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Separation, Characterization, and Stability of Products from *cis*-$PtCl_2(NH_3)_2$ and [PtCl(dien)]Cl with 9-Ethylguanine, Formed under Neutral or Alkaline Conditions. Evidence for a Migration of the Platinum Moiety from N1 to N7

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The synthesis, separation, and characterization by proton NMR of various adducts obtained from the reaction of the monofunctional [PtCl(dien)]Cl (dien denotes diethylenetriamine) and the bifunctional *cis*-$PtCl_2(NH_3)_2$ with 9-ethylguanine are reported. When the reaction is performed at pH 10.5, N1-coordinated platinum adducts are formed in addition to the N7-coordinated adducts, which are also obtained under neutral conditions. An interesting migration of the platinum moiety of the two identified N1 adducts from the N1 site to almost exclusively the N7 site is observed. This migration takes place only after protonation of the N7 site, i.e. under acidic conditions. Indications for an intramolecular migration mechanism are briefly discussed.

Introduction

Results obtained from many chemical and biochemical studies[1-4] indicate that the cellular DNA is directly involved in the working mechanism of the antitumor drug *cis*-$PtCl_2(NH_3)_2$ (abbreviated as cis-Pt; see Figure 1). For this reason, many investigations in the field of the platinum anticancer chemistry are focused on platinum–DNA[5-8] and platinum–oligonucleotide[9-12] interactions. The reaction with guanine-containing DNA fragments is of special interest, since the platinum compounds exhibit a kinetic preference for this nucleobase.[14-16] Guanines blocked at the N9 site, such as 5′-GMP, guanosine, and 9-ethylguanine (abbreviated as eGua; see Figure 1) possess three potential sites for platinum coordination:[17] N7, N1 (only after deprotonation), and N3. In most X-ray structures of platinum bis(oxopurine) complexes, synthesized under neutral or slightly acidic conditions, N7 coordination is observed.[18-21] Also N1,N7 dinuclear platinum compounds with oxopurines have been described.[20,21] Mononuclear N1 compounds are known for N7-alkylated purine deratives.[20,22] Very recently, the X-ray structure of an N1,N3,N7 trinuclear eGua adduct has been reported.[21]

Until now, little was known about the nature and stability of mononuclear platinated products of guanine at the N1 site. For this reason, eGua was reacted with cis-Pt and the monofunctional platinum analogue [PtCl(dien)]Cl (see Figure 1) under neutral (pH 7) and alkaline (pH 10.5) conditions. 9-Ethylguanine was used instead of guanine, to exclude platinum coordination to the N9 position. The reaction products were separated on charge by cation-exchange chromatography, and their identities as well as the stability of two N1 compounds were investigated by proton NMR. Special attention was paid to an interesting migration of the platinum moiety from the N1 to the N7 position, which was observed only under acidic conditions. Indications for a possible intramolecular migration mechanism are discussed.

Materials and Methods

Starting Materials. eGua was obtained from Sigma Chemicals and used without further purification. cis-Pt and [PtCl(dien)]Cl were prepared from K_2PtCl_4, according to literature procedures.[23,24] cis-Pt was purified by crystallization from DMF.[25]

Synthesis and Separation of the Products. The reactions between eGua (0.15 mmol in 50 mL of doubly distilled water) and [PtCl(dien)]Cl (0.10 mmol) or cis-Pt (0.075 mmol) were performed at both pH 7.0 and pH 10.5 for 3 days at 80 °C in the dark. After concentration to 0.2 vol by rotary evaporation, the reaction mixtures were loaded on a cation-exchange resin column (CM-Sephadex C25, Pharmacia). The various products were separated by applying a linear triethylammonium hydrogen carbonate (TEAB) gradient (fraction 1, 0 M; fraction 75, 1.6 M). The volatile TEAB salt present in the various peak fractions was removed by repeated rotary evaporation. If necessary to remove final traces of TEAB, additional desalting was performed by gel permeation chromatography (Sephadex G25, Pharmacia) using doubly distilled water as eluent.

NMR. The different reaction products were dissolved in 0.5 mL of D_2O (99.8%; Merck), together with a trace amount of tetramethylammonium nitrate (TMA; 3.18 ppm downfield from DSS) as a reference, and lyophylized. After the products were dissolved 99.95% D_2O (Merck), proton NMR spectra of the products were recorded on a Bruker WM 300 Spectrospin instrument. When necessary, the HDO signal was reduced by selective saturation. For monitoring of the pH-dependent chemical shift behavior of the eGua H8 signal in the various products, the pH as adjusted with 0.1 and 0.01 M solutions of NaOD or DCl. The pH values are not corrected for the deuterium isotope effect. The integrated spectra were recorded at 16 K with 1.8-s aquisition time.

Stability Measurements. To investigate the stability of the N1 compounds, samples were heated in the NMR tube at 80 °C in water. After

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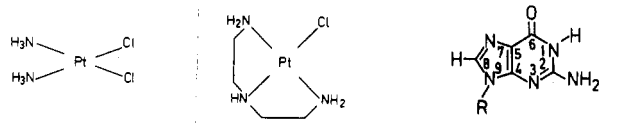

Figure 1. Schematic depiction of the antitumor active *cis*-$PtCl_2(NH_3)_2$, the monofunctional [PtCl(dien)]Cl, and the nucleobase 9-ethylguanine (R denotes an ethyl group).

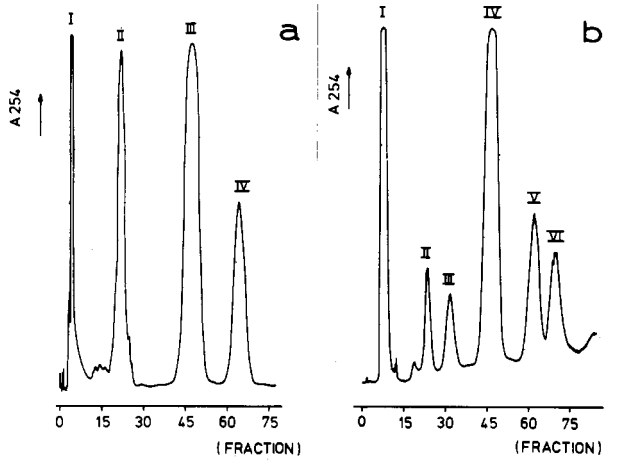

Figure 2. UV profile of the chromatographic separation of the reaction products of eGua with [PtCl(dien)]Cl (a) and *cis*-Pt (b). The reactions were performed at pH 10.5.

certain time intervals, spectra were recorded at ambient temperature after rapid chilling of the NMR tube in water (20 °C). For investigation of the migration mechanism, 0.1 vol of a 1.0 M NaCl or NH_4Cl solution in D_2O were added to the sample before heating.

Results and Discussion

The results of the reactions between eGua and [PtCl(dien)]Cl or cis-Pt are comparable to a similar study of these reactions with the guanine-like base 9-methylhypoxanthine.[20] The purine N9-position in this nucleobase is blocked by a methyl group whereas the amine group at the 2-position is replaced by a hydrogen atom (see Figure 1). Therefore, the results of this previous study[20] are briefly mentioned, and a few differences are noted. Since the charges of the various products are pH dependent (see below), they have been omitted in the structural formulas for clarity.

Products of the Reaction between [PtCl(dien)]Cl and eGua. In Figure 2a, the elution pattern of the products formed in the reaction between eGua and [PtCl(dien)]Cl performed at pH 10.5 is shown. On the basis of charge, peaks I–IV are tentatively assigned to unreacted eGua, Pt(dien)(eGua-*N*1), Pt(dien)-(eGua-*N*7), and [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7), respectively. The elution pattern of the reaction performed at pH 7.0 shows almost no product II and IV (not shown). This result is in good agreement with the above-mentioned assignment, since at pH 7.0 the N1 site of eGua is protonated,[17] in this way preventing platination at that site. Therefore, no N1 product is expected under these conditions. Under both conditions, Pt(dien)(eGua-*N*7) appears to predominate.

To prove the tentative assignment, chemical shift profiles of the nonexchangeable H8 proton of the four products as a function of pH have been performed. The results are depicted in Figure 3. Product I, ascribed to unreacted eGua, is protonated at the N7 around pH 2.5, as reflected by the large chemical shift of the H8 proton (about 1.1 ppm). At the alkaline end of the pH curve, a small part of an N1 deprotonation can be observed, also characteristic for unbound eGua.

Product II is protonated at the N7 around pH 4.5, as can be deduced from the large chemical shift of the H8 proton due to this protonation. No N1 deprotonation is observed in the curve up to pH 12. This result is in good agreement with the assignment of this product to Pt(dien)(eGua-*N*1). In this compound, N1 protonation is prevented by the platination of that site. The observed increase of the pK_a value of the N7 protonation is

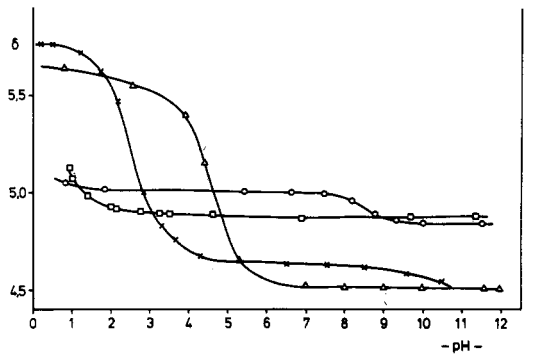

Figure 3. pH curve of the chemical shifts of the H8 proton of unbound eGua (×), Pt(dien)(eGua-*N*1) (△), Pt(dien)(eGua-*N*7) (○), and [Pt-(dien)]$_2$(μ-eGua-*N*1,*N*7) (□).

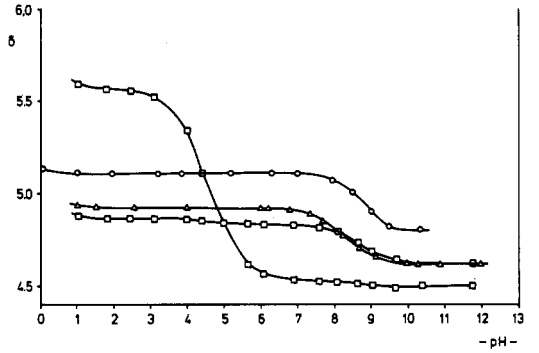

Figure 4. pH curve of the chemical shift of the H8 proton(s) of the adducts *cis*-$Pt(NH_3)_2$(eGua-*N*7)$_2$ (△), *cis*-$Pt(NH_3)_2$(eGua-*N*7)X (○), and *cis*-$Pt(NH_3)_2$(eGua-*N*7)(eGua-*N*1) (□).

comparable to the similar Pt(dien)(mHyp-*N*1).[20]

The pH curve of product III, with an N1 deprotonation around pH 8.5 is characteristic for an N7-platinated guanine residue. The pK_a of the N1 deprotonation appears to be lowered by 1.5 to 2 units compared to that of free eGua. No N7 protonation is seen at low pH. These observations clearly prove the identity of product III to be Pt(dien)(eGua-*N*7).

Product IV, assumed to be [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7), should not give any (de)protonation in the investigated pH range since both N1 and N7 are involved in platinum binding. Nevertheless, the beginning of a protonation step is clearly present at low pH. This effect can probably be ascribed to an N3 protonation, which has already been suggested.[20] The pH curve is similar to that of [$Pt(NH_3)_3$]$_2$(μ-eGua-*N*1,*N*7)[21]. We did not observe a product similar to the recently reported[22] adduct in which three $Pt(NH_3)_3$ moieties are coordinated to N1, N3, and N7.

Products of the Reaction between cis-Pt and eGua. In Figure 2b, the elution pattern of the products formed in the reaction between eGua and cis-Pt at pH 10.5 is shown. Six UV-absorbing products can be observed. When the reaction is performed at pH 7.0, elution peaks III and IV increase, while peaks II, V, and VI strongly decrease. This indicates that the latter three peaks contain platinum products in which N1-coordination is involved. Consistent with the earlier mentioned results[20] of the reaction between cis-Pt and mHyp at pH 10.5, the following adducts can be expected: *cis*-$Pt(NH_3)_2$(eGua-*N*1)$_2$, the monofunctional *cis*-Pt-$(NH_3)_2$(eGua-*N*7)X (X denotes an unknown ligand), *cis*-Pt-$(NH_3)_2$(eGua-*N*1)(eGua-*N*7), *cis*-$Pt(NH_3)_2$(eGua-*N*7)$_2$, (eGua-*N*7)(*cis*-$Pt(NH_3)_2$)(μ-eGua-*N*1,*N*7)(*cis*-$Pt(NH_3)_2$)(eGua-*N*7) (a dinuclear compound), and *cis*-$Pt(NH_3)_2$(eGua-*N*1)X.

Elution peak I appears to contain mainly unreacted eGua, as deduced from UV and NMR data. Gel filtration chromatography of this peak gives in addition to a main peak (unbound eGua) a very small peak under which probably a platinated eGua product is eluted. This is only the case when the reaction was performed at pH 10.5. The amount of this minor product is too little to characterize it by NMR. It is, however, attractive to ascribe it to either *cis*-$Pt(NH_3)_2$(eGua-*N*1)$_2$ or to a monofunctional product

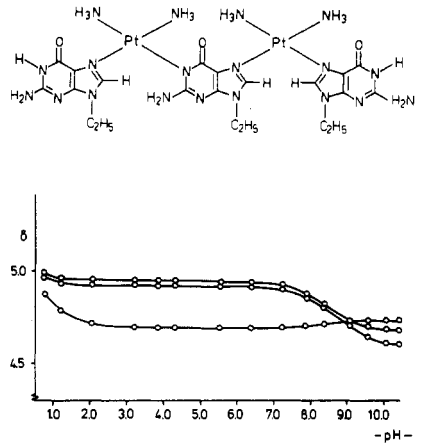

Figure 5. pH curve of the chemical shifts of the H8 protons of the dinuclear compound (eGua-*N*7)(*cis*-Pt(NH_3)$_2$)(μ-eGua-*N*1,*N*7)(*cis*-Pt(NH_3)$_2$)(eGua-*N*7) and the proposed structure.

cis-Pt(NH_3)$_2$(eGua-*N*1)X (X denoting a monoanion). Since these products are neutral under the conditions used during the cation-exchange chromatography, they are expected to elute together with the neutral eGua.

From NMR data, it is concluded that elution peak II contains the compound *cis*-Pt(NH_3)$_2$(eGua-*N*1)(eGua-*N*7) (see Figure 4). The product shows two H8 signals, and the pH dependence of these signals clearly points to the presence of both an N1- and an N7-platinated eGua. This was deduced from comparison of the NMR behavior of this compound with the above-described corresponding Pt(dien) adducts with eGua, which are monofunctionally coordinated at N1 or N7.

Elution peak III appears to contain a monofunctional adduct, denoted as *cis*-Pt(NH_3)$_2$(eGua-*N*7)X. The pH profile (Figure 4) clearly argues for N7-coordination. Since this product elutes just between species with charge +1 (vide supra) and +2 (vide infra) under neutral conditions, it is difficult to deduce the charge of the unknown group bound to the second coordination site of cis-Pt. Coordination of a hydroxo group (X), however, is unlikely since the amount of the product increases when the reaction is performed under neutral conditions.

The main product formed both at pH 7.0 and at pH 10.5, eluted under peak IV, appears to be *cis*-Pt(NH_3)$_2$(eGua-*N*7)$_2$. The pH curves of the coinciding H8 signals of this product indicates this. Compared to Pt(dien)(eGua-*N*7), an apparent upfield shift of the H8 signal of about 0.1 ppm is observed for the cis-Pt compound. This can be ascribed to a mutual shielding effect present in the latter adduct, which has been suggested earlier.[26] The same effect is also observed in product III, in which the H8 proton is shifted about 0.2 ppm downfield compared to that of *cis*-Pt(NH_3)$_2$(eGua-*N*7)$_2$. The absence of this shielding effect also indicates the absence of a second guanine in product III.

From the three H8 signals in the NMR spectrum, it is concluded that elution peak V contains a dinuclear platinum compound, containing three eGua's. The pH dependence of these signals is shown in Figure 5. By means of the information supplied by the pH plots of the corresponding Pt(dien) adducts, it was concluded that both an N1,N7-platinated eGua and two N7-platinated eGua's must be present in species V. The dinuclear compound, depicted in Figure 5 agrees with these NMR data. It is interesting to note that the observed protonation at low pH in the pH curve of [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7), which we ascribe to an N3-protonation, is also present in the N1,N7-platinated part of the trimer, i.e. the centrally positioned eGua.

NMR spectra of product VI show an irreproducible and probably unstable product, which was therefore not investigated further.

No OH-bridged platinum dimers could be characterized in our study with cis-Pt. Such species would have a higher positive overall charge than those of the characterized products. Rather precise charges can be deduced by comparing the cation-exchange profiles of the cis-Pt products with the Pt(dien) profiles. The proposed structures of the cis-Pt adducts agree with the deduced charges.

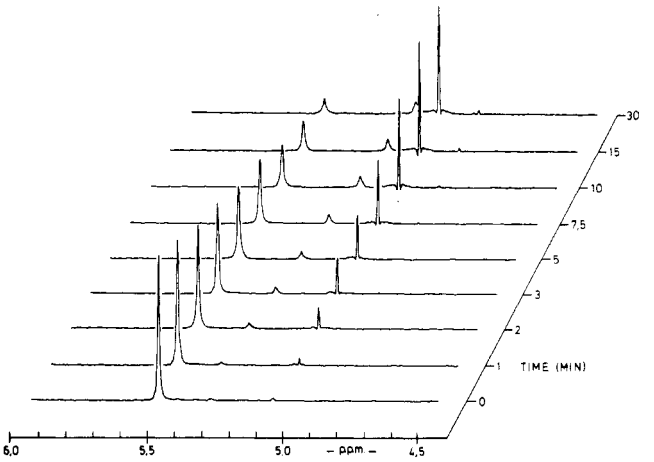

Figure 6. Aromatic region of the ^{1}H NMR spectra of Pt(dien)(eGua-*N*1), during the isomerization, monitored after certain time intervals. For conditions, see Materials and Methods.

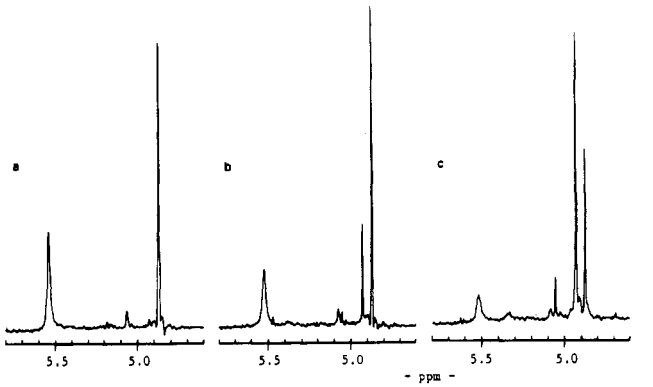

Figure 7. Aromatic region of the ^{1}H NMR spectra of *cis*-Pt(NH_3)$_2$(eGua-*N*7)(eGua-*N*1), during the isomerization, recorded at t = 0 (a), 3 (b), and 15 (c) min.

In summary, the results presented here are comparable to those obtained with the ligand mHyp. However, the expected products *cis*-Pt(NH_3)$_2$(eGua-*N*1)$_2$ and/or *cis*-Pt(NH_3)$_2$(eGua-*N*1)X could not be obtained in sufficient amounts to allow NMR characterization. The fact that also Pt(dien)(eGua-*N*1) could be isolated does not support an earlier hypothesis,[27] which implicates that N1-platination will occur only after N7-platination. In particular, the presented NMR results appear to be indispensible for the identification of several degradation products which are formed out of both identified N1 products. This interesting degradation is described in the next section.

Degradation of N1-Platinated eGua Adducts. To obtain more data about the stability of N1-platinated eGua compounds, the monofunctional compound Pt(dien)(eGua-*N*1) was heated at 80 °C for 30 min under different conditions (pH 10, 7, and 2.8). At certain time intervals, possible degradation was monitored by proton NMR. As expected from the slightly acidic character of the H8 protons in purines,[28] rapid H8 hydrogen substitution for deuterium occurred at pH 10, and to a smaller extent also at pH 7.0, during the heating experiment. Since the NMR signals of the N9-bound ethyl group are also sensitive to different platinum coordination modes, degradation was checked in these cases by monitoring the ethyl signals. The quartet has shifted 0.08 ppm upfield for the N7 adduct compared to the N1 adduct at pH 2.8.

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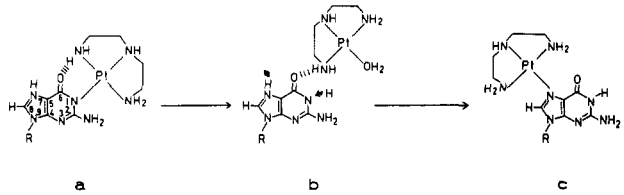

Figure 8. Schematic depiction of the intramolecular migration of the Pt(dien) moiety from N1 to N7 at pH 2.8.

Because no new ethyl signal splitting patterns arose during the heating experiment performed at pH 7 and 10, as checked by NMR at pH 2.8, it was concluded that the N1 compound is stable under these conditions. The stability of the compound under alkaline conditions is not surprising, since it is formed at high pH and 80 °C (see Materials and Methods).

At pH 2.8, on the other hand, an interesting degradation was observed. Figure 6 shows the aromatic region in the proton NMR spectra of the N1 compound recorded at certain time intervals during the heating experiments. A clear decrease in the spectrum of the N1 compound appeared to be accompanied by the formation of a new compound. After 30 min, four species can be observed in the NMR spectrum. Using the pH curves of the different Pt(dien) adducts (see Figure 4), it was possible to assign all of them. The N1 compound (5.55 ppm) had almost disappeared after 30-min heating. The predominant product appeared to be Pt(dien)(eGua-*N*7) (5.07 ppm), whereas also small amounts of nonmetalated eGua (5.43 ppm) and [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7) (4.91 ppm) can be observed. Clearly, an almost complete N1 to N7 migration of the Pt(dien) moiety had occurred. Contrary to both other conditions (pH 7.0 and pH 10.0), under which such a rearrangement is not observed, the N1 compound is largely N7-protonated at pH 2.8 (see figure 3). We therefore propose that the N7-protonation is a prerequisite for the observed instability of the N1 compound. This idea is supported by the observation that this migration is significantly slower at pH 4 (not shown). The upfield shifting of the H8 resonance of unbound eGua during the reaction reflects an pH effect of about 0.2 unit to higher value. This can be understood as the rearrangement of the nonprotonated N1 adduct (about 25% of the total N1 adduct amount at pH 2.8, see Figure 4) uses a proton (see Figure 8). The H8 resonance of unbound eGua and the N1 adduct appears to be broadened at pH 2.8. This is most likely due to a N7-protonation/deprotonation process that is slow on the NMR time scale. When the NMR spectra are recorded at pH values not in the pK_a range of the N7-(de)protonation, the signals sharpen. The same effect is observed for the N1,N7 adduct with cis-Pt. The H8 signal of the N1-bound eGua is broadened whereas this signal of the N7-bound eGua is sharp at pH 2.8 (vide infra).

It is well-known[28] that at high temperatures and especially at high pH, rapid H8 exchange in guanine residues can occur. This phenomenon seems to be enhanced for N7-platinated guanine residues.[29,30] If the rate of this exchange of hydrogen for deuterium under these conditions is different for the four platinum adducts under investigation, we would arrive at wrong conclusions about the quantities of the formed adducts, since the quantitation is based upon integration of the guanine H8 signals. Therefore Pt(dien)(eGua-*N*7), [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7), and eGua were separately incubated for 3 h at pH 2.8 and 80 °C. By comparison of the areas of the H8 signal and the ethyl signals, it was concluded that within experimental error no H8-exchange had occurred. It is evident that the deuterium exchange of the H8 proton in the N1 compound could not be monitored, due to the instability of the adduct under these conditions.

Besides H8 hydrogen exchange for deuterium, signal disappearance resulting from platinum binding is also an important factor to take into account for quantitation. Contrary to the other isotopes,[31] ^{195}Pt (34% abundance), has a nuclear spin of $^1/_2$. Scalar coupling with the H8 signal of guanine is well established for a variety of N7-platinated guanines and other oxopurine residues. This coupling results in "satellite" peaks around the H8 signal.[3,32,33] However, disappearance of these "satellites" is found when the spectra are recorded at higher magnetic fields. This is explained as the result of an field-dependent chemical shift anisotropy relaxation effect.[34] Therefore, the observed areas of the H8 signals of the N7-platinated eGua adducts, i.e. of Pt(dien)(eGua-*N*7) and [Pt(dien)]$_2$(μ-eGua-*N*1,*N*7), have to be corrected for this effect.[35]

Taking this effect into account, it was concluded that after the isomerization, Pt(dien)(eGua-*N*7) was present as the major product (yield more than 80%). The isomerization (80 °C, pH 2.8) was considered to be complete after 2 h, when the H8 signal of the N1 adduct had completely disappeared in the proton NMR spectrum. Prolonged heating gave no further change in the spectra; therefore, both other platinum adducts, i.e. the N7 and the N1,N7 adduct, were considered to be stable.

For the other N1 product, *cis*-Pt(NH$_3$)$_2$(eGua-*N*1)(eGua-*N*7), a similar migration phenomenon was observed. Heating of a sample of this adduct under the above described conditions (80 °C, pH 2.8) resulted in the disappearance of this adduct (compare Figure 4) and the formation of two new species, a major and a minor one. From the NMR data depicted in Figure 4 it was easy to assign the major product to *cis*-Pt(NH$_3$)$_2$(eGua-*N*7)$_2$ (4.92 ppm), whereas the minor product is expected to be *cis*-Pt(NH$_3$)$_2$(eGua-*N*7)X (5.08 ppm; X denotes an unknown ligand). Some typical spectra of this isomerization, recorded at 0, 3, and 15 min, are redrawn in Figure 7.

Migration Mechanism. Although we did not investigate the isomerization mechanism in great detail, a few comments will be made. For the above-described isomerizations, two different mechanisms can be distinguished, i.e. an intramolecular and an intermolecular mechanism. For the first mechanism, the platinum moiety remains coordinated to the same eGua, while in the other case it migrates from the N1 site of eGua to the N7 site of another, unbound nucleobase.

To discriminate between these mechanisms, the isomerization was followed both in the presence and in the absence of salts. Earlier observations have made clear that the reaction between cis-Pt and DNA or 5′-GMP is considerably slowed down when it is performed in the presence of a 0.1 M salt concentration.[36,37] It is likely that the same holds for [PtCl(dien)]Cl. In the case of ammonium chloride, an irreversible inactivation of the Pt(dien) moiety can be expected. Therefore, a strong increase of the amount of unbound eGua can be expected when the isomerization is performed in a 0.1 M NH$_4$Cl solution, if this process would occur intermolecularly. On the other hand, almost no inactivation effect would be expected for the intramolecular mechanism.

Isomerization of Pt(dien)(eGua-*N*1) at pH 2.8 and 80 °C in the presence of 0.1 M NaCl or NH$_4$Cl was performed together with a control experiment in which the salts were omitted. No significant differences in the adduct formation among the three experiments could be observed, based on the NMR spectra (not shown). The result that no more unbound eGua was found in the experiments performed in 0.1 M salt indicates that the isomerization largely occurs via an intramolecular mechanism.

On the molecular level, an intrabase rearrangement of the platinum moiety can be understood when it occurs via hydrogen bonding of the platinum-coordinated amine to the O6 of eGua.

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This mechanism is schematically visualized in Figure 8. After the Pt–N1 bond, which is weakened by the N7-protonation, is broken, a rapid protonation (N1) and deprotonation (N7) can take place (see Figure 8b). Meanwhile, the platinum moiety can move around from the N1 to the N7 and bind to this site, meanwhile remaining attached to the eGua by hydrogen bonding to the O6. This N1 to N7 migration will occur very rapidly, since no inactivation of the hydrogen-bonded Pt moiety by means of substitution of the coordinated water by Cl^- of NH_3 is observed. Additional support for this mechanism was obtained by the observation that the isomerization yielded more metal-free eGua when performed at pH 1.5 (data not shown). In that case, a larger amount of the intermediate compound is protonated at both the N1 and the N7, making the ultimate formation of the N7 adduct less probable.

Another migration model, assuming two head-to-tail stacked N1 adducts in which the two platinum moieties jump at the same time from the N1 to the N7 of the stacking eGua is not very likely. Stacking interactions are very sensitive to ionic strength differences, and in our case the addition of 0.1 M salt did not induce any difference in the migration rate (vide supra).

It has been reported that a platinum migration mechanism might also occur for inosine.[16] In that case, however, an N7 to N1 isomerization at pH 5–6 was described, with a dinuclear N1,N7 species as intermediate. These results are, however, not applicable to our study, as in our case an opposite migration from N1 to N7 occurs and the dinuclear compound $[Pt(dien)]_2(\mu$-eGua-*N*1,*N*7) appears to be very stable at pH 2.8. The difference therefore must originate from the amine group, which is not present at C2 in inosine.

Concluding Remarks. In this study, the synthesis and characterization of various platinum products with 9-ethylguanine have been presented. An interesting migration of the platinum moiety from N1 to N7—which probably occurs intramolecularly—could be studied in detail. Since platinum adducts with nucleobase are generally looked upon as very stable, this finding of instable mononuclear eGua-*N*1 adducts is of great importance and can be of relevance for future studies of metal–DNA interactions.

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Metal Complexes of Tetrapyrrole Ligands. 43.[1] Nickel(II) 5,15-Dialkyl-5,15-dihydro-2,3,7,8,12,13,17,18-octaethylporphyrins (Decaalkylporphodimethenes): Isolation of a New Stereoisomer and ^{1}H NMR Spectroscopy

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The synthesis and spectral characterization of novel nickel(II) 5,15-dialkyl-5,15-dihydro-2,3,7,8,12,13,17,18-octaethylporphyrins, $Ni(OEPR_2)$ (*aa*-**1**–*aa*-**4**), are described wherein the bulky 5,15-substituents (R = Me, Et, *i*-Pr, and *t*-Bu) assume the syn-axial conformation. The isolation of a hitherto unknown anti (axial-equatorial) isomer of the diisopropyl derivative $Ni(OEPPr_2)$ (*ae*-**3**) is reported, and its configuration is deduced from a detailed analysis of the NMR spectra of all the nickel chelates. The chemical shifts are influenced by ring current effects of the individual pyrrole units, the folding of the tetrapyrrole system, the saddlelike deformation of the pyrromethene halves, and long-range shielding by neighboring groups.

The occurrence of reduced heme proteins in natural systems has generated much recent interest in the chemistry of simple chlorins (2,3-dihydroporphyrins) and isobacteriochlorins (2,3,7,8-tetrahydroporphyrins).[3–5] Both are species in which hydrogenation of the porphyrin ring has occurred at peripheral double bonds without effect upon the macrocyclic conjugation. A different class of hydrogenated porphyrins are the less commonly encountered 5,15-dialkyl-5,15-dihydroporphyrins (decaalkylporphodimethenes). Here, hydrogenation has occurred at two opposite meso positions and the ring conjugation is destroyed. Porphodimethenes readily coordinate to metal ions via the four pyrrole nitrogens, and we reported most recently the synthesis and ^{1}H NMR spectra of a series of paramagnetic cobalt(II) porphodimethenes.[6] The complexes specificially discussed herein are the nickel(II) 5,15-dialkyl-2,3,7,8,12,13,17,18-octaethylporphodimethenes, $Ni(OEPR_2)$ (see Figure 1), which are best prepared from zinc(II) octaethylporphyrin, Zn(OEP), by reduction with sodium anthracenide followed by alkylation with alkyl halide,

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