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Reaction of Symmetrically and Asymmetrically Substituted Derivatives of Cisplatin with r(ApG). Influence of the Nonleaving Groups on Structures and Kinetics

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Reactions between symmetrically and asymmetrically substituted *cis*-platinum amine compounds with the dinucleotide r(ApG) have been studied. The symmetric *cis*-platinum amine compounds (general formulae *cis*-$PtCl_2(LL)_2$, L = NH_3, alkylamine, also LL = ethylenediamine; these compounds contain a C_2 symmetry element) gave only one A-N7-G-N7 adduct as a major reaction product. On the other hand, the asymmetrically substituted compounds (general formulae *cis*-$PtCl_2(LL')_2$, L = NH_3, L' = alkylamine, also LL' = *N,N*-dimethylethylenediamine) gave two products (in different ratios), as detected and separated by HPLC. Product 1 has been assigned to a A-N7-G-N7 adduct having the substituted alkyl group(s) cis to the 3'G, whereas product 2 appears to have a structure with the alkylamine cis to the 5'A. The structures of the asymmetric adducts appear to deviate slightly from the cisplatinum adduct *cis*-$Pt(NH_3)_2$(ApG-*N7,N7*). Reaction velocity constants were measured under pseudo-first-order conditions, and for both the first and second binding steps the influence of the substituents was measured in the order $NH_3 \geq RNH_2 \gg R_2NH$. In the first step the binding selectivity for either the cis or trans side to the substituted amine was found to be quite low; however, the second step differs by a factor of 2-3 for the two reactions products. Steric hindrance appears to be important to explain the reactivity. On the other hand, the reaction velocity for the second step does not seem to be related to the rate of rotation (rotational barrier) around the Pt-GN7 bond. Rate constants for the first step vary from 0.017 (for $Pt(diethylamine)_2^{2+}$) to 0.277 M^{-1} s^{-1} (for $Pt(en)^{2+}$); for the second step they vary from 4.5×10^{-7} (for $Pt(diethylamine)_2^{2+}$) to 1.84×10^{-4} s^{-1} (for $Pt(en)^{2+}$). First-step rate constants of primary amine derivatives are nearly equal to that of cisplatin, while those of the asymmetric secondary amine derivatives are small. Second-step rate constants of secondary amine derivatives are small, and the bulkyness of the alkylamine groups may interfere with the rate of chelate formation.

Introduction

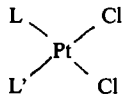
cis-diamminedichloroplatinum, also called cisplatin, is a well-known antitumor drug. It is supposed to obtain its activity by the reaction with DNA.[1] Cisplatin can form various adducts with DNA, but the major adducts are the intrastrand cross-links at adjacent purine GpG and ApG adducts.[2-4] The ApG and GpG adducts induce globally the same structural distortion in DNA, although it seems that at the nucleotide level they distort the double helix differently.[5]

The nature of the nonleaving groups (general formula *cis*-Pt-$(LL)_2X_2$, X = leaving group, L = amine = nonleaving group) seems to have some influence on the antitumor activity. In earlier studies[6] about structure-activity relationship for alkyl-substituted *cis*-platinum amine compounds the antitumor activity has been reported to decrease following the order $NH_3 \geq RNH_2 > R_2NH > R_3N$.

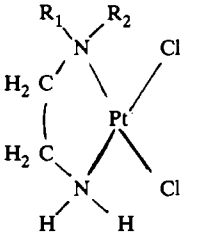
It is not yet completely clear how the influence of the substituents can be explained. Probably a change in the DNA conformation, after Pt binding, is induced by the substituents; however, also the decrease or even disappearance of hydrogen-bonding ability between the substituted amine and the nucleic acid constituents (bases, phosphates) might be important.[7] In addition, the reaction kinetics may play an important role. The substituents, giving the nonleaving groups a bulky character, will retard or hinder the binding of a platinum to purine N7. The influence of substituents on the reaction velocity of Pt(1,3-propanediamine) and 5'GMP was already reported.[8] Besides that also the chelation step (forming of a second binding after a 1:1 adduct) is likely to be important. The rotation around the Pt-GN7 bond in the intermediate adduct might be important for the intrastrand cross-linking and is supposed to be considerably influenced by a substitution in an amine group.[9,10]

The aim of the present study is to obtain a better understanding of the influence of the substituents on the reaction of substituted cisplatin analogues with certain DNA sites. To study adducts similar to a platinum-DNA adduct, r(ApG) (see Figure 1 for a schematic structure) was chosen as a reactant for a variety of platinum compounds.

Scheme I. Structure of the *cis*-Platinum Amine Complexes and Abbreviations



cisplatin	L = NH_3	L' = NH_3
Ptmma	L = NH_3	L' = NH_2CH_3
$Pt(mma)_2$	L = NH_2CH_3	L' = NH_2CH_3
Ptdma	L = NH_3	L' = $NH(CH_3)_2$
$Pt(dma)_2$	L = $NH(CH_3)_2$	L' = $NH(CH_3)_2$
Ptmea	L = NH_3	L' = $NH_2CH_2CH_3$
$Pt(mea)_2$	L = $NH_2CH_2CH_3$	L' = $NH_2CH_2CH_3$
$Pt(dea)_2$	L = $NH(CH_2CH_3)_2$	L' = $NH(CH_2CH_3)_2$



in Pten	R_1 = H	R_2 = H
in Ptdmen	R_1 = CH_3	R_2 = CH_3

The used cisplatin analogues and their abbreviations are depicted in Scheme I. A simple abbreviation is used for convenience,

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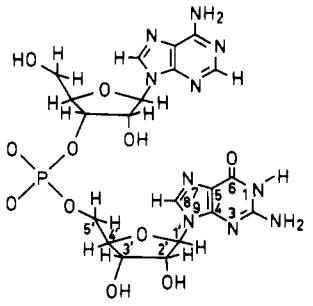

Figure 1. Schematic structure of r(ApG).

instead of the more complicated systematic names.[11] The *cis*-platinum amine compounds used can be divided into two groups, symmetric and asymmetric ones. Especially the asymmetric substituted *cis*-platinum amine compounds require special attention. Although Marzilli reported the reaction of Ptdmen with 5′AMP[10] and with d(TGGT),[12] the reaction of bis(ligand), nonbridged, asymmetric cisplatin analogues with small nucleic acid fragments has not yet been studied, to the best of our knowledge. Very recently, Arpalahti and Lippert published a study about the kinetics of the reaction between nucleosides and some symmetrically substituted platinum(II) amine compounds.[13a]

The first part of the present study concerns the assignment of the reaction products and their characterization by CD and NMR spectroscopy. The second part is a kinetic study, in which the concentrations were followed as a function of time. The obtained results are discussed, using the data of the cisplatin–r(ApG) reaction[13b] and the Pt(dmen) data[10,12] as references.

Experimental Methods

Synthesis. The complexes Pt(mma), Pt(dma), and Pt(mea) were synthesized as described by Rochon and Kong.[14] A slight improvement was found; i.e., on addition of ammonium hydroxide to the iodo dimer $[Pt(L')I_2]_2$, a stoichiometric amount was used (instead of the advised excess of it). Pt(dmen) was synthesized by a reaction, in stoichiometric ratio, of a K_2PtCl_4 solution with *N,N*-dimethylethylenediamine. Cisplatin, $Pt(mma)_2$, $Pt(dma)_2$, $Pt(mea)_2$, and $Pt(dea)_2$ were prepared as described by Dhara.[15] The purity of the formed platinum compounds was confirmed by IR spectroscopy and elemental analysis.[16] In the kinetic experiments the aquated NO_3 form of the platinum compound was used, because the slow Pt–Cl cleavage (aquation reaction) is the rate-determining step in the case of the reaction between cisplatin and r(ApG).[17,18] The aquated nitrate was prepared by a reaction with $AgNO_3$,[19] followed by passage of the reaction solution through a membrane filter to remove the AgCl(s).

NMR and CD Spectroscopy. The ammonium salt of r(ApG) was obtained from Sigma Chemicals. The reactions of the platinum complexes Pt(mma), Pt(dma), and Pt(dmen) with r(ApG) were performed at room temperature in the dark. The pH of the reaction solution was 5–6. After the reaction was completed (about 5 days), the reaction products were separated by HPLC. For the separation of Cosmosil $5C_{18}$ sorbent with an eluent of a 0.05 M KH_2PO_4 solution with methanol gradient (0–25%) was used. The separated samples were purified by passage through the HPLC column again, this time by using a low-concentrated $NaClO_4$ solution (0.01–0.005 M) with methanol. (N.B. *Caution*! Although no explosions were found in the present study, one should not evaporate the obtained perchlorate solutions.)

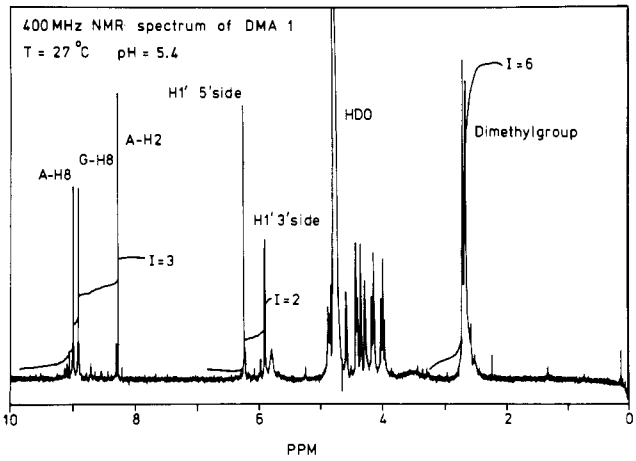

Figure 2. NMR spectrum of Pt(dma)-1, obtained at 400 MHz, 27 °C, pH = 5.4, and 3 mM concentration. The reference is TSP-d_4.

NMR samples were dissolved in 0.6 mL of 99.95% D_2O after being lyophilized three times from 99.75% D_2O (Merck). Solutions of the NMR samples were prepared in concentrations of about 3 mM (estimation). A trace of TSP-d_4 (sodium salt of 3-(trimethylsilyl)propionic acid; Aldrich) was added to the samples as an internal reference. For measurements of T_1 and 1-D NOE difference spectroscopy it was necessary that the samples were degassed under nitrogen atmosphere. All the measurements were performed at room temperature and pH 5.5. An example of a spectrum is depicted in Figure 2.

For the NMR analysis a 400-MHz JEOL apparatus, with use of standard Fourier transform techniques, was employed. For the adducts of Pt(dma) and Pt(dmen) with r(ApG), ^1H NMR spectra were recorded at several pH* values between 1.5 and 11. The pH* was adjusted with small quantities of either a NaOD or $DClO_4$ solution. The pH* values are not corrected for D_2O. The proton T_1 relaxation times were obtained by the inversion recovery method (180–*t*–90). The 1-D NOE was measured in the difference spectral mode, and the preirradiation time, 5 s, was used for complete saturation.

The CD spectra for all the Pt(mma), Pt(dma), and Pt(dmen) reaction products were measured at pH 2, 6, and 10 on a JASCO J-600 spectropolarimeter.

Kinetic Measurements. Reactions were performed under pseudo-first-order conditions; i.e., the platinum concentrations were 10–50 times in excess over that of r(ApG) (usually 0.03–0.07 mM/L was used). The reactions were performed at a constant temperature (18.3 °C), and the pH was kept steady at 3.3 by adding a concentrated HOAc solution. The reaction was started after mixing the platinum stock solution with the r(ApG) solution. The r(ApG) concentration was determined by measuring A_{254} of the r(ApG) stock solution, just before the start of the reaction; a molar extinction coefficient 18 000 M^{-1} cm^{-1} was used.[21] The reaction was followed by HPLC, and separation was achieved as described above. The signals in the HPLC chromatograms were detected by measuring the absorbance at 260 nm. Even though the binding of platinum causes a small shift in the λ_{max} for the nucleotides, the same molar extinction coefficient was assumed for all the reactants (although the molar extinction coefficient of the intermediate is not known, it is considered to have almost the same molar extinction coefficient as r(ApG)[13b]). The concentrations of the reaction products, intermediates, and r(ApG) have been determined by integration of the peak areas in the HPLC chromatograms (change of total peak area <5%).

The concentration of the platinum compound was determined, just after finishing the reaction, by AAS. A Shimadzu AA-630-12 atomic absorption/flame emission spectrophotometer was used.

The reaction velocity constants were determined by using a nonlinear curve-fitting computer program.[22] The overall reaction of the asym-

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(10) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 6785.
(11) Systematic names for the compounds are cisplatin = *cis*-diamminedichloroplatinum(II), Pt(mma) = *cis*-amminedichloro(methylamine)platinum(II), $Pt(mma)_2$ = *cis*-dichlorobis(methylamine)platinum(II), Pt(dma) = *cis*-amminedichloro(dimethylamine)platinum(II), $Pt(dma)_2$ = *cis*-dichlorobis(dimethylamine)platinum(II), Pt(mea) = *cis*-amminedichloro(ethylamine)platinum(II), $Pt(mea)_2$ = *cis*-dichlorobis(ethylamine)platinum(II), $Pt(dea)_2$ = *cis*-dichlorobis(diethylamine)platinum(II), Pt(en) = dichloro(ethylenediamine)platinum(II), and Pt(dmen) = dichloro(*N,N*-dimethylethylenediamine)platinum(II).
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(18) Segal, E.; Le Peck, J. B. *Cancer Res.* **1985**, *45*, 492–498.
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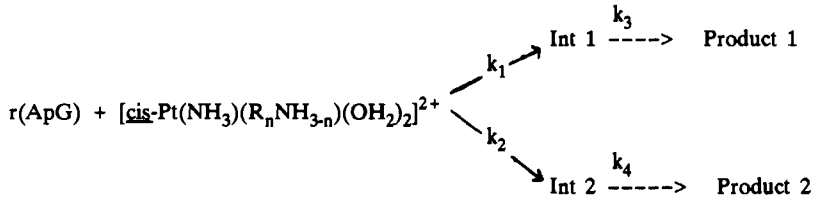
(20) Patt, S. L.; Sykes, B. D. *J. Chem. Phys.* **1972**, *56*, 3182.

(21) See ref 13b. In fact these authors used $A_{256.5}$.

Scheme II. Reactions and Kinetic Equations



$$\frac{[r(ApG)]_{t=t}}{[r(ApG)]_{t=0}} = e^{-(k_1'+k_2')t}$$

$$\frac{[Int\ 1]_{t=t}}{[r(ApG)]_{t=0}} = \frac{k_1'}{k_3-(k_1'+k_2')} * (e^{-(k_1'+k_2')t} - e^{-k_3t})$$

$$\frac{[Prod\ 1]_{t=t}}{[r(ApG)]_{t=0}} = k_1'k_3*[\frac{1}{k_3(k_1'+k_2')} + \frac{1}{(k_1'+k_2')(k_1'+k_2'-k_3)} e^{-(k_1'-k_2')t} - \frac{1}{k_3(k_1'+k_2'-k_3)} e^{-k_3t}]$$

$$\frac{[Int\ 2]_{t=t}}{[r(ApG)]_{t=0}} = \frac{k_2'}{k_4-(k_2'+k_1')} *(e^{-(k_2'+k_1')t} - e^{-k_4t})$$

$$\frac{[Product\ 2]_{t=t}}{[r(ApG)]_{t=0}} = k_2'k_4*[\frac{1}{k_4(k_2'+k_1')} + \frac{1}{(k_2'+k_1')(k_2'+k_1'-k_4)} e^{-(k_2'+k_1')t} - \frac{1}{k_4(k_2'+k_1'-k_4)} e^{-k_4t}]$$

Table I. Chemical Shifts of the Nonexchangeable Base Protons at pH = 5.5

	cisplatin[a]	Pt(mma)-1	Pt(mma)-2	Pt(dma)-1	Pt(dma)-2	Pt(dmen)-1	Pt(dmen)-2
A-H8	9.26	9.13	9.30	9.00	9.40	9.20	9.42
G-H8	8.45	8.63	8.41	8.91	8.38	8.89	8.26
A-H2	8.28	8.27	8.28	8.28	8.29	8.30	8.29

[a] Cisplatin data are taken from ref 13b.

metric platinum compounds with r(ApG) is shown in Scheme II. Under the used pseudo-first-order conditions, the concentration of each species at time t is also given in Scheme II. In this scheme k_1' and k_2' are observed rate constants; i.e., $k_1' = k_1*[Pt]$ and $k_2' = k_2*[Pt]$.

In the case of a symmetrical platinum compound, only one intermediate and one product is obtained.[13b] This became also clear after studying the pattern of the HPLC chromatograms. By use of the same pseudo-first-order conditions as before, the equations took the form as shown in Scheme III.

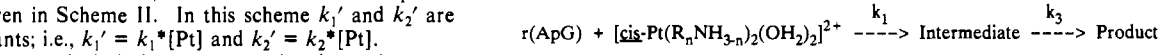
These equations are easily obtained by taking $k_2 = 0$ and $k_4 = 0$ in the case of the asymmetric platinum compounds (Scheme II), and they agree with the equation used by Chottard et al. for measuring the reaction velocity constants of cisplatin with r(ApG) and r(GpA).[13b] All the reactions were performed in triplicate, and the final reaction constants were obtained by averaging.

Results

Assignment of the Reaction Products. The reaction of asymmetric *cis*-platinum amine compounds with r(ApG) results in two major end products. They are called **1** and **2** after their HPLC elution order (with abbreviations such as Pt(mma)-1 and Pt(mma)-2). The ratio of the two products was always found to be 1:1, and only in the case of dmen was a clear preference found for one product (26:74 as determined by HPLC peak integration). After the separation of the two adducts, each product was characterized by detailed NMR spectral analysis.

The NMR spectrum of Pt(dma)-1 is shown in Figure 2. Integration of the nonexchangeable base protons and of the substituted methyl protons clearly shows that the reaction product is a 1:1 adduct. The nonexchangeable base protons of Pt(dma)-1 and Pt(dma)-2 were assigned by the pH–NMR titration (see supplementary material Figures S1 and S2). Relevant chemical shift values are listed in Table I. The titration curve of the G-H8 proton showed that the pK_a value at G-N1 is about 8.5; moreover, no protonation at G-N7 was observed. The titration curves of the A-H8 and A-H2 protons showed an inflection at pH < 3.0, as a result of the protonation at A-N1 of a N7-platinated adenine.[23,24] Discrimination between A-H8 and A-H2 was possible

Scheme III. Reactions and Kinetics for Symmetric Products



$$\frac{[r(ApG)]_{t=t}}{[r(ApG)]_{t=0}} = e^{-k_1't}$$

$$\frac{[Int]_{t=t}}{[r(ApG)]_{t=0}} = \frac{k_1'}{(k_3-k_1')} *(e^{-k_1't} - e^{-k_3t})$$

$$\frac{[Prod]_{t=t}}{[r(ApG)]_{t=0}} = k_1'k_3*[\frac{1}{k_3k_1'} + \frac{1}{k_1'(k_1'-k_3)} e^{-k_1't} - \frac{1}{k_3(k_1'-k_3)} e^{-k_3t}]$$

Table II. Relaxation Times T_1 of the Nonexchangeable Base Protons of Cisplatin–r(ApG)[a] and Pt(dma)–r(ApG) Products **1** and **2**

	cisplatin	Pt(dma)-1	Pt(dma)-2
A-H8	0.82 (s)	0.51 (s)	0.43 (s)
G-H8	0.82 (s)	0.68 (s)	0.76 (s)
A-H2	2.9 (s)	5.28 (s)	5.98 (s)

[a] Data are from ref 13b.

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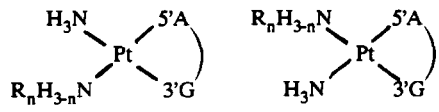
Chart I. Representations of the Two Possible Orientations of the Asymmetric *cis*-Platinum Amine–r(ApG) Adduct



Structure 1 *Structure 2*

from their T_1 values,[13b] as listed in Table II. At pH > 10 the A-H8 and G-H8 protons slowly exchange with solvent D_2O, as seen from their gradual decrease in intensity. The rate of exchange is faster for A-H8, indicating that Pt(dma)-1 and Pt(dma)-2 are A-N7–G-N7 adducts. This assignment is supported by the fact that one of the H1′ protons shows a singlet (arising from the N conformer of the furanose ring). Similar NMR spectral data were observed for the other r(ApG)–cisplatin analogues.

Cisplatin has been known to yield an A-N7–G-N7 adduct as a major reaction product in the reaction with r(ApG).[13b] The measured CD spectra of the Pt(mma), Pt(dma), and Pt(dmen) adducts with r(ApG) (data not shown) agree well with the cisplatin data,[13b] showing an A-N7–G-N7 anti,anti structure, while they maintain the overall right-handed helical arrangement of the sugar–phosphate backbone.[13b,25] For all the substituted *cis*-platinum amine analogues some minor products were detected. The yield of these minor products, however, in all cases is less than 5%. At pH < 4 where the kinetic measurements were done these products were observed even <1%. Cisplatin has a C_2 symmetry element.[13b] The lack of such a symmetry element is thought to be the origin of the two AN7–GN7 adducts (i.e. Pt(dma)-1 and Pt(dma)-2) with r(ApG). The difference between the two isomers originates from the orientation of the substituted methyl groups with respect to the orientation of the A and G bases (see Chart I). Formation of such isomers, arising from the lack of C_2 symmetry of the Pt compound, has also been found in the reaction products of Pt(dmen) and d(TGGT), as described by Marzilli.[12] They assigned the two isomers (both GN7–GN7 adducts) by using ^{31}P chemical shifts for the GpG moiety,[12] the major adduct (66%) has the Me groups cis toward 3′G, and the minor product (34%) has the Me groups cis toward 5′G.

As is known from earlier studies with cisplatin–d(GG)[26] and cisplatin–d(CGG),[27] the ^{31}P signal between the two guanine residues is shifted 0.7 ppm further downfield in the case of the d(CGG), because of the hydrogen bonding between a 5′-phosphate and the coordinated ammine.[12] Molecular mechanics[28] also supported the hydrogen bonding between the ammine and the phosphate group 5′ to the GpG moiety.

Since Pt(dmen) has a primary and a tertiary amino group, the shift of the ^{31}P can be correlated with the potential hydrogen-bonding ability of the platinum amine groups. Therefore, it was possible, even without separation, to assign the two geometrical isomers. However, in the case of r(ApG) this assignment is not possible because of the lack of such a 5′-phosphate group. Therefore, the 1-D NOE difference spectrum was measured.

In the case of Pt(dma)-2 a signal at the A-H8 position was observed after irradiation of the substituted methyl groups (see supplementary material Figure S3). No signal was observed at the G-H8 position. Therefore, Pt(dma)-2 is assigned to the structure with the methyl-substituted amine cis to the 5′A (structure 2 in Chart I). The geometrical isomer, Pt(dma)-1, is supposed to possess a structure with the substituted amine cis to the 3′G (structure 1 in Chart I). However, no NOE signal at all was observed in this case, upon irradiation of the substituted methyl groups; this may indicate a prolonged distance between the substituted methyl groups and 3′G.

The results of the T_1 measurements are shown in Table II. For the reaction products of 5′AMP with Pt(dmen), Reily and Marzilli observed that the signals for an H8 proton close to the methyl-substituted amine have lower T_1 values than those for an H8 proton close to the ND_2 group.[10] The low values for Pt(dma)-1 G-H8 and Pt(dma)-2 A-H8 support the assignment based on the data of the NOE experiment.

It is supposed that the H8 signals are mainly influenced by the coordinated platinum, orientation and nature of the nonleaving group, ring current, and relative orientation of adjacent bases; i.e., they have diversity. However, the chemical shift of the nonexchangeable base protons observed in the present work appears to have a common "shift pattern"; viz., the following results are noted: (1) The chemical shift of A-H2 does not change in all the platinum adducts. (2) Compared with cisplatin, A-H8 of the adducts **1** is always shifted upfield and that of the adducts **2** is shifted downfield. (3) The opposite relation is observed for the chemical shift of the G-H8 protons. Compared with cisplatin, the G-H8 value of the adducts **1** is always shifted downfield and that of the adducts **2** is shifted upfield. (4) The difference in the chemical shift between the A-H8 and the G-H8 becomes always larger in the case of the adducts **2** compared with adducts **1**.

From these results, it can be concluded that the chemical environment of the A-H8 and G-H8 protons of all products **1** and **2** differs in the same way, i.e. for Pt(mma), Pt(dma), and Pt(dmen).

The CD spectra of the asymmetric substituted *cis*-platinum amine–r(ApG) adducts agree with the cisplatin data, suggesting a right-handed helical arrangement of the two bases (vide supra). Such an arrangement leads to a shorter distance between $NH(CH_3)_2$ and A-H8 (Pt(dma)-2, NOE observed) compared with the distance between $NH(CH_3)_2$ and G-H8 (Pt(dma)-1, no NOE observed). However, the structure of the asymmetric *cis*-platinum amine adducts might be slightly distorted by the alkyl substituent, compared with the cisplatin adduct. The adenine base in Pt(dma)-2 can make a small clockwise turn (viewed from Pt toward A-N7) to reduce the steric repulsion between the $NH(CH_3)_2$ and the 6-NH_2 of the adenine residue. This structural change will lead to a downfield shift of A-H8 and an upfield shift of G-H8. (In this case, there may be a possibility for hydrogen bonding between the NH_3 and the O6 because of the right-handed helical arrangement.) In the case of Pt(dma)-1, the G base could make a small counterclockwise turn to reduce the steric repulsion between $NH(CH_3)_2$ and the O6, leading to the downfield shift of G-H8 and the upfield shift of the A-H8.

An interesting observation was made by studying the state of the substituted group signal. In the case of Pt(dma)-2 the substituted methyl peak appears as a doublet. This is an interesting observation because only one peak would be expected for the chemical equivalent methyl groups. Obviously, the rotation around the Pt–N(substituted amine) bond is slowed down. The methyl signal of Pt(mma)-2 gives only a singlet, suggesting fast rotation around the Pt–N bond.

For Pt(dmen) the methyl signals are observed as a doublet. Because of the "en bridge", the rotation around the Pt–N bond becomes impossible; in fact, the doublet is expected even in the case of fast puckering of the "en" ring, because of the difference in chemical environment between the up-and-down sides of the platinum coordination plane, A-N7–G-N7–Pt(en).

Kinetic Measurements. A general reaction pathway for the asymmetric substituted *cis*-platinum amine compounds was proposed after studying the HPLC chromatograms as a function of time. The plots all show the general pattern of the two intermediates and the two final adducts. The adducts **1** and **2** are called after their HPLC elution order. The intermediates are numbered in agreement with their corresponding adduct, although in fact their HPLC elution order is opposite. It is supposed that, in the

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Table III. Reaction Velocity Constants for *cis*-Platinum Amine Compounds and r(ApG) (T = 18.3 °C; pH = 3.3)

Pt complex	k_1[a]	k_2[a]	$k_1 + k_2$[a]	k_3[b]	k_4[b]	k_{av}[b]
cisplatin	0.171 (0.01)[c]		0.171	1.44 (0.09)		1.44
Pt(mma)	0.0455 (0.004)	0.0383 (0.002)	0.0838	1.73 (0.007)	1.13 (0.005)	1.43
Pt(dma)	0.0247 (0.002)	0.020 (0.002)	0.0447	1.26 (0.03)	0.54 (0.06)	0.90
Pt(mea)	0.059 (0.003)	0.067 (0.006)	0.123	2.12 (0.002)	0.81 (0.010)	1.44
$Pt(mma)_2$	0.178 (0.021)		0.178	1.62 (0.14)		1.62
$Pt(dma)_2$	0.060 (0.009)		0.060	0.15 (0.02)		0.15
$Pt(mea)_2$	0.137 (0.013)		0.137	1.27 (0.06)		1.27
$Pt(dea)_2$	0.017 (0.004)		0.017	0.0045 (0.0004)		0.0045
Pt(en)	0.277 (0.07)		0.277	1.84 (0.17)		1.84
Pt(dmen)	$0.26k_{1+2}$	$0.74k_{1+2}$				

[a] Dimensions for the first-step reaction velocity constants are M^{-1} s^{-1}. [b] Dimensions for the chelation step are 10^{-4} s^{-1}. [c] The standard deviation is given between the parentheses.

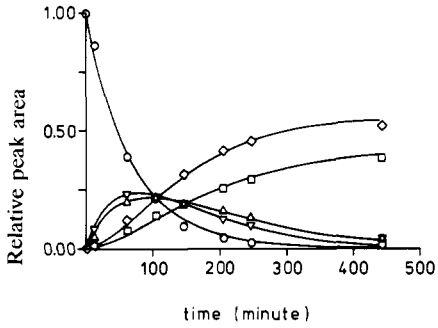

Figure 3. Species distribution as a function of time for the reaction of r(ApG) with Pt(mma): ○ = r(ApG); △ = intermediate **2**; □ = end product **2**; ▽ = intermediate **1**; ◇ = end product **1**. [r(ApG)] = 0.039 mM, [Pt] = 1.57 mM, pH = 3.3, and T = 18.3 °C.

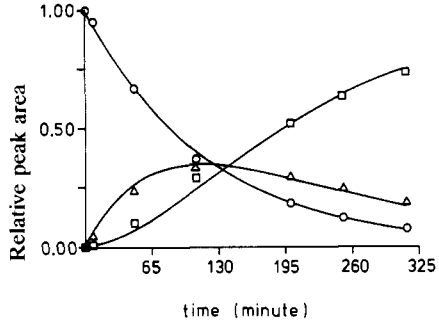

Figure 4. Species distribution as a function of time for the reaction of r(ApG) with cisplatin: ○ = r(ApG); △ = intermediate; □ = end product. [r(ApG)] = 0.046 mM, [Pt] = 2.21 mM, pH = 3.3, and T = 18.3 °C.

intermediate structure, the platinum is monofunctionally bound to G-N7.[29] The retention time of the final adducts **1** and **2** (obtained under pseudo-first-order conditions) was in complete agreement with that obtained in the preparative HPLC study and NMR spectra.

Figures 3 and 4 show the time dependence of r(ApG), the intermediates, and the final adducts, being obtained from a curve fitting using a nonlinear least-square program (vide supra). Figure 3 shows the reaction between the asymmetric Pt(mma) and r(ApG), while Figure 4 shows the reaction between the symmetric *cis*-platinum amine compounds and r(ApG). All the asymmetric *cis*-platinum amine analogues show the same pattern as Pt(mma) in Figure 3. The symmetrically substituted cisplatin analogues also show a pattern very similar to that of cisplatin in Figure 4.

By the study of Table III, it is quite clear that almost all k_1 values for the symmetric platinum compounds are nearly equal to the k_1 of cisplatin. In fact this is the case for the symmetric compounds $Pt(mma)_2$, $Pt(mea)_2$, and Pt(en). The value of k_1 for cisplatin is about 1 order of magnitude smaller than that reported[13b] by Chottard et al. at pH 5.2 and 20 °C (our values are pH 3.3 and T = 18.3 °C). In our case the low pH value (below the pK_a of A-N1, used to suppress the formation of a Pt–A-N1–G-N7 adduct) is believed to be responsible for the reduced value of k_1.

For a comparison of the kinetic data in Table III, the sum of k_1 and k_2 should be taken in the case of asymmetric compounds, because of the parallel reaction. These values represent in general the rate of conversion of r(ApG) to the platinum–r(ApG) intermediates. In the case of symmetric platinum compounds like cisplatin one has to consider k_1 and k_2, but because of the C_2 symmetry in the nonleaving group, they lead to the same intermediate. The $k_1 + k_2$ value is about equal to cisplatin for Pt(mea) although in the case of Pt(mma) a small decrease is observed. It is most likely that a monoalkyl substituent is only having a small influence on the first-step reaction. However, the value of $k_1 + k_2$ is lower in the case of the *cis*-platinum amine analogues involving the secondary amino group, Pt(dma) and $Pt(dma)_2$. In this respect it is interesting that the asymmetric Pt(dma) and $Pt(dma)_2$ have about the same reaction velocity in the first-step reaction. Therefore, it might be assumed that one $NH(CH_3)_2$ group is sufficient to slow down the reaction velocity.

In the comparison of k_1 and k_2, k_1 is not so different from k_2 in the cases of Pt(mma), Pt(dma), and Pt(mea). This means that the selectivity for either the cis or trans side to the substituted amine is quite low. Only in the case of Pt(dmen) is a clear selectivity found. The ratio of adducts **1** and **2** is 24:76; so k_2 is about 3 times larger than k_1. It is not clear what are the driving forces for formation of either adduct **1** or adduct **2**. However, either sterical hindrance (the bulkyness of a ligand may hamper the platinum to approach the G-N7 vicinity) or the loss of hydrogen-bonding ability might play an important role. In fact, these effects are expected to be responsible for the decrease of $k_1 + k_2$ in general.

Chottard et al.[30] studied the structure of the intermediate for the reaction of r(ApG) with *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$. They showed that in the pentacoordinated intermediate the hydrogen bonds are quite important. The hydrogen bondings, from the phosphate to the equatorial H_2O, are strong. There is also a weak hydrogen bond between the axial NH_3 and the phosphate. The velocity constant for the first-step reaction seems to be related to the hydrogen-bonding ability of the activated intermediate.

Pt(dmen) is the only compound with an ethylene bridge, which results in a preference for the binding side trans to the substituted amine. It can be concluded that the bulky ligands are becoming quite "fixed" in their structure because of the bridge. In the nonbridged case, for example in Pt(dma), the nonleaving groups are more free to rotate and vibrate. Therefore, when the platinum atom approaches the G-N7 binding site, it will not result in a selective hampering as in the Pt(dmen) case. In the case of Pt(dmen) it is not expected that a hydrogen bond between the tertiary amino group and the phosphate group will be formed.

For the second reaction step, some interesting phenomena were also observed. To obtain a good reference, the average value of k_3 and k_4 was taken in the case of an asymmetric platinum compound. This value of k_{av} (for chelation) represents the average

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conversion rate of the mono-G-N7-substituted intermediates to the A-N7-G-N7 adducts. That is, it is the equivalent of k_3 in the symmetric case. Quite accurate data were obtained, and as can be seen in Table III, the value of k_{av} obtained for cisplatin is about the same for Pt(mma), Pt(mma)$_2$, Pt(mea)$_2$, Pt(mea), and Pt(en) and shows good agreement with the cisplatin data obtained by Chottard et al.[13b] The only exceptions are the asymmetric Pt(dma) and the Pt(dma)$_2$ and Pt(dea)$_2$ compounds, which show a remarkable decrease in their second-step velocity. The decrease factor is about 2 for asymmetric Pt(dma), about 10 for Pt(dma)$_2$, and even about 300 for the bulky Pt(dea)$_2$.

In the case of the asymmetric compound an interesting pattern in the comparison of k_3 and k_4 is observed. Although the value for k_{av} does not change for Pt(mma) and Pt(mea), there is no equality for k_3 and k_4. In the case of Pt(mma), k_3 is 1.5 times larger than k_4, and in the case of Pt(dma) and Pt(mea), k_3 is about 2.5 times k_4. The difference of the second step becomes clear by comparing k_3 and k_4. The alkyl substitution begins to become remarkably effective in the second step of the reaction.

One would expect that the rotation around the Pt-G-N7 bond, directing the second binding place of the *cis*-platinum amine complex toward A-N7, is an important step in the second-step reaction,[9,10] which would lead to a small value of k_3 (corresponding to the conversion rate from the intermediate **1**, which has the alkyl amino group cis to the guanine residue). However, as can be seen in Table III, the value for k_3 is larger than for k_4 in all cases. Therefore, rotation around the Pt-G-N7 bond does not appear to play an important role in the kinetics of the chelation step.

It was already pointed out that although k_4 decreases, k_{av} does not decrease in the case of Pt(mma) and Pt(mea). The increase in the value of k_3 might originate from an instability of its monofunctional intermediate. As a result, the conversion to the adduct **1** might become easier.

Concluding Remarks

The reaction of substituted cisplatin analogues with r(ApG) results in one or two A-N7-G-N7 adducts as the major product, depending upon the symmetry of the platinum compound. The resulting structures seem to deviate slightly from cisplatin, as deduced from NMR and CD data. On the other hand, the substituents appear to have a marked influence on the reaction velocity of both the first binding step and the chelation step. Especially in the case of secondary amine derivatives, slow kinetics are observed.

The recently published results of Arpalahti and Lippert[13a] about the kinetics of cisplatin derivatives in reaction with nucleosides is in good agreement with our results. They found that the order of reactivity is $RNH_2 \geq NH_3 > R_2NH$, whereas we found $NH_3 > RNH_2$; this small deviation is easily understood from the fact that they use mononucleotides (for which hydrogen bonding is not so important), whereas in our case the phosphate group is likely to have the most important interaction[30] with NH_3.

It should be taken into account that for example [Pt(dien)Cl]Cl, a monofunctionally binding compound often used for mimicking the intermediate of a reaction between cisplatin and a nucleic acid,[31] is not antitumor active.[32] Therefore, also a substituted cisplatin analogue with a very slow chelation step may not exhibit any antitumor activity. Perhaps the low antitumor activity of cisplatin analogues containing bulky nonleaving groups is related to the slow kinetics. Preliminary time-dependent cytotoxicity experiments[16] seem to corroborate this hypothesis.

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Registry No. Cisplatin, 15663-27-1; Pt(mma), 105057-84-9; Pt(mma)$_2$, 15273-32-2; Pt(dma), 131657-35-7; Pt(dma)$_2$, 27928-80-9; Pt(mea), 64538-67-6; Pt(mea)$_2$, 22881-88-5; Pt(dea)$_2$, 41714-07-2; Pt(en), 14096-51-6; Pt(dmen), 41575-66-0; r(ApG), 3352-23-6.

Supplementary Material Available: Three figures (Figures S1-S3) showing titration curves and an NOE difference spectrum (3 pages). Ordering information is given on any current masthead page.

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Electrochemical Properties of Copper(II)/Copper(I)-Macrocyclic Polythia Ether Complexes: Determination of Formal Potential Values and Cyclic Voltammetric Behavior

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Extensive potentiostatic and cyclic voltammetric studies have been carried out on a series of copper-polythia ether complexes, including macrocyclic and acyclic ligand species, in both aqueous and 80% methanol (w/w) solutions. From the accumulated data, a recommended set of formal potential values are reported for the $Cu^{II/I}L$ redox couples in aqueous solution at 25 °C. On the basis of these potentials and previously measured stability constants for the $Cu^{II}L$ species, the apparent stability constants for the $Cu^{I}L$ species have been calculated. The formal potential values and $Cu^{I}L$ stability constants in aqueous solution are as follows (for 25 °C, 0.10 M ClO_4^-, listed in the following order: complexed ligand, E^f in V vs SHE, log $K_{Cu^{I}L}$): [12]aneS$_4$, <0.69 V, <12.$_8$; [13]aneS$_4$, ≈0.52 V, ≈10.0; [14]aneS$_4$, 0.59 V, 12.1$_1$; *syn*-[14]aneS$_4$-diol, 0.52 V, 9.7$_1$; [15]aneS$_4$, 0.64 V, 11.7$_9$; [16]aneS$_4$, 0.71 V, 12.0$_0$; [15]aneS$_5$, 0.69 V, 13.6$_5$; Me$_2$-2,3,2-S$_4$, ≈0.79 V, ≈13.1; Et$_2$-2,3,2-S$_4$, 0.79 V, 13.3$_4$. Anomalous cyclic voltammetric behavior was observed for the [12]aneS$_4$ system, which appears to be consistent with the previously proposed "square scheme" mechanism involving metastable intermediates of both the $Cu^{I}L$ and $Cu^{II}L$ species.

Introduction

Recently, we have reported the results of both homogeneous[1] and heterogeneous[2] electron-transfer studies involving low molecular weight copper-polythia ether complexes that appear to support the existence of a "square scheme" mechanism.

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