A Kinetic Study on the Reactions of Azolato-Bridged Dinuclear Platinum(II) Complexes with Guanosine 5′-Monophosphate

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By using ^{1}H NMR spectroscopy the reactions of [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)](NO_3)$_2$ (**1**), [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta-*N1,N2*)](NO_3)$_2$ (**2**) and [{*cis*-Pt(*R,R*-dach)}$_2$(μ-OH)(μ-pz)](NO_3)$_2$ (**3**) with guanosine 5′-monophosphate (GMP) were monitored spectroscopically in 0.1 M phosphate D_2O solutions at three different pD values (5.4, 6.9, 8.4). From the investigations some kinetic information was obtained. The reaction rate for all these complexes become higher when the pD decreases. In addition, for the reactions performed in unbuffered solutions (pD 8.2), the reactivity of all the complexes for GMP was found to increase dramatically. The 1:2 reaction products were characterized by ^{1}H, ^{195}Pt, and ^{31}P NMR spectroscopy. Reactions of **2** with GMP include an unusual ligand-binding isomerization, resulting from the Pt atom migration along the 1,2,3-triazolato ring.

Introduction

The antitumor activity of the square-planar platinum(II) complex, *cis*-diamminedichloroplatinum(II) (cisplatin), was discovered by Rosenberg[1,2] in the mid 1960's. Cisplatin was subsequently approved for clinical use in 1979 and is now one of the most well-established and most sold successful antitumor drugs. Not exceptionally for an antitumor drug, its chemotherapy involves some serious side effects[3−6] and it also may result in acquired drug resistance.[7,8] It has been generally accepted that the local DNA kink resulting from the formation of the 1,2-intrastrand crosslink at d(GpG) site is connected with cisplatin's antitumor activity.[9,10] Therefore, a successful approach to circumvent the cross-resistance is likely to be the design of a Pt^{II} complex which would react with DNA in a different way from that of cisplatin.[11,12]

Earlier reports by some of us have shown that the azolato-bridged dinuclear platinum(II) complexes, [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)](NO_3)$_2$ (pz = pyrazolate) (**1**) and [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta-*N1,N2*)](NO_3)$_2$ (1,2,3-ta = 1,2,3-triazolate) (**2**), exhibit remarkably high in vitro cytotoxicity on several human tumor cell lines[13] and largely circumvent the cross-resistance to cisplatin.[14,15] These complexes were aimed to provide a 1,2-intrastrand GG crosslink with a minimal kink of DNA. This type of DNA adducts would sufficiently induce the desired cytotoxic effect[16] and may expect a different biological consequence compared to cisplatin (Figure 1).

On the other hand, replacing the terminal ammine ligands with primary and secondary amines, such as ethylenediamine, diaminocyclohexane, and isopropylamines, this series of azolato-bridged dinuclear Pt^{II} complexes exhibits a decrease in cytotoxicity.[13,17] This tendency agrees with the general structure-activity relationship in mononuclear cisplatin derivatives. Full interpretation of this relationship requires a proper way to investigate their DNA binding (coordination) rate, and to compare them with the reported cytotoxic profiles.[13] In this paper we report a kinetic study on the reactions of these three azolato-bridged dinuclear Pt^{II} complexes with guanosine 5′-monophosphate (GMP), the most simple DNA fragment, in solution at different pH.

Results and Discussion

Reactions of compounds **1**, **2**, and [{*cis*-Pt(*R,R*-dach)}$_2$(μ-OH)(μ-pz)](NO_3)$_2$ (**3**) with GMP (stoichiometric (2 equiv.) or excess (4 equiv.) per Pt complex) were monitored by ^{1}H NMR in D_2O solution with/without 0.1 M phosphate buffer. These azolato-bridged dinuclear platinum(II) complexes react with 2 equiv. of GMP to yield the final products, **I**-(GMP)$_2$, **II**-(GMP)$_2$, and **III**-(GMP)$_2$ (see Schemes 1 and 2). Upon incubating these compounds with GMP, the ^{1}H NMR signals corresponding to azolates of **1**−**3** and an H8 signal of the free GMP decreases in intensity with time (see Figures 2−4). The supposed intermediates could hardly be traced for all the reactions, not even in

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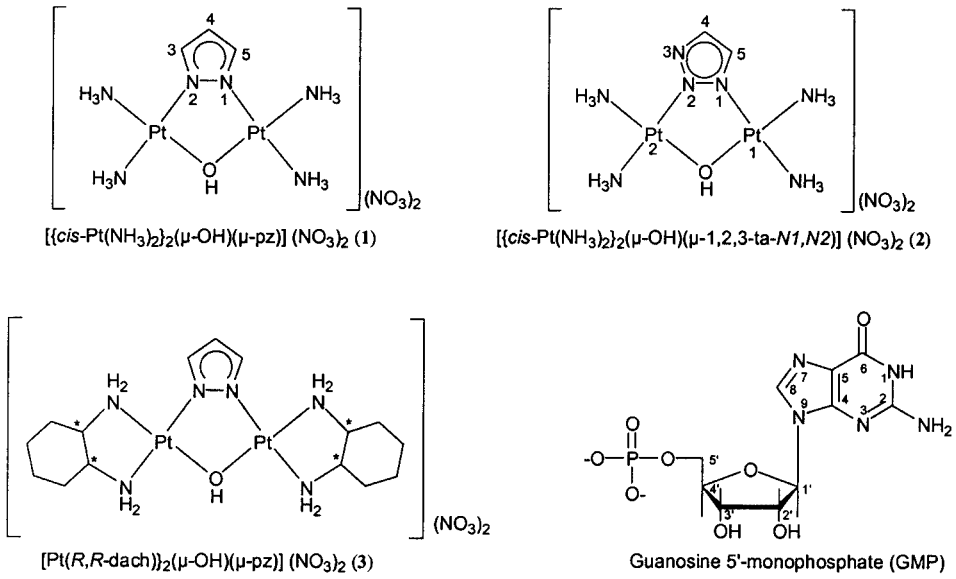


Figure 1. Schematic representation of [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-pz)](NO_3)$_2$ (**1**), [{*cis*-Pt(NH_3)$_2$}$_2$(μ-OH)(μ-1,2,3-ta-*N1*,*N2*)](NO_3)$_2$ (**2**), [Pt(*R*,*R*-dach)}$_2$(μ-OH)(μ-pz)] (NO_3)$_2$ (**3**), and guanosine 5′-monophosphate (GMP)

the reactions with stoichiometric (2 equiv.) GMP at 310 K. Therefore, the rate-determining step is obviously the reaction in which an N7 of the first GMP displaces the leaving hydroxo groups. Hence, the first substitution reaction of these complexes with GMP appears to proceed by an associative process which is followed by the second substitution reaction, in which the hydroxyl or water ligand undergoes relatively rapid substitution by the second nucleotide.

Characterization of the Reaction Products

The ^{1}H, ^{31}P, and ^{195}Pt NMR spectroscopic data for the reaction products, [{*cis*-Pt(NH_3)$_2$(GMP-*N7*)}$_2$(μ-pz)]$^-$(**I**-(GMP)$_2$], [{*cis*-Pt(NH_3)$_2$(GMP-*N7*)}$_2$(μ-1,2,3-ta-*N1*,*N3*)]$^-$ [**II**-(GMP)$_2$], and [{*cis*-Pt(*R*,*R*-dach)(GMP-*N7*)}$_2$(μ-pz)]$^-$ [**III**-(GMP)$_2$] measured in 0.1 M phosphate D_2O (pD 7.0) solution at 298 K are summarized in Table 1.

^{1}H NMR

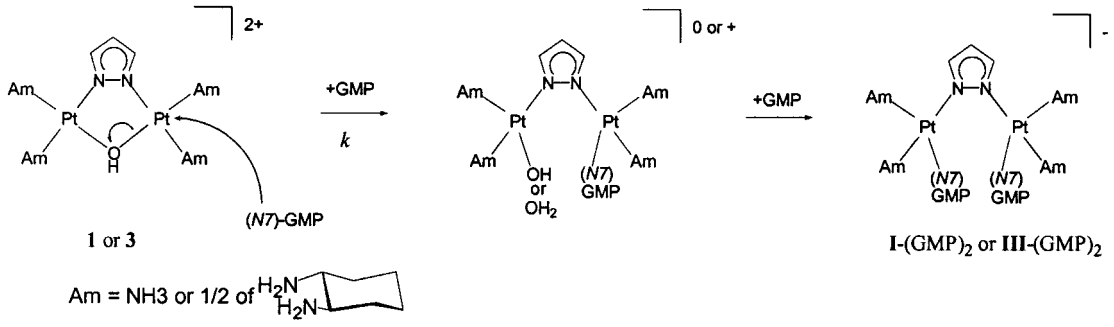
As mentioned above, **1** reacts with GMP in a bifunctional way to provide **I**-(GMP)$_2$ (Scheme 1). The signal of the H8 proton appears at slightly lower field as a completely broadened signal at 310 K, indicating a hindered rotation about the Pt−N7 bonds.[14,15] The signal becomes sharper to some extent upon increase of the temperature.[14] Referred to the H8 signal of free GMP, cisplatin-platinated GMP shows a 0.44 ppm downfield shift[18] owing to the inductive effect by platinum coordination (Figure 2). On the other hand, the H8 of **I**-(GMP)$_2$ appears at only 0.09 ppm lower field (323 K), indicating a ring-currency effect caused by well-stacked intramolecular guanine bases and by close proximity to the pyrazolate ring, which will compensate for the inductive effect.[14,15] The smaller $J_{1'-2'}$ (3.873 Hz) indicates the increase in N-type population of the sugar puckering.[19,20] Significant upfield shifts are observed for the H1′, H4′, and H5′ protons, but no significant changes are seen for H2′ and H3′. This phenomenon could have the same origin as for the H8 proton, but no proof is available as yet.

The reaction of GMP with **2** (Scheme 2), results in a sharp peak assigned to the H8 of the platinated GMP (observed at $\delta = 0.46$ ppm lower field than that of a free GMP, Figure 3), which is comparable to that of cisplatin-platinated GMP. This implies less stacked guanine ligands and allowance of a relatively free rotation about the Pt−N7

Table 1. The ^{1}H, ^{31}P, and ^{195}Pt NMR spectroscopic data of 1:2 complexes, **I**-(GMP)$_2$, **II**-(GMP)$_2$, and **III**-(GMP)$_2$ measured in 0.1 M phosphate D_2O (pD 7) solution at 298 K

Compound	δ(^{1}H), ppm H8	H1′ ($J_{1'-2'}$, Hz)	azolates	δ(^{31}P), ppm	δ(^{195}Pt), ppm
GMP	8.18	5.92 (6.036)	−	3.95	−
I-(GMP)$_2$	8.27[a]	5.73 (3.873)	6.34 [pz(4)], 7.60 [pz(3,5)]	4.07	−2393
II-(GMP)$_2$	8.64	5.89 (5.637)	7.51 (ta 4,5)	4.04	−2480
III-(GMP)$_2$	7.86	5.74 (4.959)	6.54 [pz(4)], 7.92 [pz(3,5)]	3.86	−2579

[a] Broad peaks that sharpen upon increase of temperature (323 K).



Scheme 1. Two-step reaction of compound **1** (and **3**) with 5′GMP

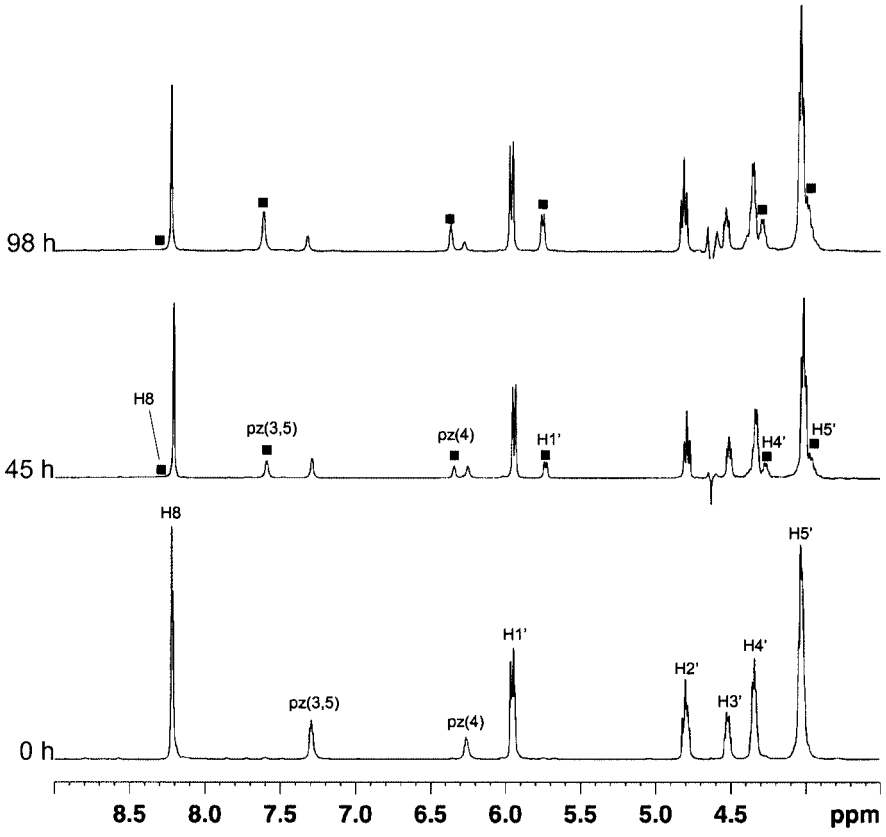
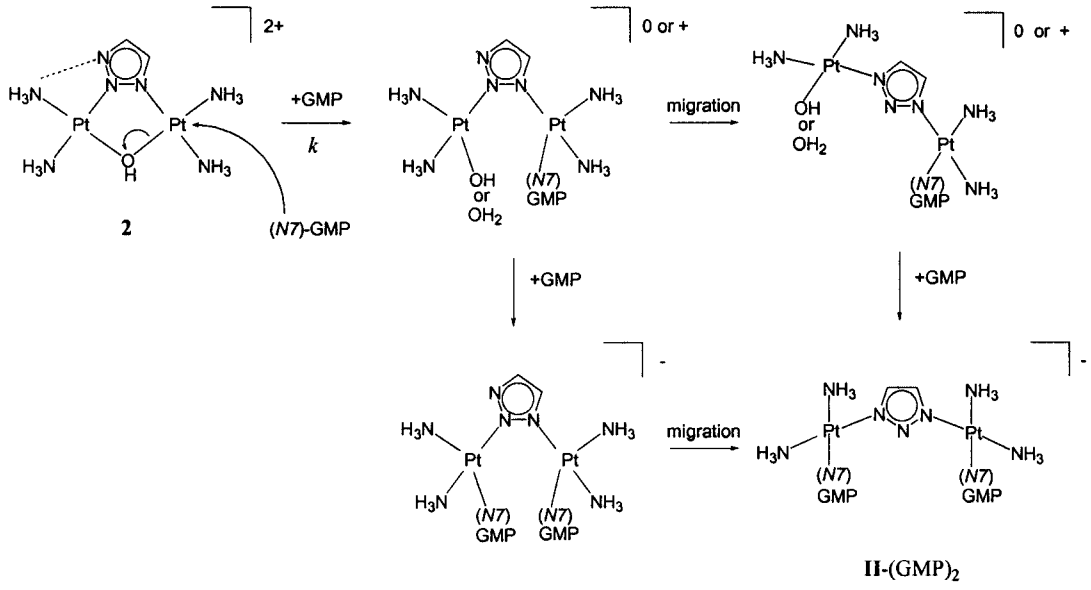


Figure 2. ^{1}H NMR spectra on the reaction of **1** with excess (4 equiv.) of GMP in unbuffered D_2O solution (pD 8.2) measured at 310 K as a function of time. The symbol (filled black squares) shows the newly appeared H8 and sugar ring proton signals (H1′, H4′ and H5′) of GMP ligands, and pz protons in a 1:2 complex, $[\{cis\text{-}Pt(NH_3)_2(GMP\text{-}N7)\}_2(\mu\text{-}pz)]^-$ (**I**-$(GMP)_2$]

bonds.[14] An interesting aspect that should be emphasized, is that the signals of ta(4) and (5) in the bridging 1,2,3-triazolate have shifted to higher field and now appear as a single peak (i.e. a highly symmetric species must be present). This phenomenon clearly indicates that the present reaction includes the same isomerization as we reported earlier for the reaction of **2** with 9EtG.[14] Thus, after the first substitution by N7 of GMP, the Pt atom initially coordinating to N2 of the 1,2,3-triazolate ring migrates from N2 to N3, to provide the symmetric 1:2 complex, **II**-$(GMP)_2$. This migration of the platinum(II) is to be seen as a novel isomerization reaction, since substitution reactions of square-planar metal complexes usually proceed via penta-coordination transition states. It seems obvious that N3 is not able to coordinate at the axial Pt position as long as N2 is coordinating to Pt2. Unlike the products of the pyrazolato-bridged complexes, no significant upfield shifts on the sugar ring proton signals are observed. The $J_{1'\text{-}2'}$ is found to be 5.637 Hz, indicating a sugar puckering with a similar N/S population as that of free GMP.[19,20]



Scheme 2. Reaction steps of compound **2** with 5′GMP

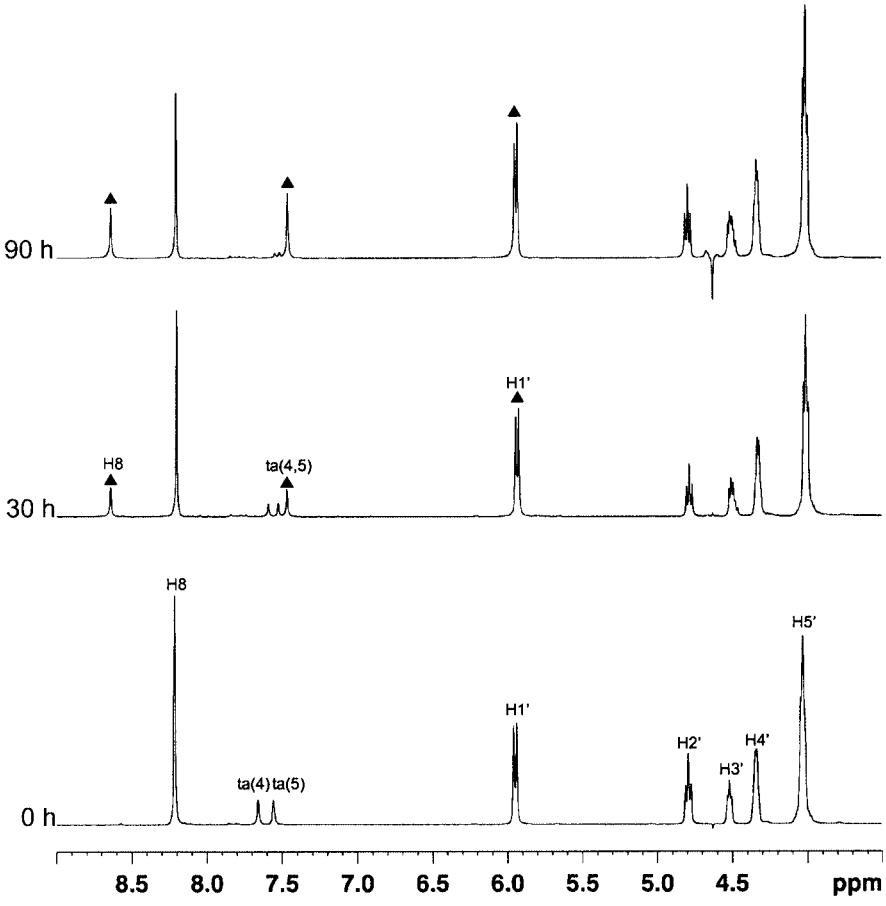


Figure 3. ^1H NMR spectra on the reaction of **2** with excess (4 equiv.) of GMP in unbuffered D_2O solution (pD 8.2) measured at 310 K as a function of time. The symbol (filled black triangles) shows the newly appeared H8 and sugar ring proton signals (H1′) of GMP ligands, and azolate protons in a 1:2 complex, $[\{cis\text{-}Pt(NH_3)_2(GMP\text{-}N7)\}_2(\mu\text{-}1,2,3\text{-}ta\text{-}N1,N3)]^-$ [**II**-$(GMP)_2$]

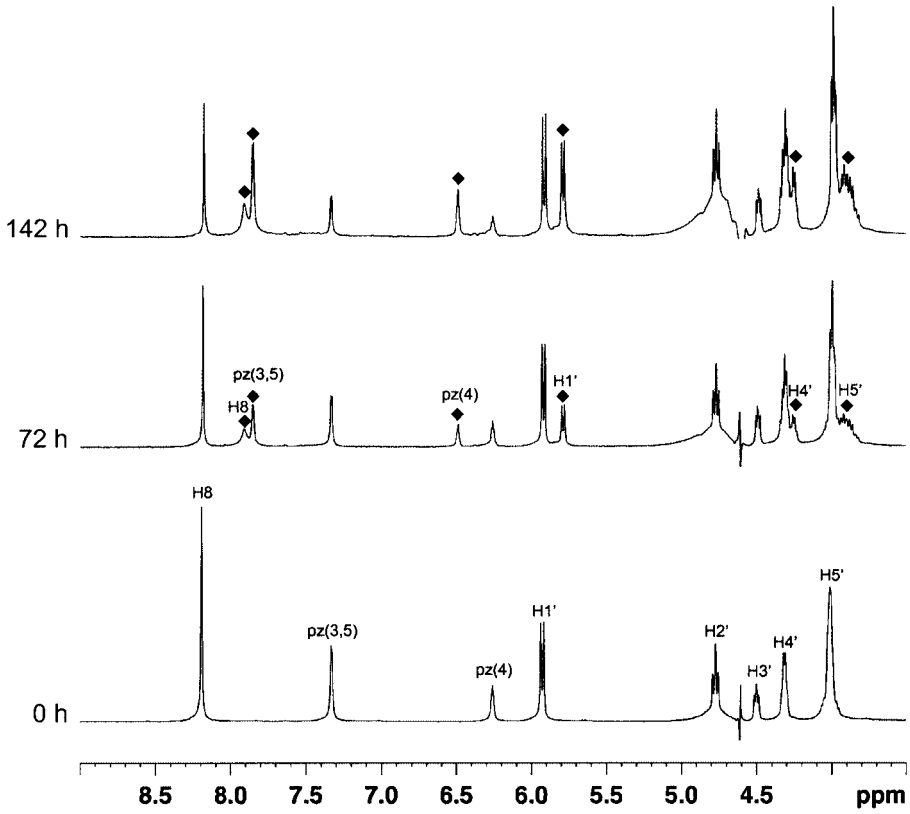


Figure 4. ^{1}H NMR spectra on the reaction of **3** with excess (4 equiv.) of GMP in unbuffered D_2O solution (pD 8.2) measured at 310 K as a function of time. The symbol (filled black diamonds) shows the newly appeared H8 and sugar ring proton signals (H1′, H4′ and H5′) of GMP ligands, and azolate protons in a 1:2 complex, [{Pt(*R*,*R*-dach)$_2$(GMP-*N7*)}$_2$(μ-pz)]$^-$ (**III**-(GMP)$_2$]

The ^{1}H NMR profiles of **III**-(GMP)$_2$ are similar to those of **I**-(GMP)$_2$ (Figure 4). A broad H8 signal is observed at higher field compared to free GMP. This upfield shift also agrees with well-stacked guanine ligands. The H1′ ($J_{1'-2'}$ = 4.959 Hz), H4′ and H5′ protons show significant upfield shifts, just as found for **I**-(GMP)$_2$.

^{195}Pt NMR

The ^{195}Pt NMR chemical shifts for the **I**-(GMP)$_2$, **II**-(GMP)$_2$, and **III**-(GMP)$_2$ are all found in the region expected for a [PtN$_4$] coordination sphere, further confirming the platinum coordination to N7 of GMP. The spectrum found for compound **2** is split into two signals,[13] owing to the two slightly different [PtN$_3$O] environments. On the other hand, the single peak observed for **II**-(GMP)$_2$, is supportive for the isomerization reaction, just as expected from the ^{1}H NMR study.

^{31}P NMR

Given that the 1:2 complexes have strong intramolecular hydrogen bonding between the ammine ligands and phosphate group, higher field shifts of their ^{31}P NMR signals, compared to free GMP (pD = 7.0), could be expected. The signals of the phosphate groups for **I**-(GMP)$_2$ and **II**-(GMP)$_2$ are observed at δ = 0.09−0.12 ppm lower field, and that for **III**-(GMP)$_2$ appeared at only 0.09 ppm higher field. These deviations from free GMP are not considered to be significant.

pH-Titration Experiments

The pH titration curves for GMP, **II**-(GMP)$_2$ and **III**-(GMP)$_2$ are depicted in Figure 5. As is clear from the plots, the N7 (*de*)protonation effects of the GMP ligands of **II**-(GMP)$_2$ and **III**-(GMP)$_2$ are absent, confirming the plati-

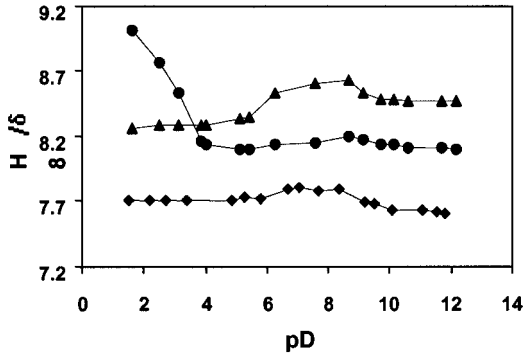


Figure 5. Plots of chemical shift (δ) of H8 resonance vs. pD for free GMP (filled black circles), **II**-(GMP)$_2$ (filled black triangles) and **III**-(GMP)$_2$ (filled black diamonds). pH titrations were performed in unbuffered D_2O solution at 298 K with adjusting pD with 0.1 M DNO_3 and 0.1 M NaOD

num coordination at the N7 site for these complexes. A pH-titration graph of H8 of **I**-$(GMP)_2$ is uninformative, due to the complete broadening at room temp. as stated above.

Kinetic Aspects

The reactions of **1**−**3** with GMP were monitored by ^1H NMR spectroscopy at 310 K. The reactions with stoichiometric quantities (2 equiv.) of GMP do fit to second-order plots (Figure 6). The reaction rates as determined for the reaction of the complexes are presented in Table 2.

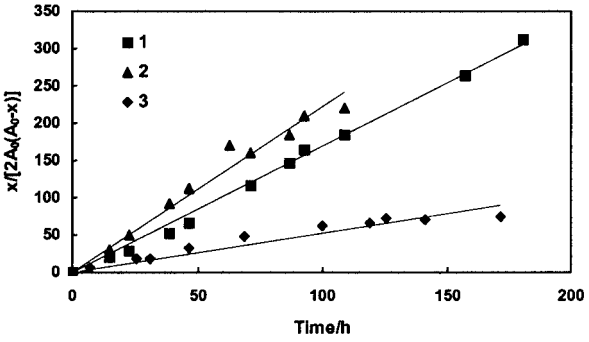


Figure 6. Second-order plots of the reactions of **1** (filled black squares), **2** (filled black triangles) and **3** (filled black diamonds) with 2 equiv. of GMP in unbuffered D_2O solution (pD 8.2) at 310 K. Values for k were calculated from the slope of the lines, as given in Equation (1)

Table 2. Obtained second-order rate constants (k in $M^{-1}s^{-1}$) of the platinum complexes for the reactions of **1**−**3** with 2 equiv. of GMP at 310 K in D_2O with or without 0.1 M phosphate

	Reaction rates k [$M^{-1}s^{-1}$]		
pD/compound	**1**	**2**	**3**
5.4[a] (2 equiv.)	$2.36\cdot10^{-4}$	$3.34\cdot10^{-4}$	$1.53\cdot10^{-4}$
6.9[a] (2 equiv.)	$1.39\cdot10^{-4}$	$2.64\cdot10^{-4}$	$1.11\cdot10^{-4}$
8.4[a] (2 equiv.)	$1.25\cdot10^{-4}$	$2.36\cdot10^{-4}$	$6.94\cdot10^{-5}$
8.2[b] (2 equiv.)	$4.67\cdot10^{-4}$	$6.15\cdot10^{-4}$	$1.46\cdot10^{-4}$

[a] Reactions were performed in 0.1 M phosphate D_2O solution. [b] Reactions were performed in unbuffered D_2O solution.

Compound **2** is found to react faster with GMP than **1**. Even though the amino groups in dach ligands of **3** should have similar *trans* effects on the leaving OH group as the ammine ligands,[21] the dach-containing complex reacts with GMP at a significantly slower rate compared to **1**. The kinetic rate of ligand-exchange reactions with associative mechanism are known to be negatively influenced by steric hindrance of the substrates, and more than those with dissociative reactions. Therefore, the observed kinetic difference could be ascribed to the steric effects of the bulky cyclohexane rings. Another contribution might be the reduced potential of dach to form hydrogen bonds compared to ammonia, since the first contact of these cationic azolato-bridged compounds is supposed to be non-covalent interactions, such as ionic and hydrogen bonds. Comparison of all the kinetic data shows that the reaction rates for all the complexes are faster when the pD decreases (see Figure 7). The pK_b value for the bridging OH group of this series of azolato-bridged dinuclear Pt complexes is very low [e.g. pK_b = 0.4 for complex **1** (unpublished observations)], and no direct contribution to the kinetics can be expected within the pD range applied for this study. However, an increase in H^+ concentration could catalytically act on the hydroxo bridge to promote the first substitution reaction.

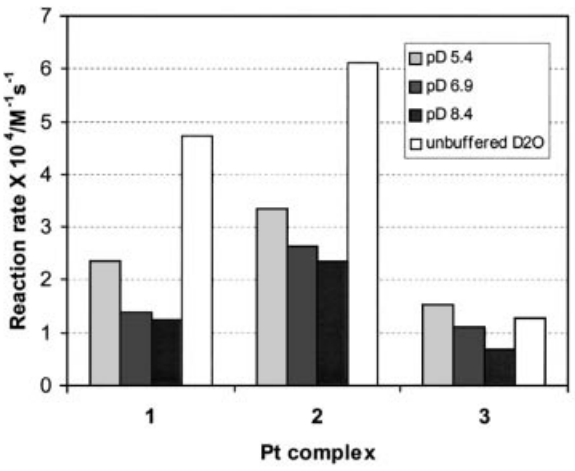


Figure 7. Column graph showing the second-order rate constants for the reaction of **1**−**3** with 2 equiv. of GMP in 0.1 M phosphate D_2O solutions with different pD (5.4, 6.9, and 8.4) and unbuffered D_2O solution (pD 8.2)

Interestingly, in unbuffered D_2O solutions, the reactivity of all complexes for GMP dramatically increases. This may be indicative for the affinity of the OH bridge and/or amine ligands of the platinum complexes for phosphate, i.e. the phosphate in the buffer competitively binds to the platinum complexes electrostatically and competes with the phosphate groups of GMP. This tendency is more pronounced in the reactions of **1** and **2**, thereby clearly demonstrating the importance of the non-covalent (dipolar) interaction with the phosphate group of the GMP as an initial step of the reaction. For confirmation, we undertook a similar kinetic study in other salts, or buffer solutions, such as MES buffer, using HPLC (data not shown). No such a large decrease in the kinetic rate compared to an unbuffered solution was found, and therefore, a high non-covalent (dipolar) affinity of these complexes can be expected also for the phosphate backbone of DNA. This interaction might be important for the processes, such as major-groove or minor-groove binding. Cisplatin, as a neutral molecule, is known to be activated and positively charged by hydrolysis (a first-order reaction with $t_{1/2}$ = 2 h[23]), and the activated species appears to coordinate to nucleic acids, promptly. Therefore, for these azolato-bridged dinuclear platinum(II) non-covalent accumulation on DNA must be more probable.

Conclusion

Interactions between transition-metal complexes and DNA have been investigated, since DNA has been believed and accepted to be one of the critical targets of cisplatin. The azolato-bridged dinuclear Pt^{II} complexes react with GMP at a relatively slow rate, and show a clear tendency to faster reactions with GMP, when the solution pH decreases. This series of Pt^{II} complexes was aimed to generate a 1,2-intrastrand crosslinks with a minimum kink of DNA. According to our recent report using the hairpin-stabilized double-stranded DNA, d(TATGGCATT$_4$ATGCCATA),[16] **1** indeed yields only the 1,2-intrastrand GG crosslink on a hairpin-stabilized double-stranded DNA, while **2** serves not only as the intrastrand crosslinker, but also generates interstrand GC crosslinks as a minor product. This behaviour appears to, depend on the different configuration about the triazolate ring, such as *N1*,*N2*- and *N1*,*N3*-coordination of the two Pt^{II} atoms. It is as yet too early to conclude that only such coordination interactions would trigger the cytotoxic effects. Accordingly, also ionic (non-coordination) interactions with DNA should also be taken into consideration[24] for the following reasons:

i) The reactions with small DNA fragments proceed at much slower rate compared with cisplatin.

ii) The complexes possess a 2+ charge.

iii) The complexes appear to have a significant affinity with the phosphate group.

Therefore, dipolar or ionic interactions, in addition to coordination, may indeed have a significant influence on the cytotoxicity of such compounds.

Experimental Section

Materials: Guanosine 5′-monophosphate (GMP) was obtained from Sigma. The azolato-bridged dinuclear platinum complexes, [*cis*-{Pt(NH$_3$)$_2$}$_2$(μ-OH)(μ-pz)](NO$_3$)$_2$ (**1**), [*cis*-{Pt(NH$_3$)$_2$}$_2$(μ-OH)(μ-1,2,3-ta-*N1*,*N2*)](NO$_3$)$_2$ (**2**) were prepared as reported before.[12,13]

Preparation of [{Pt(*R*,*R*-dach)}$_2$(μ-OH)(μ-pz)](NO$_3$)$_2$ (3): 50 mL of a water solution containing 0.224 g of Hpz (3.24 mmol) and 0.62 g of [{Pt(*R*,*R*-dach)}(μ-OH)]$_2$(NO$_3$)$_2$ (0.81 mmol) was stirred and heated at 60 °C in the dark. After 3 h incubation, the solution was filtered and the solvents evaporated to dryness, and the resulting white material was washed with 200 mL of ice-cold EtOH and diethyl ether. Recrystallization was carried out from water. Yield: 0.39 g (57%). $C_{15}H_{32}N_8O_7Pt_2{\cdot}H_2O$: calcd. C 21.33, H 4.06, N 13.27; found C 21.44, H 3.86, N 13.23%. ^{1}H NMR (D$_2$O, ppm): δ = (pz resonance) 7.39 (2 H, d), 6.30 (1 H, t), (cyclohexane resonance) 2.40 (4 H, b), 2.07 (4 H, b), 1.59 (4 H, b), 1.32 (4 H, b), 1.17 (4 H, b). ^{195}Pt NMR (D$_2$O, ppm): δ = −2272 (reference: Na$_2$PtCl$_6$).

NMR Measurements: All the NMR spectra were recorded on a Bruker DPX300 spectrometer at 300 MHz. For the monitoring of the GMP reactions, kinetic samples were measured at 310 K. Regarding the characterization for the resulting products, ^{1}H, ^{31}P, and ^{195}Pt NMR spectra were measured at 298 K in a 50 mM phosphate buffer solution (pD = 7.0) and referred to TSP and Na$_2$PtCl$_6$, and H$_3$PO$_4$ (85% in D$_2$O), respectively. For a pH titration study, the pD was adjusted with 0.1 and 1 M solutions of NaOD and DNO$_3$, and the ^{1}H NMR spectra were measured at 298 K.

Reactions in the NMR Tube: The reactions of complexes **1**−**3** with GMP were performed in the NMR tube at 310 K and followed by ^{1}H NMR spectroscopy as a function of time. The reactions of the platinum complexes (4 mM) with stoichiometric (2 equiv., 8 mM) and excess amount (4 equiv., 16 mM) of GMP were carried out in unbuffered D$_2$O solutions (pD of the reaction solution at $T = 0$ is 8.2) and 0.1 M phosphate buffered D$_2$O solutions (pD 5.4, 6.9, and 8.4). The reactions performed in pD 5.4 solutions increased the pD value by approximately 0.2 units after 1 week, and the pD values did not change for those performed in unbuffered D$_2$O solutions during the reaction.

Kinetic Analysis: To determine the time-dependent concentration of the reactants (**1**−**3**) and final product (**I**-(GMP)$_2$, **II**-(GMP)$_2$, and **III**-(GMP)$_2$], the relative integration values for the azolate protons [pz(3,5), ta(4,5), and pz(4) for **1**, **2**, and **3**, respectively) were applied. The kinetic data obtained from the stoichiometric reactions fit to the second-order kinetics [Equation (1); correlation coefficients are 0.963−0.998], Therefore, for the determination of the second-order rate constants (k), the following equation was applied:

$$x/[2A_0(A_0 - x)] = kt \quad (1)$$

where A_0 is the initial concentration of the reactant (**1**−**3**) and x is the concentration of the final product (1:2 complex) at time t. The k values obtained correspond to the first steps of the series of the reactions.

pH Titration Experiments: After each reaction of **2** and **3** with 2 equiv. of GMP in unbuffered D$_2$O solution was completed, the pD of the samples was adjusted by 0.1 M DNO$_3$ and NaOD and measured at 298 K using a PHM 80 pH meter (Radiometer) before and after each ^{1}H NMR measurement.

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