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Pyridine-Carboxylate Complexes of Platinum. Effect of *N,O*-Chelate Formation on Model Bifunctional DNA–DNA and DNA–Protein Interactions

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This paper reports on the chemistry of platinum complexes containing bidentate pyridine-carboxylate (pyAc = pyridin-2-yl-acetate and picEt = pyridine-2-ethylcarboxylate, ethylpicolinate) (*N,O*) ligands. The pyridine-2-acetate and ethylpicolinate ligands form six- and five-membered chelates, respectively, upon formation of the Pt–carboxylate bond. In all reactions with picEt with various platinum complex starting materials, spontaneous de-esterification of the pendant carboxylate ester occurs to give directly the chelates K[$PtCl_2$(pic-*N,O*)]-*trans*-[Pt(pic-*N,O*)$_2$] and *SP*-4,2-[PtCl(pic-*N,O*)(NH_3)] without any evidence of intermediates. The de-esterification is solvent dependent, and molecular modeling was used to explain this reaction. The reactions of the geometric isomers of [PtCl(pyAc-*N,O*)(NH_3)] with 5′-guanosine monophosphate, 5′-GMP, and *N*-acetyl-L-methionine, AcMet, were investigated by NMR spectroscopy. The objective was to ascertain by model chemistry the feasibility of formation of ternary DNA–Pt–protein adducts in biology. Model nucleotide and peptide compounds were formed in situ by chloride displacement giving [PtL(pyAc-*N,O*)(NH_3)]$^+$ (L = 5′-GMP or AcMet). Competitive reactions were then examined by addition of the complementary ligand L. Sulfur displacement of coordinated 5′-GMP was slow. For *SP*-4,3-[Pt(AcMet)(NH_3)(PyAc-*N,O*)]$^+$, a rapid displacement of the sulfur ligand by 5′-GMP was observed, giving *SP*-4,2-[Pt(5′-GMP-*N7*)(pyAc-*N,O*)(NH_3)]$^+$.

Introduction

The clinically used platinum anticancer drugs cisplatin, (*cis*-[$PtCl_2(NH_3)_2$], *cis*-DDP), carboplatin ([Pt(CBDCA)(NH_3)$_2$], CBDCA = 1,1-cyclobutanedicarboxylato), and oxaliplatin ([Pt(dach)(oxalato)] (dach = *R,R*-1,2-diaminocyclohexane) all belong to the general *cis*-[PtX_2(amine)$_2$] structure, where X is a leaving group such as chloride or dicarboxylate and the amine is a primary monodentate or bidentate amine or NH_3. The trans isomer of cisplatin, *trans*-[$PtCl_2(NH_3)_2$], is therapeutically inactive, but there is growing interest in chemical strategies aimed at the possible antitumor activity of complexes in the trans geometry. The synthesis of new complexes with cytotoxicity and antitumor activity complementary to the cisplatin class remains an important goal in platinum cancer chemotherapy.

Complexes containing a planar ligand such as pyridine, quinoline, or thiazole in a trans configuration show significant enhancement of the in vitro cytotoxicity over that of *trans*-[$PtCl_2(NH_3)_2$].[1] Use of the bidentate *N,O*-donor ligand pyridin-2-yl-acetate gives complexes such as *SP*-4,2-[PtCl(NH_3)(pyAc−N,O)], **2**, with enhanced aqueous solubility over the parent *trans*-[$PtCl_2(NH_3)$(L)] (L = pyridine, quinoline, etc.), Figure 1.[2] The compound **2** maintains good cytotoxicity in L1210 leukemia and is more potent than its

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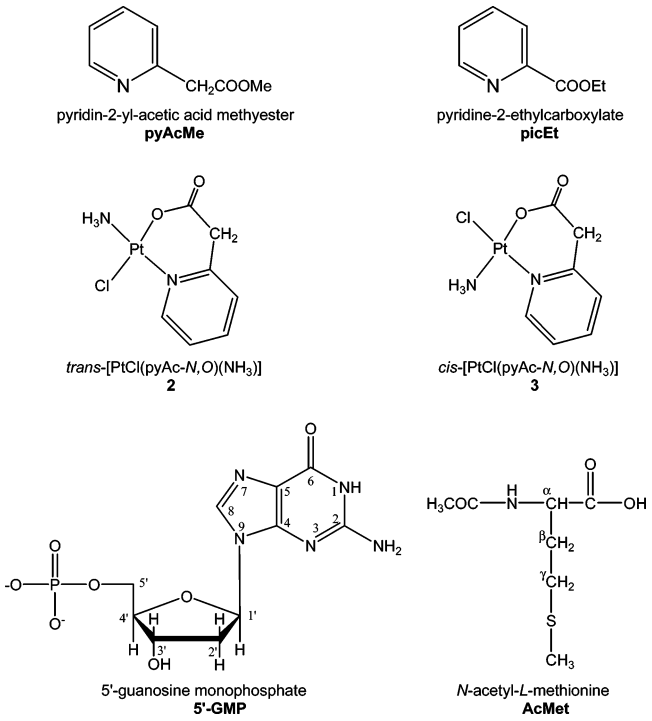

Figure 1. Structures of ligands and biological models used in this study. The previously described (see ref 2) pyridin-2-yl-acetate compounds are given as (**2**) and (**3**).

cis isomer *SP*-4,3-[PtCl(NH_3)(pyAc−N,O)], **3**. Recently, Sohn et al.[3] have used the ligands 2-picolinic (pic), nicotinic, and isonicotinic acids (isonic) to prepare complexes such as $[Pt(dmpda)(pic\text{-}N,O)]^+(pic)^-$ (dmpda = 2,2′-dimethyl-1,3-propanediamine), $[Pt(en)(pic\text{-}N,O)]^+(pic)^-$ (en = ethylenediamine), and $[Pt(dmpda)(isonic\text{-}N,N)_2]$, which show moderate in vitro cytotoxicity against mouse leukemia cell lines. These results demonstrate that the classical structure−activity relationships do not describe the full range of potentially useful platinum complexes and that the trans complexes have potential as antitumor agents.

Mechanistic work on *trans*-[$PtCl_2(NH_3)$L] (L = pyridine, quinoline, thiazole) has shown a greater propensity to form DNA−protein cross-links in cells in comparison to cisplatin.[4] The structures of the bifunctional DNA−DNA cross-links are distinct from those formed by cisplatin.[5] Further, the compounds induce protein-associated strand breaks and the formation of DNA−topoisomerase I compounds has been demonstrated in both human ovarian and murine leukaemia cell lines.[4] A possible explanation for this unique biological effect of platinum compounds is the formation of ternary Pt−DNA−protein cross-links. Model studies using *SP*-4,2-$[PtCl(9\text{-}EtGua)(NH_3)(quinoline)]^+$ showed a kinetic preference for methionine (DNA−protein model) over 5′-GMP (DNA−DNA model) binding.[6] It was therefore of interest to examine whether improved water-soluble trans compounds such as *SP*-4,2-[$PtCl(NH_3)$(PyAc-N,O)] behave in a chemical manner similar to that of the dichloride analogues.

This paper reports on the reactions of *SP*-4,2-[$PtCl(NH_3)$(pyAc-*N*,*O*)] **2**, and its geometric isomer *SP*-4,3-[$PtCl(NH_3)$-(pyAc-*N*,*O*)] **3**, (pyAc = pyridin-2-yl-acetate) with 5′-guanosine monophosphate, 5′-GMP, and *N*-acetyl-L-methionine, AcMet, investigated by NMR spectroscopy. The objective was to ascertain by model chemistry the feasibility of formation of ternary DNA−Pt−protein adducts in biology. The *N*-acetylated form of L-methionine was chosen to simulate protein-bound methionine and to prevent competitive reactions, such as *S*,*N*-chelation of platinum. The chemistry of systems containing the potentially bidentate ligand pyridine-2-ethylcarboxylate (2-ethylpicolinate, picEt) in a five-membered chelate ring was also explored.

Experimental Section

Materials. Pyridin-2-yl-acetic acid methylester (pyAcMe) and pyridine-2-ethylcarboxylate (ethylpicolinate, picEt) and other reagents and solvents were obtained from common vendors and used without prior purification. The new compounds *cis*-$[PtCl(NH_3)_2$-(pyAcH-*N*)]Cl (**1**) and *SP*-4,3-$[PtCl(NH_3)(pic\text{-}N,O)]$ (**5**) were isolated in this work for the first time. $K[PtCl_2(pic\text{-}N,O)]$ (**6**) was synthesized in a different method from the literature.[7,8] Cisplatin, $K[PtCl_3(NH_3)]\cdot H_2O$, and complexes **2**−**4** (*trans*-$[Pt(pic\text{-}N,O)_2]$) were synthesized as described in the literature.[2,7−10] *N*-acetyl-L-methionine (Sigma) was employed as the free acid, and 5′-guanosine monophosphate (Aldrich) was employed as a disodium salt.

Reactions Monitored by ^{1}H and ^{195}Pt NMR Spectroscopy. The hydrolysis reactions were carried out using a concentration of 2.5 mM in platinum complexes. The reactions of the complexes **2** and **3** with 5′-GMP and AcMet were carried out in 99.999% D_2O at room temperature (~22 °C) and at 37 °C with the following concentrations: 3 mM in platinum complexes, 3 and 6 mM in 5′-GMP and AcMet, respectively. The reactions involving AcMet were carried out at 37 °C. ^{1}H NMR spectra were recorded at the appropriate time intervals with 120 or 64 scans per time point. Quantification of the reactions was achieved by integration of signals of nonexchangeable protons (H8 in 5′-GMP). Rate constants, *k*, can be determined by the equation of the second-order rate law: $kt = x/[a_0(a_0 - x)]$, in which a_0 is the initial concentration of 5′-GMP and *x* is the concentration at the time *t*. The plot of $x/[a_0(a_0 - x)]$ versus *t*, designated by a second-order Guggenheim plot, gives *k* from the slope.[11] The values of *k* for the reactions between complexes **2** and **3** with 5′-GMP are presented in Table 3. Half-times can be calculated from the equation $t_{1/2}=1/a_0k$.

Molecular Modeling. Restricted Hartree−Fock semi-empirical minimizations were performed on the platinum drugs using the PM3 formalism in MOPAC2002.[12] The calculation was continued until the gradient norm requirement dropped to below 0.01 kcal/ mol.

Synthesis. (a) *cis*-$[PtCl(NH_3)_2(pyAcH\text{-}N)]Cl$ (1). A mixture of 3.58 g (11.92 mmol) of cisplatin and 5.40 g (35.76 mmol) of pyridin-2-yl-acetic acid methyl ester in 200 mL of water was heated

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Table 1. ^{1}H NMR Data of Free Pyridin-2-yl-acetic Acid Methylester and Ethyl Picolinate and of the Complexes **1**−**3, 5**, and **6**

compound	chemical shift (ppm)							
	H3	H4	H5	H6	CO_2H	CH_2	NH_3	CH_3
pyridin-2-yl-acetic acid methyl ester[a]	7.40 m	7.86 t	7.40 m	8.46 d		4.83 d		3.94 s
$[PtCl(NH_3)_2(pyAcH\text{-}N)]Cl$ **1**[b]	7.71 d	8.00 t	7.47 t	9.04 d	13.12	4.59−5.14 m		
SP-4,2-$[PtCl(pyAc\text{-}N,O)(NH_3)]$ **2**[b]	7.59 d	8.06 t	7.47 t	8.89 d		4.04 s	4.50 b	
SP-4,3-$[PtCl(pyAc\text{-}N,O)(NH_3)]$ **3**[c]	7.75 d	8.18 t	7.58 t	8.75 d		4.08 s	4.59 b	
ethyl picolinate[c]	8.10 m		7.75 m	8.83 d		4.45 q		1.43 t
cis-$[PtCl(pic\text{-}N,O)(NH_3)]$ **5**[b]	7.82 m	8.39 t	7.82 m	8.76 d			4.81 b	
$K[PtCl_2(pic\text{-}N,O)]$ **6**[b]	o	8.27 t	7.71 t	9.21 d				

[a] In D_2O. [b] In DMF-d_7. [c] In DMSO-d_6; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad; o, overlapped with the solvent signal.

at 90−100 °C for 2 h. To the solution was added 36 mL of concentrated HCl and heating was continued for 5 h. Concentration of this solution to a volume of 40 mL and cooling gave **1** as a pale yellow solid, which was filtered off and washed with EtOH and diethyl ether and then recrystallized from DMF/Et_2O and dried in a vacuum. Yield = 1.79 g (34%). IR: ν(C═O) 1713, ν(Pt−N_{amino}) 399, 384 (similar intensity), ν(Pt−Cl) 336 cm^{-1}. Anal. Calcd for $C_7H_{13}N_3Cl_2O_2Pt$: C, 19.22; H, 2.97; N, 9.61; Cl, 16.25. Found: C, 19.50; H, 2.98; N, 9.65, Cl, 16.07.

(b) *trans*-[Pt(pic-*N*,*O*)$_2$] (4). A mixture of 1.2 g (4 mmol) of cisplatin and 1.62 mL (12 mmol) of ethylpicolinate in 60 mL of water was heated at 90−100 °C overnight to produce a yellow precipitate, which was filtered off and washed with EtOH and diethyl ether and dried in a vacuum. Yield = 1.58 g (90%). IR: $\nu_{as}(CO_2)$ 1688 cm^{-1}. Anal. Calcd for $C_{12}H_8N_2O_4Pt$: C, 32.79; H, 1.82; N, 6.38;. Found: C, 32.76; H, 1.89; N, 7.00.

(c) *SP*-4,3-[PtCl(NH$_3$)(pic-*N*,*O*)] (5). A mixture of 0.135 g (1 mmol) of ethylpicolinate and 0.376 g (1 mmol) of $K[PtCl_3(NH_3)]\cdot H_2O$ was stirred in 3 mL of water at room temperature for 1 h. An orange precipitate formed which was filtered off, washed with water, EtOH, and Et_2O, and dried in a vacuum. Yield = 0.23 g (63%). IR: $\nu_{as}(CO_2)$ 1672, ν(Pt−Cl) 321, ν(Pt−N_{amino}) 419 cm^{-1}. Anal. Calcd for $C_6H_7N_2ClO_2Pt$: C, 19.48; H, 1.89; N, 7.57. Found: C, 19.50; H, 1.81; N, 7.41.

(d) K[PtCl$_2$(pic-*N*,*O*)] (6). A red solution of 0.415 g (1 mmol) of K_2PtCl_4 and 0.270 g (2 mmol) of ethylpicolinate in 5 mL of water was stirred at r.t. for 1 day to give an orange precipitate which was filtered off and washed with EtOH and Et_2O to give **6**. It was dried in a vacuum. Yield = 0.07 g (16%). IR: $\nu_{as}(CO_2)$ 1656, ν(Pt−Cl) 316, 356 cm^{-1}. Anal. Calcd for $C_6H_4NCl_2O_2KPt$: C, 16.86; H, 0.94; N, 3.28; Cl, 16.63. Found: C, 17.99; H, 1.00; N, 3.42, Cl, 16.26.

Physical Measurements. ^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer equipped with a 5 mm AutoSwitchable four-nucleus probe, variable temperature unit, and software for arrayed kinetic experiments. Chemical shifts were referenced to 3-trimethylsilylpropionic acid, sodium salt, used as internal standard. ^{195}Pt NMR spectra were recorded on a Varian Mercury 300 MHz NMR Spectrometer using a 10 mm broadband probe. ^{195}Pt spectra were referenced to $Na_2[PtCl_6]$. The frequency for ^{195}Pt nuclei was set at 64.32 MHz. The ^{1}H chemical shifts of new platinum complexes **1**−**3**, **5**, and **6** are given in Table 1. IR spectra were measured as KBr and CsI pellets on a Nicolet Nexus 670 FTIR spectrometer. Microanalyses were performed by QTI, Whitehouse, NJ.

Results and Discussion

Synthesis. The synthesis of complexes **2** and **3** has been described.[2] The preparative method for **2** from *cis*-$[PtCl_2(NH_3)_2]$ involves the use of the ester pyAcMe and isolation of the intermediate, *trans*-$[PtCl_2(NH_3)(pyAcH\text{-}N)]\cdot H_2O$ (**A**).

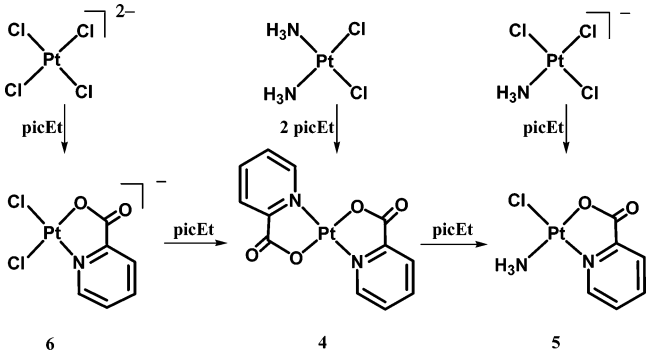


Figure 2. Structures of pyridine-2-carboxylate compounds, as well as their inter-relationships, formed from various platinum complex starting materials.

Chloride displacement and chelate closure occurs under acidic conditions. In this work, the re-examination of the preparation of **2** and modification of the conditions afforded another intermediate, *cis*-$[PtCl(NH_3)_2(pyAcH\text{-}N)]Cl$, (**1**) before the isolation of (**A**). The compound was isolated and characterized by NMR, IR, and elemental analysis, and its isolation confirmed the mechanistic pathway proposed for formation of **2**.[2]

The pyridine-2-acetate ligand forms six-membered chelates upon formation of the Pt−carboxylate bond. In principle, bifunctional substitution involves chloride displacement and opening of the chelate ring by cleavage of the Pt−O (carboxylate) bond. To examine the effect of ring size on the kinetics of possible bifunctional substitution, the synthesis of five-membered analogues of **2** and **3** was attempted. In this case, the starting ligand used was pyridine-2-ethylcarboxylate (ethylpicolinate, picEt). The products isolated are summarized in Figure 2.

In a similar synthesis to that of **2**, the reaction of picEt with *cis*-$[PtCl_2(NH_3)_2]$ afforded in high yield as the unique product the water-insoluble *trans*-$[Pt(pic\text{-}N,O)_2]$ (**4**) and no intermediates were observed or isolated. The five-membered chelate analogue of complex **3**, *SP*-4,3-$[PtCl(NH_3)(pic\text{-}N,O)]$, (**5**) was isolated through the reaction between the $[PtCl_3(NH_3)]^-$ anion and picEt. Again, no intermediate complex could be isolated, in contrast with the synthesis of **3.** Similarly, reactions using K_2PtCl_4 with picEt in water in the stoichiometric ratio of 1:2 gave an insoluble product analyzing for complex $K[PtCl_2(pic\text{-}N,O)]$ (**6**). In the presence of further equivalents of ligand, other products are observed but the final product was eventually complex **4**, and the reaction was not examined in detail. We note that **4** and **6** have been described very briefly in the literature[7,8]—in the absence of extensive data, we choose the trans configuration

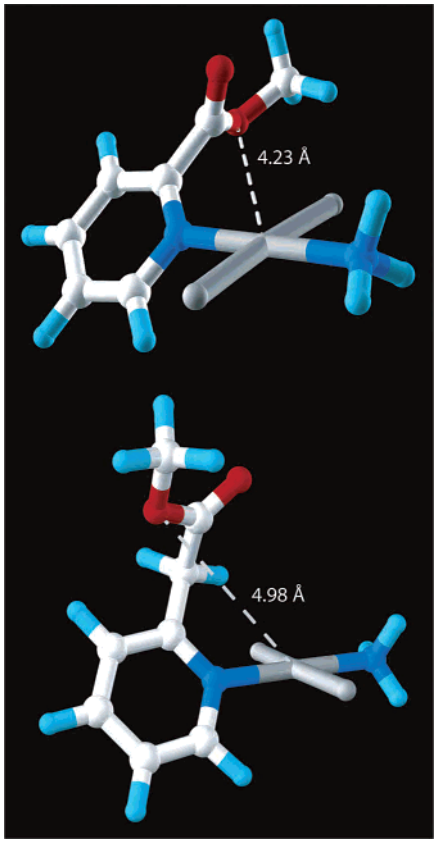


Figure 3. Molecular models of *trans*-[$PtCl_2(NH_3)$(pyr-R)] where pyr-R = pyridine-2-carboxylate, (five-membered chelate ring formation, top) and pyr-R = pyridin-2-yl-acetate (six-membered chelate ring formation, bottom).

to describe because we expect this to be the thermodynamically stable isomer.

Thus, in all reactions with picEt, spontaneous de-esterification of the pendant carboxylate ester occurs.

It was of interest to examine the origins of this difference in reactivity between the pyAcMe and picEt ligands. Molecular models of the model compounds *trans*-[$PtCl_2(NH_3)$(L)] where L = PyAcMe or PicEt were developed, and the minimized structures are shown in Figure 3. As expected, the more rigid ligand picEt gives shorter Pt−oxygen distances (OCH_3 of carboxylate group, 4.23 versus 4.98 Å) with the carboxylate ligand situated in the axial position, presenting a reasonable explanation for the susceptibility to hydrolysis. While the Pt−O distance is still too long for direct contact, a water- or solvent-mediated attack would be feasible for the picEt ligand, whereas the pyridin-2-yl-acetate ligand is more flexible and less likely to maintain a "fixed" axial position relative to platinum.

Mechanistic Studies of Pyridin-2-yl-acetate Compounds with Biologically Relevant Ligands. (a) Reaction of *SP*-4,2-[PtCl(NH_3)(pyAc-*N*,*O*)] (2) and *SP*-4,3-[PtCl(NH_3)(pyAc-*N*,*O*)] (3) with 5′-GMP. In the absence of structural analogues of both **2** and **3**, and as most compounds involving the pic-*N*,*O* ligand were only sparingly water-soluble, their reactions with biologically relevant ligands were not pursued. Mechanistic studies therefore focused on "trans" and "cis" isomers of [PtCl(NH_3)(pyAc-*N*,*O*)], **2** and **3.** Previous

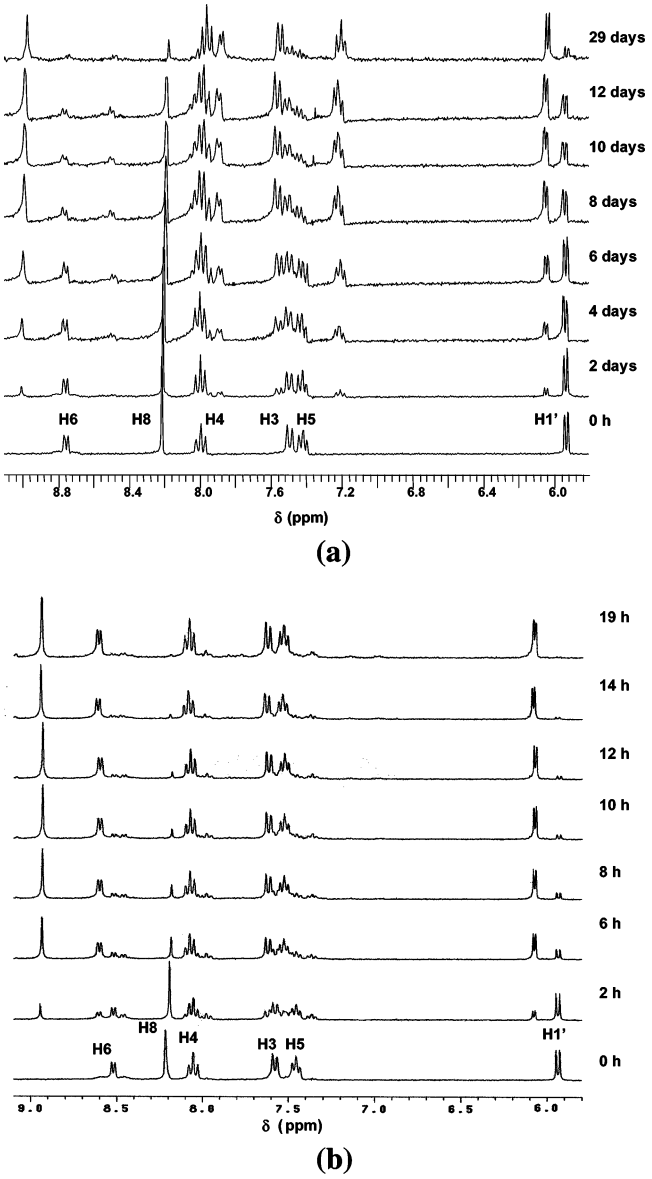


Figure 4. Evolution of the reaction between **2** (a) and **3** (b) with 5′-GMP at room temperature followed by ^{1}H NMR spectroscopy.

observations had shown that nucleotide (N7 of 5′-GMP) binding to **2** and **3** occurred without chelate ring cleavage to produce "monoadducts" on DNA.[6] The kinetics of these reactions were examined in more detail. Parts a and b of Figure 4 show the evolution of the reaction of **2** and **3** with 5′-GMP, respectively, followed by ^{1}H NMR spectroscopy.

The ^{1}H and ^{195}Pt NMR chemical shifts of the products of reaction of the platinum complexes with 5′-GMP, complexes **I** and **II**, are given in Table 2. As previously, the nature of the pyridine ligand binding can be assessed by examination of H6 and the $-CH_2-$ protons, where the latter can distinguish between monodentate and chelate binding modes.[2] In the reaction of **2** with 5′-GMP, changes in chemical shifts of the H1′ and H8 protons of 5′-GMP were observed, from 5.9 to 6.0 ppm and from 8.2 to 9.0 ppm, respectively. These results are in accordance with the coordination of 5′-GMP to the platinum centers, as well as the shifts of the $pyAc^-$ signals. The major shifts on the $pyAc^-$ protons are observed

Table 2. ^{1}H and ^{195}Pt NMR Data of Adducts **I**−**IV**[a]

compound	chemical shift (ppm)							
	H3	H4	H5	H6	H8	H1′	SCH_3	^{195}Pt
SP-4,2-[Pt(5′-GMP-*N*7)(pyAc-*N*,*O*)(NH_3)]$^-$ **I**	7.56 d	8.06 t	7.20 t	7.88d	8.98 s	6.04d		−2098
SP-4,3-[Pt(5′-GMP-*N*7)(pyAc-*N*,*O*)(NH_3)]$^-$ **II**	7.66 d	8.18 t	7.53 t	8.61 d	8.93 s	6.07 d		−2120
SP-4,2-[Pt(pyAc-*N*,*O*)(AcMet-*S*)(NH_3)]$^+$ **III**	7.63 d	8.11 t	7.54 t	8.63 d			2.56 s	−2823
SP-4,3-[Pt(pyAc-*N*,*O*)(AcMet-*S*)(NH_3)]$^+$ **IV**	7.67 d	8.12 t	7.39 t	8.50 d			2.44s	−2704

[a] In D_2O; s, singlet; d, doublet; t, triplet. All species **I**−**IV** were formed and studied in solution only.

Table 3. Half-lives, in Hours, of the Disappearance of Free 5′-GMP and Values of K, in M^{-1} s^{-1}, for the Reactions between Complexes **2** and **3** and 5′-GMP at Room Temperature and at 37 °C

	reaction of **2**		reaction of **3**	
	r. t.	37 °C	r. t.	37 °C
$t_{1/2}$ (free 5′-GMP)	220	48	3.4	0.8
$t_{1/2}$ (=$1/a_0k$)	199	50	2.8	0.8
k (s^{-1})	4.63×10^{-4}	1.85×10^{-3}	3.3×10^{-2}	1.1×10^{-1}

for the H5 and H6 protons, from 7.4 to 7.2 ppm and from 8.8 to 7.9 ppm, respectively. The complex obtained can be described as *SP*-4,2-[Pt(5′-GMP-*N*7) (NH_3) (pyAc-*N*,*O*)]$^+$ **I** in agreement with the ^{195}Pt NMR signal at −2098 ppm, characteristic of a N_3O coordination sphere.[2] For the reaction of **3** with 5′-GMP, downfield shifts were observed in the resonances of H1′ and H8 of 5′-GMP, from 5.9 to 6.1 ppm and from 8.2 to 8.9 ppm, respectively, consistent with formation of *SP*-4,3-[Pt(5′-GMP-*N*7) (NH_3) (pyAc-*N*,*O*)]$^+$ (**II**). The ^{195}Pt NMR signal observed for this species was −2120 ppm. The shifts observed for the $pyAc^-$ protons are smaller than the shifts observed for the trans isomer, from 7.4 to 7.5 ppm for H5 proton and from 8.5 to 8.6 for H6 proton.

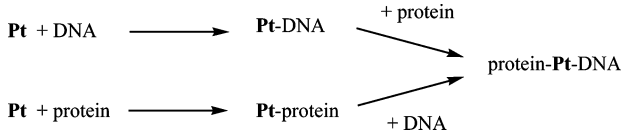
The representation of the percentage of the integrals of the 5′-GMP H8 free proton signals relative to the total value, versus time, gives the half-times of the disappearance of free 5′-GMP or of the formation of **I** or **II**. These values are presented in Table 3 and are designated by $t_{1/2}$ (free 5′-GMP). The very slow reaction of 2 suggests direct attack of 5′-GMP, without a previous hydrolysis step, as has been suggested in the analogous reaction of *SP*-4,2-[PtCl(9-EtGua-*N7*)(NH_3)quinoline]$^+$,[13] carboplatin,[14] [PtCl(en)(tmtu)]$^+$ (en = ethane-1,2-diamine, tmtu = 1,1,3,3-tetramethylurea), and [PtCl(dach)(tmtu)]$^+$ (dach = 1,2-diaminocyclohexane).[15] At 37 °C, the reaction is faster and was complete after 21 days.

(b) Reaction of *SP*-4,2-[PtCl(NH_3)(pyAc-*N*,*O*)] (2) and *SP*-4,3-[PtCl(NH_3)(pyAc-*N*,*O*)] (3) with AcMet. To understand the formation and reactivity of possible ternary DNA−Pt−protein complexes (see below), the reactions of **2** and **3** with *N*-acetylmethionine were examined. Spectral changes consistent with formation of platinum−methionine species were observed. The ^{1}H NMR spectrum of free AcMetH shows two singlets at 2.04 and 2.11 ppm corresponding to the $-COCH_3$ and $S-CH_3$ protons, respectively. Upon platination, the latter signals are shifted downfield compared to the free ligand. Platination of the thioether group produces a center of chirality on the sulfur. Diastereomers are possible whose presence may be detected by a multiplicity of NMR signals as, for example, in [PtCl(NH_3)(quinoline)-(AcMet)]$^+$ and [Pt(NH_3)$_2$(Met)$_2$].[13,16] In the cases studied here, no multiplicity was observed indicating that interconversion is too fast on the NMR time scale or that ^{1}H NMR spectroscopy is not sensitive enough in the present case to distinguish between the diastereomers. The ^{1}H and ^{195}Pt NMR chemical shifts of the products of reaction of the platinum complexes with AcMet, complexes **III** and **IV**, are given in Table 2.

In the first ^{1}H NMR spectrum registered after the addition of AcMet to **2**, the intensity of the 2.1 ppm signal assigned to the free SCH_3 decreased and a new signal at 2.6 ppm indicates that AcMet coordinates to platinum through the *S* atom. Small shifts were observed in the other signals of the AcMet protons. There was an increase in the number of signals in the $pyAc^-$ and AcMet protons region. The intensity of the H6, H4, H3, and H5 resonances of the $pyAc^-$ ligand decreased, and new signals at 8.6, 8.1, 7.6, and 7.5 ppm appeared. The chemical shift of the CH_2 protons indicates that the chelate ring (*N*,*O*)-Pt is maintained. The ^{195}Pt NMR signal observed for this species is −2823 ppm. In this way, the complex obtained was *SP*-4,2-[Pt (AcMet-*S*)(NH_3)(pyAc-*N*,*O*)]$^+$ (**III**). After 2 h, the reaction of **3** with AcMet was finished with formation of the complex *SP*-4,3-[Pt(AcMet-*S*)(NH_3)(pyAc-*N*,*O*)]$^+$ (**IV**), which shows a ^{195}Pt chemical shift at −2704 ppm. The coordination of AcMet was also confirmed by a new signal at 2.4 ppm, assigned to the SCH_3 coordinated to platinum. The H4 and H5 proton signals of the $pyAc^-$ ligand shifts from 8.0 and 7.4 ppm to 8.1 and 7.6 ppm, respectively. The other resonances did not shift significantly. The differences in the rate of the reactions with AcMet and 5′-GMP can be explained by the strong affinity of sulfur atoms to Pt^{2+} ions. This is consistent with other related studies involving platinum complexes and nucleophiles containing sulfur.[17,18] In neither case did excess of AcMet produce an observable bis(methionine) species.

Comparative Studies. DNA-Protein Formation. In principle, DNA−Pt−protein adducts could arise in two ways (Scheme1):

Scheme 1



These pathways were examined by model reactions. Upon addition of AcMet to a solution of **I**, spectral changes

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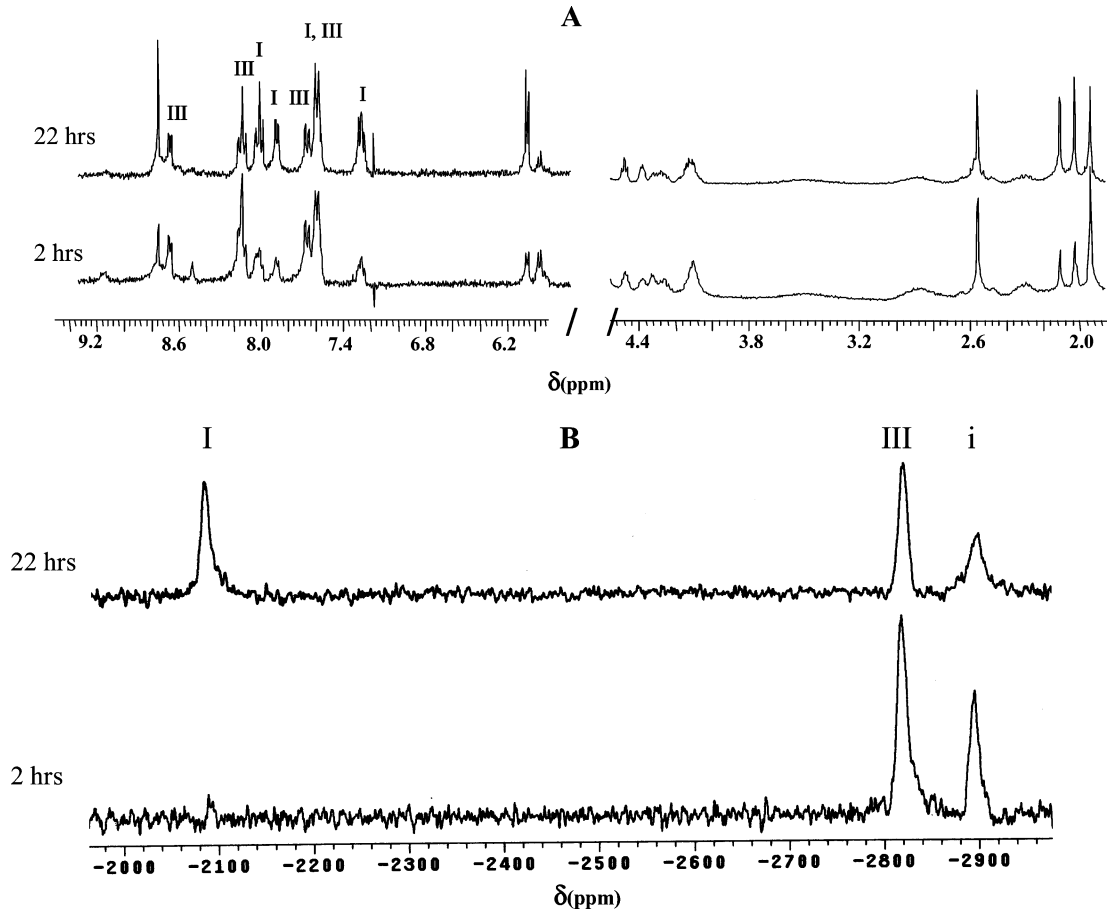

Figure 5. ^{1}H (A) and ^{195}Pt (B) NMR spectra of the reaction of *SP*-4,2-[Pt(AcMet-*S*)(pyAc-*N*,*O*)(NH_3)]$^{+}$ (**III**) with 5′-GMP after 2 (down) and after 22 h (up). The ^{195}Pt NMR spectrum shows the presence of an intermediate, i, whose chemical shift is consistent with the presence of bound methionine. See Scheme 2.

consistent with Pt−S binding occur with the appearance of a new signal at 2.6 ppm indicative of Pt−S binding. ^{195}Pt NMR spectral changes with a new signal at −2893 ppm are also consistent with Pt−S bond formation. However, the reaction is very slow and after 5 days the percent of product is still only 42%. Platinum chemical shifts can often change systematically upon ligand substitution in well-defined series such as $[PtX_n(amine)_{4-n}]^{(2-n)+}$.[19] Sulfur binding tends to give a wider range of chemical shifts, and systematic changes are not as easily noted—nevertheless, the ^{195}Pt chemical shift is consistent with an N_3S coordination sphere with cleavage of the Pt−O bond and formation of [Pt(AcMet-*S*)(5′-GMP)(NH_3) (pyAc-*N*)]$^{2+}$ (**V**). In the case of **II**, parallel spectral changes occur with the appearance of the Pt−SMet signal at 2.3 ppm and the ^{195}PtNMR signal at −2721 ppm within the first 3 h of reaction. However, as the reaction proceeds, some free pyridin-2-yl-acetate ligand, as well as free 5′-GMP, is observed. The ^{195}Pt chemical shift is similar to the N_2OS coordination sphere of **IV**. Release of free ligand and 5′-GMP could reasonably be ascribed to initial displacement of the pyridine nitrogen by sulfur followed by translabilization of the 5′-GMP. Regardless, the reaction appears to be quite complicated and was not pursued in detail.

Reactions of *SP*-4,2-[Pt (AcMet-S)(NH_3)(pyAc-N,O)]$^{+}$ (III) with 5′-GMP. The reaction of an equimolar quantity of 5′-GMP with a solution containing *SP*-4,2-[Pt(AcMet-*S*)(NH_3)(pyAc-*N*,*O*)]$^{+}$ (**III**) gave after 20 min at least two species indicative of coordinated 5′-GMP in the 8.6 ppm region. Two hours later, the amounts of these two species were equal and a peak at 2.11 ppm assigned to the SCH_3 of free AcMet was clearly visible (Figure 5A).

After 22 h, only one species with coordinated 5′-GMP was detected along with a small amount of free 5′-GMP. The ^{1}H NMR spectrum then remained essentially unchanged even after 14 days. Concomitant with the appearance of coordinated 5′-GMP, free AcMet is clearly visible. In the aromatic region of the ^{1}H NMR spectrum, while complicated, two groups of signals were observed, one assigned to *SP*-4,2-[Pt(5′-GMP-*N*7) (NH_3) (pyAc-*N*,*O*)]$^{+}$ complex **I**. The formation of **I** is confirmed by the ^{195}Pt NMR spectrum where the product has a chemical shift in the −2100 ppm region. Scheme 2 shows the possible reaction between *SP*-4,2-[Pt(AcMet-*S*)(NH_3)(pyAc-*N*,*O*)]$^{+}$ and 5′-GMP. The data are best interpreted as two competing reactions. The ternary DNA−protein intermediate in this case corresponds to [Pt(5′-GMP-*N*7)(pyAc-*N*)(AcMet-*S*)(NH_3)]$^{2+}$. The presence of

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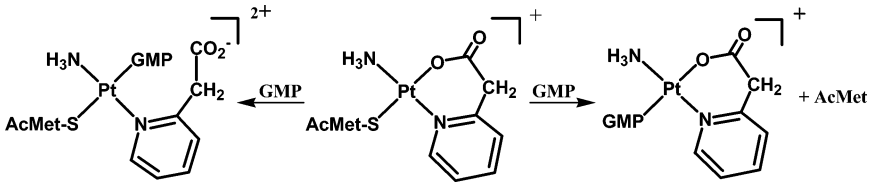
Scheme 2



this intermediate is supported by the ^{1}H NMR and ^{195}Pt NMR ($\delta = -2895$ ppm). The signal observed in the reaction between *trans*-Pt-5′-GMP and AcMet (**V**, −2893 ppm, (i, Figure 5B)) is very close to the −2895 ppm observed here.

Thus, evidence of Pt−DNA−protein ternary complex formation was seen in both model pathways but the species are quite reactive. Although no reaction was observed between *SP*-4,2-[Pt(AcMet-*S*)(NH_3)(pyAc-*N*,*O*)]$^{+}$ and AcMet, a reaction with 5′-GMP was observed, possibly due to the major thermodynamic stability of the 5′-GMP adduct. It is also possible that a scheme such as Scheme 2 for formation of a bis(methionine) species could be in rapid exchange with the chelate formation, inhibiting observation of the intermediate species in this case. The methionine substitution by 5′-GMP has a crucial importance because, even considering the formation of adducts with thioether ligands, these are easily substituted by 5′-GMP giving the thermodynamically stable Pt-5′-GMP adduct, a model for the Pt−DNA adduct responsible for the anticancer activity. The substitution of AcMet by 5′-GMP in platinum complexes has been explained by the π-withdrawal properties of the thioether groups, which are substituted by guanine-N7 bases. Lempers et al consider that the intermolecular rearrangement of Pt−thioether has important biological implications assuming a drug reservoir mechanism, in which those adducts are good intermediates for the Pt−DNA linkage.[20] Other examples of substitution of ligands containing sulfur by guanine bases are described in the literature and include [Pt(dien)(Met)]$^{2+}$,[21] [PtCl(dien)]$^{+}$,[22] [PtCl(en)(MeCO-Met-*S*)]$^{+}$ (en = etane-1,2-diamine),[23] [Pt(dien)(GSMe)]$^{2+}$, and *cis*-[Pt(NH_3)$_2$(GSMe)$_2$]$^{2+}$ complexes.[24] The reaction reported here appears considerably faster than for the [Pt(dien)(Met)]$^{2+}$ case where a half-life of 167 h is reported.[21] In the reaction between *trans*-[Pt(AcMet)(5′-GMP)(NH_3)quinoline]$^{2+}$ and excess 5′-GMP, there was no further substitution of AcMet.[6] This fact was explained by the presence of sterically hindering planar ligands.

The competitive reactions between **I**−**IV** and free 5′-GMP and *N*-AcMet may be compared with that previously studied for the system [PtCl(NH_3)(quinoline)(9-EtGua)]$^{+}$. In that case, displacement of chloride by AcMet was approximately four times faster than for 5′-GMP with an approximate half-life of 3.5 h for the sulfur ligand.[6] Further addition of excess AcMet to [Pt(AcMet)(NH_3)(quinoline)(9-EtGua)]$^{2+}$ produced some bis(methionine) species. The chloride displacement reaction of *SP*-4,2-[PtCl(AcMet)(NH_3)(quinoline)]$^{+}$ with 5′-GMP to produce the "ternary" species containing both AcMet and 5′-GMP was extremely fast due to the high trans influence of sulfur-bound methionine.[6] In the present case studied here, the chelate effect appears to remain dominant and production of ternary DNA−Pt−protein species is not significant, at least from this model chemistry. It remains to be clarified how protein-assisted DNA strand breaks are induced by *trans*-[PtCl$_2$(NH_3)(planar ligand)], but in agreement with the comparison of model studies discussed here, neither **2** nor **3** produce strand breaks in L1210 leukemia cells. The studies presented here indicate that it is likely that cytotoxicity of **2** and **3** is best mediated through monofunctional DNA binding. Nevertheless, the range of *trans*-Pt-mediated adducts giving rise to cytotoxicity is remarkably diverse.

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