New Antitumor Platinum Compounds Linked to Amino Phosphonic Acids Which Lose the Phosphonate and Tertiary Amine Ligand upon Binding to Nucleic Acids

M. J. Bloemink,† J. P. Dorenbos,† R. J. Heetebrij,† B. K. Keppler,‡ J. Reedijk,*,† and H. Zahn‡

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands, and Anorganisch-Chemisches Institut der Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany

Received August 5, 1993®

The interaction of the antitumor active platinum phosphonato complexes [*cis*-$Pt(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] (ntmp = nitrilotris(methylenephosphonic acid), dach = diaminocyclohexane) with (oligo)nucleotides has been investigated using ^1H NMR and ^{31}P NMR spectroscopy. For both complexes the formation of GN7,GN7 chelates is observed, together with the release of ntmp. First, the platinum–phosphonate bond is broken, probably by direct attack of the first G-base. The coordination of the second base is accompanied by breakage of the bond between the Pt(II) ion and the tertiary amine ligand, a very unusual observation, as N-donor ligands generally act as nonleaving groups in platinum antitumor chemistry. For this type of platinum antitumor complexes of which the strucural formula seems to violate the classical structure–activity relationships, both pH and nonleaving amine group do influence the rate of the reaction with (oligo)nucleotides significantly.

Introduction

cis-Diamminedichloroplatinum (cisplatin) is one of the most successful agents in cancer chemotherapy with activity against several solid tumors, such as testicular, ovarian, and bladder carcinomas.[1] Its therapeutic activity is thought to originate from its interaction with DNA, resulting in inhibition of DNA synthesis.[2] The main reaction product, both *in vivo* and *in vitro*, involves loss of two chloride ions and coordination of platinum to the N7 atoms of adjacent guanine bases on the same strand.[3] This d(GpG) intrastrand cross-link is uniquely formed by cisplatin, since the inactive trans isomer does not link adjacent guanine bases but, instead, forms intrastrand adducts having one or more intervening nucleotides.[4] A limitation of cisplatin in its use as an antitumor drug is its concentration-dependent nephrotoxicity,[5] besides a variety of other side effects.[6]

Numerous cisplatin analogues have been synthesized and tested as second generation antitumor drugs to obtain Pt complexes with reduced toxicity and similar or improved antitumor activity. Carboplatin, (diammine(1,1-cyclobutanedicarboxylato)platinum(II)), is a successful analog of cisplatin with clinical application because of its reduced nephrotoxicity.[7] Ultimately, carboplatin and cisplatin form the same adducts with DNA, although carboplatin is less reactive than cisplatin. Since carboplatin has a higher degree of myelotoxicity,[8] the search for new platinum complexes that possess improved therapeutic properties is continuing.

A rational approach to the development of cytostatics involves the use of carrier functions that cause a specific accumulation of the respective compounds in the target organs or in the target cells. Hormone-linked platinum complexes have been synthesized and were found to be active in experimental models.[9] DNA-targeted platinum drugs, *i.e.* platinum moieties linked to an intercalator, such as acridinecarboxamide, have shown significant activity against a P388/CP cisplatin-resistant line.[10] Complexes in which platinum(II) moieties are covalently tethered to ferrocene, a carrier which proved to navigate the drug almost entirely to the liver and the spleen, have been described by Rosenfeld *et al.*[11]

Recently, a class of platinum complexes containing phosphonic acid ligands has been reported by Klenner *et al.*,[12] and two examples, *i.e.* [*cis*-$Pt(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)], are redrawn in Figure 1, together with cisplatin and carboplatin. The rational approach to this type of compounds is based on the targeting of a cytotoxic moiety with a phosphonate function suitable to absorb onto bone surfaces, since bis(phosphonates) show a high affinity for bone and other calcified tissues.[13] The therapeutic activity of platinum phosphonate complexes *in vivo* involves both reduction of the bone tumor volume and antimetastatic activity.[14] In particular [*cis*-$Pt(NH_3)_2$(ntmp)] has been found as effective as cisplatin in osteosarcoma, but superior

† Leiden University.
‡ Universität Heidelberg.
* Abstract published in *Advance ACS Abstracts,* February 15, 1994.

(1) Loehrer, P. J.; Einhorn, L. H. *Ann. Intern. Med.* **1984**, *100*, 704.
(2) (a) Reedijk, J.; Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; van de Putte, P. *Struct. Bonding (Berlin)* **1987**, *67*, 53. (b) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293. (c) Reedijk, J. *Inorg. Chim. Acta* **1992**, *198–200*, 873.
(3) (a) 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. (b) Fichtinger-Schepman, A. M. J.; van Oosterom, A. T.; Lohman, P. H. M.; Berends, F. *Cancer Res.* **1987**, *47*, 3000.
(4) (a) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616. (b) van der Veer, J. L.; Ligtvoet, G. J.; van den Elst, H.; Reedijk, J. *J. Am. Chem. Soc.* **1986**, *108*, 3860.
(5) Daugaard, G.; Abildgaard, U. *Cancer Chemother. Pharmacol.* **1989**, *25*, 1.
(6) Von Hoff, D. D.; Schilsky, R.; Reichert, C. M.; Reddick, R. L.; Rozencweig, M.; Young, R. C.; Muggia, F. M. *Cancer Treat. Rep.* **1979**, *63*, 1527.
(7) van der Vijgh, W. J. F. *Clin. Pharmacokinet.* **1991**, *21*, 242 and references therein.
(8) Curt, G. A.; Grygiel, J. J.; Corden, B. J.; Ozols, R. F.; Weiss, R. B.; *et al. Cancer Res.* **1983**, *43*, 4470.
(9) Bednarski, P. J.; Gust, R.; Spruss, T.; Knebel, N.; Otto, A.; Farbel, M.; Koop, R.; Holler, E.; von Angerer, E.; Schönenberger, H. *Cancer Treat. Rev.* **1990**, *17*, 221.
(10) Lee, H. H.; Palmer, B. D.; Baguley, B. C.; Chin, M.; Mc Faclyer, W. D.; Wickham, G.; Thorsbourne-Palmer, D.; Wakelin, L. P. G.; Denny, W. A. *J. Med. Chem.* **1992**, *35*, 2983.
(11) Rosenfeld, A.; Blum, J.; Gibson, D.; Ramu, A. *Inorg. Chim. Acta* **1992**, *201*, 219.
(12) Klenner, T.; Valenzuela-Paz, P.; Amelung, F.; Münch, H.; Zahn, H.; Keppler, B.K.; Blum, H. In *Metal complexes in Cancer Chemotherapy*; Keppler, B.K., Ed.; VCH: Weinheim, Germany, 1993; pp 85–127.
(13) Wingen, F.; Schmähl, D. *Drug Res.* **1985**, *35*, 1565.
(14) (a) Klenner, T.; Valenzuele-Paz, P.; Keppler, B. K.; Angres, G.; Scherf, H. R.; Wingen, F.; Schmähl, D. *Cancer Treat. Rev.* **1990**, *17*, 253. (b) Klenner, T.; Wingen, F.; Keppler, B. K.; Krempien, B.; Schmähl, D. *J. Cancer Res. Clin. Oncol.* **1990**, *116*, 341.

0020-1669/94/1333-1127$04.50/0 © 1994 American Chemical Society

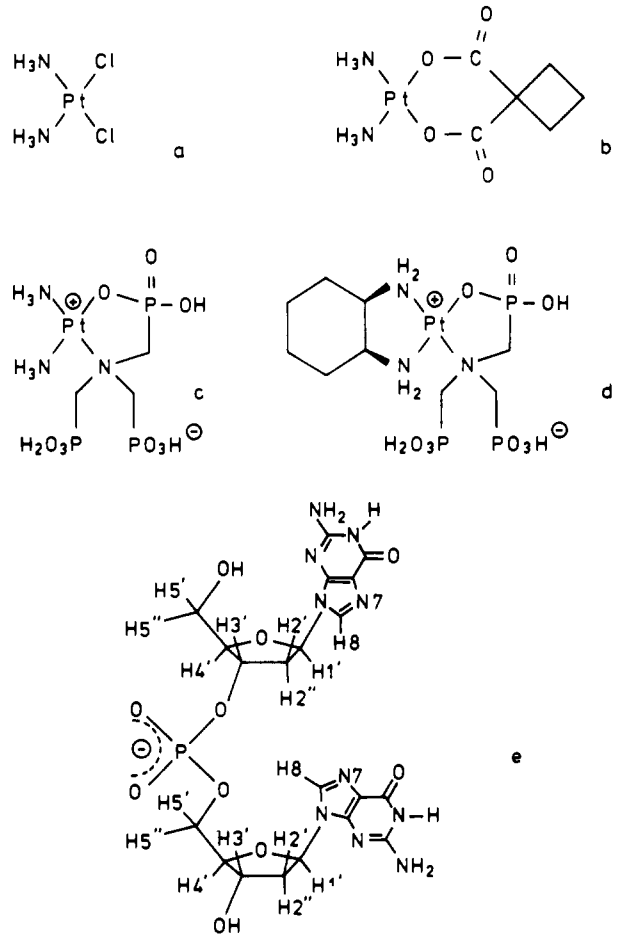

Figure 1. Structure of cisplatin (a), carboplatin (b), and the platinum phosphonate complexes [*cis*-Pt(NH_3)$_2$(ntmp)] (c) and [Pt(*R,S*-dach)(ntmp)] (d) together with a schematic representation of d(GpG) (e).

in terms of lower toxicity.[15] The platinum compounds show marked activity in a transplantable osteosarcoma, which was initially induced by the periostal administration of $^{144}CeCl_3$ to Sprague Dawley rats and later transplanted intratibially.[16] This model is relatively resistant to other chemotherapeutic agents. The compound [*cis*-Pt(NH_3)$_2$(ntmp)] effected a standstill of tumor growth during the period of therapy and a marked prolongation of the survival time.

The principal objective of the work reported here has been to investigate the mechanism of action of this type of platinum compounds, since its molecular basis is still unknown. However, because the platinum phosphonato complexes are derivatives of cisplatin, their interaction with DNA is the likely origin of their antitumor activity (*vide supra*). A biochemical study of Fruhauf *et al.*[17] has shown that the complexes [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] are capable of forming DNA cross-links in ovarian tumor cells. In this study the interaction of two platinum phosphonato complexes, *i.e.* [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)], with (oligo)nucleotides is investigated using ^{1}H NMR and ^{31}P NMR spectroscopy and will provide more insight into the fundamental inorganic chemistry underlying the antitumor activity of these platinum phosphonate complexes.

Experimental Section

Starting Materials. 5′GMP was commercially available (Sigma) and used as its sodium salt. d(GpG) was synthesized by an improved phosphotriester method[18] and used as its sodium salt. All platinum phosphonate complexes were synthesized by reaction of nitrilotris(methylenephosphonic acid) with [*cis*-Pt(NH_3)$_2$(OH_2)$_2$]$^{2+}$ or [Pt(*R,S*-dach)(OH_2)$_2$]$^{2+}$. Purity was checked by elemental analysis, infrared spectroscopy, and ^{1}H NMR and ^{31}P NMR spectroscopy.[12,19]

Reaction Conditions. The platinum complex (*i.e.* [*cis*-Pt(NH_3)$_2$(ntmp)] or [Pt(*R,S*-dach)(ntmp)]) was dissolved in deionized water at a concentration of 10^{-5} M. Either 1 equiv of (oligo)nucleotide (in case of 5′GMP or d(GpG)), or 2 equiv of mononucleotide (in the case of 5′GMP) was added to the solution, and the mixture was kept in the dark at 310 K for 7 days. Initially, the reaction was performed at pH 6.8 (near physiologically pH); however, due to the slow kinetics observed at this pH value, it was decided to study the reaction also at pH 4.8 (see results). After completion of the reaction the solvent was removed by rotary evaporation, the reaction products were dissolved in 0.5 mL of D_2O (99.8%, Merck) and the samples were lyophilized twice. In addition, all reactions were also carried out in the NMR tube (5 mM) in D_2O or in phosphate buffer (pH 4.8 or 6.8) at 310 or 323 K and followed by ^{1}H and ^{31}P NMR spectroscopy. In order to assign the products unambiguously, the reaction was also performed with cisplatin and [Pt(*R,S*-dach)Cl_2] under the same conditions.

Instrumentation. NMR Spectroscopy. Spectra were recorded on a Bruker WM 300 spectrometer equipped with a variable-temperature unit. The lyophilized products were dissolved in D_2O (99.95%, Merck). ^{1}H NMR data were collected at 297 K with TMA (tetramethylammonium nitrate, 3.18 ppm downfield from TMS) used as an internal reference.[20] ^{31}P NMR spectra were obtained at 297 K and referenced to H_3PO_4 (85%). The pH dependence of the chemical shift of several nuclei (H8, phosphate) was monitored by adding trace amounts of DCl and NaOD (0,1 and 1.0 M). The pH has not been corrected for deuterium isotope effects.[21] The relative amounts of product have been determined by integration of the H8 signals. Since H8 protons are susceptible to exchange with deuterium,[22] these are usually not suitable to use for integration. For the Pt(*R,S*-dach)–GMP adducts, fortunately, the nonexchangeable H_1' signals were nonoverlapping and could be compared to the H8 integrals; they showed an experimental variation of less than 5%, justifying the use of H8 integrals under these reaction conditions.

Results and Discussion

Identification of the Reaction Products. [*cis*-Pt(NH_3)$_2$(ntmp)]. After reaction of [*cis*-Pt(NH_3)$_2$(ntmp)] with d(GpG) in water, at both pH 4.8 and 6.8, the ^{1}H NMR spectrum shows two H8 signals, 0.3–0.6 ppm downfield compared to that of free d(GpG). The pH dependence of the base proton chemical shifts showed no N7 protonation effect around pH 2–3, whereas a clear N1 deprotonation effect is observed around pH 8.5 (data not shown). In addition, a large doublet has appeared at 0.60 ppm which can be assigned to the ligand nitrilotris(methylenephosphonic acid).[23] The ^{31}P NMR spectra show a peak at 7.8 ppm which can also be assigned to the free phosphonate ligand and a singlet at 0.17 ppm which corresponds to reacted d(GpG). This is 0.37 ppm downfield of d(GpG), a characteristic feature of Pt–d(GpG) chelates.[24] All these aspects show that the end product is the chelate d(GpG)–*cis*-Pt(NH_3)$_2$ and free nitrilotris(methylenephosphonic acid).

In reaction of [*cis*-Pt(NH_3)$_2$(ntmp)] with either 1 or 2 equiv of 5′GMP the same end product is observed for both reactions; however, in the case of the reaction with 1 equiv of 5′GMP, some unreacted [*cis*-Pt(NH_3)$_2$(ntmp)] remains present. Both ^{1}H and ^{31}P NMR spectra show the presence of nitrilotris(methylenephosphonic acid) (*vide supra*). The pH dependence of the H8

(15) Klenner, T.; Keppler, B. K.; Amelung, F.; Schmähl, D. *J. Cancer Res. Clin. Oncol.* **1989**, *115*, S54.
(16) Keppler, B. K.; Berger, M. R.; Klenner, T.; Heim, M. E. *Adv. Drug Res.* **1990**, *19*, 243.
(17) Frühauf, S.; Zeller, W. J. *Cancer Res.* **1991**, *51*, 2943.
(18) van der Marel, G. A.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Nucl. Acids Res.* **1982**, *10*, 2337.
(19) Keppler, M. K.; Münch, H.; Zahn, H. Manuscript in preparation.
(20) Hartel, A. J.; Lankhorst, P. P.; Altona, C. *Eur. J. Biochem.* **1982**, *139*, 343.
(21) Martin, R. B. *Science* **1963**, *139*, 1198.
(22) Girault, J.-P.; Chottard, J.-C.; Guittet, E. R.; Lallemand, J. Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Comm.* **1982**, *109*, 1157.
(23) Appleton, T. G.; Hall, J. R.; Mc Mahon, I. J. *Inorg. Chem.* **1986**, *25*, 726.
(24) 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.

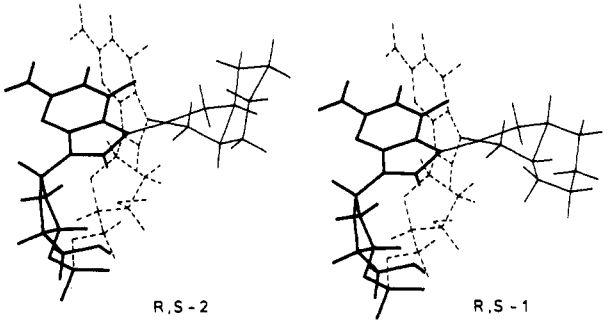

Figure 2. Structures of the Pt(*R,S*-dach){d(GpG)} adducts **RS1** and **RS2**.

proton shows no N7 protonation around pH 2–3 whereas a downfield shift is observed at pH 6.2, due to 5′-phosphate deprotonation, and an upfield shift at pH 8.5 due to N1 deprotonation (*vide infra*). All these results indicate the formation of *cis*-Pt$(NH_3)_2$(GMP-N7)$_2$, as already described by Dijt *et al.*[25] for the reaction of cisplatin with 5′GMP. In order to assign the product unambiguously, [*cis*-Pt$(NH_3)_2Cl_2$] was reacted with 2 equiv of 5′GMP and this reference product was added to the reaction product, proving both end products to be identical.

[Pt(*R,S*-dach)(ntmp)]. The reaction of the chiral platinum complex [Pt(*R,S*-dach)(ntmp)] with d(GpG) results in the formation of two N7,N7-adducts which prove to be the stereoisomers called RS1 and RS2 (see Figure 2). These were also reported by Kidani and co-workers starting from [Pt(*R,S*-dach)-Cl_2].[26] Also free ntmp is observed.

The pH dependence of the H8 chemical shifts of these stereoisomers is depicted in Figure 3A. As is obvious from Figure 3A, the two stereoisomers show no N7 protonation effect around pH 2–3 whereas a clear N1 deprotonation is observed around pH 8.5. After reaction of [Pt(*R,S*-dach)(ntmp)] with either 1 or 2 equiv of 5′GMP, only one platinated product is formed together with free nitrilotris(methylenephosphonic acid). The pH dependence of the H8 proton chemical shift of this endproduct, compared to 5′GMP, is depicted in Figure 3B showing no N7 (de)protonation effect around pH 2.3 whereas two successive titrations can still be observed. The first one (pK_a 6.2) corresponds to the titration of the 5′-phosphate group whereas the second one (pK_a 8.6) is due to the N1 deprotonation. Relative integration of the H8 protons to the H's of the cyclohexane ring shows that the ratio Pt:GMP is 1:2. As a reference Pt(*R,S*-dach)Cl_2 has been reacted with 2 equiv of 5′GMP, resulting in Pt(*R,S*-dach)-(GMP-N7)$_2$. The pH dependence of this reference complex proved to be identical to the reaction product of [Pt(*R,S*-dach)-(ntmp)] with 5′GMP; *i.e.*, the reaction of [Pt(*R,S*-dach)(ntmp)] with 5′GMP results in the complex Pt(*R,S*-dach)(GMP-N7)$_2$ together with free nitrilotris(methylenephosphonic acid).

An interesting feature is the equivalence of the two H8 protons of the adduct Pt(*R,S*-dach)(GMP-N7)$_2$ in acidic media whereas after deprotonation of the 5′-phosphate group the two H8 protons can be observed separately (see Figure 3B). Since the platinum complex is not symmetric (the two amino groups are bond to the cyclohexane ring with axial and equatorial orientations) only two H8 signals would be expected for Pt(*R,S*-dach)(GMP-N7)$_2$ if rotation around the Pt–N7 bond is fast. One H8 is from the G base at the same side of the *R*-carbon atom of the cyclohexane amine ring and the other H8 is oriented at the same side as the *S*-carbon atom, as has also been observed for Pt(R,S-dach(dGMP-N7)$_2$ at pH 6.[26] For Pt(*R,S*-dach)(dGuo-N7)$_2$ only one H8 signal

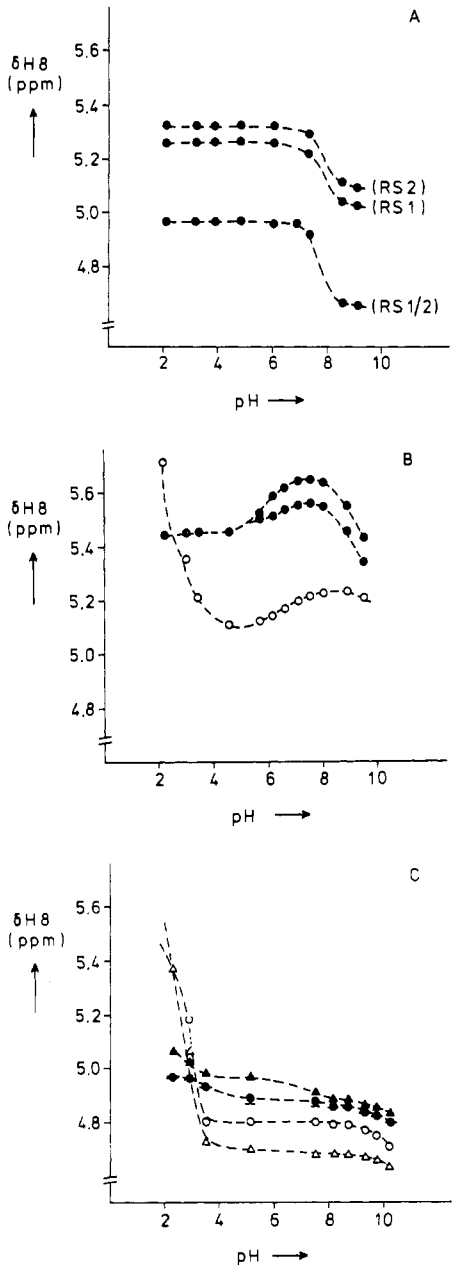

Figure 3. pH versus chemical shift profile of the H8 signals of various Pt(*R,S*-dach) adducts. (A) **RS1** and **RS2**; (B) Pt(*R,S*-dach)(GMP-N7)$_2$; (C) the reaction intermediates Pt(*R,S*-dach)(ntmp,*N*)(d(*G*pG) and Pt-(*R,S*-dach)(ntmp,*N*)(dGp*G*).

has been observed,[27] and it was concluded that, in addition to fast rotation around Pt–N7, the conformational equilibrium $\lambda \rightleftarrows \delta$ of the cyclohexane ring is fast; as a result the nonequivalence of the two guanine moieties is not reflected in the shift of H8. However, if the 5′-phosphate group of Pt(*R,S*-dach)(GMP-N7)$_2$ or Pt(*R,S*-dach)(dGMP,N7)$_2$ is deprotonated, a stabilizing H-bond from the phosphate group toward the NH-protons of the cyclohexane amine ring might retard this $\lambda \rightleftarrows \delta$ interconversion or might slow down the rotation around the Pt–N7 bond, thereby making the two G-bases chemically less equivalent. The H_1' signals can be observed separately both at acidic and basic conditions ($^3J(H_1')$ = 5.10 and 5.04 Hz).

Mechanism of Adduct Formation. To investigate the mechanism of action of platinum phosphonato complexes, [*cis*-Pt-$(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] were reacted with d(GpG) or 5′GMP and followed in time by ^{1}H NMR and ^{31}P

(25) Dijt, F. J.; Canters, G. W.; den Hartog, J. H. J.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644.

(26) (a) Inagaki, K.; Kidani, Y. *Inorg. Chem.* **1986**, *25*, 1. (b) Inagaki, K.; Nakahara, H.; Alink, M.; Kidani, Y. *Inorg. Chem.* **1990**, *29*, 4496.

(27) Gullotti, M.; Pacchioni, G.; Pasini, A.; Ugo, R. *Inorg. Chem.* **1982**, *21*, 2006.

Table 1. $t_{1/2}$ values (h) for the Reaction of a Variety of (Oligo)nucleotides with [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] in Phosphate Buffer

			$t_{1/2}$			
compound	T(K)	pH	d(GpG)	5'GMP	3'GMP	d(Guo)
[*cis*-Pt(NH_3)$_2$(ntmp)]	310	4.8	1.2	1.95		
	323	4.8	0.33	1.05	3.10	7.50
	310	6.8	17.8			
	323	6.8		33.2		
[Pt(*R,S*-dach)(ntmp)]	310	4.8	4.3			
	323	4.8		6.4	31.6	
	310	6.8	76.8			
	323	6.8		83.3		

NMR using various reaction conditions (see also Table 1). The reactions of d(GpG) and 5'GMP with [*cis*-Pt(NH_3)$_2$(ntmp)] and the formation of the products are depicted in Figure 4.

Reactions with d(GpG). The overall $t_{1/2}$ value (the time at which 50% of the starting material d(GpG) has reacted) is about 1.2 h (see Figure 4B). During this reaction two intermediate products are formed which react further resulting in *cis*-Pt(NH_3)$_2$-{d(GpG),N7(1),N7(2)} and free nitrilotrismethylenephosphonic acid. The reaction of [Pt(*R,S*-dach)(ntmp)] with d(GpG) at pH 4.8 is slower (overall $t_{1/2}$ value for d(GpG) is 4.25 h), but shows a similar profile compared to [*cis*-Pt(NH_3)$_2$(ntmp)]; *i.e.*, during the reaction two intermediate products are formed which react further resulting in the adducts RS1 and RS2 and free nitrilotris-(methylenephosphonic acid). Since the intermediate products of [Pt(*R,S*-dach)(ntmp)] with d(GpG) are more stable, compared to [*cis*-Pt(NH_3)$_2$(ntmp)], they could be more easily investigated by NMR spectroscopy. The pH dependence of the H8 protons of the intermediate products is depicted in Figure 3C. Compared with unreacted d(GpG), two H8 signals are shifted downfield whereas the shift of two H8 signals is hardly influenced by platination. The two more downfield signals show a small pH dependent effect around pH 4, due to the (de)protonation of the coordinated phosphonate ligand and can therefore be assigned to the platinated G bases whereas for the unplatinated G bases (the more upfield H8 signals) still (de)protonation of the N7-position can be observed around pH 2–3. The ^{31}P NMR data do not show a signal around 22 ppm, known to be characteristic for nitrilotris-(methylenephosphonic acid) coordinated to platinum through the oxygen.[23] So both the curvature of the intermediate products, showing phosphate deprotonation around pH 4, and the absence of a ^{31}P NMR peak at 22 ppm are evidence for N-coordination of the ntmp ligand of the intermediate products. All these observations strongly suggest the two intermediates being Pt(*R,S*-dach)(ntmp-*N*)(d(GpG),N7(1)) and Pt(*R,S*-dach)(ntmp-*N*)-(d(GpG),N7(2)). The phosphonic acid group must act as the first leaving group in reaction of [Pt(*R,S*-dach)(ntmp)] and [*cis*-Pt(NH_3)$_2$(ntmp)] with (oligo)nucleotides.

Since [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] are very stable in solution (after 24 h at 310 K no hydrolized species could be detected when the complex was dissolved in water; hardly any hydrolyzed species could be detected when the complex was dissolved in phosphate buffer), the first G base might directly attack the platinum complex. However, hydrolysis cannot be excluded if less than 5% of hydrolyzed [*cis*-Pt(NH_3)$_2$(ntmp)] would be sufficient to start the reaction. The coordination of the second G base results in the breakage of the bond between the Pt(II) ion and the tertiairy amine ligand; this is, as far as we know, a unique observation since N-donor ligands usually act as nonleaving groups in platinum antitumor chemistry.[1,2] So [*cis*-Pt(NH_3)$_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] are able to form the bifunctional chelated adduct with d(GpG). Another class of platinum antitumor active agents containing three N-donor ligands, *i.e.* [*cis*-Pt(NH_3)$_2$(N-het)Cl]$^+$, can also coordinate monofunctionally to guanine bases, but subsequent chelation does not occur.[28] The observed lability of the platinum nitrogen bond in platinum amino phosphonate complexes is probably due to the increase of steric crowding on the nitrogen; in addition the loss of the 5-membered chelate ring of ntmp, after the first G base has coordinated, might contribute to the instability.

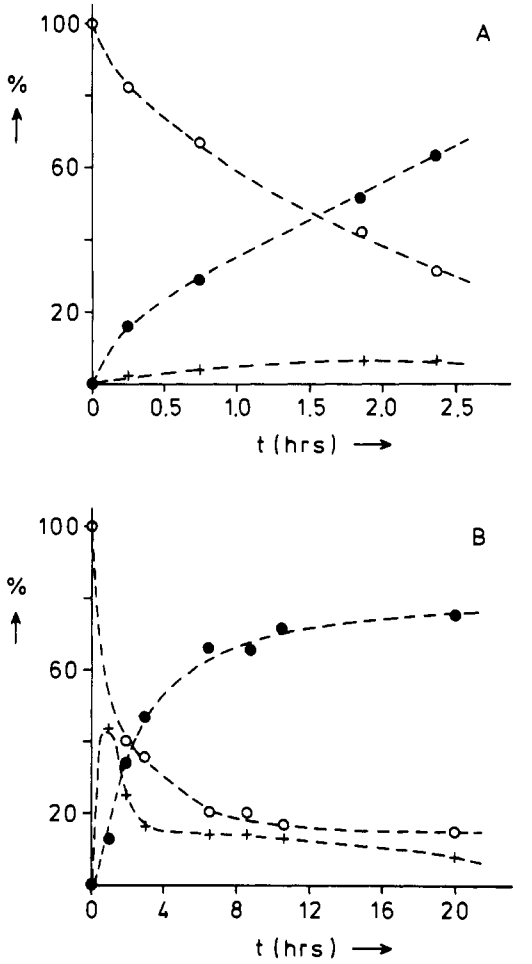

Figure 4. Formation of the products between *cis*-Pt(NH_3)$_2$(ntmp)] and (A) 5'GMP (5 mM, 323 K) or (B) d(GpG) (5 mM, 310 K) as a function of time (pH 4.8). The indicated values denote relative amounts (%) determined by intergration of the H8 signals. Keys: (○) starting material; (+) intermediate product; (●) end product.

Reactions with 5'GMP. Compared with d(GpG), 5'GMP shows a different reaction profile (see Figure 4A). During the reaction hardly any intermediate product can be detected. Figure 5 shows the ^{1}H and ^{31}P NMR spectra taken during the reaction of 5'GMP with [*cis*-Pt(NH_3)$_2$(ntmp)]. A small amount of intermediate product can be detected by ^{1}H NMR (see arrow) but the maximum never exceeds 6%.

The overall $t_{1/2}$value of 5'GMP indicates that it reacts slower compared to d(GpG). A possible reaction mechanism for the reaction of 5'GMP with [*cis*-Pt(NH_3)$_2$(ntmp)] could be that the attack of the first 5'GMP labilizes the bond between platinum and the tertiairy amine, resulting in a highly reactive intermediate. The second 5'GMP apparently preferentially attacks this intermediate rather than reacting with another [*cis*-Pt(NH_3)$_2$(ntmp)] complex, resulting in the formation of *cis*-Pt(NH_3)$_2$(GMP-N7)$_2$. This hypothesis agrees with the observation that even a substoichiometric amount of 5'GMP (0.5 equiv compared to 1 equiv of [*cis*-Pt(NH_3)$_2$(ntmp)]) results in the formation of *cis*-Pt(NH_3)$_2$-(GMP-N7)$_2$. It was thought that the 5'-phosphate group of

(28) (a) Lempers, E. L. M.; Bloemink, M. J.; Brouwer, J.; Kidani, Y.; Reedijk, J. *J. Inorg. Biochem.* **1990**, *40*, 23. (b) Hollis, L. S.; Sundquist, W. I.; Burnstyn, J. N.; Heiger-Bernays, E. W.; Lippard, S.J. *Cancer Res.* **1991**, *51*, 1866.

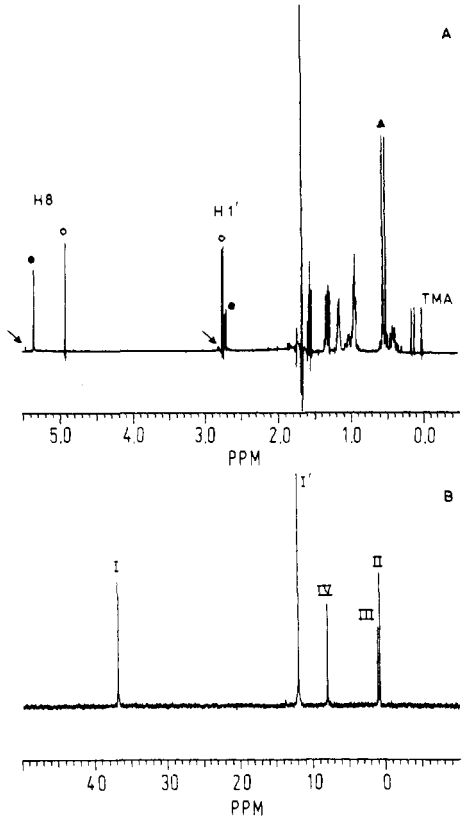

Figure 5. ^{1}H NMR (A) and ^{31}P NMR (B) spectra taken during the reation of [*cis*-Pt$(NH_3)_2$(ntmp)] with 5'GMP (10mM) at pH 4.8. Key: (A) open symbols represent 5'GMP, closed symbols represent products, arrow denotes intermediate, and ▲ is free ntmp; (B) (I and I') [*cis*-Pt$(NH_3)_2$(ntmp,N,O)], (II) 5'GMP, (III) *cis*-Pt$(NH_3)_2$(GMP,N7)$_2$, and (IV) ntmp ligand.

5'GMP could accelerate the second reaction step by intramolecular attack of the Pt–N bond. Therefore, the reaction of [*cis*-Pt$(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] with 3'GMP or d(Guo) has been followed by NMR, showing hardly any presence of intermediate product. The end products proved to be *cis*-Pt$(NH_3)_2$(3'GMP-N7)$_2$ and *cis*-Pt$(NH_3)_2$(dGuo-N7)$_2$. The overall $t_{1/2}$ value for these nucleotides is larger compared to that for 5'GMP (see Table 1) indicating that the 5'-phosphate does accelerate the first reaction step (phosphate directing effect[29]); however, it does not enhance the second reaction step since for 3'GMP and d(Guo) no large amounts of intermediates have been observed.

Comparison with Carboplatin and Cisplatin. A very recent study of Frey *et al.* describes the reaction of carboplatin with 5'GMP in aquous solution or in phosphate buffer.[30] Two reaction steps are observed: after reaction of the first 5'GMP with carboplatin a ring-opened species containing monodentate CBDCA is detected, *i.e.* [*cis*-Pt$(NH_3)_2$(CBDCA-O)(5'GMP)] (maximum amount 15%). The reaction of this ring-opened 5'GMP intermediate with a second 5'GMP results in [*cis*-Pt$(NH_3)_2$(5'GMP)$_2$] and CBDCA. The rate of the second reaction step is seven times faster than the rate of the first reaction stap,

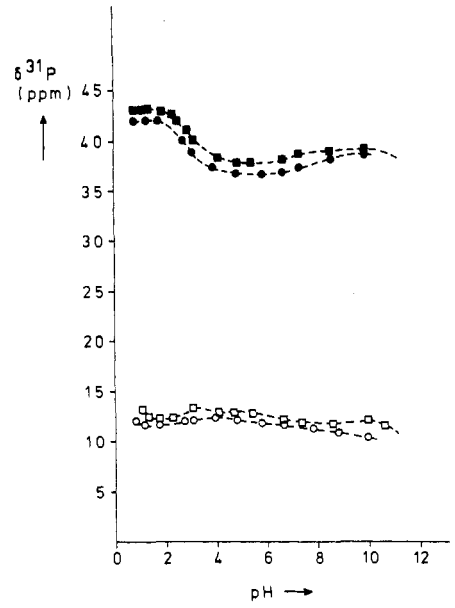

Figure 6. ^{31}P NMR titration curves of the complexes [*cis*-Pt$(NH_3)_2$(ntmp)] (○, ●) and [Pt(*R,S*-dach)(ntmp)] (□, ■). Closed symbols represent the coordinated phosphate.

resulting only in small detectable amounts of intermediate. Frey *et al.* conclude that the reaction of 5'GMP with carboplatin might involve direct attack on intact carboplatin, followed by a second faster step involving displacement of monodentate CBDCA, which is remarkably similar to the reaction profile of the Pt–phosphonate complexes with 5'GMP (*vide supra*). In contrast, for cisplatin the second reaction step is 1000 times slower than the first step.[31] The authors speculate that formation of GG cross-links on DNA will be facile for carboplatin due to the fast second reaction step. This is indeed observed in our study with the platinum phosphonate complexes, since the overall $t_{1/2}$ values of the d(GpG) reactions are considerably lower compared to the mononucleotides (see Table 1).

Effect of the Nonleaving Amine Groups. As is obvious from Table 1 the influence of the nonleaving amine group on the rate of the reaction is quite large. Compared to [*cis*-Pt$(NH_3)_2$(ntmp)], [Pt(*R,S*-dach)(ntmp)] is much less reactive, probably due to the bulky cyclohexane diamine ring. This ring is almost perpendicularly oriented with respect to the platinum coordination plane (see Figure 2), so an approach of the incoming G base might be sterically hindered, resulting in slow kinetics for [Pt(*R,S*-dach)(ntmp)] compared to [*cis*-Pt$(NH_3)_2$(ntmp)]; however, the H-bonding ability of the amine ligands[32] or alterations in their trans effects as well as cis effects[33] may also influence the rate of the reaction.

Influence of pH on the Reaction. Enhancing the pH from 4.8 to 6.8 (near physiological pH) does drastically influence the rate of the reaction as is obvious from Table 1, resulting in slow kinetics at pH 6.8 compared to pH 4.8, for both [*cis*-Pt$(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)]. This can be explained by considering the deprotonation constant of the coordinated phosphate group of [*cis*-Pt$(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)]. The ^{31}P NMR titration curves of the two platinum phosphonate complexes show for the coordinated phosphate (most downfield) at p$K_a \sim$ 4.0 (see Figure 6). At pH 6.8 this group is completely deprotonated, resulting in a negatively charged phosphate. This negative charge stabilizes the bond between the positively charged Pt(II) nucleus and the coordinated phosphate group resulting in

(29) (a) Marcelis, A. T. M.; Erkelens, C.; Reedijk, J. *Inorg. Chim. Act.* **1984**, *91*, 129. (b) Eapen, S.; Green, M.; Ismail, I. M. *J. Bioinorg. Chem.* **1985**, *24*, 233. (c) Evans, D.J.; Ford, N. R.; Green, M. *Inorg. Chim. Acta* **1986**, *125*, L39. (d) van der Veer, J. L.; van den Elst, H.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Inorg. Chem.* **1986**, *25*, 4657. (e) Van Hemelrijk, B.; Girault, J.-P.; Chottard, G.; Valadon, P.; Laoui, A.; Chottard, J.-C. *Inorg. Chem.* **1987**, *26*, 787. (f) van der Veer, J. L.; van der Marel, G. A.; van den Elst, H.; Reedijk, J. *Inorg. Chem.* **1987**, *26*, 2272. (g) Bloemink, M. J.; Lempers, E. L. M.; Reedijk, J. *Inorg. Chim. Acta* **1990**, *176*, 317. (h) van Garderen, C. J.; Bloemink, M. J.; Richardson, E.; Reedijk, J. *J. Inorg. Biochem.* **1991**, *42*, 199.

(30) Frey, U.; Ranford, J. D.; Sadler, P. J. *Inorg. Chem.* **1993**, *32*, 1333.

(31) Green, M.; Garner, M.; Orton, D. M. *Transition Met. Chem. (Weinheim Ger.)* **1992**, *17*, 164–176.

(32) (a) Laoui, A.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1988**, *27*, 2751. (b) van Kralingen, C. G. Ph.D. Thesis Delft University of Technology, 1979.

(33) Arpalathi, J.; Lippert, B. *Inorg. Chem.* **1990**, *29*, 104.

slow kinetic behavior of [*cis*-$Pt(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] near physiological pH. This stability has also been reported for platinum complexes containing phosphono carboxylate ligands, which were shown to be anionic species at neutral or basic pH, and proved to have good stability in biological media.[34] The slow kinetics, observed at physiological pH, suggest that *in vivo* an activation mechanism, perhaps induced by S-containing proteins, must take place. This activation mechanism is presently under investigation. Our observation of faster kinetic behavior of the leaving group at low pH is probably useful for designing other complexes since the pH of tumors generally is lower, compared to normal tissues.[35] A more detailed kinetic study concerning quantitative data on this observation, *i.e.* rate constants as a function of pH, will be the subject of further studies.

Concluding Remarks

The platinum complexes [*cis*-$Pt(NH_3)_2$(ntmp)] and [Pt(*R,S*-dach)(ntmp)] are capable of forming an N7,N7-adduct with d(GpG), in analogy with cisplatin, with ntmp acting as a leaving group. This is, to our knowledge, the first report of of an N-donor ligand acting as a leaving group in reactions of Pt–antitumor agents with oligonucleotides. Compared to cisplatin, the complexes react slower near physiological pH, due to deprotonation of the coordinated phosphate group. The reaction of Pt–phosphonate complexes with 5'GMP and d(GpG) are likely to involve direct attack of the nucleobase on the intact platinum complex. Studies with other antitumor platinum phosphonate complexes are in progress in order to investigate in more detail the structure–activity relationship of this type of compounds, of which the structural formula appears to violate the classical structure–activity relationships of platinum antitumor complexes.

Acknowledgment. Support for this work by the Netherlands Foundation of Chemical Research (SON) and the Foundation of Applied Technical Research (STW) (Grant 349-2293) and a loan of K_2PtCl_4 from Johnson Matthey Ltd. (Reading, England) are greatly appreciated. We acknowledge Pharmachemie BV (Haarlem, The Netherlands) for their financial support and stimulating discussions with the group of Professor Dr. J.-C. Chottard. Financial support by the European Community under Contract ERBCHRXCT920016 is thankfully acknowledged. Also support by COST Action D1-92/002 (Biocoordination Chemistry) is kindly acknowledged.

(34) Hollis, L. S.; Miller, A. V.; Amundsen, A. R.; Schurig, J. E.; Stern, E. W. *J. Med. Chem.* **1990**, *33*, 105.
(35) Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, J. R. In *Molecular Targets for Cancer Chemotherapeutic Agents*; Sartorelli, A. C., Bertino, J. R., Lazo, J. S., Eds.; Academic Press: New York, 1981; p 85.