111903-03-8; [Co(De2)(1-MeIm)], 111903-04-9; [Co(Az-P)(1,2-Me_2Im)], 111903-05-0; [Co(Az-val$\beta\beta$)(1,2-Me_2Im)], 111933-38-1; [Co(Az-piv$\beta\beta$)(1,2-Me_2Im)], 111933-39-2; [Co(Az-P)(2-MeIm)], 111933-40-5; [Co(Az-val$\beta\beta$)(2-MeIm)], 111903-06-1; [Co(Az-piv$\beta\beta$)(2-MeIm)], 111903-07-2; [Co(Az-P)(2-EtIm)], 111903-08-3; [Co(Az-val$\beta\beta$)(2-EtIm)], 111903-09-4; [Co(Az-piv$\beta\beta$)(2-EtIm)], 111903-10-7; pivaloyl chloride, 3282-30-2; pentanoyl chloride, 638-29-9; oxygen, 7782-44-7.

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Bulky Ligand Substituent Effect on the Reaction of 5'-GMP with Pt(1,3-diamine). Rotation of 5'-GMP about the Pt-N Bond and Kinetic Effects

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Substituent effects in the reaction of 5'-GMP with [Pt(1,3-diamine)$(OH_2)_2$] have been investigated. The 1,3-diamines used are 1,3-propanediamine (dap), 1,3-cyclohexanediamine (1,3-dach), 2,2,*N*,*N*-tetramethyl-1,3-propanediamine (tmdap), *N*-methyl-1,3-propanediamine (mdap), and *N*-ethyl-1,3-propanediamine (edap). The bulky substituents have a large influence on the rotation of 5'-GMP about the Pt-N7 bond and also on the rate constants for the formation of [Pt(1,3-diamine)(5'-GMP-*N7*)(OH_2)] and [Pt(1,3-diamine)(5'-GMP-*N7*)$_2$] products. In the 1:1 and 2:1 products containing tmdap, the rotation of 5'-GMP cis to the $N(CH_3)_2$ group is slow on the NMR time scale. On the other hand, the presence of only one methyl group on the coordinated nitrogen atom (i.e. $N(H)(CH_3)$) seems not to retard the rotation of 5'-GMP. The kinetics of the reaction of 5'-GMP with [Pt(1,3-diamine)$(OH_2)_2$] are sensitive to the presence of the bulky substituents. The 1:1 and 2:1 compounds containing 1,3-dach show fast rotation of 5'-GMP about the Pt-N7 bond, but the binding rate is somewhat reduced by the steric hindrance originating from the cyclohexane ring. The presence of the N-substituted group slows down the rate for binding 5'-GMP at the coordination side cis to the N-substituted group. There is almost no difference between the binding rates of 5'-GMP at the coordination side cis to the NH_2 group, except for the case of Pt(1,3-dach), where the rate is slightly decreased.

Introduction

In the antitumor properties of cis-platinum compounds (general formula *cis*-Pt(amine)$_2X_2$, X = leaving group), the nature of nonleaving ligands, i.e. the amine ligands, plays an important role. The early observation of a structure-activity relationship indicated that the activity of Pt(amine)$_2Cl_2$ decreases along the series $NH_3 \approx RNH_2 > R_2NH > R_3N$.[1] The degree of antitumor activity may depend on various kinds of factors such as solubility, stability, toxic side reactions and cell permeability of platinum compounds, and so on. Principally it might be possible to improve these properties by modification of the leaving ligand, but more likely the nonleaving ligands present an essential factor for the actual antitumor activity.

It has been generally accepted that DNA is a primary target of antitumor platinum drugs[2] and that N7 of the guanine base is a preferential platinum binding site. The interaction between the platinum compound and the guanine base, such as the intrastrand cross-link between two adjacent guanine bases,[3-8] appears to play an important role in cell killing.[9,10] Differences in the nature of the nonleaving ligands are expected to affect the reactivity between the platinum compound and the ultimate target DNA, especially when the platinum compounds have different substituents, which may result in steric effects and H-bonding differences. A bulky amine substituent in the platinum compound may induce a conformational change of the DNA after platinum modification and may lead to decrease and/or disappearance in hydrogen-bonding ability between the coordinated amino group and nucleic acid constituents (bases, phosphates). Such effects are likely to appear in a series of ligands that have only small differences.

In the present study, the reaction of guanosine 5'-monophosphate (5'-GMP) with a series of Pt(1,3-diamine) compounds containing sterically bulky substituents will be described.[11] Structures and abbreviations of the used Pt(1,3-diamine) compounds are shown in Figure 1. In the platinum complex containing 1,3-dach, the cyclohexane ring lies roughly perpendicular to the platinum coordination plane,[12] and it is likely to influence the approach of the incoming ligand, 5'-GMP. The ligand tmdap has a tertiary and a primary amino group. The two methyl groups on the coordinated nitrogen atom occupy an axial and equatorial position, with rapid interconversion of the Pt-NN chelate ring. Steric effects of the $N(CH_3)_2$ group are therefore expected to be effective below and above the Pt coordination plane. The ligands mdap and edap both have a secondary and a primary amino group. The methyl and ethyl groups may generate different steric effects because of their difference in size.

A major aim of the present study is to evaluate how the reactions between 5'-GMP and the Pt(1,3-diamine) compounds are influenced by the substituents in the ligands. The presence of the substituents may lead to restricted rotation of 5'-GMP about the Pt-N7 bond and retardation of the reaction rate between 5'-GMP and Pt(1,3-diamine). The reaction between 5'-GMP and bifunctional platinum compounds occurs via a two-step mechanism.[13,14] The first step corresponds to formation of the 1:1

(1) Cleare, M. J.; Hydes, P. C.; Malerbi, B. N.; Watkins, D. M. *Biochimie* **1978**, *60*, 835 and references therein.
(2) Robert, J. J.; Pera, M. P., Jr. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; pp 183-231.
(3) den Hartog, J. H. J.; Altona, C.; Chottard, J. C.; Girault, J. P.; Lallemand, J. Y.; de Leeuw, F. A. A. M.; Marcelis, A. T. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 4715.
(4) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352.
(5) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science (Washington, D.C.)* **1985**, *230*, 412.
(6) Admiraal, G.; van der Veer, J. L.; de Graaff, R. A. G.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1987**, *109*, 592.
(7) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707.
(8) Eastman, A. *Biochemistry* **1983**, *22*, 3927.
(9) Ciccarelli, R. B.; Solomon, M. J.; Varshavsky, A.; Lippard, S. J. *Biochemistry* **1985**, *24*, 7533.
(10) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616; *Biochim. Biophys. Acta* **1985**, *780*, 167.
(11) In this paper, the overall charges of platinum GMP compounds are omitted. For convenience we will write [Pt(1,3-diamine)(OH_2)], although at pH 6.15 for most amines the predominant species will be [Pt(1,3-diamine)$(OH)(OH_2)]^+$.
(12) Kamisawa, K.; Matsumoto, K.; Ooi, S.; Kuroya, H.; Saito, R.; Kidani, Y. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2330.
(13) Marcellis, A. T. M.; van Kralingen, C. G.; Reedijk, J. *J. Inorg. Biochem.* **1980**, *13*, 213.
(14) Reedijk, J.; den Hartog, J. H. J.; Fichtinger-Schepman, A. M. J.; Marcelis, A. T. M. In *Platinum Coordination Complexes in Cancer Chemotherapy*; Hacker, M. P., Douple, D. P., Krakoff, I. H., Eds.; Martinus Nijhoff: Boston, 1984; pp 39-50.

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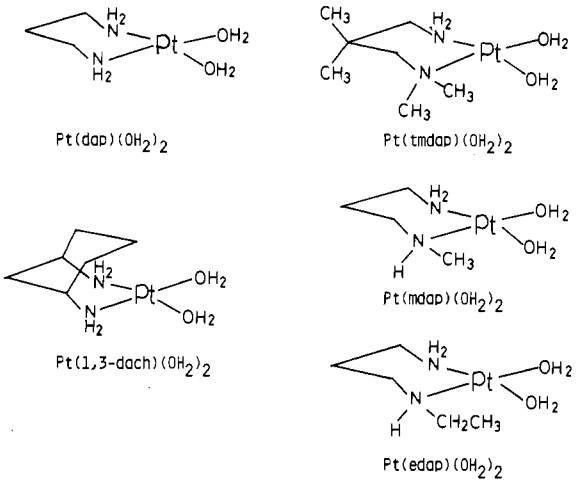

Figure 1. Structures and abbreviations of $[Pt(1,3\text{-diamine})(OH_2)_2]$.

compound [Pt(diamine)(5'-GMP-*N7*)(OH_2)] and the second step to formation of the 2:1 compound [Pt(diamine)(5'-GMP-*N7*)$_2$]. In the present paper, the rate constants for each step will be discussed in relation to the nature of the substituents.

Experimental Section

Materials. The nucleotides used were commercially available. The compounds $[Pt(1,3\text{-diamine})Cl_2]$, in which 1,3-diamine is dap, *meso*-1,3-dach, tmdap, mdap, and edap, were prepared according to the literature.[15,16] $[Pt(1,3\text{-diamine})(NO_3)_2]$ was prepared by stirring an aqueous solution of $[Pt(1,3\text{-diamine})Cl_2]$ with 2 equiv of $AgNO_3$.[17] After 24 h, the precipitate of AgCl was removed by filtration, and the filtrate was evaporated to dryness. In case of hygroscopic residues, a few milliliters of alcohol was added and the evaporation to dryness repeated. A dilute or moderately concentrated aqueous solution (<0.3 M) of $[Pt(1,3\text{-diamine})(NO_3)_2]$ appears to yield completely aquated species,[17] $[Pt(1,3\text{-diamine})(OH_2)_2]^{2+}$. It should be noted that, at pH values near those of physiological conditions, hydrolysis will occur and species like $[Pt(1,3\text{-diamine})(OH)(OH_2)]^+$ are predominant at pH 6.15 (vide infra).

NMR Measurements. The proton NMR spectra were recorded at 21 °C on a Bruker WM-300 spectrometer. A trace amount of tetramethylammonium nitrate, TMA, was added as an internal reference. All chemical shifts were measured from TMA (3.18 ppm downfield from DSS). The pH* (uncorrected meter readings in D_2O) of the solution used in this work was adjusted by adding small amounts of a D_2O solution of 1 M DNO_3 and 1 M NaOD.

Kinetic Measurements. Measurement of k_1 was carried out by UV spectrometry. The reaction[18] of 5'-GMP with $[Pt(1,3\text{-diamine})(OH_2)_2]$ was carried out in 0.03 M phosphate buffer, pH 6.15 (a value close to physiological pH with hardly any $[Pt(1,3\text{-diamine})(OH)_2]$ present). The concentration of 5'-GMP was held constant at 9.84×10^{-5} M. The concentration of $[Pt(1,3\text{-diamine})(OH_2)_2]$ was varied between 1.2×10^{-3} and 5.8×10^{-3} M. Although one could expect some interaction between phosphate and *cis*-$[Pt(amine)_2]^{2+}$, our conditions are much more dilute in platinum (30–200×) and PO_4^{3-} (20×) than those where binding was observed.[19] The reaction was monitored by changes in absorption at 294 nm. The observed rate constant, k_{obsd}, was obtained from pseudo-first-order Guggenheim plots.

The value of k_2 was obtained from NMR measurements of the time dependence of the reaction solution containing 5'-GMP (12 mM) and $[Pt(1,3\text{-diamine})(OH_2)_2]$ (5.6 mM). The concentrations of [Pt(1,3-diamine)(5'-GMP-*N7*)(OH_2)], [Pt(1,3-diamine)(5'-GMP-*N7*)$_2$, and unreacted 5'-GMP were determined by integration of their H8 signals. Integration of the H8 signals may give a small error as a result of exchange of the H8 with D_2O at high pH and coupling with the ^{195}Pt nucleus. For *cis*-$[Pt(NH_3)_2(5'\text{-GMP})_2]$, the half-life of the H8 deuterium-exchange reaction[4] has been known to be 69.3 h at pH* 10 and 37 °C. The effect of the H8 deuterium-exchange reaction appears to be negligible under our experimental conditions. To avoid coordination complication, buffer solutions were not used in this case. The pH* of the solution before and after the reaction was in the range of 6.6 and 6.4, respectively, for all cases. No chemical shift change of any species was observed throughout the reaction, confirming that there was almost no pH change during the reaction.

To determine the value of k_{2a} and k_{2b}, the solution containing Ia and Ib (see Scheme I) was prepared as follows.[20] 5'-GMP was allowed to react with an excess of $[Pt(1,3\text{-diamine})(OH_2)_2]$, [Pt]/[5'-GMP] > 2.5, for about 10 min. A slurry of Sephadex CM-25 was added to the reaction solution, and the pH of the solution was then adjusted to 6.5, to remove the unreacted $[Pt(1,3\text{-diamine})(OH_2)_2]$ by adsorption to the weak-cation-exchange resin. Immediately after filtration, the filtrate was lyophilized. For the purpose of kinetic measurement, the lyophilized

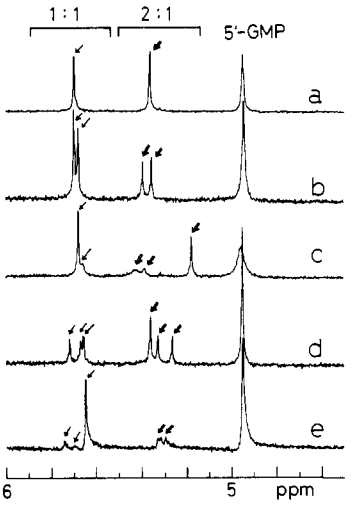

Figure 2. Downfield regions of NMR spectra of the reaction mixture of $[Pt(1,3\text{-diamine})(OH_2)_2]$ with 5'-GMP at pD 6.6: (a) Pt(dap) + 5'-GMP; (b) Pt(1,3-dach) + 5'-GMP; (c) Pt(tmdap) + 5'-GMP; (d) Pt(mdap) + 5'-GMP; (e) Pt(edap) + 5'-GMP. The arrows represent the H8 signals due to the 1:1 (←) and 2:1 (⇐) compounds. Free 5'-GMP signal occurred at 4.95 ppm.

Scheme I

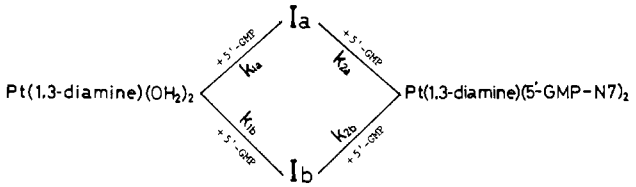

Table I. NMR Spectral Data for [Pt(1,3-diamine)(5'-GMP-*N7*)(OH_2)] and [Pt(1,3-diamine)(5'-GMP-*N7*)$_2$][a]

compds	δ(H8)	δ(H1') (*J*,Hz)
5'-GMP	4.95	2.750 (*J* = 6)
[Pt(dap)(5'-GMP-*N7*)(OH_2)]	5.69	2.850 (*J* = 3)
[Pt(dap)(5'-GMP-*N7*)$_2$]	5.36	2.710 (*J* = 5)
[Pt(1,3-dach)(5'-GMP-*N7*)(OH_2)]	5.69, 5.67	2.745 (*J* = 2.5)
[Pt(1,3-dach)(5'-GMP-*N7*)$_2$]	5.39, 5.35	2.70 (*J* = 8), 2.71 (*J* = 8)
[Pt(tmdap)(5'-GMP-*N7*)(OH_2)]	5.67, 5.65	2.85 (*J* = 3), 2.82 (*J* = 3)
[Pt(tmdap)(5'-GMP-*N7*)$_2$]	5.42, 5.38, 5.18	2.64–2.71 [6][b]
[Pt(mdap)(5'-GMP-*N7*)(OH_2)]	5.72, 5.67, 5.66, 5.65	2.84–2.88 [4]
[Pt(mdap)(5'-GMP-*N7*)$_2$]	5.37, 5.33, 5.27	2.66–2.73 [6]
[Pt(edap)(5'-GMP-*N7*)(OH_2)]	5.74, 5.70, 5.65	2.82–2.86 [4]
[Pt(edap)(5'-GMP-*N7*)$_2$]	5.34, 5.33, 5.30	2.67–2.72 [5]

[a] Conditions: pH* 6.6, 21 °C (pH* denotes uncorrected meter readings). [b] Number of observed resonance lines shown in brackets.

(15) Dhara, S. G. *Indian J. Chem.* **1970**, *7*, 335.
(16) van Kralingen, C. G. Ph.D. Thesis, Delft University of Technology, 1979.
(17) Lippert, B.; Lock, C. J. L.; Rosenberg, B.; Zvagulis, M. *Inorg. Chem.* **1977**, *16*, 1525.
(18) $[Pt(1,3\text{-diamine})(NO_3)_2]$ was dissolved in water just before the reaction, and 5'-GMP was dissolved in the phosphate buffer solution.
(19) (a) Wood, F. E.; Hunt, C. T.; Balch, A. L. *Inorg. Chim. Acta* **1982**, *67*, L19. (b) Appleton, T. G.; Berry, R. D.; Davis, C. A.; Hall, J. R.; Kimlin, H. A. *Inorg. Chem.* **1984**, *23*, 3514.
(20) Reily, M. D.; Marzilli, L. G. *J. Am. Chem. Soc.* **1986**, *108*, 6785, 8299.

Table II. Theoretical Number of H8 Resonances Expected for [Pt(1,3-diamine)(5′-GMP)] Compounds

Pt(1,3-diamine)	sym of Pt(1,3-diamine)[c]	no. of H8 resonances 1:1 fast	1:1 slow	2:1 fast	2:1 slow
Pt(dap)[a]	C_2 σ_{xz} σ_{xy}	1	2	1	2
Pt(1,3-dach)	σ_{xz}	2	4	2	4
Pt(tmdap)[a]	σ_{xy}	2	4	2	4
Pt(mdap)[b]	C_1	2	4	4	8
Pt(edap)[b]	C_1	2	4	4	8

[a] Conformational inversion in dap and tmdap is likely to be very fast. [b] In practice, Pt(mdap) and Pt(edap) are racemic mixtures so that the number of H8 resonances becomes double. [c] The axes chosen are as follows: x through Pt and middle of N–N; y through Pt and middle of N–O; z perpendicular to coordination plane.

compound was dissolved in D_2O, and a small amount of a uridine 5′-monophosphate (5′-UMP) solution of known concentration was added as an internal reference for quantification. After 5′-GMP was added, the change from the 1:1 compounds to the 2:1 compound was followed at suitable time intervals by means of NMR. The concentration of Ia and Ib was calculated from the relative integration value of the H8 signals to that of the H6 signals of 5′-UMP.

Results and Discussion

NMR Spectral Features of the 1:1 and 2:1 Compounds. Figure 2 shows the downfield region of the NMR spectra of the reaction solution of [Pt(1,3-diamine)$(OH_2)_2$] with 5′-GMP. The signals of the H8 resonances due to the 1:1 compound [Pt(1,3-diamine)(5′-GMP-*N7*)(OH_2)] appear in all cases in the range of 5.65–5.74 ppm, and those due to the 2:1 compound [Pt(1,3-diamine)(5′-GMP-*N7*)$_2$] are observed in the range of 5.18–5.42 ppm. This means that the H8 resonances due to the 1:1 and 2:1 compounds are shifted downfield by 0.7–0.8 and 0.2–0.5 ppm, respectively, compared with the H8 resonance of free 5′-GMP. This is consistent with binding through the N7 atom of 5′-GMP. The same chemical shift relationship had been observed in the corresponding compounds involving *cis*[Pt$(NH_3)_2(OH_2)_2$].[21-23] The large difference in the chemical shift permits quantification of each species by means of NMR. The H1′ sugar resonances of the 1:1 compounds appear at lower field, compared with the same resonance due to free 5′-GMP, while higher field shifts are observed for the H1′ signals of the 2:1 compound. The J(H1′-H2′) coupling constants for the 1:1 and 2:1 compounds and free 5′-GMP are in the range of 2–4, 5–7, and 6 Hz, respectively. The decreases in the coupling constants for the 1:1 compounds are relatively large, strongly suggesting an increase in population of the N-type sugar conformation.[24] These spectral features are common in all present compounds and do not depend on the Pt(1,3-diamine) moiety.

Rotation of 5′-GMP about the Pt–N7 Bond. When the amine ligand has no bulky substituents, the compounds [Pt(diamine)-(6-Opur-*N7*)$_2$] (6-Opur = 6-oxopurine) are known to exhibit fast rotation about the Pt–N7 bond on the NMR time scale.[26,27] Table II lists the theoretical number of H8 resonances expected for the compounds with Pt(1,3-diamine) and 5′-GMP. The table has been set up on the basis of the assumption that the two 5′-GMP ligands in the 2:1 compounds are in a head-to-tail arrangement.[25] A head-to-tail arrangement is the most likely orientation for such 2:1 products.[26-31]

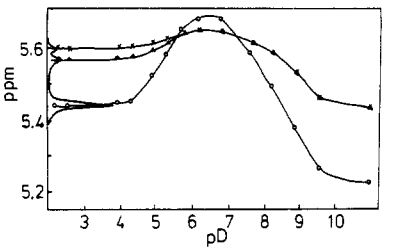

Figure 3. pH dependence of the H8 resonances of the two geometrical isomers of [Pt(tmdap)(5′-GMP-*N7*)(OH_2)].

The NMR spectra of the 1:1 and 2:1 compounds involving 5′-GMP and Pt(dap) show only a single H8 resonance (see Figure 2), suggesting fast rotation of 5′-GMP about the Pt–N7 bond on the NMR time scale. The cyclohexane ring in Pt(1,3-dach) creates different chemical environments above and below the platinum coordination plane. However, in both the 1:1 and 2:1 compounds, only two H8 resonances are observed, suggesting fast rotation of 5′-GMP about the Pt–N7 bond. The 1:1 compound involving Pt(tmdap) and 5′-GMP showed two slightly different H8 resonances (5.67 and 5.65 ppm) at pH* 6.6. Figure 3 shows the pH dependence of the H8 resonances after reaction and formation of the 1:1 compounds. The inflection centered at pH* 5.3 is mainly attributed to the change in chemical shift, resulting from the protonation of the phosphate group, although it may also involve the change due to the protonation of the hydroxo group,[32] [Pt(tmdap)(5′-GMP-*N7*)(OH)]. The pK_a value of the hydroxo group has been estimated to be 6.3–6.5.[32] The second inflection centered at pH* 8.5 is assigned to the deprotonation at the N1 atom of 5′-GMP. Figure 3 shows that three H8 resonances are observed below pH* 5.5. The peak at 5.65 ppm is thought to originate from two merged peaks. The reaction of [Pt(tmdap)$(OH_2)_2$] with 5′-GMP yields two 1:1 compounds, apparently one with 5′-GMP cis to the $N(CH_3)_2$ group and one with 5′-GMP cis to the NH_2 group. For the former species, rotation of 5′-GMP about the Pt–N7 bond is slow, whereas it is fast for the other species. In such a case, three H8 resonances should be theoretically observed, and this nicely agrees with our observation at pH* <5.5. In the case of the 2:1 compound from 5′-GMP and Pt(tmdap), three H8 resonances were observed at pH* 6.6. One of them, i.e. the peak at 5.18 ppm, is split into two peaks with equal intensity below pH* 4 and above pH* 9. This suggests that at least one 5′-GMP ligand exhibits slow rotation, likely the one cis to the $N(CH_3)_2$ group. It is not possible to conclude that the rotation of 5′-GMP cis to the NH_2 group is slow, since four H8 resonances would also be theoretically expected when the rotation is fast.

The substituted nitrogen in Pt(mdap) and Pt(edap) is chiral, having either an R or an S configuration. The [Pt(mdap)$(OH_2)_2$] and [Pt(edap)$(OH)_2$] complexes used in this study are each racemic mixtures and have no symmetry element so that the reaction of Pt(mdap) with 5′-GMP should produce four kinds of 1:1 compounds, [Pt(*R*/*S*-mdap)(5′-GMP-*N7*)(OH_2)], with 5′-GMP either cis to the $N(CH_3)_2$ group or cis to the NH_2 group. The 5′-GMP group in each compound will produce a single H8 resonance in the case of fast rotation of 5′-GMP. The four compounds are mutually nonequivalent. The presence of four H8 resonances suggests fast rotation of 5′-GMP, not only cis to the NH_2 group but also cis to the $NH(CH_3)$ group. The NMR spectrum of the 1:1 compounds involving 5′-GMP and Pt(edap) showed three H8 resonances at pH* 6.6. One of them, i.e., the peak at 5.65 ppm, is split into two peaks with equal intensity at pH* 3. The presence of the four H8 resonances suggests that the 1:1 compounds have a fast rotation of 5′-GMP, just as observed for [Pt(mdap)(5′-GMP-*N7*)(OH_2)]. In the 2:1 compound in-

(21) Marcelis, A. T. M.; Erkelens, C.; Reedijk, J. *Inorg. Chim. Acta* **1984**, *91*, 129.
(22) Dijt, F. J.; Canters, G. W.; den Hartog, J. H. J.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644.
(23) Fanchiang, Y.-T. *J. Chem. Soc., Dalton Trans.* **1986**, 135.
(24) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413.
(25) If rotation about the Pt-N7 bond is slow, two diastereomers are formed because of the presence of 5′-GMP with the chiral D-ribose.
(26) Cramer, R. R.; Dahlstrom, P. L. *J. Am. Chem. Soc.* **1979**, *101*, 3679; *Inorg. Chem.* **1985**, *24*, 3420.
(27) Marcelis, A. T. M.; van der Veer, J. L.; Zwetsloot, J. C. M.; Reedijk, J. *Inorg. Chim. Acta* **1983**, *78*, 195.
(28) Marzilli, L. G.; Chalilpoyil, P. *J. Am. Chem. Soc.* **1980**, *102*, 873.
(29) Marzilli, L. G.; Chalilpoyil, P.; Chiang, C. C.; Kistenmacher, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 2480.
(30) It has been known that [Pt(dap)(5′-GMP-*N7*)$_2$] has a head-to-tail arrangement by X-ray diffraction.[28,29] The CD spectrum[31] of [Pt(1,3-dach)(5′-GMP-*N7*)$_2$] is almost the same as that of [Pt(dap)(5′-GMP-*N7*)$_2$].
(31) Inagaki, K.; Kidani, Y. *Chem. Pharm. Bull.* **1985**, *33*, 5593.
(32) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32.

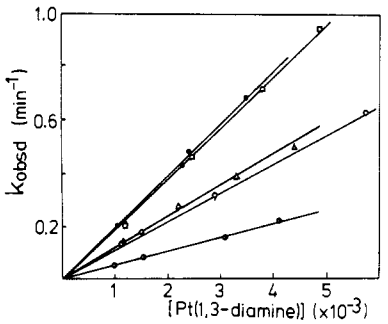

Figure 4. Observed rate constant as a function of [Pt(1,3-diamine)-$(OH_2)_2$] in the reaction with 5′-GMP ([5′-GMP] = 9.84 × 10^{-5} M; pH 6.15; 21 °C): (●) Pt(dap)–5′-GMP; (□) Pt(mdap)–5′-GMP; (△) Pt-(1,3-dach)–5′-GMP; (○) Pt(edap)–5′-GMP; (◑) Pt(tmdap)–5′-GMP.

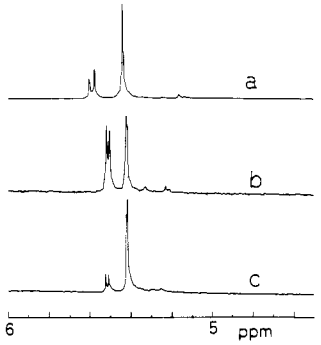

Figure 5. Downfield regions of NMR spectra of the 1:1 products (pH* 3–4; 21 °C): (a) [Pt(tmdap)(5′-GMP-*N7*)(OH_2)]; (b) [Pt(mdap)(5′-GMP-*N7*)(OH_2)]; (c) [Pt(edap)(5′-GMP-N_7)(OH_2)].

volving 5′-GMP and Pt(mdap) or Pt(edap), the NMR spectra show three H8 resonances. For [Pt(mdap)(5′-GMP-*N7*)$_2$], eight H8 resonances would be expected in the case of fast rotation because Pt(mdap) has no symmetry element and a racemic mixture of Pt(mdap) is present. This is also the case in Pt-(edap)(5′-GMP-*N7*)$_2$. Unfortunately, their chemical shift differences are not large enough to resolve all the peaks. It is not known whether the three peaks observed are due to overlapping of 8 peaks or overlapping of even 16 peaks. However, it is quite likely that rotation of 5′-GMP in these compounds is fast. In such a case, the methyl or ethyl group in these compounds may adopt an axial position of the chelate ring to facilitate the fast rotation of 5′-GMP. On the other hand, the *N*-methyl groups in [Pt-(tmdap)(5′-GMP-*N7*)$_2$] cannot occupy such a position, and therefore here slow rotation is indeed observed.

Kinetics Measurements. General Considerations. The occurring reactions can be described according to the mechanism outlined in Scheme I, in which Ia and Ib represent the 1:1 compounds. In the 1:1 compounds containing tmdap, mdap, and edap, species Ia and Ib are assigned in convenience to the compound with 5′-GMP cis to the substituted-N group and cis to the NH_2 group, respectively. They are geometrical isomers. In the case of the 1:1 compound containing dap, Ia and Ib are diastereoisomers, being produced under conditions of slow rotation of 5′-GMP about the Pt–N7 bond; however, in this case, the rotation is fast as described above, so that here Ia = Ib. Generally, the reaction between the platinum aqua complex and 5′-GMP can be regarded as an irreversible reaction because the binding between platinum and N7 of guanine base is very stable and the reverse reaction is negligibly slow.[33,34]

Rate Constants for the First Step. The formation rate of the 1:1 compound was too rapid to measure by means of NMR; therefore, its kinetics were followed by the absorption change at 294 nm of the UV spectrum, under pseudo-first-order conditions. Each observed rate constant was determined from the pseudo-first-order Guggenheim plots. Under the pseudo-first-order conditions and varying initial concentrations of [Pt(1,3-diamine)$(OH_2)_2$], a plot of k_{obsd} versus [Pt(1,3-diamine)$(OH_2)_2$] gave a good straight line passing through the origin as shown in Figure 4. The k_1 values obtained from the slope of the straight line are equal to the sum of the rate constants k_{1a} and k_{1b}. When 5′-GMP is reacted with an excess of [Pt(1,3-diamine)$(OH_2)_2$], the reaction yields almost pure 1:1 compounds. Figure 5 shows their NMR spectra, measured at pH* 3–4 to obtain maximal separation for the signals due to Ia and Ib. The ratio of the rate constants, k_{1a}/k_{1b}, was calculated from the integration values of the H8 resonances of Ia and Ib. The results are listed in Table III.

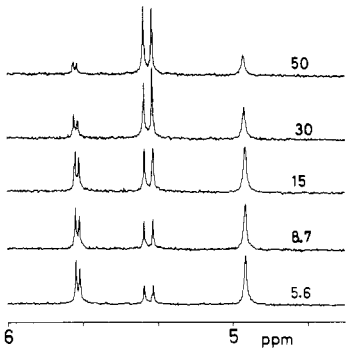

Figure 6. Time dependence of the NMR spectrum of the reaction mixture of [Pt(1,3-dach)$(OH_2)_2$] (5.6 mM) and 5′-GMP (12 mM) at pH* 6.6. Times indicated are minutes after mixing of the two solutions. Signals near 5.7 ppm are due to the 1:1 products and those near 5.4 ppm to the 2:1 products.

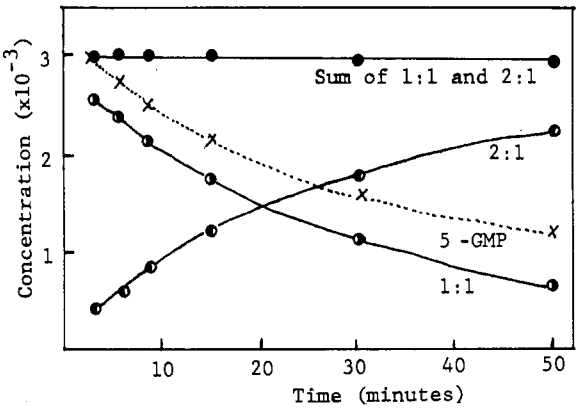

Figure 7. Time dependence of the concentration of each species in the reaction of [Pt(1,3-dach)$(OH_2)_2$] with 5′-GMP.

Rate Constants for the Second Step. Figure 6 shows the time dependence of the reaction between 5′-GMP and [Pt(1,3-dach)$(OH_2)_2$] under second-order conditions. The NMR spectrum shows doublets corresponding to the H8 resonances of the 1:1 and 2:1 compounds and one singlet due to unreacted 5′-GMP. The two peaks due to the 1:1 compounds have equal area, i.e., [Ia] = [Ib], and the ratio did not change throughout the reaction course. This indicates that there is no difference in the reactivities of Ia and Ib toward the formation of the 2:1 compound, i.e., $\vec{k}_{2a} = \vec{k}_{2b}$. In such a case, Scheme I is simplified as follows:

[Pt(1,3-diamine)$(OH_2)_2$] + 5′-GMP → [Pt(1,3-diamine)(5′-GMP-*N7*)(OH_2)]

[Pt(1,3-diamine)(5′-GMP-*N7*)(OH_2)]+ 5′-GMP → [Pt(1,3-diamine)(5′-GMP-*N7*)$_2$]

This corresponds to the reaction between 5′-GMP and [Pt-(dap)$(OH_2)_2$]. Plots of the concentration of each species versus time are shown in Figure 7. The decrease in the concentration of the 1:1 compound corresponds well to the increase of that of the 2:1 compound. It is worth noting that the sum of the concentration of the 1:1 and 2:1 compounds was constant throughout, indicating that the amount of free platinum can be neglected; i.e.,

(33) Eapen, S.; Green, M.; Ismail, I. M. *J. Inorg. Biochem.* **1985**, *24*, 233.
(34) Evans, D. J.; Ford, N. R.; Green, M. *Inorg. Chim. Acta* **1986**, *125*, L39.

Table III. Rate Constants for the Formation of the 1:1 and the 2:1 Compounds

Pt(1,3-diamine)	k_1 ($=k_{1a}+k_{1b}$),[a] M^{-1} s^{-1}	k_{1a}/k_{1b}	k_2,[b] M^{-1} s^{-1}	k_{2b}, M^{-1} s^{-1}	k_{2b}/k_{2a}
Pt(dap)	3.14 ± 0.16	1.0	0.24 ± 0.01		1.0
Pt(1,3-dach)	2.03 ± 0.15	1.0	0.12 ± 0.01	0.12 ± 0.01	1.0
Pt(tmdap)	0.89 ± 0.06	0.49		0.025 ± 0.008[d]	0.10 ± 0.04[d]
Pt(mdap)	3.05 ± 0.19	0.85		0.26 ± 0.04[e]	0.98 ± 0.14[e]
Pt(edap)	1.94 ± 0.14	0.24		0.064 ± 0.019[f]	0.24 ± 0.06[f]

[a] Conditions: pH 6.15; 20 °C. [b] Conditions: pH* 6.6; 21 °C. [c] The value was obtained from the numerical integration method by using the data from the k_2 measurement. [d] Conditions: pH* 4.5; 21 °C. [e] Conditions: pH* 5.2; 21 °C. [f] Conditions: pH* 4.8; 21 °C.

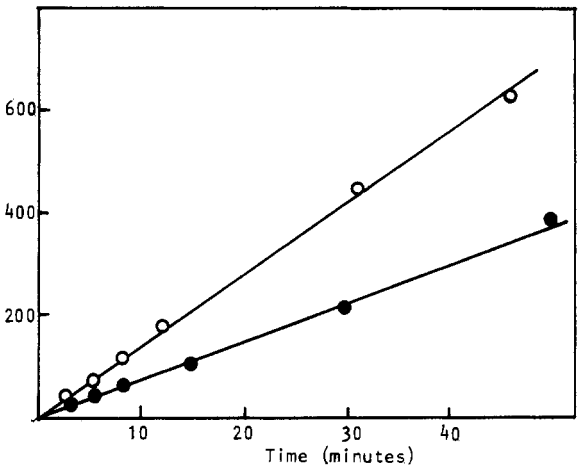

Figure 8. Second-order plot for formation of the 2:1 products with dap and dach $[Pt]_0$ = 5.6 mM; $[L]_0$ = 12 mM): (○) Pt(dap)–5′-GMP; (●) Pt(1,3-dach)–5′-GMP. The y axis represents the right-hand-side term of eq 1.

the formation of the 1:1 compound appears to be complete within 3 min under the conditions used. In such a case, the following material balance is valid:

$$[Pt]_0 = [PtL] + [PtL_2]$$

$$[L]_0 = [L] + [PtL] + 2[PtL_2]$$

where $[Pt]_0$ and $[L]_0$ are the initial concentrations of [Pt(1,3-dach)$(OH_2)_2$] and 5′-GMP. Under these conditions, the following experimental rate expression can be obtained:

$$k_2 t = \frac{1}{[L]_0 - 2[Pt]_0} \ln \frac{[Pt]_0([L]_0 - [Pt]_0 - [PtL_2])}{([L]_0 - [Pt]_0)([Pt]_0 - [PtL_2])} \quad (1)$$

A plot of the logarithmic part of eq 1 versus time yields a straight line passing through the origin as shown in Figure 8. The value of k_2 was obtained from the slope of the straight line.

On the other hand, eq 1 cannot be applied for the reaction between 5′-GMP and [Pt(tmdap)$(OH_2)_2$], [Pt(mdap)$(OH_2)_2$], or [Pt(edap)$(OH_2)_2$] because in these cases [Ia] is not equal to [Ib]. The rate constants in these cases have to be determined from the rate expression[35] of the competition reactions

$$Ia + 5'\text{-GMP} \rightarrow [Pt(1,3\text{-diamine})(5'\text{-GMP-}N7)_2]$$

$$Ib + 5'\text{-GMP} \rightarrow [Pt(1,3\text{-diamine})(5'\text{-GMP-}N7)_2]$$

The ratio of the two rate constants is

$$\ln([Ib]/[Ib]_0) = (k_{2b}/k_{2a}) \ln([Ia]/[Ia]_0)$$

The rate constants k_{2a} and k_{2b} were calculated by numerical integration of the equation

$$k_{2a}t = \int_{[Ia]}^{[Ia]_0} 1/\{X([L]_0 - [Ia]_0 - [Ib]_0 + X + [Ib]_0 X^{k_{2b}/k_{2a}} [Ia]_0^{-k_{2b}/k_{2a}})\}\, dX$$

In this equation, $[Ia]_0$ and $[Ib]_0$ are the initial concentrations of Ia and Ib and X is the variable with respect to which the integration is carried out. In order to obtain k_{2a} and k_{2b}, isolation

(35) Emanuel, N. M.; Knorre, D. G. *Chemical Kinetics*; Wiley: New York, 1973; pp 207–218.

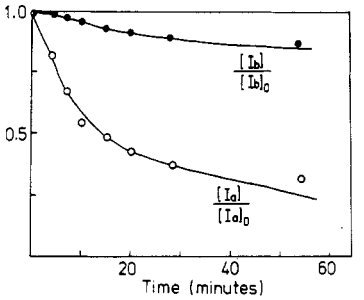

Figure 9. Plot showing the decrease of Ia and Ib in the reaction of Pt(tmdap)(5′-GMP-N7)(OH_2) with 5′-GMP ([5′-GMP] = 3.4 × 10^{-3} M; $[Ia]_0$ = 1.9 × 10^{-3} M; $[Ib]_0$ = 4.4 × 10^{-3} M; pH* 4.5; 21 °C).

Table IV. Rate Constant for Each Side in the Formation of the 1:1 and 2:1 Compounds (M^{-1} s^{-1}, Calculated from Table III)

Pt(1,3-diamine)	k_{1a}	k_{1b}	k_{2a}	k_{2b}	k_{1b}/k_{2a}
Pt(dap)	1.57	1.57	0.24	0.24	6.4
Pt(1,3-dach)	1.02	1.02	0.12	0.12	(8.7)
Pt(tmdap)	0.29	0.60	0.25	0.025	(2.4)
Pt(mdap)	1.40	1.65	0.27	0.26	6.2
Pt(edap)	0.39	1.56	0.26	0.064	5.9

of Ia and Ib is required, which was done according to the method reported by Reily and Marzilli.[19] Figure 9 shows the time dependence of Ia and Ib for the reaction of [Pt(tmdap)(5′-GMP-*N7*)(OH_2)] with 5′-GMP. Apparently, there is a large difference between the reactivities of Ia and Ib toward the formation of the 2:1 compound. It is also clear that Ib is the compound with 5′-GMP cis to the NH_2 side. This suggests that an approach of the second 5′-GMP to the side cis of $N(CH_3)_2$ is greatly retarded as a result of steric hindrance. The rate constants thus obtained are given in Table III, whereas Table IV lists the rate constants for binding to each side (Ia, Ib).

From Tables III and IV the following conclusions can be drawn.

(1) In the first step of the reaction between 5′-GMP and [Pt(dap)$(OH_2)_2$], [Pt(mdap)$(OH_2)_2$], or [Pt(edap)$(OH_2)_2$], there is almost no difference between the rate constants, k_{1b}. This suggests that the substituted-N group does not impede a binding of the first 5′-GMP to the side cis to the NH_2 group. While a significant difference was observed for the binding rate of 5′-GMP to the side cis to the substituted-N group (k_{1a}). The presence of the *N*-ethyl group serves to slow down the rate by a factor of 4, whereas the *N*-methyl group affects the rate to only a slight degree (less than 15%).

(2) In the reaction between [Pt(tmdap)$(OH_2)_2$] and 5′-GMP, a decrease in the rate constants was observed at either side (k_{1a} and k_{1b}) and the rate to the side cis to the NH_2 group (k_{1b}) is 2 times faster than that to the side cis to the $N(CH_3)_2$ group. The decrease in the rate at the NH_2 side (k_{1b}) for tmdap, compared to the rate for e.g. mdap, may be due to the 1,3-interaction between $N(CH_3)_2$ and $C(CH_3)_2$ in the chelate ring. Because of 1,3-diaxial steric repulsion between the methyl groups, the solution conformation of the chelate ring may result in an increase in population of skew-boat and boat conformers. Molecular models indicate that the boat conformation results in an approach of the $C(CH_3)_2$ group to the NH_2 group.

(3) [Pt(1,3-dach)$(OH_2)_2$] has a symmetry plane that passes through Pt, C2, and C5. The presence of the two diastereoisomers Ia and Ib arises from the chirality of the ribose moiety of 5′-GMP.

Therefore, it is not likely to result in a difference between the reactivities at either sides of $[Pt(1,3\text{-dach})(OH_2)_2]$. The decrease in the rate for 1,3-dach, compared with the rate obtained in the case of Pt(dap), suggests that the cyclohexane ring of Pt(1,3-dach) somewhat retards the approach of 5'-GMP.

(4) In the second-step reaction, the rates for binding of the second 5'-GMP to the side cis to the NH_2 group (i.e. k_{2a}) are almost equal within experimental error, except for the case of Pt(1,3-dach), just as seen for the first binding step (i.e. k_{1b}). Thus, binding of the second 5'-GMP to the side cis to the N-substituted group is greatly hampered by the $N(CH_3)_2$ and $N(CH_2CH_3)$ groups. The decrease in k_{2a} and k_{2b} in the case of Pt(1,3-dach) arises from the steric hindrance due to the cyclohexane ring.

(5) In the second step of the reaction, the methyl group on Pt(mdap) hardly seems to impede an approach of 5'-GMP. In fact, the NMR spectrum showed that each H8 resonance due to the 1:1 compounds decreases with time by almost the same velocity to yield the corresponding 2:1 compound.

(6) In comparisons of of the first- and second-step reactions for the binding of 5'-GMP to the side cis to the NH_2 group, the rate constant for the second 5'-GMP is almost 6 times smaller than that for the first 5'-GMP. This is likely to result from the steric effect of the bound 5'-GMP in the 1:1 compounds, making the approach of the second slower. It should be noted that changes in the amine ligands might have some small influence on the pK values of the hydrolysis and that some contribution to the different K values may originate from such a statistical contribution.

Concluding Remarks. In this paper the rotation of 5'-GMP about the Pt-N7 bond—an important process in the intrastrand reaction with DNA—in $[Pt(1,3\text{-diamine})(5'\text{-GMP-}N7)(OH_2)]$ and $[Pt(1,3\text{-diamine})(5'\text{-GMP-}N7)_2]$ has been described. The presence of the $N(CH_3)_2$ group significantly slows down the rotation of 5'-GMP, whereas the presence of the $NH(CH_3)$ group in Pt(mdap) and the cyclohexane ring in Pt(1,3-dach) still allows fast rotation of 5'-GMP. Our conclusions agree with those reported earlier by Marcelis et al.[21] and Cramer and Dahlstrom[26] on two related ligands.

Our kinetic data appear to agree also with our results of the rotation study. The kinetics would not necessarily have to yield the same effects because steric hindrance in the kinetics would involve a five-coordinated intermediate, whereas the rotation of 5'-GMP is influenced by the structure in a square-planar complex.

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Registry No. $Pt(dap)(OH_2)_2^{2+}$, 73946-66-4; $Pt(1,3\text{-dach})(OH_2)_2^{2+}$, 111348-91-5; $Pt(tmdap)(OH_2)_2^{2+}$, 89172-79-2; $Pt(mdap)(OH_2)_2^{2+}$, 111348-92-6; $Pt(edap)(OH_2)_2^{2+}$, 111348-93-7; 5'-GMP, 85-32-5.

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"Multilayer" Activity and Implications of Hydrogen Peroxide in the Catalytic Reduction of Dioxygen by a Dicobalt Cofacial Bis(porphyrin) (Co_2FTF4)

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Highly purified samples of the dicobalt face-to-face porphyrin dimer Co_2FTF4 spontaneously adsorb onto graphite electrodes as "multilayer" assemblies. Coverages corresponding to 30 or more monolayers on the geometric electrode area are electroactive. These modified electrodes electrocatalytically reduce dioxygen to water at moderately high potentials in acidic solutions; however, not all of the electroactive film participates in the catalytic reaction. A quantitative assessment of the potential dependence of the product distribution (H_2O vs H_2O_2) is presented; Co_2FTF4 reduces O_2 exclusively to H_2O at high potentials but also produces H_2O_2 at potentials negative of the $Co^{III}Co^{II}/Co^{II}Co^{II}$ formal potential. The catalyst-coated electrode also reduces H_2O_2 to H_2O in acidic media; this finding is discussed in the context of the mechanism of O_2 reduction and the efficiency of the system as an oxygen cathode. Strategies are presented for increasing the efficiency of the system.

Introduction

One promising application of chemically modified electrodes is electrocatalysis.[1,2] Many metal complexes of porphyrins,[3-5] phthalocyanines,[6,7] and other macrocycles[8,9] have been irreversibly adsorbed onto graphite electrodes to yield electrochemically and electrocatalytically active assemblies. Some of these modified electrodes are capable of the electrocatalytic reduction of O_2 to H_2O without the intermediate production of H_2O_2; such catalysts may be useful for fuel cell applications. This four-electron pathway has been demonstrated, with varying degrees of success, for a few metal-containing macrocycles.[10,11]

In a series of publications, Collman et al. have detailed the synthesis, characterization, and electrochemical properties in both aqueous and nonaqueous solutions of Co_2FTF4 (Figure 1); many

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(1) Murray, R. W. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1984; Vol. 13, p 192.
(2) Zak, J.; Kuwana, T. *J. Electroanal. Chem. Interfacial Electrochem.* **1983**, *150*, 645.
(3) Collman, J. P.; Marrocco, M.; Denisevich, P.; Koval, C.; Anson, F. C. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *101*, 117.
(4) Lennox, J. C.; Murray, R. W. *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *78*, 395.
(5) Bettelheim, A.; Chan, R. J. H.; Kuwana, T. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *110*, 93.
(6) Zagal, J.; Sen, R.; Yeager, E. *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *83*, 207 and references therein.
(7) Tachikawa, H.; Faulkner, L. R. *J. Am. Chem. Soc.* **1978**, *100*, 4379.
(8) Yeager, E. *Electrochim. Acta* **1984**, *29*, 1527.
(9) van der Putten, A.; Elzing, A.; Visscher, W.; Barendrecht, E. *J. Chem. Soc., Chem. Commun.* **1986**, 477.
(10) Hahnke, H.; Schonborn, M.; Zimmermann, G. *Top. Curr. Chem.* **1976**, *61*, 135.
(11) Schiffrin, D. J. *Electrochemistry* **1983**, *8*, 126.

0020-1669/88/1327-0387$01.50/0