Experimental Section

Sulfite oxidase was isolated from chicken liver by using the method of Kipke et al.[17] The purified enzyme used for the experiments had heme to protein ratios (A_{413}:A_{280}) of 0.60 and above. Analysis of representative enzyme preparations gave Mo:heme ratios of 1.03:1:00. Sulfite oxidase activity was assayed as previously described[18] by measuring the reduction of cytochrome c (0.20 M Tris-HCl, pH 8.5, with 2×10^{-4} M EDTA, 2×10^{-4} M cytochrome c, and 0.040 M sodium sulfite). Activity measurements were carried out at 25 °C by using a Uvikon 810 spectrophotometer. The concentration of sulfite oxidase active centers was determined spectrophotometrically by using $\epsilon_{413nm} = 0.0999$ M^{-1} cm^{-1} for the oxidized form of the enzyme.[3] The enzyme activity was 35 enzyme units/mg. A 0.020 M universal buffer containing equal molar concentrations of Bis-Tris, Tris base, and Bis-Tris propane was used for both the low-pH (6.00) and high-pH (9.00) experiments. Adjustment of the pH was performed by the addition of acetic acid. The pH 6.00 buffer and the 0.020 M potassium phosphate buffer contained 0.10 M KCl as electrolyte. The pH 9.00 buffer contained sodium p-toluenesulfonate, a relatively noncoordinating anion (0.10 M), as electrolyte.

Microcoulometry was performed as described previously[9] in the presence of the following mediators (each present at 1.67×10^{-4} M): 2,6-dichlorophenol-indophenol (E_o' = 0.217 V), 1,4-naphthoquinone-2-sulfonate (E_o' = 0.110 V), toluidine blue (E_o' = 0.034 V), pyocyanine (E_o' = 0.060 V), indigo disulfonate (E_o' = -0.124 V), anthraquinone-1,5-disulfonate (E_o' = -0.170 V), anthraquinone-2-sulfonate (E_o' = -0.225 V), safranine T (E_o' = -0.280 V), benzyl viologen (E_o' = -0.360 V), and methyl viologen (E_o' = -0.440 V). Each point was obtained by using duplicate 5.00×10^{-6} or 10.00×10^{-6} L samples of enzyme (~3.00×10^{-9} mol of heme). The reduction potentials were obtained by using a nonlinear least-squares curve-fitting program based on the theoretical Nernst equation for three one-electron reductions: $n = (1 + e^{(E-E_1)F/RT})^{-1} + (1 + e^{(E-E_2)F/RT} + e^{(E_3-E)F/RT})^{-1} + 2(1 + e^{(E-E_3)F/RT} + e^{(2E-E_2E_3)F/RT})^{-1}$ ($E_1 = E$[Fe(III)/Fe(II)]; $E_2 = E$[[Mo(VI)/Mo(V)]; $E_3 = E$[Mo(V)/Mo(IV)]). The SCE reference electrode was checked before and after each run against a SCE and a saturated AgCl electrode used only for calibration and found to be within ±0.002 V of 0.244 V vs NHE. The error in the reduction potentials was estimated to be ±0.015 V.

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Registry No. Mo, 7439-98-7; Cl, 16887-00-6; $H_2PO_4^-$, 14066-20-7; sulfite oxidase, 9029-38-3; heme, 14875-96-8.

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Aqueous Chemistry of Mixed-Amine Cis- and Transplatin Analogues. Intramolecular Preference for a Kinetic Six-Membered Ring over a Thermodynamic Five-Membered Ring Ortho-Platination Product

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A series of mixed-amine cis- and transplatin analogues, containing benzylamine and 2-phenylethylamine functionalities, were synthesized and the aqueous solution chemistry investigated. The *cis*- and *trans*-dichloroplatinum(II) isomers having the neutral ligands ammine and 1,2-bis(4-methoxyphenyl)ethylamine (**1** and **4**), ammine and 2-(4-methoxyphenyl)-1-phenylethylamine (**2** and **5**), and ammine and bis(4-methoxyphenyl)methylamine (**3** and **6**) were synthesized in >98% isomeric purity. With the aim of investigating the pharmacoactivation of this class of compounds, a reversed-phase HPLC assay was developed for determining the rates of Pt-Cl hydrolysis of the cis-configured isomers. The precolumn addition of KBr to the reaction solutions trapped the aquachloro- and diaquaplatinum hydrolysis products as their bromo adducts. The separations of dichloro-, bromochloro-, and dibromoplatinum complexes allowed the quantification of their time-dependent concentrations, and the hydrolysis rate constants for **1-3** could be determined. It was found that, following Pt-Cl hydrolysis, an intramolecular ortho-platination occurred with **1-6**. Proton NMR studies showed that, for the trans-configured **4** and **5**, the kinetically favored, six-membered ring cycloplatinated products were formed specifically over the thermodynamic, five-membered ring ones. For the cis-configurated **1**, the kinetic product was formed selectively. The six-membered cycloplatinated ring could be converted into the five-membered, thermodynamically favored one by heating in dilute KCl. The implications of these novel findings are discussed from both mechanistic chemical and pharmacological points of view.

Introduction

For reasons of their remarkable antineoplastic activity, cis-coordinated diaminedichloroplatinum(II) compounds have come under recent scrutiny. *cis*-Diamminedichloroplatinum(II) (cisplatin) is currently one of the most successful chemotherapeutic agents in the treatment of a variety of tumors, with particular activity against testicular and ovarian carcinomas, as well as squamous cell carcinoma of the head and neck.[2] In an effort to develop more potent and less toxic agents with a broader spectrum of antitumor activity, a wide variety of amine ligands have been substituted for the NH_3 groups of cisplatin.[3] Surprisingly, ligands containing benzylamine or 2-phenylethylamine groups have not received much attention.[4] The lack of antineoplastic data on cisplatin analogues possessing amine ligands with these aromatic functionalities prompted us to synthesize the series of cis and trans mixed-amine dichloroplatinum(II) compounds **1-6**.

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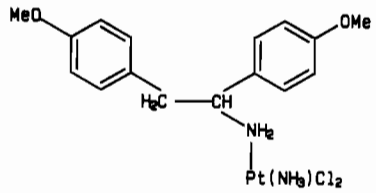

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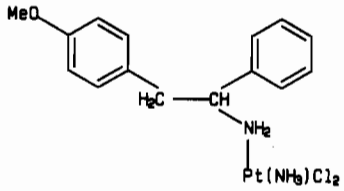

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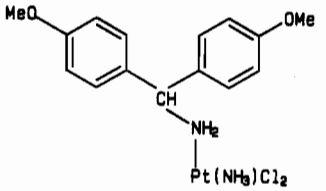


CIS: 3

TRANS: 6

Activation of the relatively inert cisplatin molecule is considered obligatory for the expression of antineoplastic activity.[3e] This pharmacoactivation process is believed to occur when the Pt–Cl bonds are hydrolyzed in the cell cytosol. Due to the low chloride concentrations present there, the DNA-reactive aquachloro- and diaquaplatinum species might accumulate.[5] Since the hydrolysis of the Pt–Cl bonds is considered necessary for the pharmacological activity of this class of compounds, the aqueous solution chemistry of compounds **1–6** was explored. The results of these investigations are the topic of this publication.

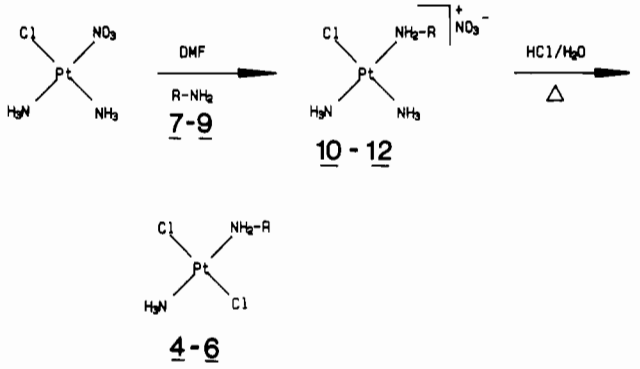
This paper reports a new HPLC assay for determining the Pt–Cl hydrolysis rate constants of cisplatin analogues in the micromolar range. Subsequent investigations showed that, following Pt–Cl hydrolysis, cisplatin analogues containing certain aromatic functionalities can undergo intramolecular ortho-platination reactions. Furthermore, evidence is presented which indicates that, in aqueous solutions of low chloride concentrations, the kinetic six-membered ring cycloplatinated product is preferentially formed over the thermodynamic five-membered one.

Results

Synthesis and Characterization of the *cis*- and *trans*-Dichloroplatinum(II) Complexes. For the syntheses of the cis coordinated mixed-amine platinum(II) compounds **1–3**, 1:1 mixtures of the amine ligands (**7–9**) and $K[PtCl_3(NH_3)]$ were reacted in methanol for several days at room temperature. The precipitated dichloroplatinum complexes were easily isolated, requiring no further purification. Attempts to synthesize the cis mixed-amine complexes through iodo-bridged platinum(II) dimers as previously described were unsuccessful.[6] The syntheses of the trans-coordinated platinum(II) complexes **4–6** are shown in Scheme I. Reaction of the amine ligands **7–9** with *cis*-diamminechloro(nitrato)platinum(II) gave the *cis*-$[PtCl(NH_3)_2(amine)]NO_3$ compounds (**10–12**), which could be reacted further without isolation to the trans mixed-amine dichloroplatinum complexes by heating in a HCl/KCl solution. The syntheses of **1–6** all gave products that were chromatographically pure as determined by reversed-phase HPLC.

Crucial for our interpretation of the ensuing experiments was the synthesis of isomerically pure cis and trans mixed-amine

Scheme I. Synthesis of *trans*-Dichloroplatinum(II) Complexes **4–6**



compounds. For this reason, effort was invested confirming the isomeric purity of the synthesized complexes. The method of Kurnakow,[7] traditionally used to determine cis/trans stereochemistry of square-planar transition-metal complexes, is cumbersome and insensitive for the detection of trace contaminations of the oppositely configured isomer in preparations of mixed-amine platinum complexes. Alternative spectroscopic and chromatographic methods were investigated for their usefulness in determining isomeric purity. Although characteristic differences between the cis and trans isomers could be found by using infrared (IR), ^1H NMR (see Table I), and fast-atom-bombardment mass spectral (FAB-MS) techniques, these three methods suffered from a lack of sensitivity in detecting trace amounts of the oppositely configured isomers in the synthetic preparations.

Reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection was the method of choice for the qualitative and quantitative analysis of cis/trans isomeric purities in the reaction products **1–6**. Only microgram quantities were necessary for analysis. Arpalahti and Lippert have similarly used reversed-phase HPLC to quantify mixtures of cis and trans isomers of platinum complexes; however, their procedure requires precolumn treatment of the platinum complexes with thiourea.[8] Our method provided an excellent chromatographic separation of cis and trans isomers directly with good sensitivity. The ratios of the retention times for trans to cis isomers (retention time trans/retention time cis) were ca. 1.5. The presence of a greater Cl–Pt–Cl dipole in the cis than in the trans isomer is the explanation for the longer retention times of the latter compounds on the hydrophobic RP-18 HPLC column.[9] The limits of detection were 2 mol % trans complex in preparations of the cis complex and 1 mol % cis complex in preparations of the trans isomer. All syntheses gave isomerically pure products.

Hydrolysis Kinetics of *cis*-Dichloroplatinum(II) Complexes. A reversed-phase HPLC assay with UV detection was developed to monitor the formation rates of the hydrolysis products from aqueous solutions of compounds **1–3**. Due to their very rapid hydrolyses, the trans complexes were not suitable for similar investigations by this method. Although the relatively inert dichloroplatinum(II) compounds were found to elute quantitatively from the end-capped, RP-18 column, the highly reactive, electrophilic aquachloro- and diaquaplatinum species did not. This was probably due to irreversible reactions between the aquaplatinum species and residual silanol groups of the RP-18 stationary phase. Consequently, it was necessary to trap these hydrolysis products by addition of Br^-, converting them again to neutral, dihaloplatinum complexes that eluted with a quantitative recovery relative to the injected dichloroplatinum complexes. As expected, the stepwise substitutions of a chloro for a bromo ligand gave complexes with increasingly longer column retention times,[10] allowing for the separation and quantification of the reaction

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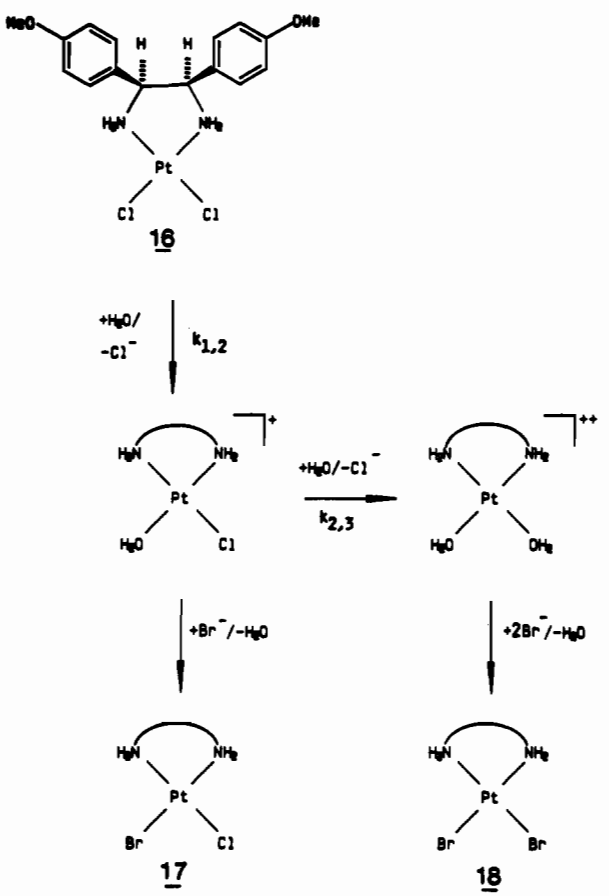
Table I. 250-MHz ^{1}H NMR Data for Compounds **1–6** (in DMF-d_7)[a]

	^{1}H NMR data								
						α-aromatic		β-aromatic	
compd	NH_3	NH_2	CH	CH_2[b]	OMe	*o* to CH[c]	*m*/*p* to CH[c]	*o* to CH_2[c]	*m* to CH_2[c]
1	4.2, s	5.36, br d (6)	4.38, m	3.05, q (13.4, 11.4) 4.15, q (13.4, 4.15)	3.71, s 3.76, s	7.30 (8.7)	6.86 (8.7)	7.03 (8.7)	6.75 (8.7)
2	4.2, s	5.44, br s	4.44, m	3.06, q (13.3, 11.1) 4.17, q (13.5, 4.30)	3.71, s	7.30, m		7.01 (8.7)	6.74 (8.7)
3	3.8, s	5.75, t (7.1)	5.47, t (7.1)		3.47, s	7.49 (8.7)	6.94 (8.7)		
4	3.8, s	4.57, m 4.87, m	4.40, m	3.07, q (13.4, 10.5) 4.00, q (13.3, 4.3)	3.72, s 3.76, s	7.28 (8.8)	6.84 (8.8)	7.01 (8.8)	6.76 (8.8)
5	3.8, s	4.63, m 4.96, m	4.45, m	3.07, q (13.3, 10.1) 4.00, q (13.3, 4.3)	3.71, s	7.32, m		7.00 (8.7)	6.76 (8.7)
6	3.8, s	5.13, d (7.0)	5.49, t (7.0)		3.79, s	7.46 (8.7)	6.92 (8.7)		

[a] Values are reported in the following order: chemical shift (ppm), peak multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), and (in parentheses below) coupling constants (Hz). [b] AB portion of an ABX system. [c] AA′BB′ coupled; *J* = ortho coupling.

Scheme II. Hydrolysis Scheme for Complex **16**



mixture components (Table II). Under the conditions of KBr addition (0.5 M, 5 min, 37 °C), a complete exchange of Br^- for coordinated water was attained. No evidence for a direct exchange of Br^- for Cl^- was seen. In contrast, the addition of KCl to the same aged solutions gave a single substance eluting with a retention time identical with that of the parent dichloroplatinum complex. The addition of KI, on the other hand, led to a release of the free monoamine ligand from the mixed-amine platinum complexes.

To validate this analytical method, the symmetrically substituted complex *meso*-[1,2-bis(4-methoxyphenyl)ethylenediamine]di-

Table II. HPLC Retention Times for the Dihaloplatinum Complexes

	HPLC	adj retention times,[b] min			
compd	elution[a] conditions	Cl_2	ClBr	BrCl	Br_2
1	A	23.7	25.5	28.8	30.0
2	A	21.5	23.8	28.8	29.8
3	A	22.1	24.6	28.7	30.2
16	B	19.0	22.7		24.8

[a] See Experimental Section for elution conditions. [b] Adjusted retention times (t'_R) are the retention time of the substance (t_R) minus the elution time of the column void volume (t_0).

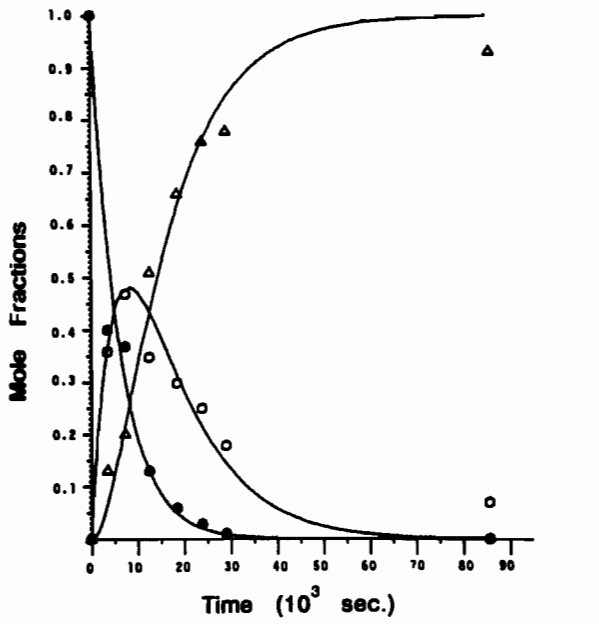


Figure 1. Time-dependent concentrations of trapped dihaloplatinum complexes from the hydrolysis of **16** at 37 °C. Data points were measured according to the HPLC assay described in the text. The solid lines represent the best-fit values calculated by the Gear–Simplex algortihm. Key: (●) dichloroplatinum complex; (○) bromochloroplatinum complex; (△) dibromoplatinum complex.

chloroplatinum(II) (**16**) was chosen. Hydrolysis of this compound should follow two consecutive pseudo-first-order reactions (Scheme II). Due to the very low chloride concentrations at the beginning of the incubations, unidirectional kinetics could be assumed. (For

Table III. Pseudo-First-Order Hydrolysis and Apparent Cycloplatination Rate Constants

compd	conc, mM	T, °C	method of determ	rate const[a] × 10^4, s^{-1}						
				$k_{1,2}$	$k_{2,3}$	$k_{1,3}$	$k_{2,4}$	$k_{3,4}$	$k_{2,5}$	$k_{4,5}$
1	0.02	37	HPLC	0.20 ± 0.06		1.09 ± 0.10	0.65 ± 0.57	1.62 ± 0.17	1.61 ± 0.50	5.30 ± 1.06
2	0.02	37	HPLC	0.30 ± 0.19		0.79 ± 0.02	0.65 ± 0.53	1.08 ± 0.13	2.2 ± 1.05	5.73 ± 2.24
3	0.02	37	HPLC	0.23 ± 0.05		0.64 ± 0.09	0.70 ± 0.22	0.56 ± 0.09	0.96 ± 0.55	0.93 ± 0.10
16	0.02	37	HPLC	1.36 ± 0.15	0.83 ± 0.05					
Pt(en)Cl_2[b]	0.3–0.5	35	UV and pH-stat	1.1 ± 0.04	0.73 ± 0.09					
cisplatin[c]	1.0	37	potentiometry	1.1 ± 0.5	0.42 ± 0.02					

[a] Values are the average of three to four determinations ± standard deviations. [b] Ref. 13. [c] Ref. 14.

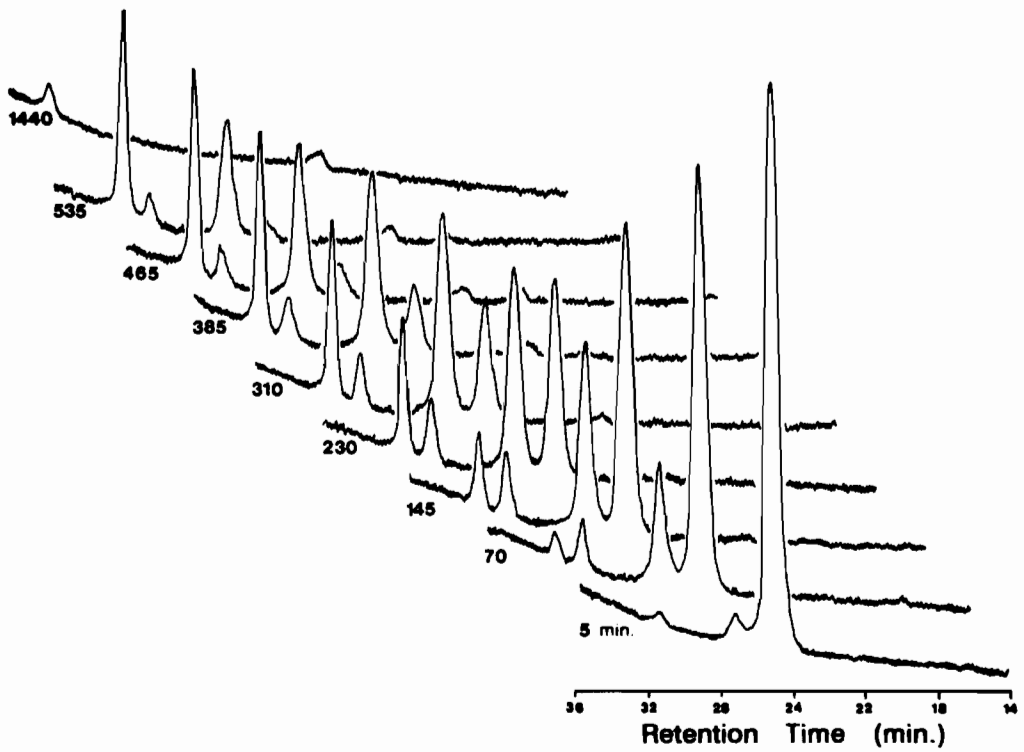


Figure 2. Time-dependent HPLC chromatograms obtained from the aqueous reactions of 20 μM **3** (37 °C) quenched with 0.5 M KBr.

reasons of simplicity, the equilibra between the aquaplatinum and the hydroxoplatinum species have been left out.) The distinctive concentration versus time plot of the hydrolysis products, trapped as their bromochloro- and dibromoplatinum complexes (**17** and **18**, respectively), was in agreement with the expected results (Figure 1).[11] The loss of dichloroplatinum complex **16** was equally matched with the appearance of bromochloro- (**17**) and dibromoplatinum species (**18**).

To calculate the hydrolysis rate constants $k_{1,2}$ and $k_{2,3}$, the system of coupled first-order differential equations was integrated numerically by using a Gear algorithm program, which is based on the Runge–Kutta method.[12] These rate constants were found to compare closely with those previously reported for dichloro(ethylenediamine)platinum(II) (Pt(en)Cl_2)[18] (Table III). The algorithm generated curves fitted well to the experimentally determined concentrations of platinum(II) species at the early time points (Figure 1). The increase in released chloride concentrations at later times (i.e. > 8 h) resulted, however, in an equilibrium between the aquachloro- and the diaquaplatinum species. As found with both Pt(en)Cl_2[18] and cisplatin,[14] the hydrolysis of the first Pt–Cl bond of compound **16** proceeded approximately twice as fast as the hydrolysis of the second Pt–Cl bond.

A temporal HPLC-profile of the bromide-trapped hydrolysis products from mixed-amine complex **3** is shown in Figure 2. Scheme III describes the system of reactions used in modeling the aqueous chemistry of compounds **1**–**3**. Again, all reactions were assumed to be unidirectional, pseudo-first-order reactions. The computer generated curves fitted well to the experimentally determined concentration–time points (Figure 3).

The HPLC chromatograms of the trapped hydrolysis products from aqueous solutions of the mixed-amine complexes displayed an additional component (Figure 2). This feature was attributed to the presence of two stereoisomeric bromochloroplatinum complexes, since the hydrolysis of this first Pt–Cl bond can occur cis or trans to the coordinated alkylamine (Scheme III). Due to the steric hindrance of the bulky (alkylaryl)amine group, the trans-chloro ligand should be selectively replaced before the substitution

(11) Two isomeric bromochloroplatinum species for **17** would be expected to form owing to the *R,S*-configuration of the diphenylethylenediamine ligand; the first Br^- can coordinate cis or trans to the *R*-configured carbon. However, no chromatographic separation of this isomeric pair was observed, and for all practical purposes, this technicality can be ignored.

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(15) The cycloplatinated products could be eluted from the column if the pH of the phosphate buffer was dropped from 5.0 to 3.3. However, their retention times were then too similar with those of the dihaloplatinum complexes and hence interfered in the quantification of the hydrolysis products.

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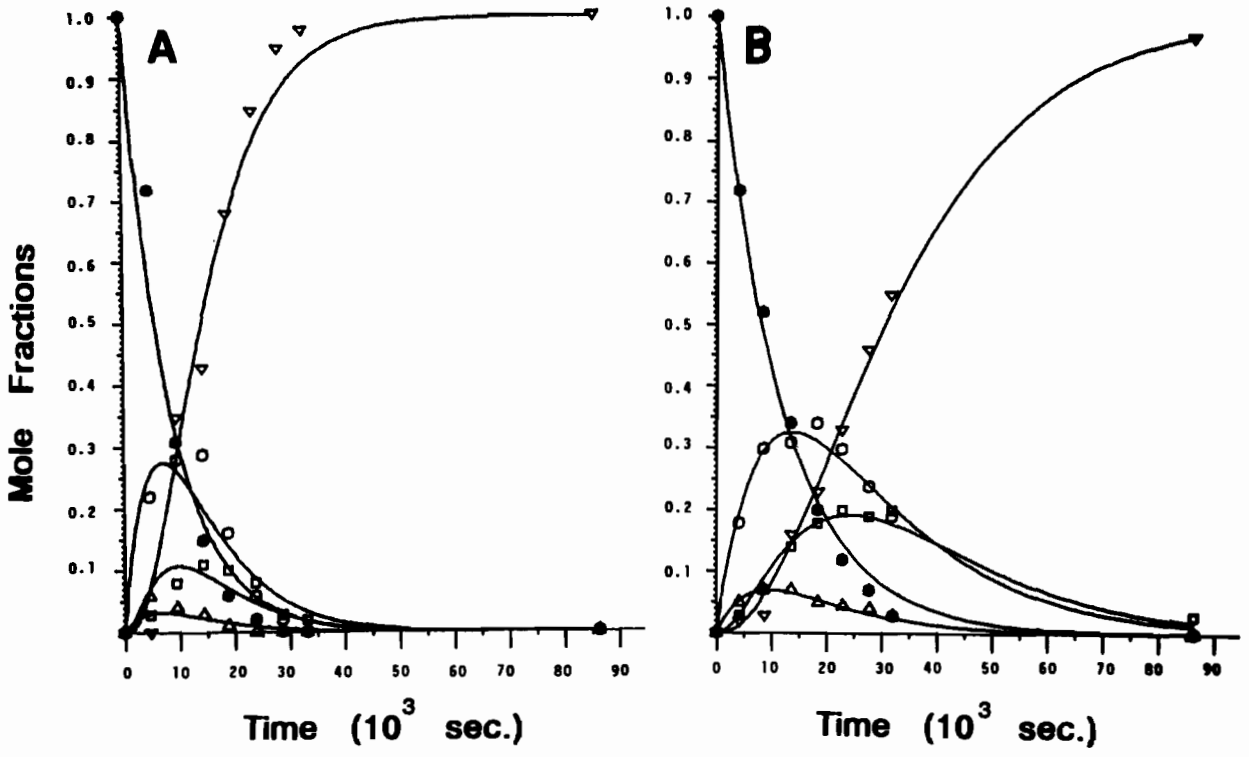
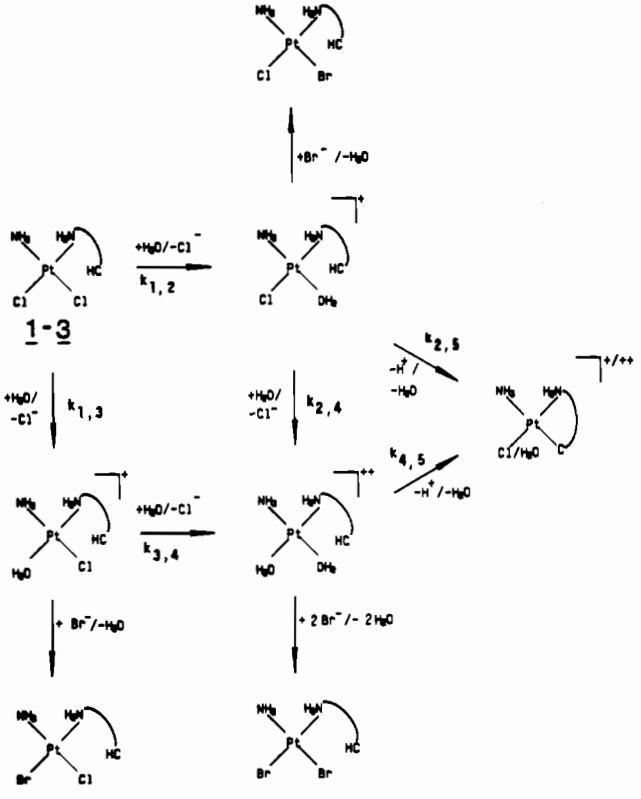


Figure 3. Time-dependent concentrations of trapped dihaloplatinum complexes from the hydrolysis of 20 μM **1** (A) and **3** (B) at 37 °C. Key: (●) *cis*-dichloroplatinum complex; (○) *a*-bromo-*b*-chloro-*c*-amine-*d*-ammineplatinum complex; (△) *a*-bromo-*b*-chloro-*c*-ammine-*d*-amineplatinum complex; (□) *cis*-dibromoplatinum complex; (▽) cycloplatinated products.

Scheme III. Hydrolysis Scheme for Complexes **1–3**



of the cis-chloro ligand. The two rate constants for the hydrolysis for the first Pt–Cl bond were not equal (Table III; compare $k_{1,2}$ with $k_{1,3}$ for compounds **1** and **3**). Hence, the larger hydrolysis rate constant corresponds to the hydrolysis of the trans-Pt–Cl bond. This rationale allows for the tentative assignment of the absolute configuration to the bromochloroplatinum isomeric pairs; the bromochloroplatinum complex with the bromo ligand substituted trans to the amine ligand eluted prior to the oppositely configured bromochloroplatinum analogue in the HPLC chromatograms (Figure 2).

Since cisplatin possess two identical Pt–Cl bonds, the hydrolysis rate constant $k_{1,2}$ (1.1×10^{-4} s^{-1})[14] is actually the sum of two identical Pt–Cl hydrolyses, each with a rate constant of 0.55×10^{-4} s^{-1}. With this in mind, it was apparent that the hydrolysis of the first Pt–Cl bond in the ethylamine complexes **1** and **2**, trans to the (alkylaryl)amine ($k_{1,3}$, Table III), went faster than the hydrolysis of the first Pt–Cl bond of cisplatin. The analogous Pt–Cl bond in the methylamine analogue **3** hydrolyzed at a rate comparable to that for the first Pt–Cl bond of cisplatin. The hydrolysis rates of the first cis-Pt–Cl bonds for **1–3** ($k_{1,2}$'s) were comparable to each other and nearly 50% of the rate reported for cisplatin. As expected, increasing the ionic strength of the solutions from 0.0 to 0.15 M (Na_2SO_4) had no effect on the hydrolysis rate constants of either compound.[13]

Unlike the chelated 1,2-diphenylethylenediamine complex **16**, compounds **1–3** displayed a time-dependent loss of *all* observable species from the aqueous reaction medium (Figure 2), suggestive of the participation of at least one species in a further, unidentified side reaction. There was no evidence that the free amines were being released from the platinum complexes before or after the addition of KBr. This time-dependent loss of hydrolysis products proceeded significantly faster for the ethylamine complexes **1** and **2** than for the methylamine analogue **3**. Thus, although the Pt–Cl hydrolyses of **1** and **2** were approximately twice as fast as for **3**, the rapidity of the ensuing side reaction was so much greater for **1** and **2** than for **3** that higher peak concentrations of hydrolysis products were actually reached by the later compound (Figure 3).

UV-Difference Spectroscopy Experiments. The nature of the side reaction was first alluded to through UV-difference spectroscopy experiments with the aqueous reactions of compounds **1–5**. Due to the low solution concentrations (20 μM), an intermolecular reaction was considered unlikely. The results summarized below implicated an irreversible, intramolecular substitution of platinum to the ortho position of the aromatic ring.

Observed was a time-dependent increase in the UV absorption less than 340 nm (Figure 4). In contrast, the chelated (diphenylethylenediamine)platinum(II) complex **16** showed no such

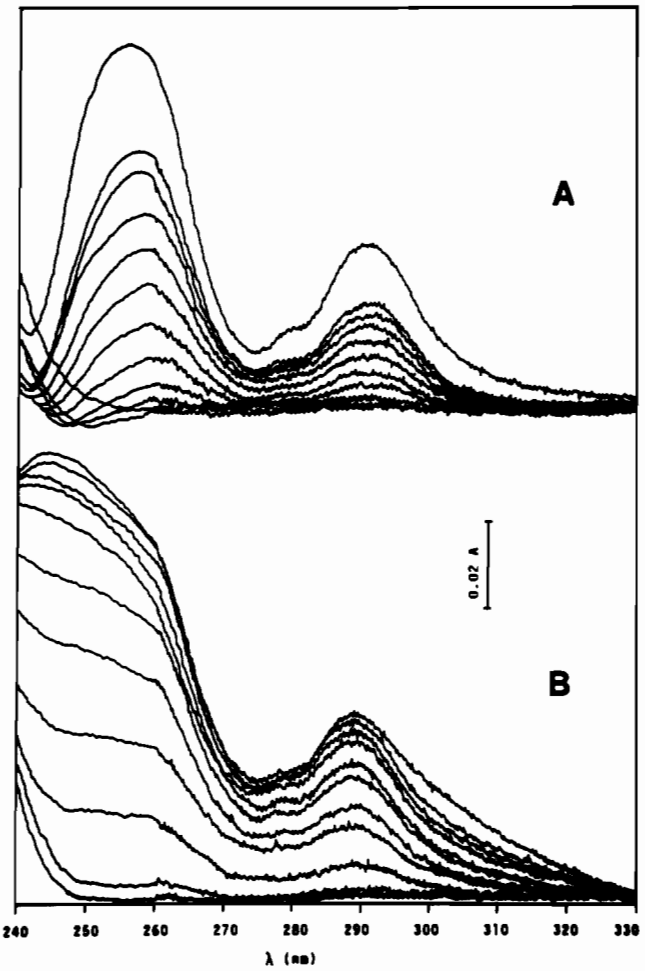


Figure 4. UV-difference spectra for the aqueous reactions of 20 μM **3** (A) and **1** (B) at 37 °C. Following the addition of the Pt complex, measurements were taken at 1-h intervals for the first 8 h and then at 24 h.

UV changes under the same aqueous conditions. The trough centered at ca. 270 nm corresponded to the loss of one *p*-methoxyphenyl ring system. The absence of isosbestic points in the UV-difference spectra is indicative of a multistep reaction pathway. Although all the compounds displayed increases in the absorption centered at 290 cm^{-1}, the hypsochromic absoption increases were distinctive of the type of amine ligand coordinated to platinum. Complex **3**, possessing only α-substituted phenyl rings, gave an absorption maximum at 255 cm^{-1} (Figure 4A), while **1**, **2**, **4**, and **5**, compounds possessing, in addition to an α-phenyl, a β-substituted phenyl group, had just reached an absorption maximum at ca. 240 cm^{-1} (Figure 4B).

The comparative kinetics of the UV-difference spectra for the cis-configured **1** and **3** in aqueous solutions showed the former compound inducing spectral changes more rapidly than the later. For both compounds a close correlation was seen between the kinetics of these spectral changes and the time-dependent losses of chromatographically determineable dihaloplatinum complexes from the aqueous reactions mixtures (Figure 5).

Difference spectra kinetics were approximately 100 times faster for the trans complexes than for their cis isomers, suggesting the involvement of a trans effect in the reaction. A change of solvent from water to methanol slowed the reaction of the trans complex **5** from minutes to hours, evidence for a solvent-assisted, associative substitution reaction to platinum. Likewise, the presence of increasing NaCl concentrations slowed the formation of the UV-difference spectra, but once formed, the spectra were stable to the addition of chloride, evidence for an irreversible reaction. These results indicated that the hydrolysis of one of the Pt–Cl bonds was a prerequirement for the ortho-platination reaction.

Kinetics of Cycloplatination. The cycloplatinated products did not elute from the HPLC column under the conditions of the assay but their quantification could be made indirectly by determining the fraction of chromatographable substance lost from the solutions.[15] Since the UV spectral changes associated with orthometalation had paralled these losses (Figure 5), it was assumed that the lost material went directly to cycloplatinated products. With these assumptions in mind, computer modeling of the kinetic pathways to Scheme III allowed for an estimation of the apparent rate constants of cycloplatination for compounds **1**–**3**. This reaction was found to proceed faster for the complexes possessing an aromatic ring β-substituted to the coordinating nitrogen (**1** and **2**) than for the complex possessing only α-substituted rings (**3**) (Table III; compare the $k_{2,5}$'s and $k_{4,5}$'s for **1** and **3**). When the algorithm was run by using a kinetic scheme in which the cycloplatination reaction was routed instead through the oppositely configured aquachloroplatinum species, the fit between the com-

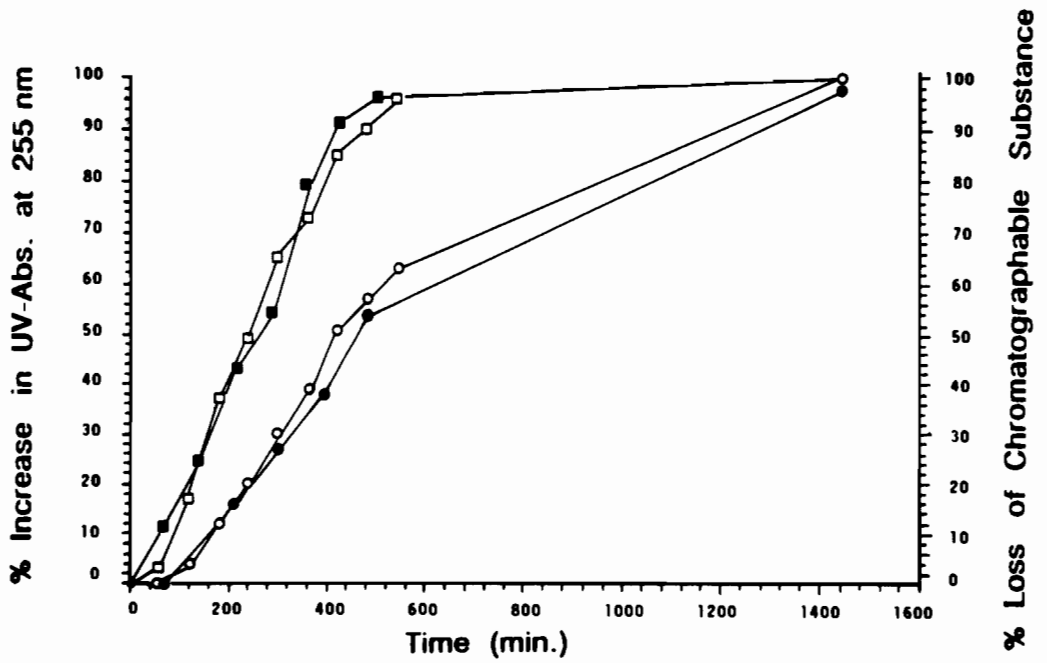


Figure 5. Comparison of the time-dependent UV-difference changes to the losses of chromatographable substance from the aqueous reactions of (□, ■) **1** and (○, ●) **3**. Open symbols are increases in UV absorbtions; closed symbols are losses of chromatographable substance.

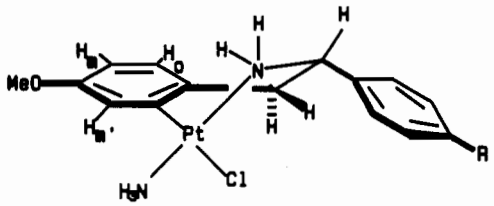
Table IV. ^{1}H NMR Data for Compounds 13–15[a]

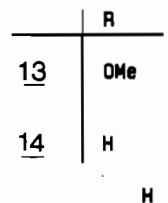
	^{1}H NMR data											
						α-aromatic			β-aromatic			
compd	NH_3	NH_2	CH	CH_2	OMe	*o* to CH	*m*/*p* to CH	*m*′ to CH	*o* to CH_2[c]	*m* to CH_2[c]	*m*′ to CH_2[c]
13	4.0, s	3.59, s 5.82, s	4.40, m	2.66, q (13.7, 2.3) 3.22, q (13.4, 11.1)	3.73, s 3.82, s	7.54, d[b] (8.7)	6.96, d[b] (8.7)		6.74, d (8.0)	6.41, q (8.0, 2.6)	6.82, d (2.6)
14	4.1, s	3.61, s 5.95, s	4.41, m	2.70, q (13.2, 1.0) 3.23, q (13.2, 1.0)	3.73, s	7.63, d[b] (6.9)	7.40, m[b]		6.75, d (8.1)	6.41, q (8.0, 2.6)	6.83, d (2.6)
15	4.1, s	4.95, s 6.18, s	5.13, t (5.7)		3.70, s 3.81, s	7.58, d[b] (8.8)	6.93, d[b] (8.8)				
							6.42, m[c]	6.70, d[c] (2.1)			

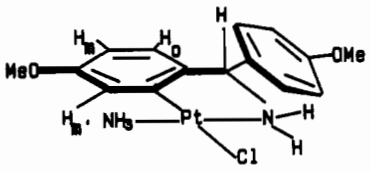
[a] See Table I for abbreviations. [b] AA′BB′ coupled. [c] ABC coupled.

puter-generated curves and the experimental points worsened. This was taken as further evidence that the more rapidly eluting bromochloroplatinum complex arose out of the trans aquaplatinum species, since only the cis aquaplatinum species could have ortho-metalated the aromatic ring. Increasing the ionic strength of the solutions from deionized water to 0.15 M Na_2SO_4 had no effect on the cycloplatination rate constants of **1** and **3**.

Isolation of Cycloplatination Products. Typically, aqueous concentrations of 0.2 mM for the trans complexes **4–6** could be reached at 37 °C, so that a clear solution was maintained throughout the course of the reaction. Most of the structural analysis was done with compounds **13–15**, which were the products







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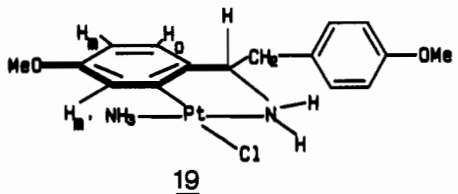

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from the *trans*-dichloroplatinum complexes, because they could be obtained in a pure form. The isolation of the aqueous reaction products was achieved directly by lyophilization of the aqueous reaction medium.

Charaterization of Cycloplatination Products. The most revealing structural information of the reaction lyophilates was gained from ^{1}H NMR. The proton absorptions for compounds **4–6** and **13–15** are compared in Tables I and IV. NOE experiments assisted in assigning the various proton resonances of compound **4**.

For the lyophilates originating from the reactions of the *trans*-dichloroplatinum complexes **4** and **5**, the AA′BB′ proton splitting pattern observed for the 1,4-disubstituted aromatic rings of **4** and **5** had been specifically replaced with a characteristic ABC splitting pattern, suggesting ortho substitution to only *one* of the aromatic rings (Figure 6A). Protons H_o and H_m are ortho-coupled (J_o = 8.0 Hz) and proton H_m is meta-coupled to proton $H_{m'}$ (J_m = 2.6 Hz). The para coupling constants between H_o and $H_{m'}$ were too small to be measured. Two independent experiments provide convincing evidence that ortho-metalation occurs regiospecifically in the β-substituted aromatic ring of the ethylamine complexes **4** and **5**. First, for the unsymmetrically aryl-substituted (ethylamine)platinum complex **14**, only the β-substituted methoxyphenyl ring experienced proton loss and displayed the same ABC proton splitting pattern for the analogous ring of **13** (Figure 6B). The monosubstituted phenyl ring α to the coordinated amine still integrated for five protons between δ 7.3 and 7.7 ppm. Second, the ABC proton splitting pattern observed for the ortho-metalated aromatic ring of **15** was not comparable to that seen in **13** (Figure 6C). Compound **15** could only have possessed a five-membered cycloplatinated ring. Interestingly, while giving a three proton integration for the platinated aromatic ring, protons H_o and H_m of that ring were shifted 1.16 and 0.51 ppm upfield, respectively, to the same frequency (δ 6.42 ppm). Only the AA′BB′ proton splitting pattern of the unplatinated α-methoxyphenyl rings of both **13** and **15** were similar. These results were interpretated to mean that in aqueous solutions of the *trans*-dichloroplatinum complexes **4** and **5**, a six-membered ring cycloplatinated product formed specifically over the five-membered ring one.

In comparison, the ^{1}H NMR of the lyophilates from the aqueous reaction of a cis complex, **1**, showed a 2:1 mixture of two different ortho-substituted aromatic ring systems. The main component of the mixture was assigned by a doublet at 7.59 ppm (J = 8.0 Hz), which corresponded to the ortho proton of the unplatinated α-aromatic ring. Thus, of the two possible cycloplatination products in the mixture, the six-membered ring compound predominated over the five-membered ringed one.

The ^{1}H NMR spectra showed the methylene protons in the six-membered ring cycloplatinated compounds **13** and **14** to be diastereotopic (Table IV). This was an indication of the conformational stability of the six-membered ring. Ring flipping did not occur upon warming, as evidenced by the stability of the NMR spectra between 25 and 120 °C.

The heating of arylplatinated compounds in dilute HCl is known to facilitate the exchange of Pt(II) for H^+ in the aromatic ring.[16] The warming of compound **13** in 2 N HCl to 70 °C resulted in a precipitate that redisplayed IR and NMR spectra identical with those of the starting material **4**, further evidence that the *o*-aryl substituent was a platinum(II) atom.

The identity of the remaining two ligands to platinum was elucidated from the positive ion FAB-MS of **13–15**. A typical example was the spectrum of **13**, which showed a weak M + H^+ ion at m/z 504. The low intensity of this ion is due to the labile

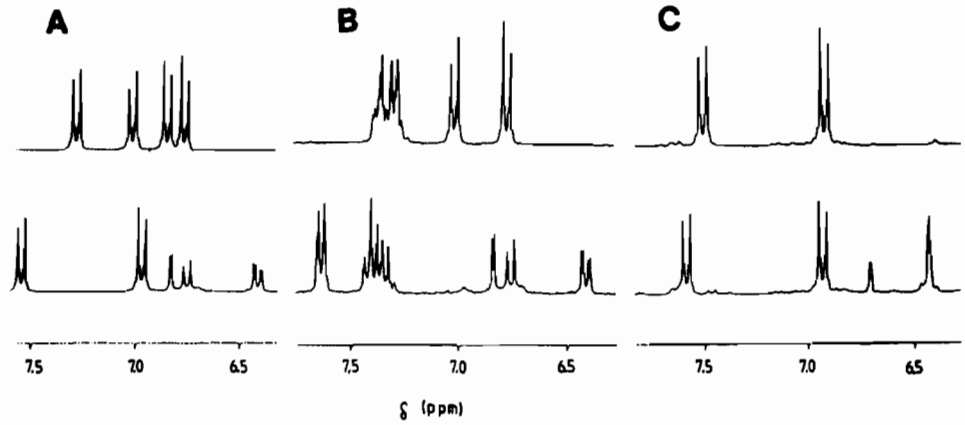

Figure 6. ^{1}H NMR (250 MHz) spectra of the aromatic regions of (A) **4** and **13**, (B) **5** and **14**, and (C) **6** and **15**. Upper spectra are for the *trans*-dichloroplatinum(II) complexes; lower spectra are for the cycloplatinated complexes.

nature of the coordinated chloro ligand. The rapid loss of HCl from the molecule gave rise to the parent peak at m/z 468. This scheme is consistent with NH_3 and Cl^- being the third and fourth coordinated ligands. The elemental analyses were also in agreement with these structures. The further loss of the NH_3 ligand explains a portion of the ion cluster centered at m/z 450. Substitution of the chloro ligand for matrix DMSO accounts for the ion cluster at m/z 546.

Conversion of the Kinetic into the Thermodynamic Product. After first allowing the cycloplatinated complex **13** to form, we investigated the ability of the product to convert from the kinetic into the thermodynamic cycloplatinated product. Warming of a 0.2 mM solution of **13** at 65 °C in the presence of 10 mM KCl induced the intramolecular aryl ligand exchange that converted the six-membered ring **13** into the five-membered ring cycloplatinated **19**. This gradual conversion was evident from the ^{1}H NMR spectra of the lyophilates of the reaction after 3 and 7 days (Figure 7).

In addition to the proton absorptions of the parent compound **13**, the presence of a second compound was apparent. Although the resonances of several aromatic protons were somewhat obscured by the peaks of the parent compound, the proton splitting pattern and integration strongly suggested the presence of the five-ring cycloplatinated product **19**. The pair of doublets centered at 7.31 ppm (J = 8.7 Hz) and at 6.94 ppm (J = 8.6 Hz) characterized the new para-disubstituted aromatic ring. The chemical shifts of these protons were similar to but not identical with those of the β-substituted methoxyphenyl ring of **4**. The appearance of a broad doublet at 3.06 ppm integrated as two protons and most likely corresponded to the new pair of benzylic methylene protons. The increase in the intensity of this doublet paralleled the loss of the ABX pattern assigned to the methylene protons of the six-membered cycloplatinated ring.

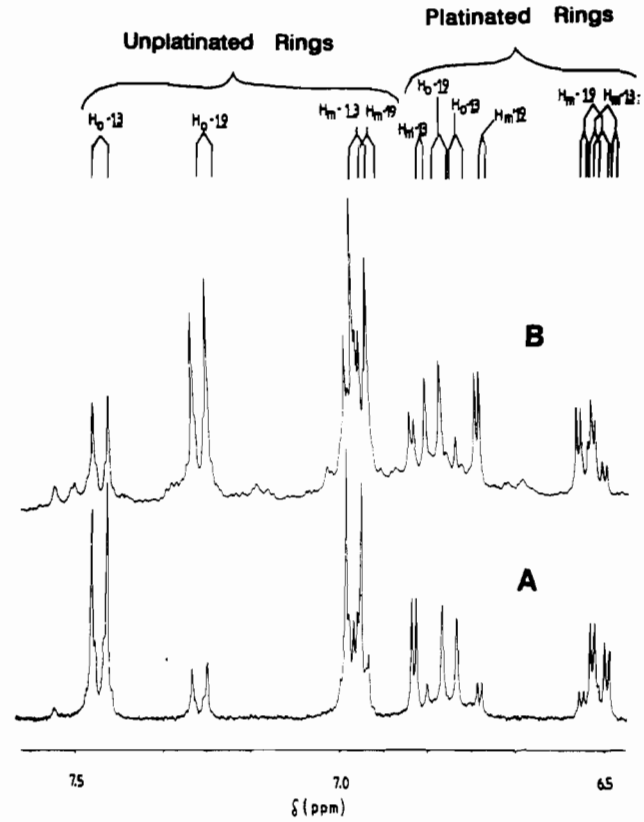

Figure 7. ^{1}H NMR (250 MHz) spectra of the aromatic region after heating **13** in 10 mM KCl at 65 °C for 3 (A) and 7 days (B).

Discussion

It has long been accepted that a requirement for the expression of cisplatin-antitumor activity is the hydrolyses of Pt–Cl bonds. In the Cl^- low cytoplasm of the cell, these hydrolysis products accumulate. This chemical transformation of a rather inert, neutral transition-metal complex into a highly reactive bifunctional electrophile facilitates the covalent binding of the compound to the presumed bionucleophilic target DNA.[17] Intrastrand cross-links through the N-7 positions of adjacent guanine bases with the metal atom are believed to be the molecular event responsible for the antineoplastic activity of cisplatin.[18] The lack of a systematic approach to study the pharmacoactivation of new cisplatin analogues vis-à-vis the hydrolysis of the Pt–Cl bond led us to investigate this reaction with our mixed-amine dichloroplatinum(II) complexes. In the course of these investigations with model compounds **1–3**, it became apparent, however, that more was happening than simple hydrolysis reactions.

Rates of Pt–Cl hydrolysis for cisplatin have typically been measured by UV spectrophotometric,[19] pH titration,[13] molar conductivity[14] and, recently, ^{195}Pt NMR[20] methods. All of these methods have their drawbacks, the main one being that solutions of relatively high Pt complex concentrations (0.1–1.0 mM) must be used for the determinations. The poor water solubility of many cisplatin analogues prohibits the preparation of solutions at these concentrations, and hence hydrolysis reactions cannot be followed for many platinum complexes. Furthermore, the first three mentioned methods do not take into account other reactions that might be occurring in addition to hydrolysis. Reversed-phase HPLC with UV detection appeared well suited for measuring rates of Pt–Cl hydrolysis in diluted solutions of mixed-amine cisplatin analogues **1–3**.

Reversed-phase HPLC has been attracting notice as a valuable analytical method for studying inorganic reactions. In this work,

(19) See: Miller, S. I.; House, D. A. *Inorg. Chim. Acta* **1989**, *161*, 131, and references therein.
(20) (a) Lippard, S. T. *Science* **1982**, *218*, 1075. (b) Bancroft, D. P.; Lepre, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 6860.

an HPLC assay was developed to quantify the time-dependent concentrations of hydrolysis products from *cis*-dichloroplatinum(II) complexes. Reversed-phase HPLC has been used before to determine an apparent rate constant for the first Pt–Cl hydrolysis of cisplatin,[21] but to our knowledge this is the first report of HPLC being used to measure the rates of both Pt–Cl bond hydrolyses for a series of cisplatin analogues. Aqueous solutions with platinum complex concentrations of 20 μM were typically used. This concentration is considerably lower than those commonly used in the determination of hydrolysis rate constants by the above mentioned methods.

The hydrolysis patterns of the mixed-amine dichloroplatinum complexes **1–3** provided some interesting surprises. Hydrolysis of the first Pt–Cl bond can occur either cis or trans to the substituted amine (Scheme III). Steric hindrance due to bulky chelated ligands is known to greatly influence the rates of substitution of square-planar transition-metal complexes.[22] In accordance with this, different hydrolysis rates between the two isomeric Pt–Cl bonds of the mixed-amine platinum complexes **1–3** were observed. Electronic differences between the ammine and the benzylamine ligands might also contribute to these differing rates of Pt–Cl hydrolysis.

Each pair of bromochloroplatinum stereoisomers, formed by trapping the aquachloroplatinum complexes with Br^-, were separated very well from one another by the RP-18 column. A good explanation for their widely differing chromatographic behavior is not obvious, but is probably a result of dipole and molecular size effects. By the arguement that the trans-Pt–Cl bond should hydrolyzed faster than the cis-configured Pt–Cl bond, a tentative assignment of the absolute stereochemistry to the pairs of bromochloro isomers could be made. Thus, a further unforseen advantage of the HPLC methodology for the determination of kinetic rate constants of mixed-amine platinum(II) complexes are the ability to differentiate stereoisomeric hydrolyses. If the traditional methods mentioned above were used, such distinctions would have been difficult if not impossible to make.

The kinetic behavior of the hydrolysis products from **1–3**, trapped as their Br^- adducts, was unexpected. It was found that the concentrations of the dibromoplatinum complexes plateaued with time and then declined until no chromatographically detectable material could be measured in the solution. This was not simply an artifact of the assay since the hydrolysis kinetics of the (1,2-diphenylethylenediamine)platinum complex **16** had showed no such time-dependent losse of the dibromoplatinum product. With the aid of UV, ^1H NMR, and mass spectral methods, the reason for these losses was attributed to an intramolecular cycloplatination reaction.

Concerning the mechanism of ortho-metalation by transition metals, a considerable knowledge has been gained since the discovery of the reaction by Cope and co-workers[23] over 2 decades ago. The vast majority of these reactions were reported to involve the formation of five-membered ring transition states, intermediates, or products.[24] Fewer examples of the less stable six-membered ring exist, and then only when a pathway to five-ring products was not available.[25] Given a choice between closing a five- or six-membered ring, it was generally believed that ortho-metalation would follow the pathway to give the thermodynamically more stable five-membered ring product. This led Omae to propose the five-membered ring structural theory.[26] Recently, however, evidence has been presented that a six-membered ortho-metalated ring can form over a five-membered one; the cyclopalladation of *N*-phenylbenzamidine by Pd^{2+} salts proceeded at the Ph–N group and not at the Ph–C group.[27] The experimental findings reported here independently confirm these results. Both the kinetics of the cycloplatination for **1–3** as well as the NMR experiments with **13–15** indicate the prefered formation of the kinetic six-membered ring products in aqueous, chloride-free medium.

The most convincing evidence for a kinetically controlled reaction comes from the comparative ^1H NMR experiments of the products from the aqueous reactions of trans complexes **4–6**. Platinum was found to substitute specificly into the ortho position of the β-substituted aromatic, forming exclusively a six-membered cycloplatinated ring (**13**). Confirmation of the regiospecificity of the reaction was found by making comparisons of the NMR spectra from **13–15**.

Most interestingly, following cycloplatination, the proton absorptions of the ortho-platinated aromatic rings of the five-ring compounds **15** and **19** were shifted further upfield than the analogous aromatic protons of the six-membered ring compounds **13** and **14**. These differences are attributed, in part, to varying extents of metal-to-ligand back-bonding between the filled t_{2g} orbitals of platinum and the vacant π^* orbitals of the aromatic ring.

X-ray crystal studies have shown that, for five-membered ring cyclopalladinated compounds, the coordination plane of palladium lies coplanar with the coordinated aromatic ring.[28] This allows for a significant trans-influence between the Pd and carbon atom, as determined from bond length measurements. This was used as evidence for palladium-to-aryl π-back-bonding. Proton NMR supported this find; due to the flow of electron density from the d orbitals of palladium atom into the aromatic ring, shielding of all aromatic protons resulted.[29] For the five-membered ring cycloplatinated complexes **15** and **19**, upfield shifts were also recorded for *all* protons of the platinated aromatic rings. In **15**, the protons H_o, H_m, and $H_{m'}$ were upfield shifted 1.04, 0.50, and 0.22 ppm, respectively, relative to the same protons in the unplatinated compound **6**. Likewise, the same protons in **19** were upfield shifted 0.51, 0.41, and 0.17 ppm relative to those protons in **4**.

The six-membered ring of compounds **13** and **14** prohibits the same coplanar arrangement of platinum with the aromatic ring. Assuming a ca. 90° C–Pt–NH_3 bond angle, models show that the deformed six-membered ring turns the coordination plane of platinum approximately 60° out of the plane of the aromatic ring. This dihedral angle would orient the aromatic π^* orbitals between the lobes of the t_{2g} metal orbitals, an arrangement unfavorable for orbital overlap. Due to the diminished d to π^* back-bonding, less shielding of the aromatic protons would be expected. The chemical shift data for protons H_o, H_m, and $H_{m'}$ of **13** and **14** support this proposal. Nuclei H_o and H_m are upfield shifted only 0.25 and 0.35 ppm, respectively, relative to the same protons in **4** and **5**. Proton $H_{m'}$ is downfield shifted 0.07 ppm relative to the same proton in the unplatinated compounds.

Kinetic modeling, based on the disappearance of chromatographicable substance from the reaction solutions, allowed for a determination of the apparent rates of cycloplatination for the cis-configured, mixed-amine platinum complexes. Since NMR studies of the cycloplatinated products of the cis-configured 1,2-diphenyl-substituted (ethylamine)platinum(II) complex **1** showed an approximate 1:2 mixture of both five- and six-ring compounds, these rate constants should only be intreperted qualitatively; the cycloplatination rate constants derived for these compounds are an average of the rates of both five- and six-ring formation. In spite of this, it is apparent that the pathway to

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six-ring products proceeded faster than to five-ring ones, judging by the relative rate constants for compounds **1–3**. Changes in ionic strength had no effect on the apparent cycloplatination rate constants, suggesting that the rate-determining transition state(s) of cycloplatination do not involve charge changes relative to the reactants.

The kinetic/thermodynamic control of ortho-palladation for an unsymmetrically substituted bifunctional benzylamine has been demonstrated by Ryabov.[30] Under kinetic controlled conditions ($CHCl_3$, 20 °C), the bifunctional (benzylamine)palladium complex underwent ortho-metalation in the electron-rich 3,4-dimethoxyphenyl ring, while under thermodynamic conditions (CH_3COOH, 70 °C) cyclopalladation took place in the electron-poor, *p*-nitrophenyl ring. Furthermore the kinetic product could be converted into the thermodynamic one by heating it in acetic acid. These studies prompted us to investigate the kinetic/thermodynamic nature of five- versus six-membered ring ortho-platination. The six-membered ring cycloplatinated complex **13** was surprisingly stable and required extended heating at 65 °C in a dilute KCl solution for the compound to interconvert to the five-membered ring one, as determined by ^1H NMR (Figure 7). Newkome and co-workers have compared the X-ray crystal structures of analogous five-membered with six-membered ring cyclopalladated complexes.[25c] They found that the six-membered ring can readily accommodate the square-planar central metal and that no unusual or unfavorable geometrics or interactions were found compared to the five-membered ring complexes. Thus, in light of our findings that the five-membered ring ortho-platinated complex **19** is indeed the thermodynamic product, it would appear that the enhanced stability of the five-membered ring complex is related to factors other than ring strain. One likely reason could be the comparative Pt–C bond energies between the two different cycloplatinated rings; the greater extend of d to π^* back-bonding in the five-membered than in the six-membered ring type could result in a more stable organometallic bond.

One proposed intermediate of ortho-metalation for transition metals is an intramolecular η^2-bonded arene, which rearranges through an η^1-arenium transition state to form a σ-carbon–metal bond with concomitant elimination of a proton.[31] Although suggested almost 20 years ago, evidence for such intramolecular η^2-bonded intermediates has been largely lacking. An example of a weak, intramolecular η^2-bond between an aromatic and a transition metal has been characterized.[32] The X-ray crystal structure of the isolated compound showed an aromatic ring, β-substituted to coordinating nitrogen, rigidly fixed above the empty coordination site of the molybdenum(II) atom. The difficulty in isolating such an intermediate with an aromatic ring α-substituted to the coordinated nitrogen was attributed to the facile formation of the nearly unstrained five-membered ring.

If an η^2-bonded intermediate is involved in orthoplatination, then the comparative geometries of the π-bond in this transition state intermediate might reveal why platinum reacts faster with a β-substituted aromatic ring than with an α-substituted one. Models indicate that the bond between carbons C-1′ and C-2′ of a phenyl ring, β-substituted to the coordinated nitrogen, can attain a conformation perpendicular to and directly below the fourth liganding position of the square-planar platinum atom (Figure 8A). This geometry allows for a η^2-arene intermediate with optimal d–π^* orbital overlap. In contrast, for a phenyl ring α-substituted to the coordinating nitrogen, the fourth liganding position of platinum cannot be oriented perpendicularly above and between the same two aromatic carbon atoms (Figure 8B). The ensuing π-bond to the transition metal lacks optimal orbital overlap and is less stable than the η^2-arene intermediate formed with the β-ring. Thus, we postulate that the presence of a kinetic product is the result of a more favorable orbital geometry in the η^2-bonded transition state intermediate of the six-membered relative to the five-membered ring ortho-platinated product. Ongoing investigations are attempting to answer this question.

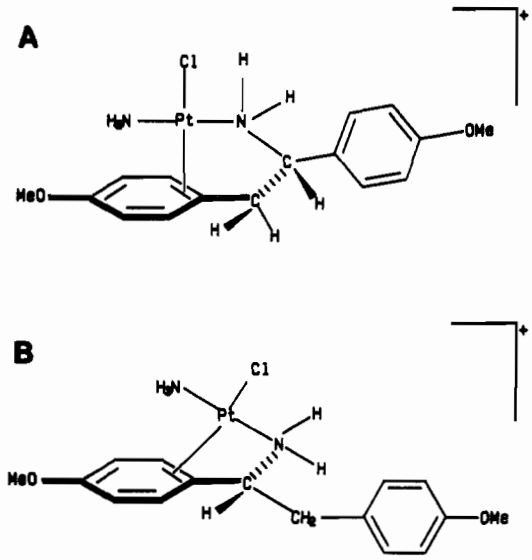

Figure 8. Proposed transition-state intermediates leading to either (A) six- or (B) five-membered ring cycloplatinated complexes.

From a pharmacological perspective, these results describe the first reported examples of an intramolecular ortho-platination reaction occurring with cisplatin analogues under physiological conditions. Although slightly slowed, the reaction still proceeded in the presence of intracellular concentrations of chloride (4 mM) at pH 6.9. These observations illustrate the need for an appreciation of the chemistry pertaining to the pharmacoactivation of new cisplatin analogues. As the complexity of these transition-metal compounds increases, such detailed investigations are even more advisable. Certain ortho-metalated complexes of Pd and Pt have been shown to covalently bind to DNA;[33] however, results from cell culture experiments indicate that cycloplatination leads to the loss of cytotoxic activity for compounds **1–3**. The potential reactivity of aquated platinum(II) toward an aromatic moiety present together in the same molecule should, therefore, be an important consideration in the future design of cisplatin analogues.

An additional aspect is the role that ortho-platination might play in the metabolism and toxicity of cisplatin. Traditionally, biomolecules containing sulfur, nitrogen, or oxygen donor atoms have been considered as potential ligands for reactions with cisplatin in vivo. The possibility that carbon could serve as a donor atom in reactions with cisplatin has been proposed,[34] but thus far only one example of an organometallic metabolite of a cisplatin analogue, formed under aqueous conditions, has appeared in the literature. An ascorbic acid adduct of *cis*-(1,2-diaminocyclohexane)platinum(II) has been charaterized and found to be coordinated to the metal through the C2 carbon atom and a deprotonated hydroxy group of ascorbate, forming a six-membered chelate ring.[35]

The reactivity of biomolecules containing donor atoms such as sulfur and nitrogen toward cisplatin would be expected to be greater than that of a nucleophilic carbon. For this reason, it would be unlikely that an activated carbon could effectively compete with these traditional ligands in bimolecular reactions for cisplatin. However, many biomolecules are bidentate (i.e. amino acids) containing both traditional liganding groups (i.e. amine, carboxylic acid) as well as aromatic ring systems. Cisplatin is known to coordinate with amino acids through either the amino or carboxylate groups, or both.[36] Aromatic amino acids and

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biogenic amines represent an interesting class of compounds due to their 2-phenylethylamine structure. With the new awareness that such aromatic functionalities can react with aquated platinum(II) in an intramolecular fashion, it would be of interest to know if following the formation of such cisplatin–monoamino acid adducts ortho-platination occurs. For example, we have found that the cisplatin monoadduct of the dimethoxy derivative of dopamine, coordinated to platinum through the amino group, undergoes a rapid ortho-platination reaction under physiological conditions.

Experimental Section

Materials and Methods. Cisplatin and $K_2[PtCl_4]$ were generously supplied by the Degussa AG (Frankfurt a. M., FRG). $[Pt(NH_3)_4]Cl_2$ was purchased from Alfa Chemicals (Karlsruhe, FRG). $K[PtCl_3(NH_3)]$[37] and *meso*-[1,2-bis(4-methoxyphenyl)ethylenediamine]dichloroplatinum(II)[38] (**16**) were synthesized as previously described. Amines **7–9** were prepared by literature methods[39,40] and were deemed of acceptable purity by elemental analysis, ^1H NMR, and HPLC methods. Where reported, methanol (Baker, Deventer, Holland) and DMF (Aldrich, Steinheim, FRG) were HPLC-grade. Water was deionized by means of a Millipore Milli-Q Water System to a resistivity of 1.8 MΩ cm.

Proton NMR were recorded in DMF-d_7 with TMS as the internal standard by using a 250-MHz Bruker WM 250 spectrometer. NMR data for the compounds described below are presented in Tables I and IV. FAB mass spectra were recorded on a Varian MAT 311A instrument. Samples were dissolved in a glycerol/DMSO (1:1) matrix immediately prior thereto. UV-difference spectra were recorded with a dual-beam Kontron Uvikon 810 spectrophotometer equipped with a thermostatically controlled cuvette holder. Infrared spectra were measured (as KBr pellets) between 250 and 4000 cm^{-1} by using a Beckman IR 4240 instrument. HPLC analysis was done with an Altex 110A high-pressure mixing-gradient system fitted with a Rheodyne 7125 sample injector having a 500-μL injection loop. A 0.4 × 25 cm Nucleosil-100 RP-18 column (Macherey-Nagel, Düren, FRG) with a 0.4 × 3.0 cm precolumn was used for the chromatography and a Kontron Uvikon 720LC variable-wavelength UV–vis detector set at 272 nm was used for sample detection. Elemental analyses were performed by the Microanalysis Lab at the University of Regensburg.

Syntheses of *cis*-Dichloroplatinum(II) Complexes 1–3. As 75 mg (0.2 mmol) of $K[PtCl_3(NH_3)]$ was stirred in 4 mL of methanol, 0.22 mmol of amine (**7–9**) in 4 mL of methanol was added. The reaction was allowed to stir at room temperature for approximately 72 h in the dark. The cream-colored precipitate was collected by suction filtration and washed with methanol, and the product was dried in vacuo over P_2O_5.

Syntheses of *trans*-Dichloroplatinum(II) Complexes 4–6. As 150 mg (0.5 mmol) of cisplatin was stirred in 5.0 mL of DMF, 85 mg (0.5 mmol) of $AgNO_3$ in 2.0 mL of DMF was added at once. After the mixture was stirred overnight at room temperature in the dark, the AgCl was removed by suction filtration and the clear, yellowish solution of *cis*-$PtClNO_3(NH_3)_2$ was added to a solution of 0.5 mmol of amine (**7–9**) in 2.0 mL of DMF. After being allowed to react overnight, the reaction was concentrated to ca. 1 mL by vacuum distillation (40 °C), and then added slowly to 400 mL of a 1% HCl/5 g KCl solution. For the syntheses of compounds **4** and **5**, warming at 80 °C for 1 h was sufficient to substitute Cl^- for NH_3. For compound **6**, however, heating to 95 °C was necessary. After reaction was cooled to ca. 50 °C, the cream-colored precipitated was collected by suction filtration, and dried in vacuo over P_2O_5.

Syntheses of Cycloplatinated Compounds 13–15. As 500 mL deionized water was stirred at 40 °C, 0.1 mmol of trans-Pt complex (**4–6**) in 1.0 mL of DMF was added dropwise over 30 min. After addition, the reaction was allowed to stir overnight at 40 °C, then frozen and lyophilized to give a white powder.

HPLC Determination of Cis/Trans Isomeric Purity. Solutions of **1–6** (10^{-2} M) in DMF were prepared immediately prior to use. The DMF solutions were diluted 500× into a 0.1 M NaCl solution with constant stirring. A 500-μL probe of the aqueous solution was loaded onto the HPLC column and eluted with 60:40 MeOH/20 mM KH_2PO_4 at 0.7 mL/min.

HPLC Pt–Cl Hydrolysis Assay. All reactions were performed in silylanized 25-mL glass vials. Solutions of **1–3** and **16** (10^{-2} M) in DMF were prepared immediately prior to use. The DMF solutions were diluted 500× into 20 mL of deionized water with constant stirring at 37 °C, giving a final concentration of 20 μM Pt complex. Immediately following this addition and then at ca. 1-h intervals thereafter, 1.0 mL aliquots were removed from the aqueous reactions and added to 65 mg of KBr. After 5 min at 37 °C, 500 μL of the KBr-quenched solution was loaded onto the HPLC column and eluted with one of the following systems: (A) 20 min 50:50 MeOH/20 mM KH_2PO_4 of 0.7 mL/min, 10 min linear gradient 50–65% MeOH, 5 min 65% MeOH; (B) 40:60 MeOH/20 mM KH_2PO_4 of 0.7 mL/min.

For **1–3**, gradient system A was used. For **16**, the isocratic system B was sufficient. In a typical kinetic experiment, 7–8 time points were measured with the last measurement being taken 24 h after addition. Integration of the peak areas was done by multipling the peak height by the peak width at half the peak height. Mole fractions were calculated by dividing the peak area at the various time points by the peak area of the dichloroplatinum(II) complex at T_0. Rate constants were calculated from samples taken during the first 8 h by using a Gear-Simplex algorithm (Serena Software, Bloomington, IN) according to the kinetic schemes described in the text.

The relatively large extinction coefficient of the *p*-methoxyphenyl group of the liganding amines (λ_{max} at 272 nm, $\epsilon \approx 1500\ cm^{-1}\ M^{-1}$) made the changes in the UV absorption associated with the exchange of coordinated Cl^- for Br^- small by comparison. At 272 nm, the molar absorbance differences between *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[PtBr_2(NH_3)_2]$ are $\approx -100\ cm^{-1}\ M^{-1}$, which would constitute an error of ca. −4% in the quantification of the dibromoplatinum complexes **1**, **3**, and **19**, and ca. −8% in the quantification of the dibromoplatinum complex **2**. These errors had only a minimal effect on the values of the kinetic rate constants. Since the interexperimental deviations between rate constants were greater than the deviations between the rate constants calculated from the observed data compared to those calculated from the corrected data, we chose to report the rate constants calculated from the uncorrected data.

UV-Difference Spectroscopy. DMF solutions of **1–5** (10^{-2}) were diluted 500× into deionized water at 37 °C with stirring. Half of the solution was added to the sample cuvette (l = 1.0 cm), the other half being added to the reference cuvette containing enough KCl to give a 0.1 M chloride solution. The cuvettes were maintained at 37 °C through out the course of the experiment. For the cis-configured isomers, spectra were recorded every hour for the first 8 h then again at 24 h. After this time, no further changes in the difference spectra were observed. For the trans isomers, the time span between spectra was reduced to 3 min, the last spectra being taken 30 min after the experiment was begun.

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Supplementary Material Available: Tables giving yields and IR, FAB-MS, and elemental analysis data for compounds **1–6** and **13–15** (3 pages). Ordering information is given on any current masthead page.

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