Ternary Pt(II)–amino acid–nucleotide complexes: kinetics of formation

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Abstract

Ternary complexes of Pt(II) with the nucleotides 5′-GMP, 3′-GMP and 5′-dGMP (GMP = guanosinemonophosphate), and with the amino acids N_α-BOC-L-histidine, N_α-BOC-L-methionine and 1-methylimidazole (1-MeIm) were studied as models for Pt mediated DNA–protein crosslinks. The triamine complexes $[PtAm_2(L)Cl]^+$ (where Am_2 = *cis*- or *trans*-$(NH_3)_2$ or ethylenediamine and L = 1-MeIm or N_α-BOC-L-his-*N*3) react readily with the mononucleotides 5′-GMP, 3′-GMP and 5′-dGMP to form the ternary crosslinked complexes $PtAm_2(L)$(nucleotide). The 5′-nucleotides react faster than their 3′ counterparts towards either triamine complex. Kinetic studies by ^1H NMR show that *cis*-$[PtAm_2(1\text{-MeIm-}N3)Cl]^+$ reacts with 5′-GMP faster than the *trans* isomer (second order rate constants $k_2 = 0.756$ and 0.358 M^{-1} s^{-1}, respectively) and that the ethylenediamine complex is faster than both ($k_2 = 1.09$ M^{-1} s^{-1}).

Key words: Kinetics and mechanism; Platinum complexes; Amino acid complexes; Nucleotide complexes

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP**, cisplatin) is a clinically effective antitumor agent which is in wide use against testicular, ovarian, and head and neck cancers [1, 2]. Cisplatin is believed to exert its cytotoxicity by binding to the DNA and arresting its replication [3, 4]. The major adduct of *cis*-DDP with DNA, a GpG intrastrand crosslink, has been identified and structurally characterized [5–7]. In addition to forming inter- and intrastrand crosslinks platinum complexes form DNA–protein crosslinks which have been detected, both *in vitro* and *in vivo*, subsequent to administration of the anticancer drug [8–12]. While some reports suggest that Pt mediated DNA–protein crosslinks may be involved in the mechanism of action of the anticancer drug, the biological role of these crosslinks is not clear [13].

Many other metal ions promote the formation of ternary complexes involving the metal ion and two biopolymers [14–16]. The formation of metal mediated DNA–protein crosslinks is not surprising in view of the high reactivity of metal ions towards proteins and peptides on the one hand and the close association of DNA and proteins, especially in the nucleosome core, on the other hand [17].

While various model systems for these crosslinks have been reported, only recently have ternary complexes of Pt(II) and Pd(II) with DNA and protein models received more systematic and serious attention. Hadjiliadis and Lippert have prepared models for DNA protein crosslinks and characterized them both by NMR spectroscopy and by X-ray crystallography [18–22].

This manuscript reports the preparation, characterization and kinetics for the formation of models for Pt mediated DNA–protein crosslinks. The models used

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**Abbreviations: DDP, diamminedichloroplatinum(II); 1-MeIm, 1-methylimidazole; GMP, guanosinemonophosphate; Guo, guanosine; His, histidine, Met, methionine, BOC, t-butyloxycarbonyl; 1-MeC, 1-methylcytosine; en, ethylenediamine; Am_2, $(NH_3)_2$ or en.

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in this study differ from the previously reported ones in two major aspects: (i) the choice of amino acids and imidazole reflects the reported affinities of the amino acids towards the platinum atom (methionine, histidine and lysine) [23]; (ii) the amino acids were constrained to bind the metal atom through the side chains (as is more likely to happen with proteins) and not through terminal groups.

Experimental

Materials and equipment

cis-DDP, *trans*-DDP and K_2PtCl_4 were purchased from Aldrich Chemical Company, Inc. The nucleotides and amino acids were purchased from Sigma Chemical Company, Inc. $Pt(en)Cl_2$ was prepared from K_2PtCl_4 by the method of Dhara [24]. The hexanucleotide dATGCAT was prepared by Professor B. Gaffney, Department of Chemistry, Rutgers University [25]. All reagents and solvents were used without further purification.

All ^1H spectra were measured on a Varian XL 400 spectrometer equipped with a 5 mm dedicated proton probe. The spectra were obtained in D_2O at pD 6.5–7.0 (measured with an Orion Research digital pH/millivolt meter 611 equipped with a Fisher electrode) and were referenced to TSP, which was used as an internal standard. ^{195}Pt NMR spectra were obtained at 85.836 MHz using a 5 mm broadband probehead. K_2PtCl_4 was used as an external reference at -1624 ppm. The data were processed using a 200 Hz line broadening.

Reactions were monitored by HPLC chromatography using a Waters 6000A with an Autochrom CIM, to allow a single pump gradient, and an analytical Waters C-18 Nova-Pak cartridge (8×100 mm). Elution was carried out using a linear gradient of 0.1M TEAA (triethylammonium acetate buffer, pH 6.8) and acetonitrile (0–20% over 10 min using a flow rate of 4 ml/min and detection at 280 nm). Large scale purification of triamines was performed with a Waters C-18 Nova-Pak cartridge (25×100 mm) eluting isocratically with 0.1M TEAA for 30–60 min using a flow rate of 4 ml/min. Ternary complexes were purified using a 2–20% linear gradient of TEAA and acetonitrile over 60 min using a 4 ml/min flow rate.

Preparation of the triaminemonochloroplatinum(II) complexes

The chloride or nitrate salts of the triaminemonochloroplatinum(II) complexes were all prepared following the methods of Lippert *et al.* [26] and Hollis *et al.* [27]. Briefly, the diaminedichloroplatinum(II) complexes were reacted in DMF with one equivalent of silver nitrate to yield the desired compounds (see Scheme 1(a)). When necessary, the triamine complexes were purified by HPLC. The preparation of *cis*-$[Pt(NH_3)_2(Guo\text{-}N7)Cl](NO_3)$ (**1**) has already been detailed [22].

trans-[Pt(NH$_3$)$_2$(Guo-N7)Cl](NO$_3$) (2)

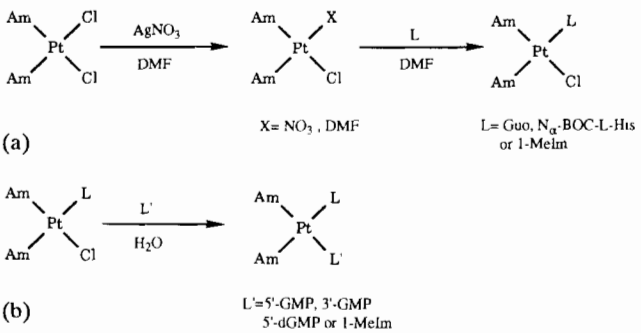
300 mg (1 mmol) of *trans*-DDP were dissolved in 30 ml of DMF. 169 mg of $AgNO_3$ (1 mmol) were added and the solution was stirred overnight at room temperature in the dark. The AgCl was filtered off and 283 mg (1 mmol) of guanosine hydrate were added and the reaction was allowed to continue for 14 h. The DMF was evaporated and the complex was precipitated by stirring with excess CH_2Cl_2. The light yellow powder was crystallized from water to yield 442 mg of *trans*-$[Pt(NH_3)_2(Guo\text{-}N7)Cl](NO_3)$. X-ray quality crystals were obtained by slow evaporation from aqueous solution [28]. *Anal.* Calc. for $C_{10}H_{19}N_8O_8ClPt \cdot H_2O$: C, 19.13; H, 3.37; N, 17.85. Found: C, 19.08; H, 3.67; N, 17.79%.

cis-[Pt(NH$_3$)$_2$(1-MeIm-N3)Cl](NO$_3$) (3)

300 mg (1 mmol) of *cis*-DDP were dissolved in 30 ml of DMF. 169 mg of $AgNO_3$ (1 mmol) were added and the solution was stirred overnight, at room temperature in the dark. The AgCl was filtered off and 82 mg (1 mmol) of 1-MeIm were added and the reaction was allowed to continue for 17 h. The DMF was evaporated and the complex was precipitated out by stirring with excess CH_2Cl_2. The light yellow powder was crystallized from water to yield 297 mg of *cis*-$[Pt(NH_3)_2(1\text{-MeIm-}N3)Cl](NO_3)$. Slow evaporation of the aqueous solution yielded clear X-ray quality crystals [28]. *Anal.* Calc. for $C_4H_{12}N_5O_3ClPt$: C, 11.75, H, 2.95; N, 17.14. Found: C, 11.96; H, 2.93; N, 17.58%.

trans-[Pt(NH$_3$)$_2$(1-MeIm-N3)Cl](NO$_3$) (4)

The same procedure was followed for the *trans* isomer, yielding 288 mg of *trans*-$[Pt(NH_3)_2(1\text{-MeIm-}N3)Cl](NO_3)$. *Anal.* Calc. for $C_4H_{12}N_5O_3ClPt$: C, 11.75; H, 2.95; N, 17.14. Found: C, 11.79; H, 2.72; N, 17.02%.



Scheme 1.

[Pt(en)(1-MeIm-N3)Cl](NO$_3$) (5)

Similarly, a reaction with 326 mg of Pt(en)Cl$_2$ resulted in 255 mg of crude [Pt(en)1-MeIm-*N*3)Cl](NO$_3$), which was recrystallized from water. *Anal.* Calc. for $C_6H_{14}N_5O_3ClPt$: C, 16.57; H, 3.22; N, 16.11. Found: C, 16.66; H, 3.11; N, 15.98%.

cis[Pt(NH$_3$)$_2$(N$_\alpha$-BOC-L-His-N1,Nα)] (6a) and cis-[Pt(NH$_3$)$_2$(N$_\alpha$-BOC-L-His-N3)Cl] (6b)

The same procedure was followed beginning with 300 mg (1 mmol) of *cis*-DDP and adding an equivalent amount of N$_\alpha$-BOC-L-His (255 mg, 1 mmol). Following precipitation by CH_2Cl_2, the solid was dissolved in EtOH and filtered to remove unreacted *cis*-DDP. Evaporation of the ethanolic solution gave 519 mg of white powder, containing a mixture of materials. A 200 mg portion was subjected to preparative HPLC from which the major components (**6a**) (52 mg) and (**6b**) (67 mg) were obtained from desalted samples by crystallization from aqueous solution. *Anal.* Calc. for $C_{11}H_{21}N_5O_4Pt \cdot 2H_2O$ (**6a**): C, 26.39; H, 5.01; N, 13.46. Found: C, 26.31; H, 4.79; N, 13.23%. Calc. for [$C_{11}H_{22}N_5O_4PtCl$] (**6b**): C, 25.46; H, 4.29; N, 13.49. Found: C, 25.31; H, 4.79; N, 13.23%.

Preparation of the ternary tetraamine complexes

The ternary tetraamine complexes were obtained by reacting the triaminemonochloro complexes with the nucleotide or amino acid in aqueous solution at pH 6.8 with gentle warming (see Scheme 1(b)). The ternary complexes were isolated by crystallization from H_2O/EtOH mixtures.

cis-Pt(NH$_3$)$_2$(1-MeIm-N3)(5'-GMP-N7) (7)

382 mg of *cis*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)Cl](NO$_3$) were dissolved in 10 ml of water and 407 mg of the disodium salt of 5'-GMP were added. The reaction mixture was warmed to 50 °C for 50 h. Any insoluble material was filtered off and the solution was concentrated under reduced pressure. Absolute ethanol was added to precipitate a white solid (400 mg, 60% yield). *Anal.* Calc. for $C_{14}H_{24}N_9O_8PPt \cdot 4H_2O$: C, 22.59; H, 4.33; N, 16.93. Found: C, 22.98; H, 4.36; N, 17.13%.

trans-Pt(NH$_3$)$_2$(1-MeIm-N3)(5'-GMP-N7) (8)

The same procedure was applied to the *trans*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)Cl](NO$_3$) and yielded 414 mg of white powder. *Anal.* Calc. for $C_{14}H_{24}N_9O_8PPt \cdot 5H_2O$: C, 22.05; H, 4.49; N, 16.53. Found: C, 22.21; H. 4.87; N, 16.83%.

The ternary complexes *trans*-Pt(NH$_3$)$_2$(1-MeIm-*N*3)(3'-GMP-*N*7) (**9**), *trans*-Pt(NH$_3$)$_2$(1-MeIm-*N*3)(5'-dGMP-*N*7) (**10**), *cis*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)(Guo-*N*7)]$^{2+}$ (**11**), *trans*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)(Guo-*N*7)]$^{2+}$ (**12**), [Pt(en)(1-MeIm-*N*3)(5'-GMP-*N*7)]$^{2+}$ (**13**), *cis*-Pt(NH$_3$)$_2$(Nα-BOC-L-His-*N*3)(5'-GMP) (**14**) and *cis*-Pt(NH$_3$)$_2$(Nα-BOC-L-His-*N*3)(3'-GMP) (**15**) were prepared *in situ* for the kinetic studies but were not isolated. These ternary complexes were prepared in water at 37 °C and the reactions were monitored by ^{1}H and ^{195}Pt NMR spectroscopy. Typically, approximately 20 mM solutions of the reactants in 700 μl of D_2O were used for these experiments.

Kinetic measurements

The kinetic studies were performed by following the changes in the ^{1}H NMR spectrum of the appropriate triaminemonochloroplatinum(II) complex or of the free ligand upon addition of the nucleotide or amino acid. Platinum binding causes a downfield shift of certain proton resonances of the free ligand (up to 0.6 ppm compared with the free ligand) affording a facile way of monitoring the reaction kinetics. All measurements were performed on 20 mM solutions in D_2O at 37 °C and a pD range of 6.5 to 7.0. The data were processed as second order kinetics and the rate constants were obtained by plotting the reciprocal concentration as a function of time. A linear regression was applied to obtain the best fit to the experimental data. Typical correlation coefficients were in the range 0.992–0.999. The estimated error in the second order rate constants is 4–7%.

Biological studies

Recently, a series of Pt(II) complexes containing three nitrogen donors have been found to possess *in vivo* antitumor activity [27]. Hollis *et al.* found that the most active analogues of their triamine series contained the heterocycle pyridine or one of its derivatives. This suggests that the influence of heterocyclic amine ligands on the biological properties of Pt complexes should be examined further. Thus, the *in vitro* biological activities of **3** and **4** against the L1210/0 cell line were determined in this lab by standard assays [29]. In brief, L1210 cells were grown in Fischer's medium supplemented with 10% horse serum and were exposed to varying concentrations of the platinum complexes in H_2O. After 72 h the cell concentrations were determined and ID_{50} values were obtained from the counts. *cis*-DDP was used as a control drug.

Results and discussion

Schematic structures of the triamine Pt(II) complexes **1–6** are given in Fig. 1; those of the ternary tetraamine Pt(II) complexes **7–15** in Fig. 2.

Synthesis

The reaction of *cis*-DDP with N$_\alpha$-BOC-L-His resulted in a material which displayed two distinct resonances

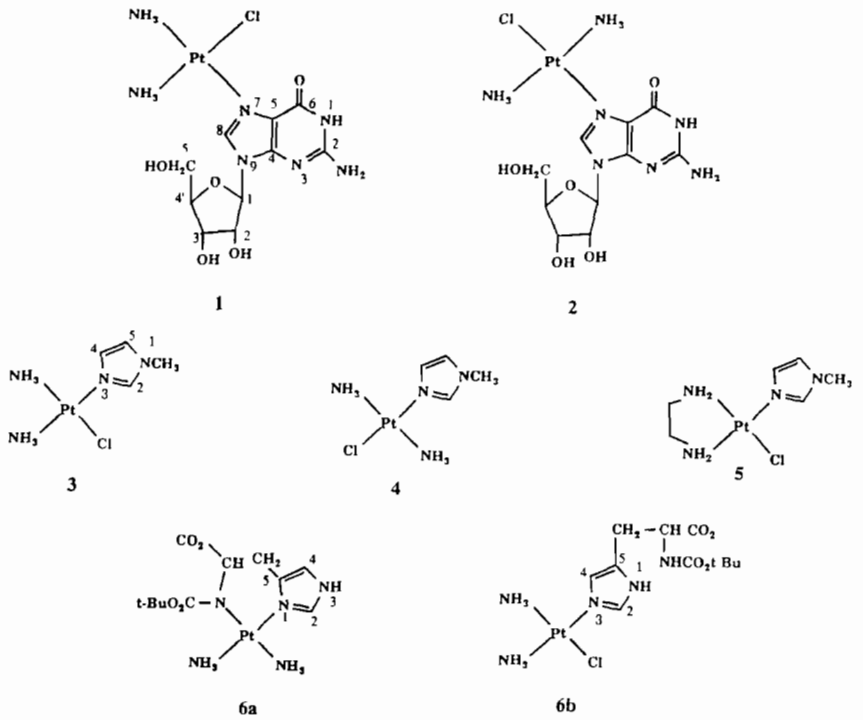


Fig. 1. Schematic structures of the triamine Pt(II) complexes **1–6**.

in the ^{195}Pt NMR. Both peaks (−2309 and −2324 ppm) are in the N_3Cl coordination region and can be ascribed to a mixture of *cis*-Pt(NH_3)$_2$(N_α-BOC-L-His-*N*1)Cl and *cis*-Pt(NH_3)$_2$(N_α-BOC-L-His-*N*3)Cl. This type of linkage isomerism has been observed by NMR spectroscopy [30] and by an X-ray crystallography study of the binding of platinum complexes to bovine carboxypeptidase A, in which the Pt is bound to the N3 of His 29 and to the N1 of His 303 [31]. Upon standing in solution *cis*-Pt(NH_3)$_2$(N_α-BOC-L-His-*N*1)Cl rearranges to form the chelate complex **6a**, *cis*-Pt(NH_3)$_2$(N_α-BOC-L-His-*N*1,N_α) which has been characterized by single crystal X-ray diffraction [32].

The reaction of the water soluble [PtAm$_2$(1-MeIm-*N*3)Cl]$^+$ complexes with the various nucleotides (5′-GMP, 3′-GMP and 5′-dGMP) proceeds smoothly in aqueous solutions at ambient temperatures and at 37 °C, yielding the desired ternary complex. As evident from the kinetic values (*vide supra*), these reactions are essentially complete after about 18 h at 37 °C and do not require the harsher conditions which had been originally employed. The reactions of *cis*- and *trans*-[Pt(NH_3)$_2$(Guo-*N*7)Cl]$^+$ with 1-MeIm are not as facile and require either higher temperature or longer reaction times.

NMR spectroscopy

NMR spectroscopy was employed both to characterize the complexes prepared and to monitor the reaction rates. Selected data from ^{195}Pt NMR spectra appear in Table 1 and they clearly display the N_3Cl coordination sphere for the triamine monochloro precursors and the N_4 coordination sphere for the ternary complexes [33].

Select ^{1}H data for the free ligands, the triamino-monochloro complexes and the ternary complexes are presented in Table 2. It is clear that Pt binding shifts the H8 and H1′ resonances of the nucleotides to lower field (approximately 0.3–0.6 ppm) affording easy detection of Pt binding. As can be seen for the PtAm$_2$(1-MeIm-*N*3)(nucleotide) complexes, the resonances of the *cis* isomers occur at a higher field than their *trans* counterparts. This can be attributed to ring current effects which result in mutual shielding between the imidazole and purine rings in the *cis* configuration. It indicates that the reaction between these Pt triamines and the nucleotides proceeds without isomerization at the metal center. The existence of several rotamers has been described for some Pd(II) complexes and for Pt(II)–1-MeC complexes [34], but this was not the case for PtAm$_2$(1-MeIm-*N*3)(nucleotide). Previously published data demonstrate that the purine ligands exhibit a free rotation around the Pt–N7 bond which is fast on the NMR time scale when the other ligands do not provide significant steric hindrance [35]. The results for PtAm$_2$(1-MeIm-*N*3)(nucleotide) complexes studied here are in agreement with this observation.

^{1}H NMR spectra of the histidine complexes **6a** and **6b** reveal N1 and N3-imidazoyl binding, respectively (see Fig. 1). Assignment of the H2 and H4 chemical shifts can be made by comparison with those of similar

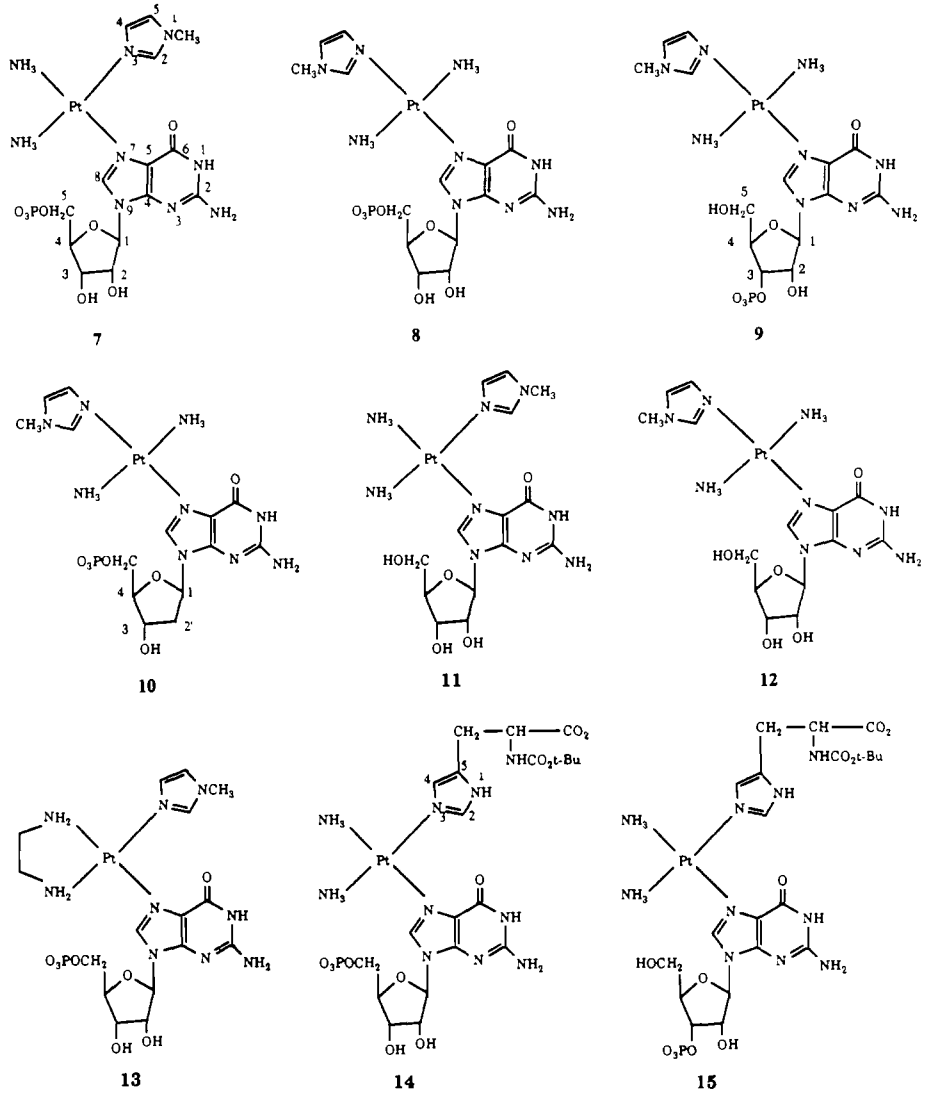


Fig. 2. Schematic structures of the ternary tetraamine Pt(II) complexes **7–15**.

histidine complexes in which the resonances of the N1 isomer were found to appear downfield relative to those of the N3 isomer [30]. However, ^{1}H NMR spectra of the ternary complexes formed from the reaction of **6b** with guanosine derivatives, unlike those of the imidazole complexes, provide evidence that rotation about the Pt–N bonds is hindered in complexes with the bulkier histidine ligand. As shown in Fig. 3, for example, at room temperature the H8 of 5′-GMP as well as the H2 and H4 of N_α-BOC-L-His in complex **14** display broad signals which appear split. As the temperature is increased the signals sharpen and coalesce.

Kinetic measurements

We examined the kinetics of the reaction between *trans*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)Cl]$^+$ and the nucleotides 5′-GMP, 3′-GMP and 5′-dGMP to assess the relative reactivities of the different nucleotides. We also studied the reactions between *cis*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)Cl]$^+$, [Pt(en)(1-MeIm-*N*3)Cl]$^+$ and *cis*-[Pt(NH$_3$)$_2$(N_α-BOC-L-His-*N*3)Cl]$^+$ with 5′-GMP, which affords comparison between the reaction kinetics of these four complexes with the same nucleotide. A typical time course progression of the reaction is depicted in Fig. 4. The changes in the chemical shifts of the H8 and H1′ protons of 5′-GMP as a result of the Pt binding are easily seen. Whenever possible, the H1′ protons were integrated and used for the numeric calculations. The H1′ are preferable to the H8 protons, which are broad to begin with and are further broadened by ^{195}Pt coupling and exchange with D$_2$O.

The results of these kinetic studies appear in Table 3 and a typical plot of the second order kinetics is depicted in Fig. 5. *cis*-[Pt(NH$_3$)$_2$(1-MeIm-*N*3)Cl]$^+$ reacts with 5′-GMP approximately twice as fast as the *trans* isomer. The reaction rates of 5′-GMP and the deoxy

TABLE 1. ^{195}Pt NMR chemical shifts for triamine and tetraamine Pt complexes

Triamine complexes	$-\delta$ (ppm)
cis-Pt(NH_3)$_2$(Guo)Cl (**1**)	2306
trans-Pt(NH_3)$_2$(Guo)Cl (**2**)	2294
cis-Pt(NH_3)$_2$(1-MeIm)Cl (**3**)	2312
trans-Pt(NH_3)$_2$(1-MeIm)Cl (**4**)	2313
Pt(en)(1-MeIm)Cl (**5**)	2506
cis-Pt(NH_3)$_2$(N_α-BOC-L-His)Cl (**6**)	2309, 2324
Ternary tetraamine complexes[a]	
cis-Pt(NH_3)$_2$(1-MeIm)(5′-GMP) (**7**)	2469
trans-Pt(NH_3)$_2$(1-MeIm)(5′-GMP) (**8**)	2478
cis-Pt(NH_3)$_2$(Guo)(1-MeIm) (**11**)	2460
trans-Pt(NH_3)$_2$(Guo)(1-MeIm) (**12**)	2467
Pt(en)(1-MeIm)(5′-GMP) (**13**)	2671

[a]^{195}Pt NMR chemical shifts were not determined for **9** and **10** because of the small amount of material prepared.

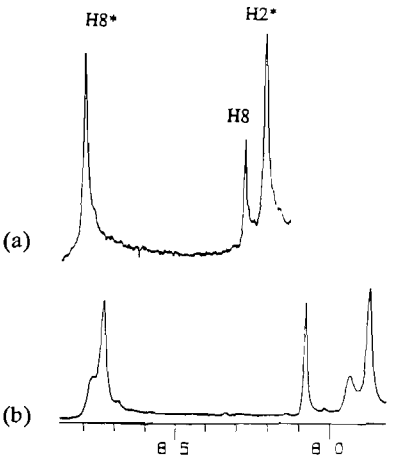


Fig. 3. ^{1}H NMR spectrum of **14** from the reaction of **6b** and 5′-GMP at (a) 80 °C and (b) 20 °C. Resonances marked with an asterisk designate ligand protons in the ternary complex.

analog, 5′-dGMP, with **2** are comparable while 3′-GMP reacts significantly slower. This trend was further corroborated by comparing the binding rates of *cis*- and *trans*-[Pt(NH_3)$_2$(1-MeIm-*N*3)Cl]$^+$ in preliminary experiments with the double stranded hexanucleotide dATGCAT. Both isomers react with the hexanucleotide in aqueous solution at 37 °C to yield a major (>90%) and several minor components (see Fig. 6). Since the sequence of the oligonucleotide was specifically designed to contain a single G site and no AG sites and since the [Pt(NH_3)$_2$(1-MeIm-*N*3)Cl]$^+$ has only one labile coordination site, it is reasonable to assume that the main product observed in the HPLC chromatogram is Pt(NH_3)$_2$(1-MeIm-*N*3)(dATG*CAT) where the Pt is bound to the N7 of the guanine [36].

Pt(en)(1-MeIm-*N*3)Cl]$^+$ forms crosslinks with 5′-GMP even faster than *cis*-[Pt(NH_3)$_2$(1-MeIm-*N*3)Cl]$^+$. This is interesting because it is reported that Pt(en)Cl_2 does not form DNA–protein crosslinks *in vivo* [37]. Our results clearly indicate that [Pt(en)]$^{2+}$ is capable of crosslinking amino acids and nucleotides. The lack of *in vivo* crosslinking by Pt(en)Cl_2 may be attributed

TABLE 2. Selected ^{1}H chemical shifts for triaminemonochloroplatinum(II) and ternary tetraamine complexes

	H8	H1′	H2	H4	H5
5′-GMP	8.177	5.922			
3′-GMP	8.021	5.933			
5′-dGMP	8.148	6.312			
1-MeIm			7.628	7.025	6.953
Triamine complexes					
cis-Pt(NH_3)$_2$(Guo)Cl$^+$ (**1**)	8.470	5.971			
trans-Pt(NH_3)$_2$(Guo)Cl$^+$ (**2**)	8.574	5.977			
cis-Pt(NH_3)$_2$(1-MeIm-*N*3)Cl$^+$ (**3**)			7.932	7.168	7 083
trans-Pt(NH_3)$_2$(1-MeIm-*N*3)Cl$^+$ (**4**)			7.975	7.182	7.105
Pt(en)(1-MeIm-*N*3)Cl$^+$ (**5**)			7.927	7 168	7.063
cis-Pt(NH_3)$_2$(Nα-BOC-L-His-*N*1,*N*α) (**6a**)			7.929	7.097	
cis-Pt(NH_3)$_2$(Nα-BOC-L-His-*N*3)Cl$^+$ (**6b**)			7.858	6.879	
Ternary tetraamine complexes					
cis-Pt(NH_3)$_2$(1-MeIm)(5′-GMP) (**7**)	8.865	5.976	7.928	7.086	7.056
trans-Pt(NH_3)$_2$(1-MeIm)(5′-GMP) (**8**)	8.921	6.048	8.166	7.270	7.246
trans-Pt(NH_3)$_2$(1-MeIm)(3′-GMP) (**9**)	8.639	6.022	8.141	7.270	7.261
trans-Pt(NH_3)$_2$(1-MeIm)(5′-dGMP) (**10**)	8.788	6.410	8.152	7.262	7 245
cis-Pt(NH_3)$_2$(1-MeIm-*N*3)(Guo) (**11**)	8.427	5.946	7.915	7.185	7.057
trans-Pt(NH_3)$_2$(1-MeIm-*N*3)(Guo) (**12**)	8.611	6.036	7.855	7.320	7.272
Pt(en)(1-MeIm-*N*3)(5′-GMP) (**13**)	8.829	5.986	7.830	7.092	6.977
cis-Pt(NH_3)$_2$(Nα-BOC-L-His-*N*3)(5′-GMP) (**14**)	8.837	5.965	8.023	6 903	
cis-Pt(NH_3)$_2$(Nα-BOC-L-His-*N*3)(3′-GMP) (**15**)	8.746	6.163	8.075	7.095	

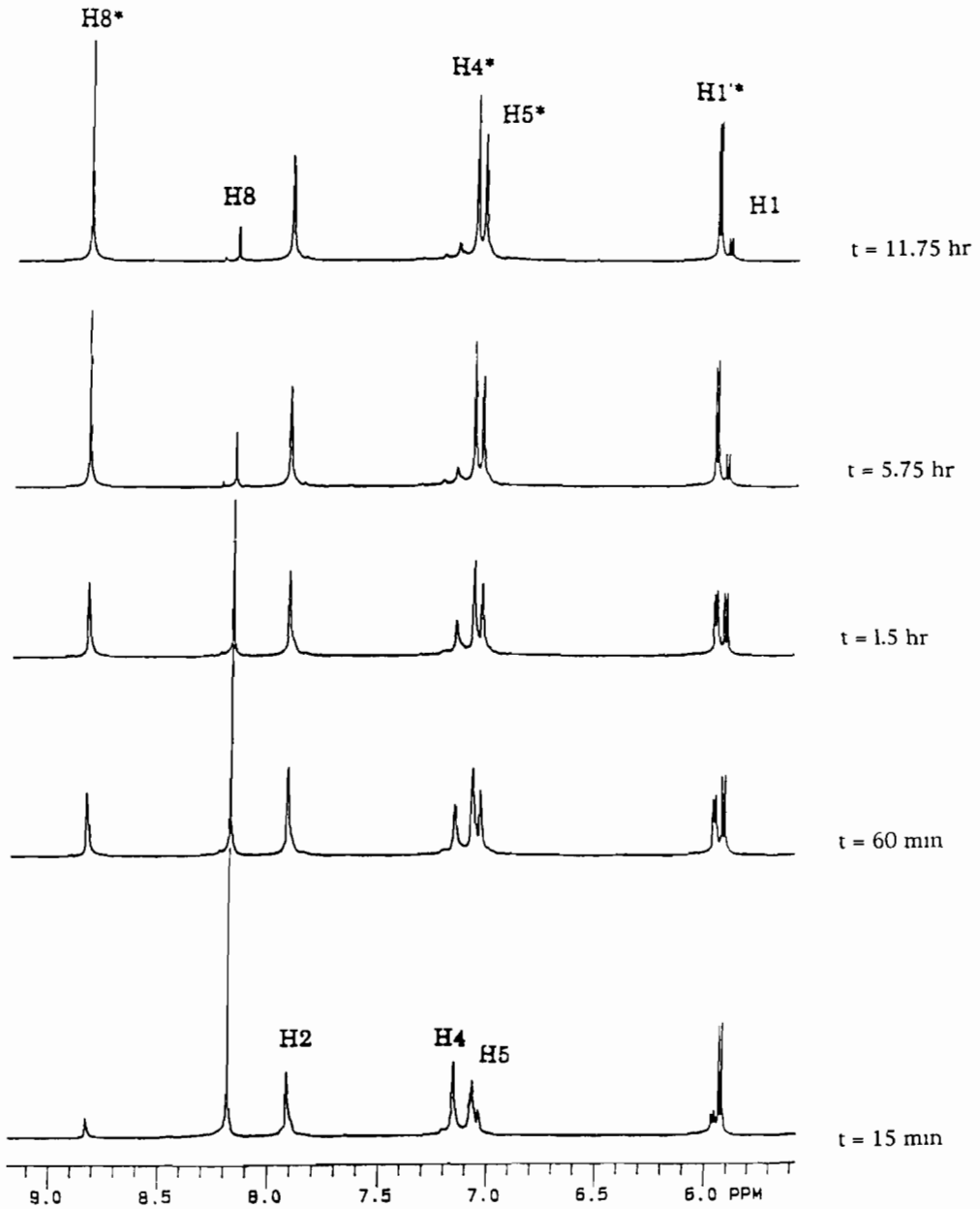


Fig. 4. Representative ^{1}H NMR kinetic data (with peak assignments), showing the downfield region for the reaction between *cis*-$[Pt(NH_3)_2(1\text{-MeIm-}N3)Cl]^+$ and 5′-GMP at various time intervals. Resonances marked with an asterisk designate ligand protons in the ternary complex.

to the non-lability of the chelating moiety. Thus, it is possible that the formation of stable Pt mediated DNA–protein crosslinks *in vivo* may involve sulfur containing amino acids which are capable of *trans* labilizing the coordinated amine ligand but not the chelating ethylenediamine. The chemistry of crosslink models with sulfur-containing amino acids is currently being studied in this lab.

Reactions of *cis*-$[Pt(NH_3)_2(N_\alpha\text{-BOC-L-His-}N3)Cl]^+$ with 5′-GMP and 3′-GMP parallel those of the $[Pt(NH_3)_2(1\text{-MeIm-}N3)Cl]^+$ isomers. The peak heights for H2 and H4 of **6b** decrease with time and are replaced by the corresponding signals for the ternary crosslink which appear about 0.2–0.3 ppm downfield. As one might expect, the slightly larger histidine-containing triamine complex reacts somewhat slower than the platinum imidazole species. Like the *trans*-imidazole complex (**4**), the histidine complex was found to react more rapidly with 5′-GMP than with 3′-GMP.

The reactions between *cis*- and *trans*-$[Pt(NH_3)_2(Guo\text{-}N7)Cl]^+$ and 1-MeIm or protected amino acids are in general much slower than those between $[PtAm_2(1\text{-}$

TABLE 3. Results of the rate determinations for the formation of the ternary complexes

Compound 1	Compound 2	k_2 (M^{-1} s^{-1})
trans-$Pt(NH_3)_2$(1-MeIm)Cl (**4**)	5′-GMP	0.358
trans-$Pt(NH_3)_2$(1-MeIm)Cl (**4**)	3′-GMP	0.235
trans-$Pt(NH_3)_2$(1-MeIm)Cl (**4**)	5′-dGMP	0.400
cis-$Pt(NH_3)_2$(1-MeIm)Cl (**3**)	5′-GMP	0 756
Pt(en)(1-MeIm)Cl (**5**)	5′-GMP	1.09
cis-$Pt(NH_3)_2$(Nα-BOC-L-His-*N3*)Cl^+ (**6b**)	5′-GMP	0.269
cis-$Pt(NH_3)_2$(Nα-BOC-L-His-*N3*)Cl^+ (**6b**)	3′-GMP	0.225

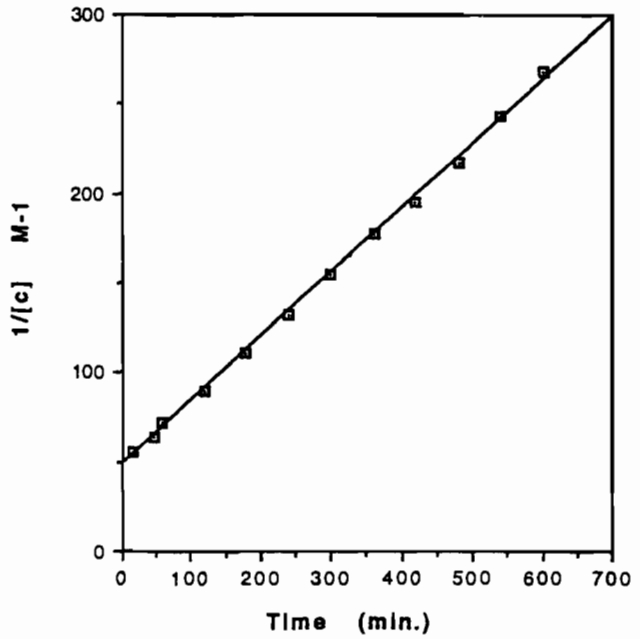


Fig. 5. Representative kinetics plot depicting the reciprocal concentration as a function of time for the reaction between *cis*-$[Pt(NH_3)_2$(1-MeIm-*N3*)Cl$]^+$ and 5′-GMP in D_2O.

MeIm-*N3*)Cl$]^+$ and the various nucleotides. After 5 days the ^{195}Pt NMR spectra displayed two broad peaks, at −2487 and −2498 ppm, growing in for the N-BOC-L-His and two peaks, at −2864 and −2935 ppm, for the N-BOC-L-Met. The ^{1}H NMR showed that several compounds were formed and that these reactions did not proceed cleanly to yield one product. HPLC chromatography showed that each of these mixtures consisted of several well resolved peaks. Isolation and identification of the various products has begun.

Biological studies

Complexes **3** and **4** were examined for cytotoxicity in murine L1210 leukemia cells sensitive to cisplatin; the ID_{50} for the complexes were determined to be 61 and 171 mM, respectively. These values are substantially higher than that of cisplatin (0.33 mM) and also of neutral complexes containing the 1-methylimidazole ligand [38]. It appears that although certain cationic platinum triamine complexes have demonstrated antitumor activity [27], it is unlikely that **3** or **4** will show significant *in vivo* cytotoxicity against leukemia cell lines.

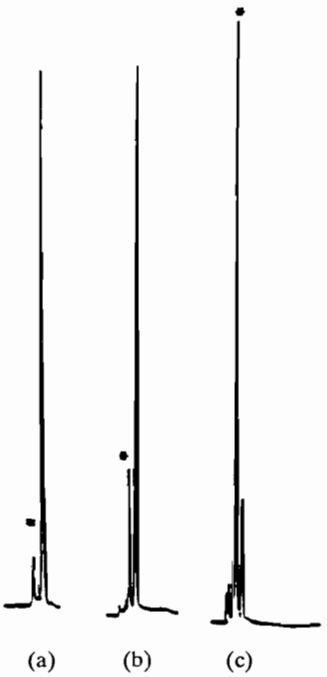


Fig. 6. HPLC chromatograms depicting the reaction between *trans*-$[Pt(NH_3)_2$(1-MeIm-*N3*)Cl$]^+$ and dATGCAT after (a) 28 min, (b) 3 h and (c) 23 h. Product peak marked with an asterisk.

Conclusions

This examination of the reaction of triamine complexes $[PtAm_2$(1-MeIm-*N3*)Cl$]^+$ with the mononucleotides 5′-GMP, 3′-GMP and 5′-dGMP shows that they readily form the ternary crosslinked complexes $PtAm_2$(1-MeIm-*N3*)(nucleotide). The 5′ nucleotides react faster than their 3′ counterparts. *cis*-$[PtAm_2$(1-MeIm-*N3*)Cl$]^+$ reacts faster than the *trans* isomer towards these mononucleotides and these imidazole complexes react faster than *cis*-$[Pt(NH_3)_2(N_\alpha$-BOC-L-His-*N3*)Cl$]^+$. Additional crosslink model studies involved both isomers of $[Pt(NH_3)_2$(Guo-*N7*)Cl$]^+$. Although these react with 1-methylimidazole to yield one product, their reaction was not as rapid as those of the triamine complexes $[PtAm_2$(1-MeIm-*N3*)Cl$]^+$. The N_α protected amino acids His and Met are even slower to react with the platinum triamine complexes of guanosine and lead to mixtures of ternary complexes. The faster means of forming crosslinks *in vitro*, and perhaps also *in vivo*, appears to be binding of Pt(II) to the amino acid first and subsequently to the nucleotide.

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