Modification of Platinum(II) Antitumor Complexes with Sulfur Ligands. 2. Reactivity and Nucleotide Binding Properties of Cationic Complexes of the Types [PtCl(diamine)(L)]NO_3 and [{PtCl(diamine)}$_2$(L-L)]$(NO_3)_2$ (L = Monofunctional Thiourea Derivative; L-L = Bifunctional Thiourea Derivative) in Relation to Their Cytotoxicity

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The reactions of [PtCl(en)(tmtu)]NO_3 (**1**) and [PtCl(dach)(tmtu)]NO_3 (**2**) (en = 1,2-ethanediamine, dach = racemic *trans*-1,2-cyclohexanediamine, tmtu = 1,1,3,3-tetramethylthiourea) and [{Pt(en)Cl}$_2${μ-C_2H_4(NMeCSNMe$_2$)$_2$-*S*,*S*′}]$(NO_3)_2$ (**3**) and [{Pt(en)Cl}$_2${μ-C_6H_{12}(NMeCSNMe$_2$)$_2$-*S*,*S*′}]$(NO_3)_2$·0.5EtOH (**4**) with 5′-GMP and *r*(GpG) and their chemistry in aqueous solution have been investigated by ^{1}H and ^{195}Pt NMR spectroscopy. **1** and **2** only form the monofunctional adducts [Pt(en)(5′-GMP-*N7*)(tmtu)] (**I**) and [Pt(dach)(5′-GMP-*N7*)(tmtu)] (**II**), irrespective of an excess of free nucleotide. ^{195}Pt NMR chemical shifts of −3003 and −2982 ppm, respectively, confirm a [N_3S] mixed-donor environment of platinum. The bulky tmtu ligand in **1** and **2** decreases the rate of hydrolysis of the Pt−Cl bond and the rate of nucleotide binding compared to analogous reactions for cisplatin and structural analogues. The dinuclear complexes **3** and **4** exhibit an unusual rapid *intra*molecular disproportionation in solution ($t_{1/2}$ = 2.5 and 12 h, respectively) which yields [$PtCl_2$(en)] and [Pt(en)(L-L)]$^{2+}$ (L-L = chelating bifunctional thiourea derivative; δ_{Pt} −3454 with L-L = C_2H_4(NMeCSNMe$_2$)$_2$). Accordingly, **3** forms the mononuclear adducts [Pt(en)(5′-GMP-*N7*)$_2$] (**III**) and [Pt(en){*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**IV**). Due to the considerably slower rate of decomposition, **4** gives both the dinuclear adduct [{Pt(en)}$_2${μ-C_6H_{12}(NMeCSNMe$_2$)$_2$}{μ-*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**V**) (70%) and **IV** (30%). The 5′ sugar residue of *r*(GpG) in **IV** exhibits an N-type conformation, as commonly observed in bifunctional adducts that are formed between Pt(II) antitumor complexes and dinucleotides. The absence of this structural feature in **V** supports the formation of a conformationally less restricted macrochelate. Cytotoxicity data for **1**−**4** in L1210 leukemia are in accordance with the nucleotide-binding properties of **1** and **2** and the aqueous solution chemistry of the dinuclear compounds **3** and **4**. The results indicate that structurally modified thiourea ligands may be interesting for their use as alternative, strongly coordinating carrier groups in platinum(II) antitumor complexes.

Introduction

The continued interest in platinum-based antitumor compounds is stimulated by the fact that certain tumors are resistant to the clinically used drug cisplatin (*cis*-diamminedichloroplatinum(II), *cis*-DDP). The rationale behind the design of new ("third generation") drugs is that their novel structural features would result in an alternative mechanism of action distinct from that of *cis*-DDP and its direct analogues.[1] Second-generation drugs such as carboplatin[2] (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) and [$PtCl_2$(dach)][3] (dach = *trans*-1,2-cyclohexanediamine) form the same type of DNA adducts as *cis*-DDP and thus are unlikely to overcome resistance that is associated with an increased repair of these adducts.

To reduce cisplatin resistance and widen the overall spectrum of activity, nonclassical platinum(II) antitumor compounds have been developed. Among these are mononuclear and dinuclear complexes of general formulas [PtCl(diamine)(sulfoxide-*S*)]$^+$[4,5] and [{PtCl$(NH_3)_2$}$_2$-μ-{$H_2N(CH_2)_nNH_2$}]$^{2+}$.[6] Complexes that carry a sulfoxide ligand instead of the second chloride in the classical compounds show enhanced cytotoxicity in cisplatin-resistant cell lines. These findings have been attributed to an altered metabolism (reactivity and cellular uptake) which may be related to the hydrolytic behavior of the Pt−S bond and the stereochemical requirements of the sulfur ligand.[4] Model studies on the DNA-binding properties, however, imply that such species form *cis*-DDP-like adducts on DNA.[7] It can be concluded that after loss of chloride and sulfoxide, platinum will covalently bind to the N7 positions of adjacent guanine bases (1,2 intrastrand cross-link). The structural impact of this lesion is a directed bend (∼30°) of the double helix toward the major groove, as has been demonstrated for a platinated dodecanucleotide in the solid state.[8] In contrast, dinuclear complexes where the Pt(II) centers are bridged by an aliphatic diamine linker of variable chain length form a different array of adducts (1,3 and 1,4 GG interstrand cross-links) that are not

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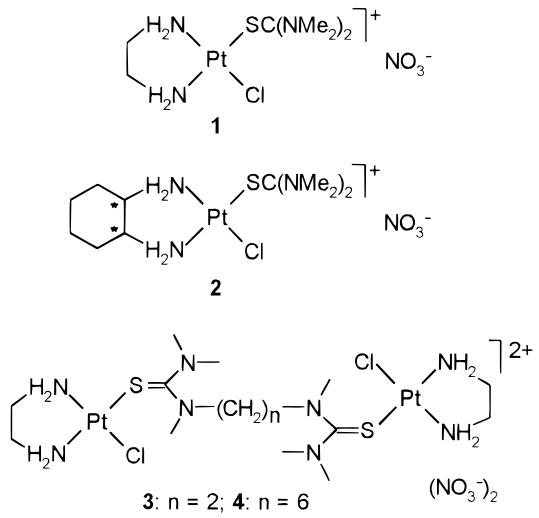
Chart 1



accessible to mononuclear platinum complexes.[6,9,10] These long-range interactions have been shown to effect an irreversible conversion of right-handed *B*-form to left-handed *Z*-form DNA, a structural impact that might be relevant for the distinct biological activity of this type of complex.[11]

In a search for alternative ligand systems, we are currently examining thiourea derivatives as carrier groups in Pt(II) antitumor compounds. Toward this objective, the mononuclear complexes [PtCl(en)(tmtu)]NO_3 (**1**) and [PtCl(dach)(tmtu)]NO_3 (**2**) (en = 1,2-ethanediamine, dach = *trans*-1,2-cyclohexanediamine, tmtu = 1,1,3,3-tetramethylthiourea) and the dinuclear species [{Pt(en)Cl}$_2${μ-C_2H_4(NMeCSNMe$_2$)$_2$-*S*,*S*′}]$(NO_3)_2$ (**3**) and [{Pt(en)Cl}$_2${μ-C_6H_{12}(NMeCSNMe$_2$)$_2$-*S*,*S*′}]$(NO_3)_2$·0.5EtOH (**4**) have been synthesized and fully characterized (Chart 1).[12] The aim of the present study was to investigate the effects of thiourea coordination on the solution chemistry and nucleobase chemistry of **1**−**4**. Simple model reactions were performed utilizing 5′-guanosine monophosphate (5′-GMP) and the dinucleotide *r*(GpG) to monitor the affinity of platinum for guanine-*N7*, the preferred binding site for Pt-based drugs.[13] The results of this model study are related to in vitro cytotoxicity data obtained for **1**−**4** in cisplatin-sensitive and -resistant L1210 leukemia cells. Reactivity features and nucleotide-binding modes observed at this model nucleobase level proved to be instructive for the ongoing design of thiourea-containing platinum drugs and for their mechanism of action.

Experimental Section

Materials and Procedures. Complexes **1**−**4** were prepared by the published procedures.[12] *cis*-[$PtCl_2(NH_3)_2$] and [$PtCl_2$(en)] were synthesized by following a method described by Dhara.[14] The mononucleotide 5′-GMP was employed as its disodium salt (Aldrich). The dinucleotide *r*(GpG) was available as its triethylammonium salt·5.5H_2O (Sigma) which was dissolved in a minimum amount of D_2O and lyophilized. All other reagents were purchased from common vendors and used as supplied.

Instrumentation. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Gemini-300 NMR instrument equipped with a variable-temperature unit and software for arrayed kinetic experiments. Except for nucleotide-binding studies and variable-temperature studies, spectra were obtained at 294 K. Chemical shifts (δ, ppm) are referenced to DSS (4,4-dimethyl-4-silapentanesulfonic acid). Proton-decoupled ^{195}-Pt NMR spectra were taken on a General Electric QE-300 spectrometer at 64.54 MHz with a spectral window of 166 000 Hz. A 0.1 M solution of $K_2[PtCl_4]$ in D_2O served as the external standard. Shifts were recalculated for $[PtCl_6]^{2-}$ standard (δ vs $Na_2[PtCl_6] = \delta$ vs $K_2[PtCl_4] - 1631$).[15]

Chloride ion concentrations and pH values were determined at 294 K with an Accumet 925 pH/mV meter equipped with a chloride-sensitive electrode (Fisher Scientific solid-state ISE, Ag/AgCl reference electrode) and a Beckman combination electrode, respectively. All pH values taken of D_2O solutions are not corrected for the deuterium isotope effect and will be stated as pH* throughout this paper. Where necessary, pH values were adjusted with 0.1 M NaOD and DCl solutions. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ.

Reactions in the NMR Tube. The reactions between the platinum complexes **1**−**4** and the mono- and dinucleotide (5′-GMP, *r*(GpG)) were performed in 99.996% D_2O at 310 K and were followed by ^{1}H NMR spectroscopy. All solutions were unbuffered (pH* 6.6−6.9). ^{1}H NMR spectra were recorded at appropriate time intervals with 64 scans per time point. The first spectrum was acquired when the sample had adopted the nominal temperature (ca. 10 min). Arrayed spectra were recorded using the PAD command available in the Varian software. Quantitation of the progress of the reactions was achieved by integration of the nonexchangeable H8 proton(s) of the nucleobase(s) and the H1′ proton(s) of the ribose residue(s). The following reactions were studied: **1** (10 mM) + 5′-GMP (10 mM, 20 mM); **2** (10 mM) + 5′-GMP (10 mM, 20 mM); **3** (5 mM) + 5′-GMP (10 mM); **3** (5 mM) + *r*(GpG) (5 mM); **4** (5 mM) + *r*(GpG) (5 mM). Furthermore, the solution stability of **3** and **4** was monitored at 310 K: **3** (5 mM, 50 mM) in D_2O and methanol; **4** (5 mM, 50 mM) in D_2O.

Larger amounts of the Pt−nucleotide adducts sufficient for ^{195}Pt NMR studies ($c_{Pt} \approx 50$ mM) were synthesized in water according to the above-mentioned conditions and lyophilized.

Cytotoxicity Studies. The in vitro cytotoxicity studies were performed using standard assays described previously.[16] **1** and [$PtCl_2$(en)] were dissolved in DMF (HPLC grade) and diluted by serial dilution in saline solution to a final concentration of 0.5% in DMF. All other compounds were dissolved in saline solution.

Results

General Remarks. The reactions and stability tests reported in this paper were carried out at 310 K near physiological pH. For reactions involving *r*(GpG), a pH drop of about 0.5 pH unit was observed. No attempts were made to control the pH with a phosphate buffer to avoid the formation of Pt−phosphate intermediates.[17] Platinum coordination to the N7 positions (Figure 1) of the guanine bases has been established for the adducts **I**−**V**: ^{1}H NMR spectra of these species show signals for the H8 base protons shifted to lower field relative to those of the unplatinated nucleobases. Furthermore, these signals do not show a downfield shift in spectra taken at low pH. This is in contrast to the "free" nucleotides, where this effect is observed due to protonation of unplatinated N7 ($pK_a = 2.4$).[18] A representative pH titration (pH 2−10) is shown for adduct **I** in Figure S1 (Supporting Information). For diplatinated *r*(GpG) (in adducts **IV** and **V**) no downfield shift of the H8 resonances

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Table 1. Selected ^{1}H and ^{195}Pt NMR Data[a] for the Adducts **I**−**V** Formed in the Reactions between **1**−**4** and the Nucleotides 5′-GMP and *r*(GpG)

adduct, nucleotide	$\delta(^1H)$/ppm ($^3J_{H1'-H2''}$/Hz) H8	H1′	$\delta(^{195}Pt)$/ppm
[Pt(en)(5′-GMP-*N7*)(tmtu)] (**I**)[b]	8.77	5.95 (6.0)	−3003
[Pt(dach)(5′-GMP-*N7*)(tmtu)] (**II**)[b]	8.748/8.751	5.94 (6.3)	−2982
[Pt(en)(5′-GMP-*N7*)$_2$] (**III**)[c]	8.71	5.96 (4.2)	−2648
5′-GMP[b]	8.18	5.92 (6.1)	
[Pt(en){*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**IV**)[d]	8.19/8.43	5.87 (7.2)/6.04 (0.0)	n.d.[e]
[{Pt(en)}$_2${μ-C$_6$H$_{12}$(NMeCSNMe$_2$)$_2$-*S*,*S*′}{μ-r(GpG)-*N7*(*1*),*N7*(*2*)}] (**V**)[f]	8.58/8.63	5.95 (6.1)/5.97 (4.8)	n.d.[e]
r(GpG)[d]	7.94[g]/8.01[h]	5.81 (5.0)/5.89 (5.1)	

[a] 294 K, D_2O solution. [b] pH* 6.8. [c] pH* 6.7; see also ref 7. [d] pH* 6.2. [e] Insufficient solubility. [f] pH* 6.0. [g] Assigned to 3′ guanine; see ref 26. [h] Assigned to 5′ guanine, see ref 26.

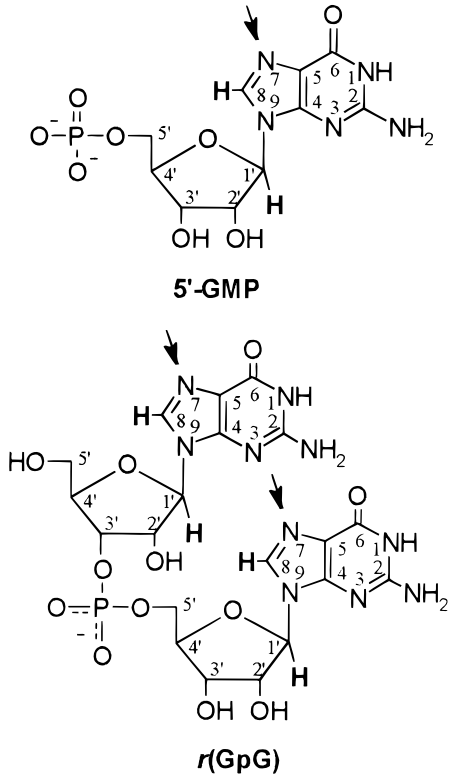


Figure 1. Structures of 5′-GMP and *r*(GpG) giving atom numbering. The arrows indicate the preferred binding site for Pt-based drugs, the N7 position of guanine.

is observed on going from low to neutral pH. This is in agreement with the absence of a deprotonation step of the phosphodiester group, unlike the situation for the 5′ phosphate (pK_a = 6.2) in the mononucleotide.[18] Selective NMR data for the final products of the reactions described below are given in Table 1.

Reactions of [PtCl(en)(tmtu)]NO$_3$ (1) and [PtCl(dach)(tmtu)]NO$_3$ (2) with 5′-GMP. The reaction of **1** and **2** with 5′-GMP at equimolar concentrations of platinum complex and nucleotide results in the displacement of the chloro ligand by the nucleobase, giving [Pt(en)(5′-GMP-*N7*)(tmtu)] (**I**) and [Pt(dach)(5′-GMP-*N7*)(tmtu)] (**II**) (Scheme 1). The half-times of product formation are 9 and 11.5 h, respectively. **I** and **II** are the only adducts formed with no detectable (aqua) intermediates appearing during the reaction. The progress of the reaction is depicted for the formation of **I** in Figure 2. The rates of N7 binding for **1** and **2** prove to be considerably slower than those found for chloro−am(m)ine complexes of Pt(II) ($t_{1/2}$ = 4−5 h) under analogous conditions.[19] This effect may be attributed to the presence of the bulky tmtu ligand, which should be unfavorable for a nucleophilic attack of the nucleobase on

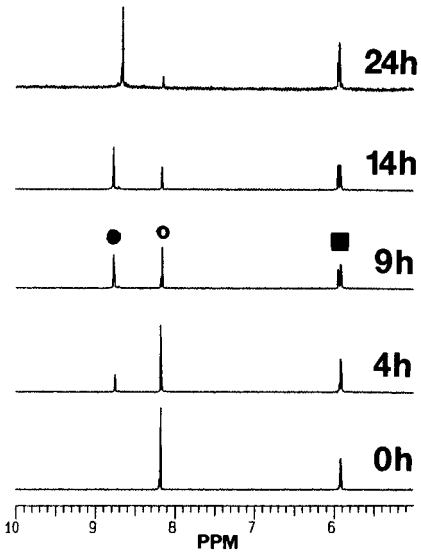
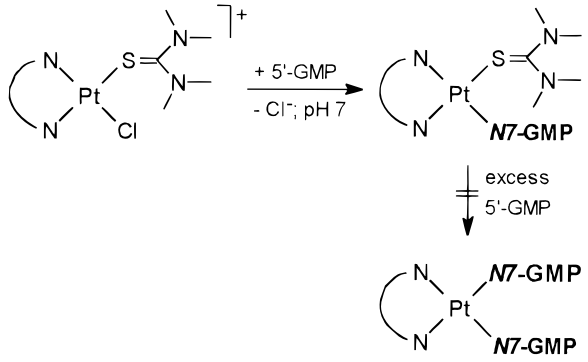


Figure 2. Progress of the reaction of 5′-GMP (10 mM) with [PtCl(en)(tmtu)]NO$_3$ (**1**) (10 mM) followed by ^{1}H NMR spectroscopy. Conditions: 310 K, D_2O solution, pH* 6.8. Assignments: (○) H8 of unplatinated 5′-GMP; (●) H8 of platinated 5′-GMP in [Pt(en)(5′-GMP)(tmtu)] (**I**); (■) overlapping H1′ doublets of free and platinated 5′-GMP.

Scheme 1



platinum. Furthermore, ion-sensitive measurements indicate that tmtu profoundly affects the hydrolytic behavior of the Pt−Cl bonds in **1** and **2**. At room temperature, 1 mM aqueous solutions of **1** and **2** slowly release ca. 0.5 mM (50% hydrolysis) of chloride within 15 h. This is in contrast to the degree and rate of hydrolysis of cisplatin under similar conditions.[20] This altered aqueous solution chemistry of **1** and **2** may also contribute to their relative kinetic inertness in reactions with 5′-GMP. Aqua cations constitute reactive intermediates in the

(19) Bierbach, U.; Farrell, N. Unreported experiments for the monofunctional complex [PtCl(dien)]Cl (dien = diethylenetriamine).

two-step mechanism of platinum binding to nucleic acid fragments (rate-determining hydrolysis of the Pt−Cl bond followed by substitution of the aqua ligand by the nitrogen base).[21]

Despite the bulkiness of the peralkylated thiourea derivative in the position cis to the nucleobase in **I** and **II**, free rotation about the Pt−N7 bonds and bonds of the thiourea moieties is observed. This results in sharp ^{1}H NMR signals for both the H8 singlets and the H1′ doublets of the sugar residues (see Table 1) and the methyl protons of tmtu at 3.10 ppm. Platinum coordination to N7 of guanine in mononucleotides is known to cause a change in the sugar ring conformation from S-type (C2′-*endo*, C3′-*exo*) to N-type (C3′-*endo*, C2′-*exo*).[22] In **I** and **II**, this transition is not observed, as can be deduced from $^3J_{H1'-H2'}$ coupling constants (Table 1). A plausible reason for this effect cannot be given, but steric effects of the thiourea ligand may play a role. Two H8 signals for **II**, separated by only 0.003 ppm, are consistent with the formation of a 1:1 mixture of two diastereomers, produced by the chirality of the R-ribose and the presence of the *R,R* and *S,S* forms of the dach ligand. ^{195}Pt chemical shifts for **I** and **II** around −3000 ppm (vs $[PtCl_6]^{2-}$) are in agreement with a $[N_3S_{thiourea}]$ environment of platinum(II).[23]

No reaction is observed when the monofunctional platinum−nucleotide adducts **I** and **II** are incubated with a second equivalent of 5′-GMP at 310 K for 2 weeks. Similarly, in experiments that employ a 1:2 molar ratio of platinum and nucleotide, half of the applied 5′-GMP remains unreacted in solution. Species **I** and **II** do not bind a second nucleobase. Neither a displacement of the tmtu ligand nor a ring opening of the diamine chelate (labilization of the Pt−N bond trans to sulfur) is observed during the long-term incubations at physiological pH.

Solution Behavior of the Dinuclear Complexes 3 and 4. Stability tests on aqueous solutions of **3** initially led to the conclusion that this species shows an increased rate of hydrolysis compared to the mononuclear species **1** and **2** (ion-selective measurements). ^{1}H NMR spectra taken of **3** in D_2O at 310 K at appropriate time intervals (Figure 3) show the disappearance of signals at 2.71 ppm (en) and at 3.16, 3.31, and 4.18 ppm (bridging thiourea ligand) while new signals appear in the 2.6−2.8 ppm region (en) and at 3.25 and 3.37 ppm. An almost unrecognizably broadened signal is observed at 3.84 ppm that slightly sharpens at temperatures above 330 K. From aqueous solutions with an initial concentration in **3** higher than 50 mM, bright-yellow needles precipitate which are identified as $[PtCl_2(en)]$ (analyses, IR). The remaining solution contains a species that gives a ^{195}Pt chemical shift of −3454 ppm, indicative of a $[N_2S_2]$ environment of platinum(II).[23] According to these findings, **3** slowly disproportionates into the mononuclear species $[PtCl_2(en)]$ (which in fact rapidly hydrolyzes) and $[Pt(en)\{C_2H_4(NMeCSNMe_2)_2\text{-}S,S'\}]^{2+}$, the half-time of this process being 2.5 h (see caption of Figure 3 for further explanations). Analogous products are obtained for the dinuclear complex **4**, but in this case the reaction proceeds much slower ($t_{1/2} \approx 12$ h).

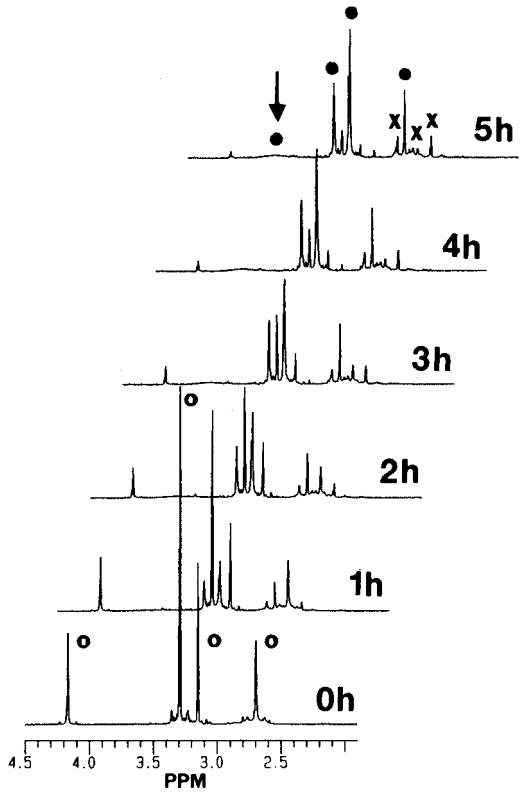
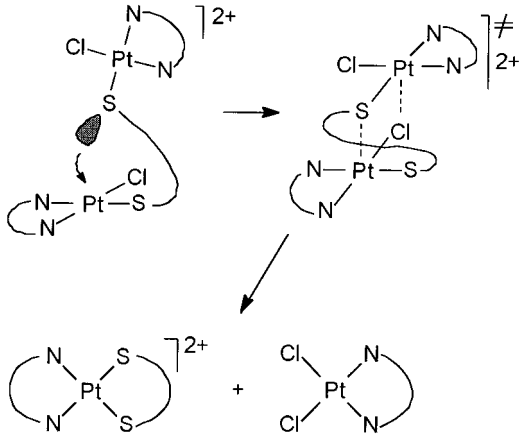


Figure 3. Disproportionation of $[\{PtCl(en)\}_2\{\mu\text{-}C_2H_4(NMeCSNMe_2)_2\text{-}S,S'\}](NO_3)_2$ (**3**) followed by ^{1}H NMR spectroscopy. Conditions: 310 K, $c = 5$ mM, D_2O solution. Assignments: (○) thiourea and en protons of **3**; (●) signals of the decomposition product, $[Pt(en)\{C_2H_4(NMeCSNMe_2)_2\text{-}S,S\}](NO_3, Cl)$; (×) signals of the en ligand in $[PtCl_2(en)]$ and its hydrolysis products. Signal broadenings observed for the bis(thiourea) chelate (●) are attributed to the slow dynamics of interconversion of different chelate ring conformations. The arrow indicates the broad signal assigned to the central ethylene protons of the thiourea ligand, which are affected most.

Scheme 2. Proposed Mechanism of Decomposition for **3** and **4**



Considering the experimental data, we propose a mechanism for the decomposition of **3** and **4** (Scheme 2) that involves an intramolecular nucleophilic attack of coordinated thiourea sulfur. A transition state with the two platinum centers bridged by a sulfur atom is suggested, which finally rearranges into the mononuclear complexes. This mechanism is supported by the following observations: (i) the fact that mononuclear **1** and **2** do not disproportionate at the same concentrations in platinum and **4** reacts slower than **3** (separation of the Pt centers by an extended linker) points to an *intra*molecular process; (ii) the

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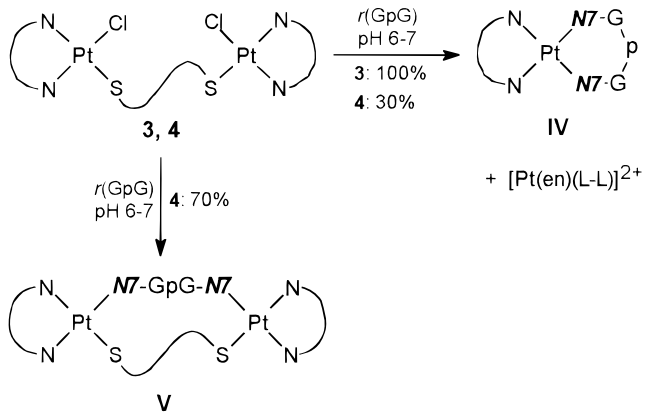
Scheme 3



reaction does not require hydrolysis of the Pt−Cl bond (the same reactivity is observed in nonaqueous solutions), which should favor a nondissociative, concerted mechanism and a direct attack of coordinated sulfur utilizing its vacant lone pair (Scheme 2). Bridging thiourea sulfur (here in a putative five-coordinate transition state) is a well-documented binding mode in polynuclear transition metal complexes.[24]

Reaction of [{Pt(en)Cl}$_2${μ-C$_2$H$_4$(NMeCSNMe$_2$)$_2$-*S,S*′}](NO$_3$)$_2$ (3) with 5′-GMP and *r*(GpG). When **3** is incubated with 2 equiv of 5′-GMP, the H8 signal of the free nucleotide decreases in intensity ($t_{1/2}$ = 5.5 h) and a single downfield-shifted H8 peak appears at 8.71 ppm. The only adduct formed proves to be [Pt(en)(5′-GMP-*N7*)$_2$] (**III**), in accordance with published ^{1}H NMR data[7] and a ^{195}Pt shift of −2648 ppm[25] (Table 1). H8 resonances at 8.62 and 8.79 ppm that transiently appear during the early phase of the reaction are assigned to the intermediates[21] [PtCl(en)(5′-GMP-*N7*)] and [Pt(D$_2$O)(en)-(5′-GMP-*N7*)].

The analogous reaction of **3** with *r*(GpG) solely yields the mononuclear adduct [Pt(en){*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**IV**) (Scheme 3), which is in agreement with the results obtained for 5′-GMP. Figure 4 depicts the progress ($t_{1/2}$ = 2 h) of the reaction as monitored by characteristic changes in the H8 and H1′ proton resonances. ^{1}H NMR data for **IV** such as chemical shift differences between the H8 protons of the 5′ and the 3′ guanine base and values for $^3J_{H1'-H2'}$ (Table 1) appear to be almost identical with those reported for the analogous *N7*,*N7*-chelate formed by *cis*-[PtCl$_2$(NH$_3$)$_2$].[26] The absence of the latter vicinal coupling is indicative of a switch in the sugar conformation of the 5′ ribose residue from S-type to N-type (^3J = 0 Hz; see Figure 4b) upon platination. This is a well-established structural feature for bifunctional adducts that are formed between *mononuclear* platinum complexes and adjacent nucleobases in dinucleotides.[27]

Reaction of [{Pt(en)Cl}$_2${μ-C$_6$H$_{12}$(NMeCSNMe$_2$)$_2$-*S,S*′}](NO$_3$)$_2$·0.5EtOH (4) with *r*(GpG). The reaction of *r*(GpG) with **4** proceeds significantly slower ($t_{1/2} \approx$ 5 h for the disappearance of free nucleotide) than with **3**. The H8 region of the ^{1}H NMR spectrum taken after 48 h (Figure 5a) shows four signals downfield of those of free *r*(GpG). The two least downfield-shifted H8 signals and the signals detected for the

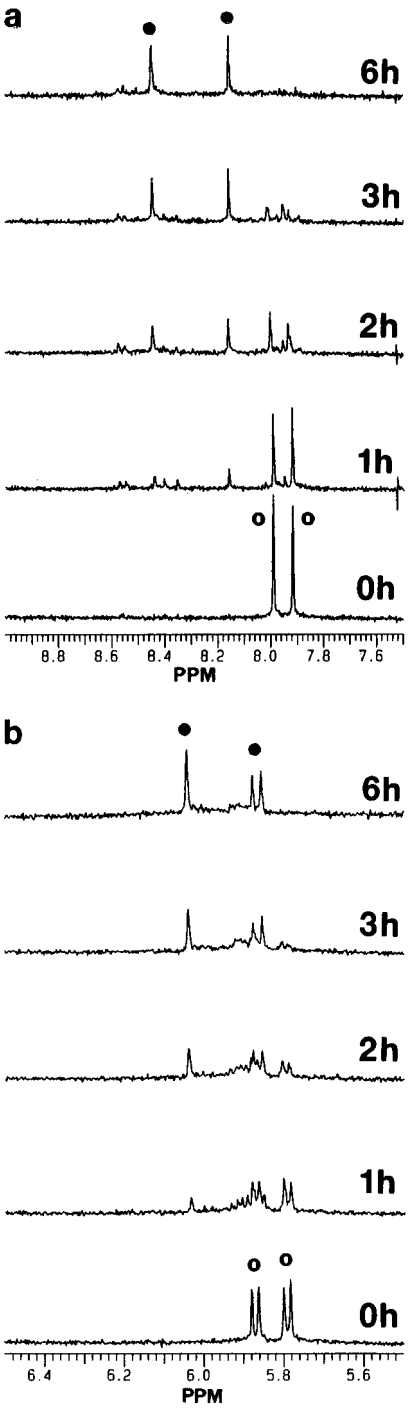


Figure 4. Progress of the reaction of *r*(GpG) (5 mM) with [{PtCl(en)}$_2${μ-C$_2$H$_4$(NMeCSNMe$_2$)$_2$-*S,S*′}](NO$_3$)$_2$ (**3**) (5 mM) followed by ^{1}H NMR spectroscopy: (a) H8 proton region; (b) H1′ proton region. Conditions: 310 K, D$_2$O solution, pH* 6.8−6.2. Assignments: (○) free *r*(GpG); (●) platinated *r*(GpG) in adduct **IV**. Note the absence of the vicinal ($^3J_{H1'-H2'}$) coupling for the 5′ sugar H1′ proton, indicating a fully N-type ribose conformation.

H1′ sugar protons at 5.87 and 6.04 ppm (Figure 5b) confirm the formation of [Pt(en){*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**IV**) (Table 1). In addition to the mononuclear adduct **IV** (30%), a new species has formed (70%), as evidenced by H8 signals at 8.58 and 8.63 ppm and two overlapping H1′ doublets at 5.95 and 5.97 ppm. We propose the formation of the macrochelate [{Pt(en)}$_2${μ-C$_6$H$_{12}$(NMeCSNMe$_2$)$_2$-*S,S*′}{μ-*r*(GpG)-*N7*(*1*),*N7*(*2*)}] (**V**) (Scheme 3), in agreement with the integral intensity ratio observed for H8, H1′ and the en protons at 2.82 ppm. Unlike

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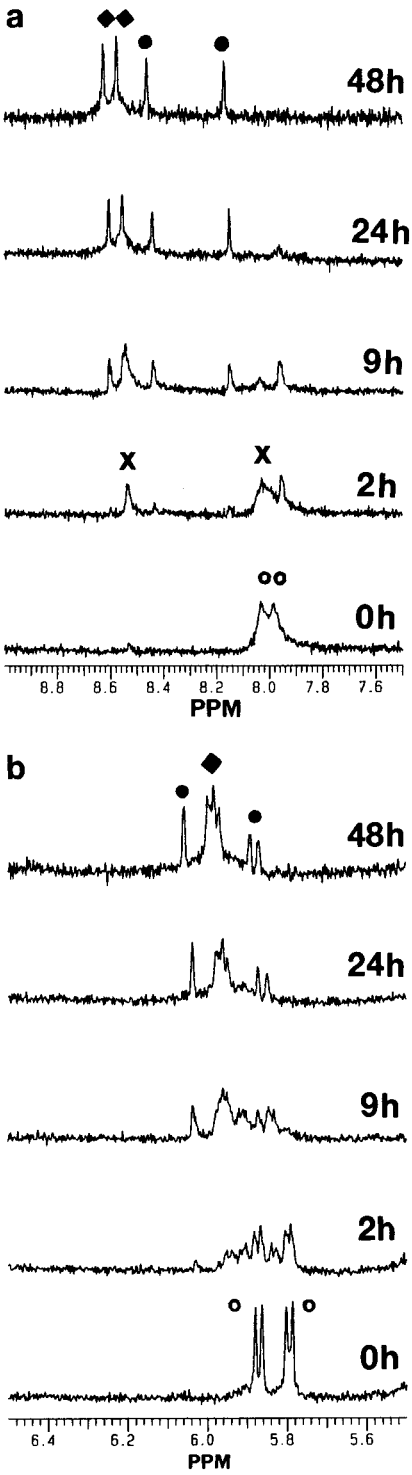

Figure 5. Progress of the reaction of *r*(GpG) (5 mM) with [{PtCl(en)}$_2${μ-C_6H_{12}(NMeCSNMe$_2$)$_2$-*S*,*S*′}](NO$_3$)$_2$·0.5EtOH (**4**) (5 mM) followed by ^{1}H NMR spectroscopy: (a) H8 proton region; (b) H1′ proton region. Conditions: 310 K, D_2O solution, pH* 6.6−6.0. Assignments: (○) free *r*(GpG); (●) platinated *r*(GpG) in the mononuclear adduct **IV**; (◆) platinated *r*(GpG) in the macrochelate **V**; (×) monoplatinated intermediate; see text. The broadening of the peaks is caused by a small amount of precipitate (of unknown nature) which forms immediately after combining the reactants in the NMR tube but slowly redissolves as the reaction proceeds. The formation of polymeric species of the type −[Pt−Pt−N7−*r*(GpG)−N7−]$_n$ is not observed. The broad bases of the H8 signals for **V** result from unresolved ^{195}Pt−^{1}H couplings.

the situation for **IV**, the $^3J_{H1'-H2'}$ coupling constants (Table 1) confirm the absence of a S→N conformational change of the 5′ ribose residue as commonly observed in (deoxy)oligonucleotides

Table 2. In Vitro Cytotoxicity of **1**−**4** in L1210 Leukemia Cells[a]

complex	ID$_{50}$/μM[b] L1210/0	L1210/DDP
[PtCl(en)(tmtu)]NO$_3$ (**1**)	>50	>50
[PtCl(dach)(tmtu)]NO$_3$ (**2**)	>50	>50
cis-[PtCl$_2$(NH$_3$)$_2$] (cisplatin)	0.19	11.63 (61)[c]
[{PtCl(en)}$_2${C_2H_4(NMeCSNMe$_2$)$_2$}](NO$_3$)$_2$ (**3**)	4.15	39.58 (9.5)
[{PtCl(en)}$_2${C_6H_{12}(NMeCSNMe$_2$)$_2$}](NO$_3$)$_2$ (**4**)	6.93	56.63 (8.2)
[PtCl$_2$(en)]	1.25	>20 (>16)

[a] Complexes in saline solution except for **1** and [PtCl$_2$(en)], which were in 0.5% DMF. [b] Drug concentration required to inhibit cell growth by 50%. [c] Resistance factor, defined as ID$_{50}$(resistant)/ID$_{50}$(sensitive), is given in parentheses.

modified with dinuclear platinum complexes.[28,29] The stepwise reaction of **4** with *r*(GpG) should involve intermediates with one platinum bound to either the 3′ or the 5′ guanine base, followed by closure to the macrochelate (and polymer formation; see caption of Figure 5). Interestingly, the H8 signal of 5′ guanine in free *r*(GpG) decreases in intensity more rapidly (Figure 5a) than that of the 3′ base. This observation and the fact that only one major intermediate H8 signal is observed after 2 h indicate that **4** preferentially binds to the 5′ N7 position in the first reaction step. The final bifunctional adduct is stable in solution under the applied conditions. This proves that the mononuclear chelate **IV** is not a decomposition product of the macrochelate **V** but is formed by the direct reaction with [PtCl$_2$(en)].

Cytotoxicity Studies. The cytotoxicity of complexes **1**−**4** was studied both in cisplatin-sensitive and in cisplatin-resistant murine L1210 leukemia cells (Table 2). Solutions of the dinuclear complexes **3** and **4** were freshly prepared and incubated immediately after dilution to take the instability of the drugs into account. Two experiments were performed. In the first series of incubations, the mononuclear complexes **1** and **2** were studied and compared with cisplatin. In the second series, the dinuclear complexes **3** and **4** were studied together with [PtCl$_2$(en)], their potential decomposition product. Preliminary results are given in Table 2. **1** and **2** show no drug response at drug concentrations <50 mM in both cisplatin-sensitive (L1210/0) and cisplatin-resistant (L1210/DDP) cells and thus have to be considered inactive. The dinuclear complexes **3** and **4** are fairly active in the sensitive cell line but show a marked decrease in cell growth inhibition in resistant cells. **3** appears to be slightly more active than **4** with the cross-resistances to cisplatin being similar for both complexes. [PtCl$_2$(en)] shows slightly greater efficacy than **3** and **4** but proves to be less active than cisplatin and does not overcome cisplatin resistance in L1210/DDP.

Discussion

The nucleotide-binding studies on [PtCl(en)(tmtu)]NO$_3$ (**1**) and [PtCl(dach)(tmtu)]NO$_3$ (**2**) unequivocally show that tmtu does not act as a leaving group in reactions with guanine, which implies that **1** and **2** will form monofunctional adducts on double-stranded DNA. This observation seems plausible since the reverse of this reaction has been observed: (i) thiourea (SC(NH$_2$)$_2$, tu) and its partially *N*-alkylated derivatives have been shown to slowly displace guanosine (Guo) and 5′-GMP from

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the bifunctional adducts *cis*-$[Pt(NH_3)_2(Guo)_2]^{2+}$ ($k \approx 10^{-5}$ s^{-1})[30] and *cis*-$[Pt(NH_3)_2(5'\text{-GMP})_2]$;[31] (ii) thiourea is used as a mechanistic probe which traps monofunctional Pt−DNA adducts and as a reagent that effectively removes Pt from DNA.[32] At least in the case of *cis*-DDP related drugs, the critical lesion that leads to cytotoxicity is thought to be the 1,2 intrastrand cross-link of guanine bases. We therefore suggest that the inability of **1** and **2** to form this adduct could be the reason for the observed inactivity of these complexes in vitro. Monofunctional Pt−DNA adducts such as those formed by the inactive complex $[PtCl(dien)]^+$ (dien = diethylenetriamine) are known to be ineffective in blocking DNA replication.[33]

The ligand properties of tmtu clearly distinguish **1** and **2** from analogous cationic complexes $[PtCl(diamine)(SORR')]^+$ which carry a (chiral) S-bound sulfoxide ligand. The substitution lability of the latter sulfur ligands is undoubtedly a prerequisite for the biological activity of these species.[4,5,7] At this point, a comparison of ligand properties of different sulfur nucleophiles that are relevant to platinum antitumor chemistry seems worthwhile. It appears that in these systems ligands with π-acceptor properties[34,35] (thioether, sulfoxide) are substituted by N7 of guanine to give the thermodynamically more stable Pt−nucleobase adduct. The distinct reactivity of the $Pt-S_{thioether}$ bond in Pt−methionine adducts in competition reactions with guanine-*N7* and the possible biological implications should be noted.[36,37] On the other hand, for sulfur ligands that lack acceptor properties but are strong (σ and π) donors, such as thiourea[12,38] and thiolate,[39] obviously the reverse reactivity is observed.

The rationale behind the development of the dinuclear complexes **3** and **4** was that these would act as cross-linking agents with the two platinum subsites binding monofunctionally to DNA. This is a well-established binding mode for analogous complexes of the formula $[\{PtCl(NH_3)_2\}_2(H_2N-R-NH_2)]^{2+}$ (R = linear aliphatic chain), which allows the targeting of larger DNA sequences for bifunctional adduct formation.[6] Accordingly, in **3** and **4** the bridging thiourea derivatives should act as tightly bound, nonreplaceable spacers that determine the "bite" on DNA. Initial nucleotide-binding studies on **3** and **4** were performed to monitor the ability of these complexes to form *stable* dinuclear units containing the motif [N7−Pt−{μ-bis(thiourea)−*S*,*S*′}−Pt−N7]. Although reactions with *r*(GpG) should also yield information about the geometric requirements for the specific 1,2 intrastrand cross-link for these complexes, it was not the intention of this study to favor or disfavor this adduct over alternative long-range adducts.

In reactions between **3** and the nucleotides, both 5′-GMP and *r*(GpG) exclusively react with $[PtCl_2(en)]$, the rapidly formed and most reactive species in the reaction media. The short half-life of **3** under the conditions of the nucleotide-binding experiments apparently controls the nature of the final adducts. For this reason, the formation of a "macrochelate" with dinuclear **3** coordinating to the dinucleotide seems unfavorable, irrespective of the stereochemical feasibility of this adduct. In vitro cytotoxicity data for **3** are in agreement with $[PtCl_2(en)]$ being the only biologically active species. The results may also explain the observed cross-resistance of **3** to cisplatin, based on a similar mechanism of action and an identical array of DNA adducts for $[PtCl_2(en)]$. Despite the increased stability of the analogous dinuclear complex **4** and the ability to form a stable macrochelate (at least in our model studies), an overall similar activity is found in L1210 leukemia. The cytotoxicity data for **4** imply that in this case mononuclear $[PtCl_2(en)]$ also is the only "surviving" species that reaches the target DNA but basically do not rule out the possibility of bifunctional adducts of (intact) **4** with DNA in vitro. The frequency of such cross-links may be low and/or these adducts may not significantly contribute to the biological activity of **4**.

Conclusions and Perspectives

The specific incorporation of thiourea derivatives as S-donor ligands into platinum antitumor complexes drastically changes the reactivity of the Pt(II) center toward the biologically relevant target guanine-*N7*. Tmtu in $[PtCl(en)(tmtu)]NO_3$ (**1**) and $[PtCl(dach)(tmtu)]NO_3$ (**2**) behaves as a typical nonleaving group due to the *thermodynamic* stability of the Pt−S bond. Although undesired in the case of **1** and **2**, this structural motif proves to be extremely valuable for the design of the dinuclear species **3** and **4**, where platinum has the same ligand environment. However the inherent reactivity of these species appears to be unfavorable for their use as anticancer drugs. Our ongoing research will focus on how the mononuclear compounds can be activated despite their monofunctional covalent binding mode and on structural modifications of the dinuclear compounds to increase their stability. With the use of bis(thiourea) ligands that are conformationally more rigid than those in **3** and **4**, it should be possible to avoid chelation of a single Pt center and consequently intramolecular disproportionation.

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Supporting Information Available: A plot of chemical shift of H8 vs pH* for [Pt(en)(5′-GMP-*N7*)(tmtu)] (adduct **I**) (1 page). Ordering information is given on any current masthead page.

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