. A series of spectra was recorded over the temperature range 275–360 K at 5 day K intervals for two concentrations. Exact temperatures were determined from the chemical shift of the HDO peak relative to TMA as described earlier.[17]

The proton T_1 relaxation times were obtained by the inversion recovery method (180°–τ–90° sequences) at 20 °C. The delay between the sequences was 10 s.

^{31}P-NMR spectra were recorded at several temperatures on a Bruker WM-300 spectrometer, operating at 121.5 MHz (concentration 5 mM). Heteronuclear proton-noise decoupling was used throughout. Trimethylphosphate (TMP) was used as an internal reference. The ^{31}P resonances were assigned by selective decoupling of the two phosphorus signals at 295 K.

The 2D-relayed COSY spectrum[18a] was measured at 310 K, and a phase-sensitive 2D-NOE (mixing time 0.5 s) was performed at 280 K as previously described.[18b]

Crystal Growth. Crystals of Pt(dien)[d(ApGpA)-*N*7(2)] were grown from solutions of 10 mM Pt(dien)[d(ApGpA)-*N*7(2)] in H_2O at pH 8.0. After a few weeks needle-shaped crystals appeared (size 0.05 × 0.05 × 0.5 mm^3), which were found suitable for X-ray diffraction.

Crystallographic data of Pt(dien)[d(ApGpA)-*N*7(2)] are as follows: space group $P2_12_12_1$, $Z = 8$, $a = 20.094$ (5) Å, $b = 21.418$ (5) Å, $c = 29.631$ (6) Å, $V = 12752$ Å^3, resolution = 1.15 Å; apart from two Pt(diene)[d(ApGpA)-*N*7(2)] molecules 18 well-ordered water molecules are present in the asymmetric unit.

Data Collection. Intensities were measured on an Enraf-Nonius CAD4 using monochromated Mo Kα radiation at 20 °C: ω–2θ scan, scan range (0.52 + 1.04 tan θ)°; slit width (0.35 + 1.05 tan Θ)°; slit height 1.0°, maximum θ = 18°, resolution = 1.15 Å. Total number of independent reflections 4855, 2905 reflections having $I > 2\sigma(I)$. Reflections were corrected for absorption (Monte Carlo absorption correction method,[19a] μ = 20.3 cm^{-1}), decay (maximum decay = 10%), and Lorentz polarization and 4855 reflections were used in the refinement.

Structure Determination and Refinement. The positions of the two independent platinum atoms were obtained from a Patterson synthesis, after which a series of weighted $2F_o - F_c$ Fourier maps (weights were used of $2F_o - F_c$ from Main)[19b] resulted into the location of the remaining non-hydrogen atoms. In addition 27 water molecules were found. Because of disorder nine water oxygen atoms were refined with an occupancy of 0.5. Constrained least-squares refinement was carried out with bond distances put in at values derived from the literature.[19c] In the last refinement cycles also bond angles of sugars and bases had to be con-

(5) Eastman, A. *Biochemistry* **1982**, *22*, 3927–3933.
(6) Dijt, F. J.; Chottard, J.-C.; Girault, J.-P.; Reedijk, J. *Eur. J. Biochem.* **1989**, *179*, 333–344.
(7) van Hemelryck, B.; Girault, J.-P.; Chottard, G.; Valadon, P.; Laoui, A.; Chottard, J.-C. *Inorg. Chem.* **1987**, *26*, 787–795.
(8) Schaller, W.; Reisner, H.; Holler, E. *Biochemistry* **1987**, *26*, 943–950.
(9) van der Veer, J. L.; van der Elst, H.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Inorg. Chem.* **1986**, *25*, 4657–4663.
(10) (a) den Hartog, J. H. J.; Altona, C.; Chottard, J.-C.; Girault, J.-P.; Lallemand, J.-Y.; de Leeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucl. Acids Res.* **1982**, *10*, 4715–4730. (b) Herman, F.; Kozelka, J.; Stoven, V.; Guittet, E.; Girault, J.-P.; Huynh-Dinh, T.; Igolen, J.; Lallemand, J.-Y.; Chottard, J.-C. *Eur. J. Biochem.* **1990**, *194*, 119–133. (c) Laoui, A.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1988**, *27*, 2751–2753.
(11) (a) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368–7381. (b) Coll, M.; Sherman, S.; Gibson, D.; Lippard, S. J.; Wang, A. H. J. *J. Biomol. Struct. Dynam.* **1990**, *8*, 315.
(12) Admiraal, G.; van der Veer, J. L.; de Graaff, R. A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592–594.
(13) van Garderen, C. J.; Altona, C.; Reedijk, J. *Eur. J. Biochem.* **1988**, *178*, 115–121.
(14) Watt, G. W.; Cude, W. A. *Inorg. Chem.* **1968**, *7*, 335–338.
(15) van der Marel, G. A.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Nucl. Acids Res.* **1982**, *10*, 2337–2351.
(16) Ernst, R. R. In *Advances in Magnetic Resonance*; Waugh, J. S., Ed.; Academic Press: New York, 1966; Vol. 2., pp 1–135.
(17) Hartel, A. J.; Lankhorst, P. P.; Altona, C. *Eur. J. Biochem.* **1982**, *129*, 343–357. Note that the conversion factor *a* for the 0–52 °C temperature range should read *a* = 177.6.
(18) (a) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3731–3732. (b) van den Hoogen, Y. Th.; Erkelens, C.; de Vroom, E.; van der Marel, G. A.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1988**, *173*, 295–303.
(19) (a) Kinneging, A. J.; de Graaff, R. A. G. *J. Appl. Crystallogr.* **1984**, *17*, 364. (b) Main, P. *Acta Crystallogr.* **1979**, *A35*, 779–785. (c) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1985. (d) LeBreton, P. R.; Xu Yang; Shigeyuki, U.; Fetzer, S.; Min Yu; Leonard, N. J.; Kumar, S. *J. Am. Chem. Soc.* **1990**, *112*, 2138–45. (e) Tretyak, S. M.; Mitkevich, V. V.; Sukhodub, L. F.; Wierzchowski, K. L. *Acta Crystallogr., C* **1989**, *45*, 1961–3.

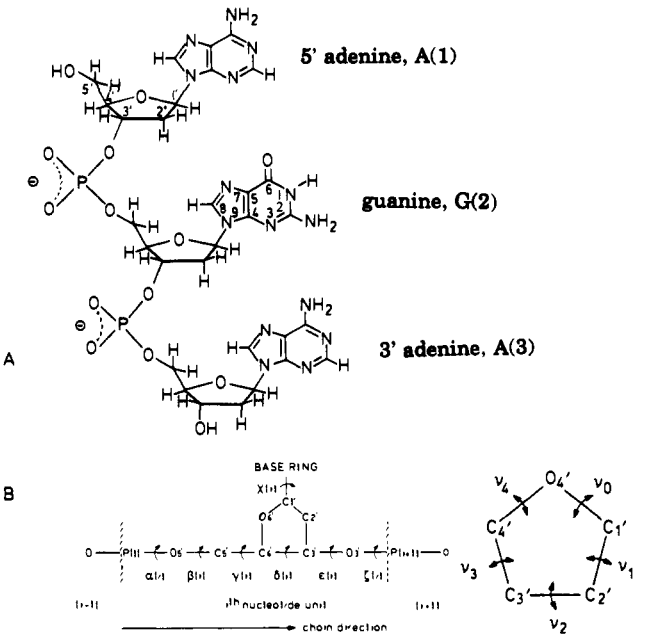


Figure 1. (A) Schematic structure of d(ApGpA), with the numbering of the purine and sugar atoms. (B) Conformational notation of the nucleic acid torsion angles. The angle χ is defined as C(4)–N(9)–C(1′)–O(4′) as given in ref 20.

Table I. pK_a Values of Non-Platinated Guanine and Adenine

	pK_a (N7)	pK_a (N1)
guanine	2.4	9.5
adenine	<0.0	3.8

strained as several ring bond angles deviated more than 15° from their usual values. All 4855 reflections were used in the refinement. Platinum, phosphorus, and water oxygen atoms were refined anisotropically, while the other atoms were refined with isotropic thermal parameters. The least-squares matrix consisted of 3 blocks, one block for each Pt molecule and one block for all water oxygen atoms. Final weighted R value was $R_w = 0.087$ (defined as $R_w = \{[(|F_o| - |F_c|)^2]/|F_o|^2\}^{1/2}$, with $w = [1/\sigma^2(F)]$).

Due to disorder in the 3′ sugar of molecule 1, reflected by the high thermal parameters of C3′ and O3′, it was not possible to refine C2′ with occupancy of 1.0. Therefore an occupancy of 0.5 for this atom was used, which gave coordinates in good agreement with electron density. Attempts to resolve the disorder did not succeed. The final difference Fourier map showed the largest peak near a Pt atom of only 1.09 e A^{-3}. The maximum positional shift/error was 0.161 for the last refinement cycle. Final atom positions are available as supplementary material, together with tables for F(obs) and F(calc).

Nomenclature. The common nomenclature for inorganic compounds is combined with that on nucleic acid constituents and abbreviations.[20] The purine and sugar atom numbering is indicated in Figure 1A. The nucleotide residues are numbered (1) to (3) starting at the 5′ terminus and the molecule number (only in the crystal structure) comes after the residue number, i.e. A(1,1) means the 5′ adenine of molecule 1, A(1,2) is the 5′ adenine of molecule 2, etc. Figure 1B shows the labeling of the backbone torsion angles (α to ξ) and the sugar ring torsion angles (ν_0 to ν_4) of a DNA fragment.

Results and Discussion

Identification and Proton Assignment of the Reaction Products in Solution. The monofunctional platinum compound [PtCl(dien)]Cl and the trinucleotide d(ApGpA) were allowed to react for 4 days at 37 °C. After evaporation the reaction products were separated by gel permeation chromatography, which resulted in two products. The first product was characterized by NMR spectroscopy as unreacted d(ApGpA). For the identification of the second product of pH dependence of the chemical shift of the five non-exchangeable base protons was monitored (Figure 2).

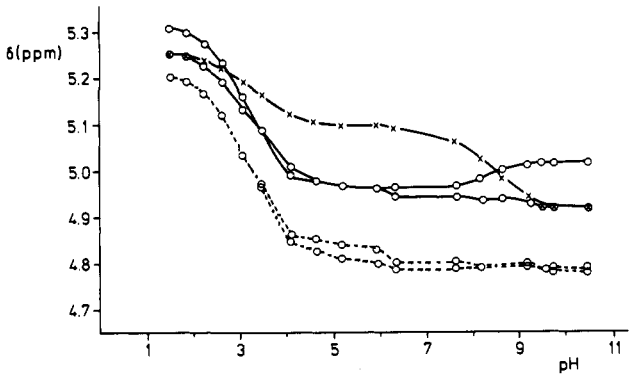


Figure 2. pH dependence of the chemical shift (δ) of the non-exchangeable base protons of Pt(dien)[d(ApGpA)-*N*7(2)]: (○) adenine protons; (×) guanine proton; (—) H8 resonances; (---) H2 resonances. Spectra were recorded at 293 K. Shift are reported relative to TMA.

A pH dependence study is a powerful tool to obtain information on the binding site of platinum.[21,22] Binding of platinum to the N7 of guanine or to the N7 or N1 of adenine leads to a change in the pK_a values (Table I).

Four signals in Figure 2 show the same curvature, with a pK_a of 3.8. These curves can only be assigned to the H8 (downfield signals) and H2 (upfield signals) of non-platinated adenine.[23] The remaining curve is characteristic for an N7-platinated guanine: an N1 protonation at pH 8.5[24-27] and no N7 protonation effect at pH 2.3.[23] Therefore the product was identified unambiguously as Pt(dien)[d(ApGpA)-*N*7(2)].

The sugar protons were assigned with the aid of a 2D-relayed COSY experiment. In this experiment, apart from two-band and three-band couplings, also four-band couplings can be observed. Figure 3 shows a part of the contour plot of the 2D-relayed COSY. The H5′(1), H5″(1), and H3′(3) protons could be easily assigned, because of their lacking a 5′-P, 5″-P, and 3′-P coupling, respectively.

In spite of the strong overlap in the H2′/2″ resonance area, the H1′ signals could be unambiguously assigned because of the four-band coupling between H1′ and H3′ protons. To distinguish between the H8 resonances of the two adenines, a 2D-NOE experiment was performed (Figure 4). A clear NOE between the most downfield H8 and the H2′(3) assigned those resonances to the H8 of the 3′-adenine. The smallest H8 resonance is correlated with the sugar ring protons of guanine. The guanine base proton exchanges with deuterium at the high pH value used (pH 9). This fast H/D exchange under basic conditions is characteristic of a N7-platinated guanine.[24,27] The high pH value was needed because of the poor solubility of the platinated compound under neutral and acid conditions. This interesting feature of Pt(dien)[d(ApGpA)-*N*7(2)] was applied for the crystallization.

Conformation around the Glycosidic Bond. Another aspect shown by the 2D-NOE spectrum is the conformation around the glycosidic bonds, the torsion angle χ, in the trinucleotide. From Figure 4 it is seen that the 3′ adenine residue is in an *anti* position; the NOE between H8(3) and H2′(3) is more intense than the NOE between H8(3) and H2″(3).[28] The NOE between H8(3)

(20) IUPAC-IUB Joint Commission on Biochemical Nomenclature. Recommendations *Eur. J. Biochem.* **1983**, *131*, 9–15.

(21) Girault, J.-P.; Chottard, J.-C.; Guittet, E. R.; Lallemand, J.-Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157–1163.

(22) Marcelis, A. T. M.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 2664–2665.

(23) Martin, R. B.; Mariam, Y. H. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1979; Vol. 8, pp 57–124.

(24) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *J. Am. Chem. Soc.* **1980**, *102*, 5565–5572.

(25) Marcelis, A. T. M.; Canters, G. W.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 391–392.

(26) Inagaki, K.; Kidani, Y. *J. Inorg. Biochem.* **1979**, *11*, 39–47.

(27) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352–1356.

(28) Scheek, R. M.; Russo, N.; Boelens, R.; Kaptein, R.; van Boom, J. H. *J. Am. Chem. Soc.* **1983**, *105*, 2914–2916.

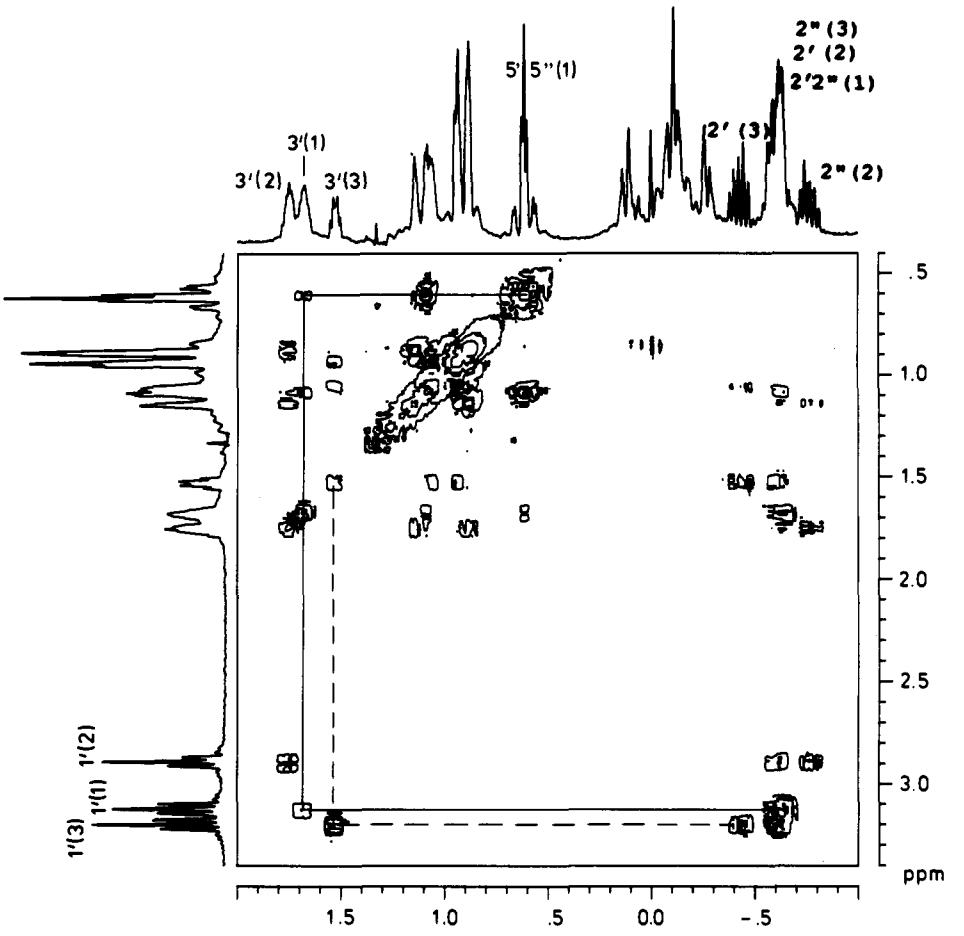


Figure 3. Part of the 300-MHz 2D-relayed COSY experiment of Pt(dien)[d(ApGpA)-*N*7(2)] at 330 K: (—) assignment of the 5′ residue, A(1); (---) assignment of the 3′ residue, A(3).

and H2″(2) demonstrates a normal B-DNA structure. There are no NOE's observed between the neighboring base protons, and the absence of an intraresidue NOE between 8(2) and H2′(2) is an indication of a change in the syn/anti equilibrium, or in the conformation around χ, of the guanine residue. Orbons et al.[29] collected inter- and intranucleotide proton–proton distances in pyrimidine–purine sequences in the A, B, and Z form and found that the guanine residue in the B form appears to be in an anti position, whereas in the Z form it occurs in a syn conformation. The rotation to the syn conformation results in a two times larger H8–H2′ distance for the guanines in Z DNA, which explains the absence of the H8–H2′ intraresidue NOE. A larger H8–H2′ distance is also expected for the high anti conformation. This conformation is reported for the guanine residue in both molecules of the crystal structure (vide infra). Therefore, most likely the guanine residue of the molecule in solution is in a high-anti conformation. Because of the isochronicity of the H2′ and H2″ protons of the 5′ adenine the conformation around the glycosidic bond of this residue cannot be obtained from the NOE spectrum. Therefore, a T_1 relaxation time experiment was executed and the results given in Table II.

The T_1 values of the base and H1′ protons of d(ApGpA) and Pt(dien)[d(ApGpA)-*N*7(2)] have been determined. The protons of the unbound trinucleotide are assigned in analogy with the results of Dijt et al.[6] on d(ApG) and d(pGpA). Because of the overlap of the two H2 resonances in the platinated fragment, an experiment at lower temperatures was performed. In this case the H2 resonances were separated and the T_1 values obtained were almost the same for both protons. The large T_1 values for the H2 protons indicate an anti conformation of both adenine residues.[30] The relaxation time of the H8 of guanine is increased on N7-platination, suggesting a change in the chemical environment of the guanine H8. This result is also found in *cis*-Pt-$(NH_3)_2$[d(ApG)-*N*7(1),*N*7(2)][6] and Pt(dien)[d(CpGpT)-*N*7(2)],[13] where there is a shift in the conformational equilibrium of the guanine residues toward the syn position. This implies, as does the NOE experiment, that platination of the guanine N7 of d(ApGpA) results in complete or partial rotation of the guanine around the glycosidic bond from an anti via high-anti toward a syn conformation.

Table II. Longitudinal Relaxation Times T_1 of Several Resonances of d(ApGpA) and Pt(dien)[d(ApGpA)-*N*7(2)] Determined at 293 K by a Nonselective Inversion–Recovery Experiment at 300 MHz[a]

	T_1, s	
proton	free d(ApGpA)	Pt(dien)[d(ApGpA)-*N*7(2)]
A(1)		
H8	1.36 (±0.02)	1.10 (±0.04)
H2	5.88 (±0.03)	3.86 (±0.02)[b]
H1′	0.84 (±0.01)	0.89 (±0.01)
G(2)		
H8	0.71 (±0.02)	0.91 (±0.05)
H1′	0.99 (±0.02)	0.86 (±0.04)
A(3)		
H8	1.08 (±0.02)	0.90 (±0.02)
H2	5.64 (±0.03)	3.86 (±0.02)[b]
H1′	1.28 (±0.01)	1.07 (±0.03)

[a] Values in parentheses are standard deviations. [b] The two H2 resonances are isochronous, and the apparent T_1 value is a mean value (see text).

(29) Orbons, L. P. M.; van der Marel, G. A.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1986**, *161*, 131–139.

(30) Tran-Dinh, S.; Neumann, J. M.; Huynh-Dinh, T.; Igolen, J.; Kan, S. K. *Org. Magn. Reson.* **1982**, *18*, 148–152.

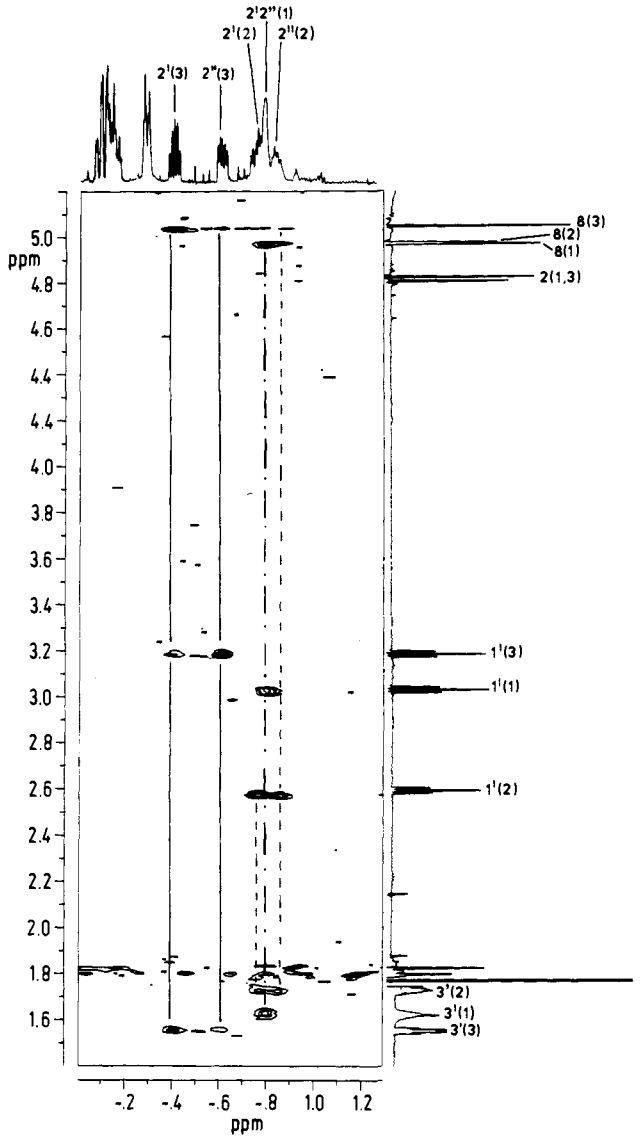


Figure 4. Part of a 600-MHz 2D-NOE spectrum of Pt(dien)[d(ApGpA)-*N*7(2)] at 280 K. The intraresidual crosspeaks are connected: (···) A(1); (---) G(2); (—) A(3). Assignment of the sugar protons was possible with the aid of the 2D-relayed COSY experiment shown in Figure 3.

Concentration and Temperature Effects on ^{1}H and ^{31}P Chemical Shifts. The intermolecular interactions of Pt(dien)[d(ApGpA)-*N*7(2)] are studied by determination of the concentration dependence of the ^{1}H chemical shifts. The chemical shifts appear to be independent of the concentration. In contrast with the strong intermolecular interactions in the crystal structure (see below), these interactions do not occur in solution.

The intramolecular interactions, often indicated as stacking interactions, are illustrated by the temperature dependence of the chemical shifts of the H1′ protons and phosphorus resonances, as shown in Figure 5. The chemical shift of the P(3) resonance, the phosphorus nucleus located between the guanine and 3′ adenine residue, appears to be virtually independent of the temperature (Figure 5A). This means that the G(2)–A(3) stacking interaction is reduced upon platination. The temperature dependence of P(2), the phosphorus located between A(1) and G(2), suggests normal melting, i.e. the intramolecular interaction between the 5′ adenine and the guanine does not change upon platinum binding. The same results, but less pronounced, are obtained from the temperature dependence of the chemical shift of the H1′(1) versus that of the H1′(3) proton (Figure 5B).

Comparison with the interactions within d(GpApGpA), as studied by Rinkel et al.,[31] shows that the 5′ stack is the same in

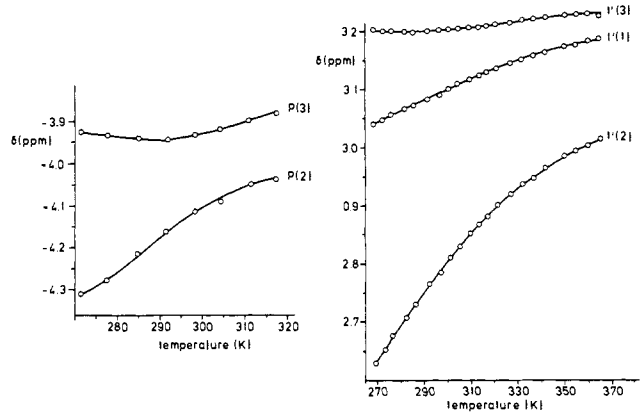


Figure 5. Chemical shift (δ) vs temperature profiles of (A, left) the phosphorus signals (δ reported relative to TMP) and (B, bottom) the H1′ protons (shifts relative to TMA) of Pt(dien)[d(ApGpA)-*N*7(2)].

the platinated trinucleotide as in d(GpApGpA), while the 3′ stacking interaction is decreased as a result of platination of the guanine-N7. These changes in the stacking interactions differ from those found in Pt(dien)[d(CpGpT)-*N*7(2)].[13] Platination of the guanine residue in d(CpGpT) results in reduction of the stacking of the 5′ side (the dC-dG stack), whereas the 3′ stack (dG-dT) remains intact.[13] This difference in behavior is probably related to the sequence of these DNA fragments. In d(CpGpT) a pyrimidine–purine and purine–pyrimidine interaction is present, while in d(ApGpA) only purine–purine interactions occur. Figure 5B also shows a large temperature dependence of the H1′ proton of guanine, just as in the N7 platinated d(CpGpT).[13] The chemical shift of this proton is a result of a combination of factors: intramolecular interactions from both sides, the 5′ and 3′ side; platination of the N7; and the conformation of the guanosine around the glycosidic bond and of its sugar ring. The temperature effect is different for all these factors, and (until now) the cause of this large temperature dependence, also found in related systems, e.g. the H1′(2) in *cis*-Pt(NH$_3$)$_2$[d(CpGpG)-*N*7(2),*N*7(3)],[32] remains to be explained.

Conformation of the Deoxyribose Rings of Pt(dien)[d(ApGpA)-*N*7(2)]. The sugar ring in DNA molecules occurs in a rapid pseudorotational equilibrium (on the NMR time scale) between S type (roughly C2′-endo, C3′-exo) and N type (roughly C2′-exo, C3′-endo) conformers.[33] This equilibrium can be characterized by the population of the S conformer (% S). In B-DNA fragments commonly 80–100% S is found, except for the sugar ring of the 3′ residue. This residue has more conformational freedom and occurs in a 60–70% S/40–30% N equilibrium.[33,34] This holds also for d(GpApGpA), in which the sugar conformation of the first three purine residues appeared to be 90–100% S, while the sugar ring of 3′ adenine is around 70% S.[31]

Simulation of the proton signals of Pt(dien)[d(ApGpA)-*N*7(2)] to get accurate coupling constants was not possible because of the isochronicity of the H2′ and H2″ protons of A(1) and a strong overlap of resonances in the H2′/2″ proton area (Figures 3 and 4). According to Rinkel et al.[34] the shape, the splitting pattern, and the distance (Hz) between the outer peaks ($\Sigma 1' = J_{1'2'} + J_{1'2''}$) of the H1′ resonances can be used to estimate the population of the S conformer. From investigations of these features a small preference of the S conformer (50–60% S) for all three of the sugar rings is predicted. In comparison with the unmodified tetranucleotide d(GpApGpA), the population of the S conformer is significantly reduced upon platinum binding.

(31) Rinkel, L. J.; van der Marel, G. A.; van Boom, J. H.; Altona, C. *Eur. J. Biochem.* **1987**, *166*, 87–101.
(32) den Hartog, J. H. J.; Altona, C.; van der Marel, G. A.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371–379.
(33) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413–133.
(34) Rinkel, L. J.; Altona, C. J. *Biomol. Struct. Dyns.* **1987**, *4*, 621–649.
(35) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205–11.

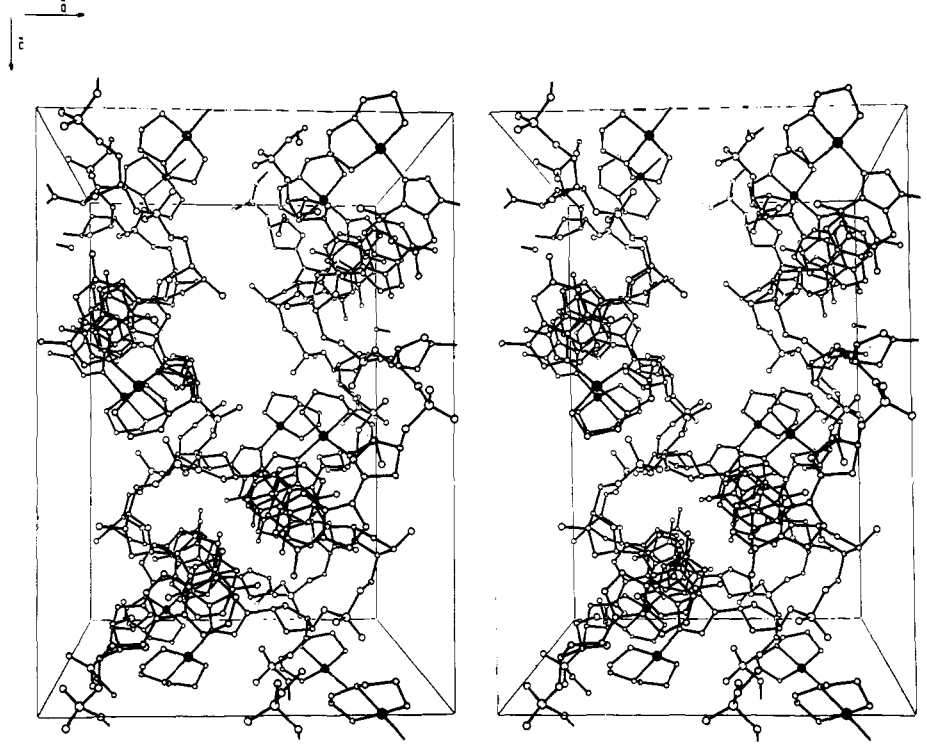


Figure 6. Stereoprojection along *b* of the unitcell contents of solid Pt(dien)[d(ApGpA)-*N*7(2)].

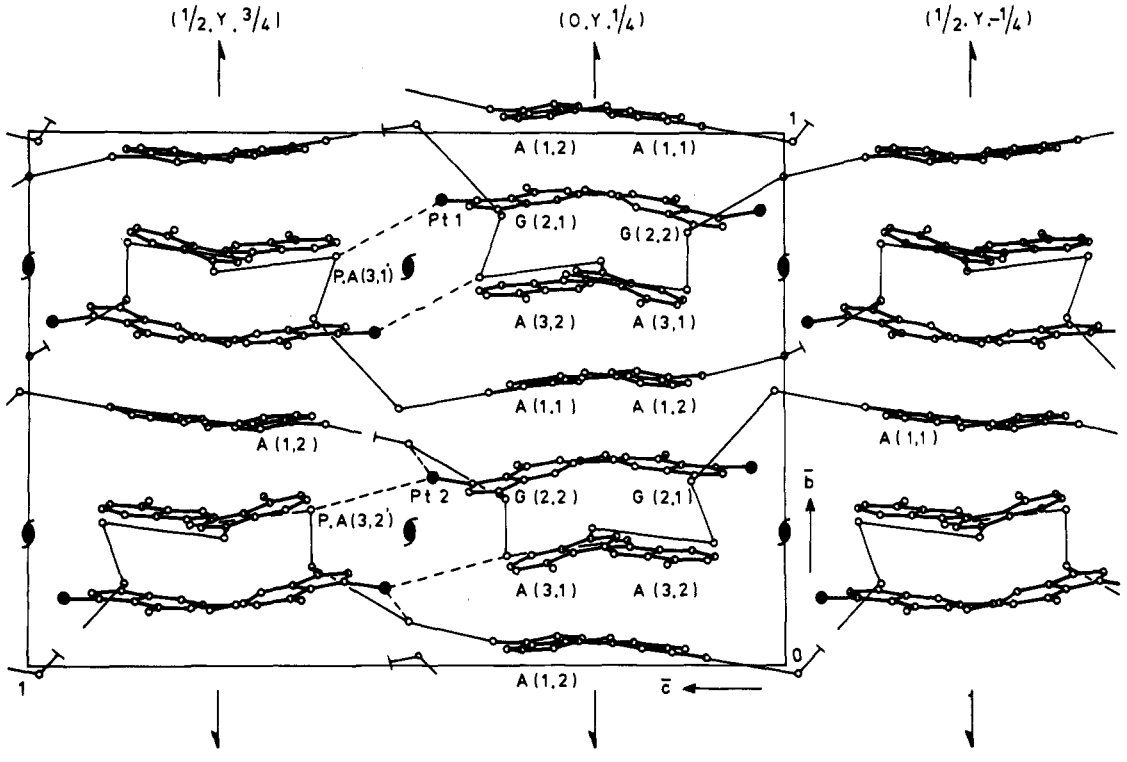


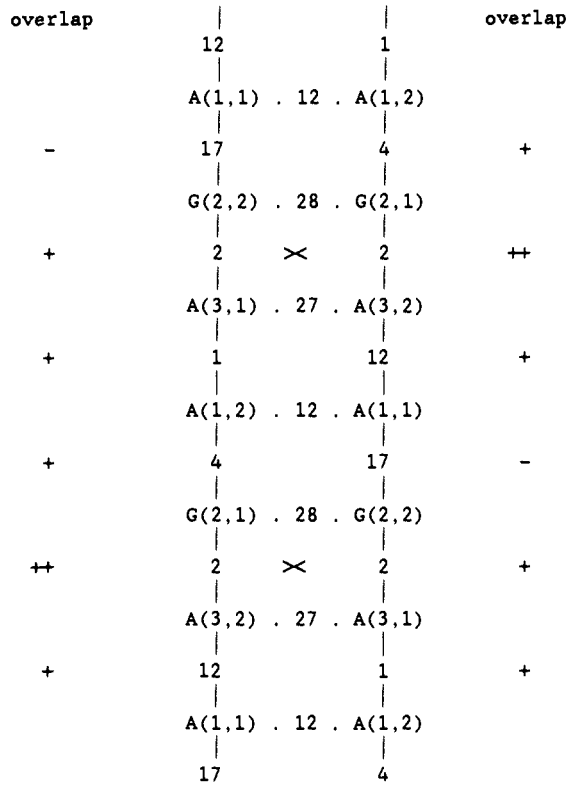
Figure 7. Stacking of bases along 2-fold screw axes $(0,y,{}^1/_4)$ and $({}^1/_2,y,{}^3/_4)$: (–O–) sugar phosphate backbone; (–O–|) sugar phosphate backbone connecting bases of stack at $(O,y,{}^1/_4)$ and bases of stack at $(-{}^1/_2,y,{}^3/_4)$. (---) phosphate dien–Pt H-bond interactions (dien omitted).

In d(CpGpT) only the sugar conformation of the guanine residue is changed upon GN7-platination with [PtCl(dien)]Cl.[13] In d(ApGpA) the conformations of all the sugar rings are changed. This is probably also a result of the different sequence of the two trinucleotides.

The Crystal Structure of Pt(dien)[d(ApGpA)-*N*7(2)]. Overall Packing Features. Figure 6 gives a stereoprojection of the structure along the *b* axis. The figure shows large channels along $(0,y,{}^3/_4)$ and along $({}^1/_2,y,{}^1/_4)$. A few ordered water molecules could be located inside these channels (omitted from Figur 6 for clarity), and for the rest electron density is low and water molecules are disordered. All bases are intermolecularly base-paired and stacked along $(0,y,{}^1/_4)$ or $({}^1/_2,y,{}^3/_4)$ (see Figure 6); a scheme of stacking is given in Figure 7. Angles and overlap between bases are given in Table III; H-bonding distances between G·G and A·A base pairs are given in Table IIIB. These hydrogen bonds are of type I (N1–N6 and N6–N1 for A(1) pairs), type II (N7–N6 and N6–N7 for A(3) pairs), and type IV (N2–N3 and N3–N2 for G(2) pairs),

Table III. Angles between Base Planes and H-Bonding Distances

A. Angles (deg) between Base Planes:
Stack Along $(0,y,^1/_4)$ in Solid Pt(dien)[d(ApGpA)-*N*7(2)][a]



B. H-Bonding Distances (Å) in Base Pairs between Bases in Solid Pt(dien)[d(ApGpA)-*N*7(2)][b]

	donor atom (D)	acceptor atom (A)	
Pairing of bases	A(1,1)..A(1,2)		D...A
	N6 (D)	N1 (A)	3.04(2)
	N1 (A)	N6 (D)	2.80(2)
Pairing of bases	G(2,1)..G(2,2)		
	N2 (D)	N3 (A)	2.84(2)
	N3 (A)	N2 (D)	2.99(2)
Pairing of bases	A(3,1)..A(3,2)		
	N6 (D)	N7 (A)	2.99(2)
	N7 (A)	N6 (D)	2.96(2)

[a] ++ = very significant overlap; + = good overlap; − = small overlap. [b] Schematic drawings are given in Figure 9B.

as classified by Saenger.[19c] The N···N contact distances vary from 2.80 to 3.04 Å for the several pairs. Similar H-bond contacts have been observed for GG[19d] and AA[19e] pairs, although not with metals coordinated to the bases.

The Pt(dien) part does not disturb the stacking interactions, which is probably due to the fact that the Pt(dien) part points away from the base stack. A schematic view concerning the packing of the Pt(dien) part is given in Figure 8, which shows interactions on Pt(dien) with phosphate groups and with 5′ sugars. The two independent molecules, molecule 1 and molecule 2, have base pairing and base stacking interactions between the guanine bases and 3′-adenine bases. Because of these interactions and additional H bonding these molecules can be considered to form a dimer (detailed H bonding distances are given in the supplementary tables for the interactions between molecules 1 and 2).

Molecular Geometry. Figure 9A shows a projection of molecules 1 and 2. The molecules are in a rather extended conformation and intramolecular base stacking is not observed. The conformations appear to be highly determined by intermolecular forces, since there are only a few intramolecular interactions, i.e. one H bond for molecule 1 and two H bonds for molecule 2. Most contacts are intermolecularly as illustrated in Figure 9B. Apart

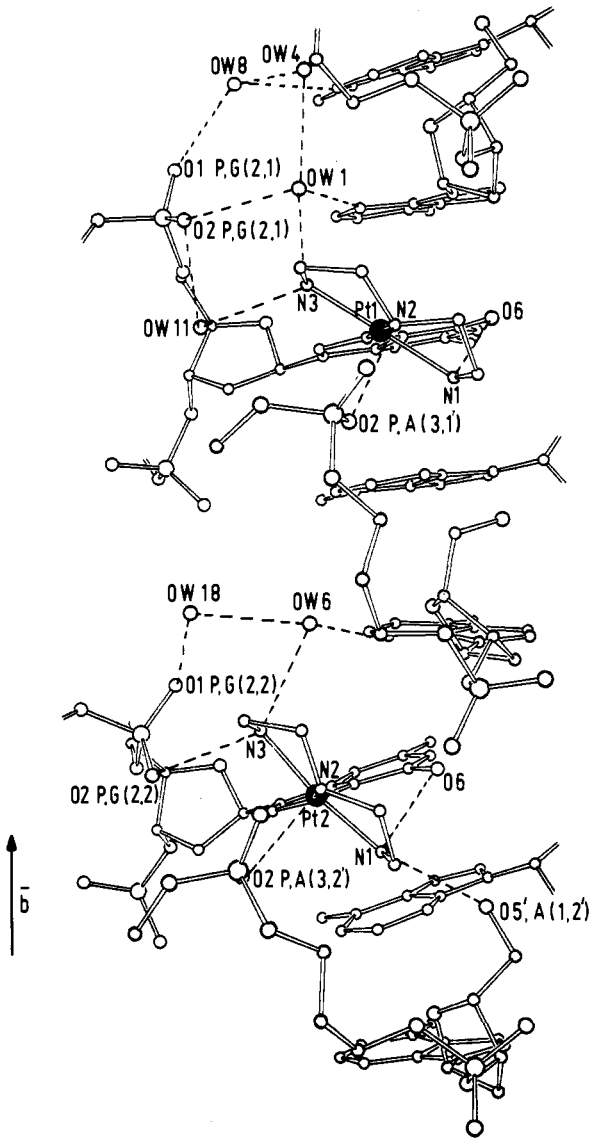


Figure 8. Orientation of the Pt(dien) moieties relative to the guanine bases, showing hydrogen bonds to the phosphate and water oxygens.

from these direct H bonds, the conformations are also stabilized by H bonds via water molecules in the lattice. Supplementary tables show intramolecular H bonds and H bonds to water molecules. The H bonds via water concern O2–P3···W's···N3 of A(1) and O2–P2···W···N3 of the Pt(dien) part.

Although the distances could not be determined accurately (see supplementary material for detailed information), the Pt–N distances were all found to be between 2.05 and 2.07 Å, usual for such contacts.[11,12]

From backbone torsion angles (Table IV; for the assignment of the labeling see Figure 1B) it is clear that the backbone conformation, especially for molecule 1, agrees very well with that of B-DNA. The sugar rings, for which torsion angles are given in Table V, have an S-type conformation, except for the sugar ring of the 3′ residue of molecule 1, which has an N-type conformation.

The orientation of the base relative to the deoxyribose ring is given by the glycosyl torsion angle χ. The 3′-adenine bases in molecules 1 and 2 are both in an anti position. The 5′ adenine of molecule 1 is in a syn position, while the χ of the 5′ adenine of molecule 2 is near −90°; falling within the "high-anti" range. The guanine bases in molecules 1 and 2 are also in a "high-anti" position. Relative to the χ of guanine in a B-DNA conformation, the guanine bases in molecules 1 and 2 are rotated toward a syn conformation. Except for the χ of the 5′ adenine of molecule 1, the conformation around the glycosidic bonds is in good agreement

Table IV. Torsion Angles of the Backbone of Pt(dien)[d(ApGpA)-*N*7(2)] and of B-DNA in the Solid State[a]

molecule		α	β	γ	δ	ε	ζ	χ
1	A	–	–	62	132	−107	67	−60 (syn)
	G	81	169	59	141	−174	156	−80 (syn/high-anti)
	A	−51	146	41	89	–	–	−166 (anti)
2	A	–	–	179	150	−84	106	−84 (syn/high-anti)
	G	70	139	50	154	−144	157	−84 (syn/high-anti)
	A	−64	168	52	143	–	–	−130 (anti)
B-DNA		−63	171	54	123	−169	−108	−117 (anti)
fiber-DNA		−41	136	38	139	−133	−157	−102 (ref 19c, p 266)

[a] See Figure 1B for the definitions of α–χ.

Table V. Torsion Angles of the Sugar Rings in Solid Pt(dien)[d(ApGpA)-*N*7(2)][a]

molecule		ν_0	ν_1	ν_2	ν_3	ν_4	P[b]	Φ_m
1	A	−24	30	−24	11	8	200	30
	G	−30	39	−34	17	8	151	40
	A	5	−24	34	−31	17	10	35
2	A	1	18	−29	30	−20	200	31
	G	−15	32	−36	28	−8	174	38
	A	−14	28	−29	22	−5	171	31

[a] Torsion angles ν_0–ν_4 and the pseudorotational parameters P and Φ are defined according to the conventions of refs 20 and 35. [b] Theoretical: C2′ endo (S type) P = 162°, C3′ endo (N type) P = 18°.

with the results of the T_1 and NOE experiments of the compound in solution (see above).

The dihedral angle of the plane formed by Pt and the four coordinated N atoms (PtN_4) and the guanine base plane is 42.3 (7)° for molecule 1 and 64.6 (7)° for molecule 2. Intra- and intermolecular interactions of N1 and N3 which are H-bond donors, together with packing interactions, will contribute to the observed dihedral angles (Figure 8). The Pt(dien) part shows significant interactions with the 5′ phosphate. In molecule 2 a weak H bond between the ammine ligand N3 and O2–P exists (Table VI). Stronger interactions between ammine N3 and 5′ phosphate are H bonds in which water molecules are involved. In molecule 1 the interactions are N3···OW1···O2—P and N3···OW11···O2—P, while in molecule 2 the interaction is N3···OW6···OW18···O1—P. Furthermore, there is a H bond between the ammine ligand N1 and O6,G(2) in molecule 1 (see Table VI). It appears that the dihedral angle Guanine/PtN_4 is highly determined by the 5′P···N3 ammine interaction and the O6,G(2)···N1 H-bond interaction (see Figure 8).

Conclusion

Platination of d(ApGpA) with a monodentate platinum compound simulates the first binding step of cisplatin to DNA. Investigations of the temperature dependence of the ^1H and ^{31}P chemical shift of Pt(dien)[d(ApGpA)-*N*7(2)] in aqueous solution shows the purine–purine interaction at the 5′ side of d(ApGpA) remaining intact, while the stacking of the 3′ side is reduced. The conformation of the sugar ring shifts to a 50–60% S/50–40% N equilibrium. These changes make it easier to chelate with the adenine residue at the 5′ side of the guanine than that at its 3′ side. This is in good agreement with the known observation that

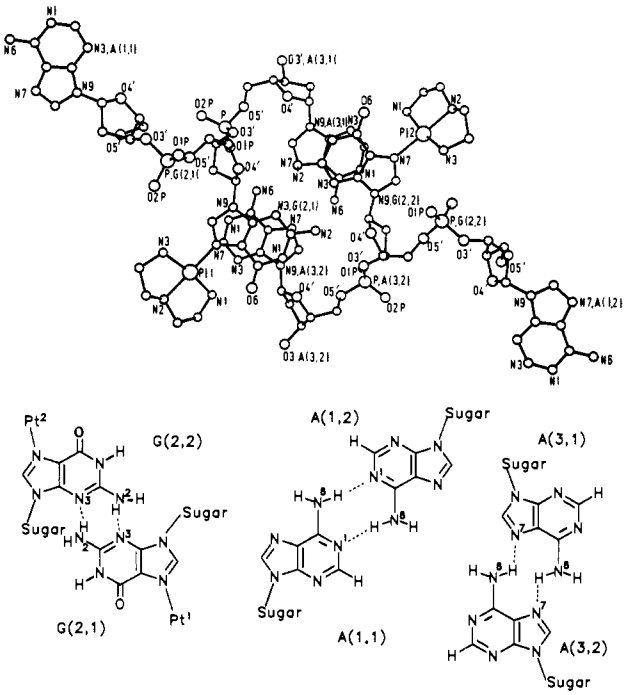


Figure 9. (A, top) Projection of the two crystallographically independent molecules (along *b*) of Pt(dien)[d(ApGpA)-*N*7(2)] showing the intermolecular AG and GA stacking pattern and (B, bottom) schematic drawing of the G:G and A:A base pairings as tabulated in Table IIIB.

AG adducts can be formed in DNA, but GA adducts have not been found.[2,5]

In Pt(dien)[d(CpGpT)-*N*7(2)] only the sugar conformation of the guanosine residue was found to change upon platination with a decrease in the 5′ side stacking interaction.[13] The difference in conformation between the trinucleotides Pt(dien)[d(ApGpA)-*N*7(2)] and Pt(dien)[d(CpGpT)-*N*7(2)] seems to be related to the nature of the bases. In d(CpGpT) a pyrimidine–purine and a purine–pyrimidine stacking interaction occurs, while in d(ApGpA) only purine–purine interactions exist.

The NOE and T_1 experiments indicate for the guanine a shift in the syn/anti equilibrium toward the syn conformation. As reported before, this syn preference is only found in small DNA fragments and does not seem to be a prerequisite for the antitumor activity of cDDP.[13]

Table VI. Interactions of d(ApGpA) with the Pt(dien) Part in Solid Pt(dien)[d(ApGpA)-*N*7(2)]

molecule	interaction	distance, Å *a*	*b*	*c*
	with the rest of the molecule			
1	N1·[a]O6,G(2)	2.84 (4)		
	N3···O2P,G(2)	4.00 (3)		
2	N1···O6,G(2)	3.11 (3)		
	N3···O2P,G(2)	3.16 (4)		
	with the 5′ P via water molecules			
1	N3·[a]OW1·[b]O2P,G(2)	2.95 (4)	2.89 (4)	
	N3···OW11···O2P,G(2)	2.88 (5)	2.91 (7)	
2	N3·[a]OW6·[b]OW18·[c]O1P,G(2)	2.87 (5)	2.86 (6)	2.61 (1)

The solid-state structure of Pt(dien)[d(ApGpA)-*N*7(2)] differs from the solution structure for the nonplatinated part, as one would expect for such a compound where intermolecular packing, stacking, and hydrogen bonds are likely to predominant in the solid.

Comparison between the solid-state structure and the solution structure reveals first of all that the solid-state structure is mainly determined by intermolecular interactions, with hardly any intramolecular interactions present. In the solution structure, on the other hand, intramolecular effects are more important.

Pt(dien)[d(ApGpA)-*N*7(2)] simulates the structure of the intermediate in the chelate formation of cDDP to DNA. The changes found in this adduct probably direct the DNA backbone, for instance by hydrogen bonding of the platinum amino groups to the phosphates and bases, to a better position for the chelation step.

Acknowledgment. Mr. C. Erkelens and Mr. J. Joordens are acknowledged for their skilfull technical assistance at the 300- and 600-MHz facility, respectively, Mr. H. van der Elst for the synthesis of the trinucleotide, and Mrs. Dr. J. M. L. Pieters for stimulating discussions. The authors thank the Netherlands Organization for the Advancement of Research (NWO) for several travel grants to visit the EMBL station in Heidelberg. We are indepted to Dr. G. Bosshard (EMBL, Heidelberg) for assistance with the graphics system. The authors acknowledge EEC-support (Grant No. ST2J-0462-C) allowing regular scientific exchange with the group of Prof. Dr. J.-C. Chottard. Johnson and Matthey Ltd. (Reading, UK) is thanked for their generous loan of K_2PtCl_4.

Supplementary Material Available: Listing of atomic fractional coordinates and isotropic thermal parameters and tables with intermolecular hydrogen bond information (11 pages); listing of *F*(obs) and *F*(calc) for the title compound (18 pages). Ordering information is given on any current masthead page.

Use of Excited-State and Ground-State Redox Properties of Polyoxometalates for Selective Transformation of Unactivated Carbon–Hydrogen Centers Remote from the Functional Group in Ketones

Lucille A. Combs-Walker and Craig L. Hill*

Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia 30322.
Received September 11, 1989. Revised Manuscript Received September 23, 1991

Abstract: Two types of processes are described which involve the selective transformation of unactivated carbon–hydrogen bonds in a ketone, *cis*-2-decalone, *cis*-**1**, which possesses conventionally far more reactive bonds. The first type of process involves irradiation of decatungstate, $W_{10}O_{32}^{4-}$ in the presence of *cis*-**1** producing, *trans*-2-decalone, *trans*-**1**, the product resulting from epimerization of an unactivated tertiary C–H bond remote from the carbonyl group, in high selectivity at high conversion of substrate, eq 1. The second type of reaction involves irradiation of the heteropolytungstate, α-$P_2W_{18}O_{62}^{6-}$ or α-$PW_{12}O_{40}^{3-}$, in the presence of *cis*-**1** producing two monounsaturated ketones (octalones) in high selectivity with the nonthermodynamic isomer, **2**, in comparable or greater quantity than the conventional thermodynamic (conjugated) isomer, **3**, eq 2. Both types of processes are independent of wavelength over the principal range of absorption of the complexes (250–380 nm). The reactions are first order in $W_{10}O_{32}^{4-}$ under optically dilute conditions. The primary kinetic isotope of the corresponding decalin hydrocarbons evaluated under the same reaction conditions as eq 1, $k_{cis\text{-decalin-H}_{18}}/k_{cis\text{-decalin-D}_{18}} \sim 2$, and the solvent kinetic isotope effect, $k_{CH_3CN}/k_{CD_3CN} = 1.0$. The photochemical reaction of decatungstate with $\alpha,\alpha,\alpha',\alpha'$-$D_4$-*cis*-**1** leads exclusively, even at moderate conversion of substrate (25%), to $\alpha,\alpha,\alpha',\alpha'$-$D_4$-*trans*-**1**. These data, an isotope crossover experiment in which decatungstate was irradiated in the presence of a 50/50 molar mixture of deuterated and protiated *cis*-decalin in CD_3CN, reactions of *cis*-**1** and *cis*-decalin with an authentic localized ground-state radical, *t*-BuO·, and a number of other experiments are consistent with initial H atom abstraction in all cases. The dramatically different products seen with the different polyoxometalate systems are dictated by the relative rates of epimerization, oxidation, and escape of the cisoid tertiary bridgehead radicals in the initial radical cage and, to a lesser extent, by the rates of conventional radical–radical reactions and other processes.

Introduction

Some classes of early transition-metal–oxygen anion clusters, or polyoxometalates, exhibit a unique combination of physical and chemical properties that make them attractive for probing a number of problems either long standing in chemistry or associated with emerging technological issues.[1-9] The systematic exploitation of the extensive redox properties of the ground and/or excited states of some polyoxometalates has already lead to new processes

(1) Reviews on early transition-metal polyoxometalates: General reviews: (a) Pope, M. T. *Heteropoly and Isopoly Oxometalates*; Springer Verlag: New York, 1983. (b) Day, V. W.; Klemperer, W. G. *Science* **1985**, *228*, 533. (c) Pope, M. T.; Müller, A. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 34. Polyoxometalate nomenclature: (d) Jeannin, Y.; Fournier, M. *Pure Appl. Chem.* **1987**, *59*, 1529. Polyoxometalates in catalysis: (e) Misono, M. *Catal. Rev.-Sci. Eng.* **1987**, *29*, 269. (f) Kozhevnikov, I. V.; Matveev, K. I. *Russ. Chem. Rev. (Engl. Transl.)* **1982**, *51*, 1075. (g) Kozhevnikov, I. V.; Matveev, K. I. *Appl. Catal.* **1983**, *5*, 135. (h) Hill, C. L. In *Metal Catalysis in Hydrogen Peroxide Oxidations*; Strukul, G., Ed.; Reidel: 1991, Chapter 8, in press.

(2) (a) Renneke, R. F.; Hill, C. L. *J. Am. Chem. Soc.* **1988**, *110*, 5461. (b) Shul'pin, G. B.; Kats, M. M. *Bull. Acad. Sci. USSR, Div. Chem. Sci.* **1989**, *38*, 2202.

(3) Radical versus carbonium generation in photochemical alkane functionalization by polyoxotungstates: Renneke, R. F.; Pasquali, M.; Hill, C. L. *J. Am. Chem. Soc.* **1990**, *112*, 6585.

(4) Role of polyoxometalate protonation on ground-state and excited-state redox processes in alkane functionalization: Renneke, R. F.; Kadkhodayan, M.; Pasquali, M.; Hill, C. L. *J. Am. Chem. Soc.* **1991**, *113*, 8357.

(5) Catalytic carbon–carbon bond formation from unactivated carbon–hydrogen bonds: Prosser-McCartha, C. M.; Hill, C. L. *J. Am. Chem. Soc.* **1990**, *112*, 3671.

(6) (a) Heller, A. and co-workers, patents pending. (b) Okamoto, H.; Iwayanagi, T.; Mochiji, K.; Umezaki, H.; Kudo, T. *Appl. Phys. Lett.* **1986**, *49*, 298. (c) Kudo, T.; Ishikawa, A.; Okamoto, H.; Miyauchi, K.; Murai, F.; Mochiji, K.; Umezaki, H. *J. Electrochem. Soc.* **1987**, *134*, 2607. (d) Okamoto, H.; Ishikawa, A.; Kudo, T. *J. Photochem. Photobiol.* **1989**, *49*, 377. (e) Yoshimura, T.; Ishikawa, A.; Okamoto, H.; Miyazaki, H.; Sawada, A.; Tanimoto, T.; Okazaki, S. *Microelec. Eng.* **1991**, *13*, 97.

(7) Thermal reductive dehalogenation of CBr_4 by reduced heteropolytungstates: Eberson, L.; Ekstrom, M. *Acta Chem. Scand.* **1988**, *B42*, 101.

0002-7863/92/1514-938$03.00/0 © 1992 American Chemical Society