Linkage Isomerism in Square-Planar Complexes of Platinum and Palladium with Histidine and Derivatives

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Reactions of three histidine-containing ligands (L-histidine, *N*-acetyl-L-histidine, glycyl-L-histidine) with monofunctional Pd^{II} and Pt^{II} (M) species ((dien)M^{II}, (trpy)M^{II}, *cis*-$[(NH_3)_2Pt(1\text{-}MeU)(H_2O)]^+$, *cis*-$[(NH_3)_2Pt(1\text{-}MeC)(H_2O)]^{2+}$, *trans*-$[(NH_2CH_3)_2Pt(1\text{-}MeC)(H_2O)]^{2+}$) have been studied by applying primarily ^1H NMR and occasionally ^{195}Pt NMR spectroscopy. Depending on reaction conditions (pH; M:ligand ratio), different products are formed which, in the case of *N*-acetylhistidine and (dien)Pt^{II}, for example, include monodentate coordination through N1 of imidazole (**3a**), N3 of imidazole (**4a**), and O of carboxylate (**5a**) or bidentate bridging via N1,O (**6a**), N3,O (**7a**) and N1,N3 (**8a**). With L-histidine and (dien)Pt^{II}, in addition, formation of an isomer with coordination through the amino group is observed (**13**), which takes place by a migration process from the initially favored O site (**12**). In the case of the ternary nucleobase/*N*-acetylhistidine complexes of Pt^{II}, N1 and N3 linkage isomers have been separated using HPLC and isolated in a few cases (**16–18**) and their acid/base equilibria determined. The formation of N1 and N3 linkage isomers, which correspond to the metallated forms of the respective tautomers, is *the* outstanding feature of this study. The differentiation of tautomers of the Pt compounds in many cases is straightforward in spectra recorded at low magnetic field (80–100 MHz) when ^{195}Pt–^1H (imidazole) couplings are observable. In addition, the methine resonances of the two isomers differ, with those of the N3 isomers downfield relative to those of the N1 isomer. On the basis of published data on the tautomer distribution and the measured distribution of (dien)Pd^{II} over the two imidazole sites, it is estimated that Pd^{II} complex formation with N1 is slightly favored over N3 in the case of *N*-acetylhistidine, but substantially more so in the case of L-histidine. As for (dien)Pt^{II}, the distribution of imidazole-bound isomers reflects primarily kinetic rather than thermodynamic factors.

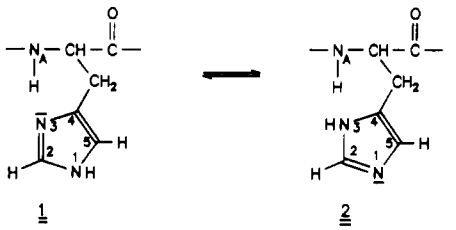
Introduction

It is now well-established, from measurements with a variety of spectroscopic techniques,[2,3] that histidine (his) and most of its derivatives exist, in aqueous solution over the pH range where the imidazole ring is monoprotonated, in two tautomeric forms shown in Scheme I, with the N1 protonated tautomer, **1**, predominating.[4]

In most of its complexes, histidine[4] acts as a tridentate ligand, through N_A, N3, and the carboxylate oxygen, e.g. in Ni(hisH-N_A,*N3*,*O*)$_2$,[5] or bidentate, either through N_A and N3, e.g. in Pt(hisH-N_A,*N3*)$_2$,[6] less commonly, through N_A and carboxylate O, e.g. in $[Cu(hisH_2\text{-}N_A,O)_2(H_2O)_2](NO_3)_2$.[7] Relatively few complexes have been well characterized in which his or a derivative acts as a unidentate ligand, such as in $[Ru(NH_3)_5(hisH_2\text{-}N1)]Cl_3\cdot H_2O$, for example.[8] The particular crystal selected for structure determination contained the N1-bound his isomer, but other isomers, in particular that with N3-bound his, may have been formed as well. Saudek et al.[9] have proposed structures for numerous products obtained from reaction of *cis*-$(NH_3)_2PtCl_2$ with his, including some with unidentate his coordination through amine or imidazole N atoms. In a NMR study of the reaction between *cis*-$[(NH_3)_2Pt(H_2O)_2]^{2+}$ and his in acidic solution, Appleton et al.[10] showed that his initially coordinates through carboxylate oxygen only, followed by formation of a N_A,O chelate ring. There the acidic conditions prevent involvement of the imidazole nitrogens (N_I) in metal coordination. Upon addition of sufficient base and imidazole deprotonation, a N_A,N3 chelate forms rapidly.

Kostic et al.[11,12] have reported that $[Pt(trpy)Cl]Cl^4$ reacts selectively with the imidazole side-chains in his-containing proteins and have also studied reactions of a number of small peptides and other histidine derivatives as "model compounds". While the UV–visible spectra obtained conclusively reveal that the $Pt(trpy)^{2+}$ moiety is bound to an imidazole nitrogen, they do not allow a

Scheme I



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(4) The IUPAC convention rather than the biochemical one is used for numbering atoms in the imidazole ring. N_A will be used to refer to amine or amide nitrogen, and N_I to refer to imidazole N atoms generally. His is used for L-histidine of unspecified charge, $hisH_2$ refers to the neutral ligand, hisH to the monoanion (cf. also Figure 1). Other abbreviations used: trpy = 2,2′,6′,2″-terpyridine; dien = diethylenetriamine; 1-MeC = 1-methylcytosine; 1-MeC$^-$ = 1-MeC deprotonated at the exocyclic amino group; 1-MeU = 1-methyluracil anion. In formulas and in the text "H" will be taken as referring to either ^1H or ^2H(D).
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differentiation between N1 and N3. Some ^{1}H NMR data were also obtained, which were interpreted in terms of coordination through N1 only.[11]

On the other hand, Jordan et al., in stopped-flow kinetic studies of the reaction of $[Ni(H_2O)_6]^{2+}$ with histidine[13] and histamine[14] have interpreted their results[14] in terms of initial competitive reactions of Ni^{II} with N3, which is followed by N3,N_A chelation, and with N1, which cannot lead to a chelate complex for steric reasons.

There are numerous metalloproteins in which a metal ion is bound to a histidine imidazole nitrogen through N1 only (e.g. hemoglobin[15]), but there are also metalloproteins in which a metal at the active site is bound through N3 of his (e.g. Zn in SOD, superoxide dismutase,[16] or Cu in plastocyanin[17]) or has a mixed donor site sphere (e.g. Cu in SOD: $(N3)_2$, $N1_{term}$, $N1_{bridge}$[16]) or where identical metals display different environments (e.g. $Cu_1(N3)_2$ and $Cu_3(N1)_8$ in ascorbate oxidase[18]).

In the light of these data, there did not appear to be any compelling steric or electronic reason why square-planar platinum(II) and palladium(II) complexes offering one relatively labile coordination site to his or a derivative of it should not show, under appropriate conditions, N1/N3 linkage isomerism. ^{1}H NMR spectroscopy provides a useful tool for studying this phenomenon as previously demonstrated by ourselves in the Pt/uracil[19] and Pt/thymine[20] systems as well as by Reedijk et al.[21-23] in their studies on Pt^{II} complexes with imidazole derivatives. Specifically, differences in coupling constants of the ^{195}Pt isotope ($I = {}^1/_2$; 34% natural abundance) with aromatic protons can be applied to assign binding sites.

In this paper, the reactions of the classic monofunctional complexes $[M(dien)(H_2O)]^{2+}$ (M = Pt, Pd)[4] with his and some of its derivatives (Figure 1) will be described. As well, reactions of $[Pt(trpy)Cl]^+$ will be reexamined, and some reactions of the model nucleobase[4] complexes *cis*-$[(NH_3)_2Pt(1\text{-}MeC)(H_2O)]^{2+}$ and *cis*-$[(NH_3)_2Pt(1\text{-}MeU)(H_2O)]^+$ will be described. It was actually results obtained with these simple ternary complexes that led us to the discovery of linkage isomers of *N*-acetylhistidine and prompted subsequent studies with other his ligands and other metal species.

Experimental Section

Starting Materials. The following materials were used as supplied: L-histidine and *N*-acetyl-L-histidine monohydrate (Aldrich) and glycyl-L-histidine dihydrate (Sigma). [Pt(dien)I]I,[24] [Pd(dien)I]I,[25] and [Pt(trpy)Cl]Cl·2H$_2$O,[26] were prepared by literature methods; *cis*-$[(NH_3)_2Pt(1\text{-}MeC)Cl]Cl$,[27] *cis*-$(NH_3)_2Pt(1\text{-}MeU)Cl\cdot H_2O$,[28] and *trans*-$[(CH_3NH_2)_2Pt(1\text{-}MeC)Cl]Cl$,[29] as described. The corresponding nitrato and/or aqua complexes were obtained by reacting the halogeno compounds with the necessary amounts of $AgNO_3$. After several hours of stirring in the dark, AgI or AgCl was removed by filtration and the solutions were brought to dryness and/or concentrated to a small volume. In the case of the $[M(dien)(ONO_2)]NO_3$ species, the resultant solids were washed with acetone and air-dried.

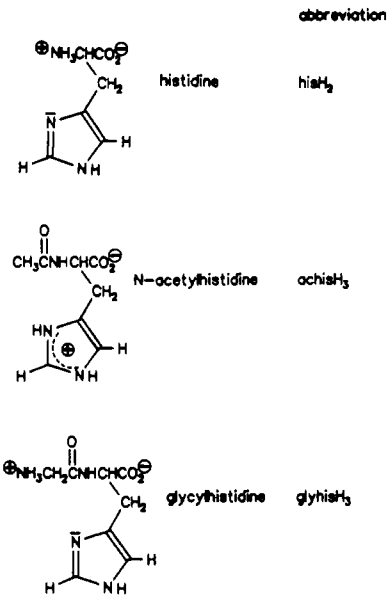

Figure 1. Ligands used, depicted in their major tautomeric forms. Hs in abbreviations refer to number of protons potentially replaced by a metal ion.

Mixed Nucleobase/Acetylhistidine Compounds. Reactions of *cis*-$[(NH_3)_2Pt(1\text{-}MeU)(H_2O)]^+$ and *trans*-$[(NH_2CH_3)_2Pt(1\text{-}MeC)(H_2O)]^{2+}$ with a 1-fold excess of achisH$_3$ were carried out on a ^{1}H NMR scale prior to preparative work. According to ^{1}H NMR spectroscopy, reactions in both cases were practically quantitative at pD 7 with ratios of N1:N3 isomers being ca. 10:6 in the case of 1-MeU compound and ca. 10:8 in the case of the 1-MeC compounds. Preparative work was carried out with a 1:1 Pt achisH$_3$ ratio at pD 7 or a small scale (semipreparative HPLC). Low isolated yields are due to this fact. In principle, isolation of the individual linkage isomers in yields close to those observed in the ^{1}H NMR studies should be possible, but was not attempted in this work. *cis*-$[(NH_3)_2Pt(1\text{-}MeU)H_2O]NO_3$, prepared from *cis*-$(NH_3)_2Pt(1\text{-}MeU)Cl\cdot H_2O$ and $AgNO_3$ in situ, was mixed with 1 equiv of achisH$_3$ with the pH adjusted to 7 by means of 0.1 M NaOH and kept at 40 °C for 3 d. After concentration to a small volume, the mixture was separated by means of a HPLC (Waters system) using a RP 18 (MN Nucleosil 10 C 18) column and applying a nonlinear H_2O/MeOH (30%) gradient elution. *cis*-$(NH_3)_2Pt(1\text{-}MeU)(achisH_2\text{-}N1)\cdot H_2O$ (**16**) eluted first, followed by *cis*-$(NH_3)_2Pt(1\text{-}MeU)(achisH_2\text{-}N3)\cdot 3H_2O$ (**17**). Isolated yields were 11% (**16**) and 4% (**17**). Anal. Calcd (found) for $C_{13}H_{25}N_7O_7Pt$ (**16**): C, 26.6 (26.8); H, 4.3 (4.1); N, 16.7 (16.5). Anal. Calcd (found) for $C_{13}H_{27}N_7O_8Pt$ (**17**): C, 25.8 (26.0); H, 4.5 (4.4); N, 16.2 (16.3).

trans-$[(NH_2CH_3)_2Pt(1\text{-}MeC)(achisH_2\text{-}N1)]NO_3\cdot 3H_2O$ (**18**) was obtained from *trans*-$[(NH_2CH_3)_2Pt(1\text{-}MeC)Cl]Cl\cdot H_2O$, $AgNO_3$, and achisH$_3$ (1 equiv) in water (48 h, 47 °C), concentration, HPLC separation and evaporation to dryness as a microcrystalline compound. No attempts were made to optimize the isolated yield of 7.5%. Anal. Calcd (found) for $C_{15}H_{31}N_9O_{10}Pt$: C, 26.0 (26.0); H, 4.5 (4.8); N, 18.2 (17.9).

NMR Spectra. ^{1}H NMR spectra were run on D_2O solutions containing sodium trimethylsilylpropane-3-sulfonate (TSP) as internal reference. Spectra were routinely run at 200 MHz on a Bruker AC-200 instrument and occasionally at 300 MHz on a Bruker AM-300 instrument. To allow observation of ^{195}Pt satellite peaks, spectra of representative samples were also run at lower magnetic fields, e.g. on JEOL FX-100 and Bruker AC-80 instruments. Possible intermolecular stacking interactions of trpy ligands, which should primarily affect the chemical shifts of trpy resonances, were not studied. For ^{1}H-decoupled 42.8-MHz ^{195}Pt NMR spectra, shifts are reported relative to $PtCl_6^{2-}$ (external standard, −1630 ppm for K_2PtCl_4). Spectra were run typically with 50 000 transients, approximately 0.2 s apart, with a magnetization vector flip angle of 30°. Spectra were run with a width of 130 KHz, and a line broadening factor of 10–100 Hz was used in processing the data. The phase-sensitive DQF-COSY (200 MHz) was recorded with the Bruker Microprogram "COSYPDHG.AU" (sweep width 400 Hz, aromatic region only,

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Table I. ^{1}H NMR Data for Diethylenetriamine and Terpyridyl Complexes

compound	no.	solution pD	Imidazole Protons δ (*J*(Pt–H))[a] H(2)	Imidazole Protons δ (*J*(Pt–H))[a] H(5)	methine protons δ (line spacings)[b]	CH_3[c] δ
achisH$_3$		4.5	8.61	7.27	4.55 (8.7, 5.1)	1.99
[Pt(dien)(achisH$_3$-*O*)]$^{2+}$	**5**	4.5	8.59 (0)	7.25 (0)	4.55 (8.7, 5.1)	1.96
[Pt(dien)(achisH$_2$-*N3*)]$^+$	**4a**	3.5	7.97 (19)	7.11 (9)	5.25 (9.5, 5.4)	1.99
[Pt(dien)(achisH$_2$-*N1*)]$^+$	**3a**	3.5	7.86 (20)	6.86 (18)	4.51 (10.6, 5.3)	1.96
[Pd(dien)(achisH$_2$-*N3*)]$^+$	**4b**	3.5	7.94	7.08	5.20 (9.1, 5.6)	1.99
[Pd(dien)(achisH$_2$-*N1*)]$^+$	**3b**	3.5	7.75	6.78	4.54 (8.0, 5.4)	1.97
{[Pt(dien)]$_2$(μ-achisH$_2$-*N3,O*)}$^{3+}$	**7a**	4.5	7.98	7.07	5.35 (10.2, 4.7)	*q*
{[Pt(dien)]$_2$(μ-achisH$_2$-*N,O*)}$^{3+}$	**6a**	4.5	7.87	6.83	4.54 (8.9, 5.1)	*q*
{[Pt(dien)]$_2$(μ-achisH-*N1,N3*)}$^{2+}$	**8a**	9.0	7.32	6.70	5.0[e,f]	1.96
{[Pd(dien)]$_2$(μ-achisH-*N1,N3*)}$^{2+}$	**8b**	7.0	7.17	6.57	≈4.9[f]	1.97
[Pt(trpy)(achisH$_2$-*N*$_1$)]$^+$ (major isomer)		5.5	8.53	7.49	4.50 (8.3, 5.1)	1.79
[Pt(tryp)(achisH$_2$-*N*$_1$)]$^+$ (minor isomer)		5.5	8.42[h]	7.36	4.53 (10.2, 5.1)	2.05
hisH$_3^+$		3.5	8.67	7.39	4.05 (6.6, 6.6)	
[Pt(dien)(hisH$_3$-*O*)]$^{3+}$	**12**	2.5	8.69 (0)	7.39 (0)	4.18 (7.1, 6.5)	
[Pt(dien)(hisH$_3$-*N*$_A$)]$^{3+}$	**13**	1.5	8.66 (0)	7.39 (0)	3.84 (7.9, 5.7)	
{[Pt(dien)]$_2$(μ-hisH$_2$-*N*$_A$,*O*)}$^{4+}$	**14**	1.5	8.66	7.35	3.67 (≈8, ≈6)	
[Pt(dien)(hisH$_2$-*N3*)]$^{2+}$	**11a**	6.5	8.04 (19)	7.25 (10)	4.26 (6.4, 6.4)	
[Pt(dien)(hisH$_2$-*N1*)]$^{2+}$	**10a**	6.5	7.92 (20)	6.94 (20)	3.94 (6.3, 6.3)	
{[Pt(dien)]$_2$(μ-hisH-*N3*,*N*$_A$)}$^{3+}$	**16a**	7.0	7.86	7.08	*e*	
[Pd(dien)(hisH$_2$-*N3*)]$^{2+}$	**11b**	6.8	8.00	7.20	4.19 (6.4, 6.4)	
[Pd(dien)(hisH$_2$-*N1*)]$^{2+}$	**10b**	6.8	7.80	6.84	3.93 (6.3, 6.3)	
{[Pd(dien)]$_2$(μ-hisH-*N3*,*N*$_A$)]$^{3+}$	**16b**	6.8	7.93	7.20	*e*	
{[Pd(dien)]$_2$(μ-hisH-*N1*,*N*$_A$)}$^{3+}$	**15b**	6.8	7.58	7.1	*e*	
[Pt(trpy)(hisH$_2$-*N*$_1$)]$^{2+}$ (major isomer)		6.5	8.54	7.65	4.16 (6.5, 6.5)	
[Pt(trpy)(hisH$_2$-*N*$_1$)]$^{2+}$ (minor isomer)		6.5	*d*	7.24	4.07 (6.5, 6.5)	
[Pt(dien)(glyhisH$_3$-*N3*)]$^{2+}$		6.5	8.34 (20)	7.17 (9)	5.01 (8.3, 6.1)	
[Pt(dien)(glyhisH$_3$-*N*$_1$)]$^{2+}$		6.5	7.85 (20)	6.84 (20)	4.45 (6.2, 6.2)	

[a] Pt–H coupling constants (Hz) given for selected platinum complexes whose spectra were run at 100 MHz. [b] Line spacings in methine signal at 200 MHz; see text. [c] For *N*-acetylhistidine complexes. [d] Obscured by signals from trpy ligand. [e] Obscured by other methine signals. [f] Obscured by HDO peak. [g] Not clearly resolved from other CH_3 peaks. [h] Taken from 2D spectrum.

Table II. Imidazole Resonances of Mixed Nucleobase/*N*-Acetylhistidine Complexes of Pt^{II} [a]

compound	no.	pD	imidazole protons δ (*J*(Pt–H))[b] H2	imidazole protons δ (*J*(Pt–H))[b] H5
cis-(NH$_3$)$_2$Pt(1-MeU)(achisH$_2$-*N1*)·H$_2$O	**16**	6.8	7.80 (21.0)	6.75 (21.3)
cis-(NH$_3$)$_2$Pt(1-MeU)(achisH$_2$-*N3*)·3H$_2$O	**17**	6.8	7.90 (20.1)	6.95 (7.9)
trans-[(NH$_2$CH$_3$)$_2$Pt(1-MeC)(achisH$_2$-*N1*)]NO$_3$·3H$_2$O	**18**	8.3	8.18 (21.5)	7.17 (21.5)
cis-[(NH$_3$)$_2$Pt(1-MeC)(achisH$_3$-*O*)]$^{2+}$	**19**	4.4	8.56 (0)	7.16 (0)
cis-[(NH$_3$)$_2$Pt(1-MeC)(achisH$_2$-*N1*)]$^+$ [c]	**20**	5.2	7.86[d]	6.85[d]
cis-[(NH$_3$)$_2$Pt(1-MeC)(achisH$_2$-*N3*)]$^+$ [c]	**21**	5.2	8.05[d]	6.99[d]

[a] Shifts in ppm; D_2O. [b] Coupling constants (Hz) obtained from 80-MHz spectra. [c] Assignment from comparison with **16** and **17**. [d] Coupling constants not determined.

relaxation delay 1 s. 1K × 1K data points). A Gauss apodization was applied in F_2; an unshifted sine bell apodization, in F_1.

Typical Preparation of NMR Sample. Reaction of [Pt(dien)(D_2O)](NO_3)$_2$ with excess *N*-acetylhistidine was carried out as follows. A solution of *N*-acetylhistidine monohydrate (0.01028 g, 0.0478 mmol) in 0.6 mL of D_2O was added to solid [Pt(dien)(ONO_2)]NO_3 (0.01227 g, 0.0291 mmol). The mixture was shaken to dissolve the solid. A small quantity of TSP was added. After the mixture was allowed to stand at 22 °C, the ^{1}H NMR spectrum was obtained. When necessary, the pD of the solution was adjusted by means of DNO_3 or NaOD solutions in D_2O, with narrow-range indicator strips (Merck) used to monitor the pD. ^{195}Pt and ^{1}H NMR spectra were run on the same solutions.

Results

NMR data for dien and trpy complexes are presented in Table I and for 1-MeU and 1-MeC complexes in Table II.

In most of our spectra, the small coupling (ca. 1–3 Hz) between H2 and H5 of the imidazole ring was resolved. This caused the H2 signal, to lower shielding, to appear as a doublet. For H5, to higher shielding, long-range coupling to the methylene protons was sometimes also resolved, and whether resolved or not, this coupling usually caused some broadening of the peak envelope for this proton, making the peak height lower than that for H2 in the same compound. This provided a useful check that the H2 and H5 assignments in our complexes, based on the usual chemical shift order, remained valid. These small couplings did not vary much for the compounds studied and are not listed in our tabulated data.

It was frequently possible to assign resonances for the methine proton to particular isomers. The shifts are tabulated, together with the line separations observed in the four-line methine signal at 200 MHz. It should be emphasized that these methine signals each represent the X-part of an ABX spectrum, so that the line separations do not correspond to coupling constants and will be field-dependent. However, by providing these splittings, we do allow the appearance of this multiplet in the 200-MHz spectrum to be reconstructed.

The methylene regions of the spectra were relatively complex, and, with dien complexes, overlapped with peaks from the dien ligand. No attempt was made to analyze these parts of the spectra.

The coupling constants from ^{195}Pt to the imidazole protons were useful in making structural assignments but were in most cases not obtainable from ^{1}H NMR spectra measured at 200 MHz. Because of rapid relaxation of the ^{195}Pt nuclei by the chemical shift anisotropy mechanism (CSA),[30] ^{195}Pt satellite peaks were not usually observed in spectra run at this (or higher) magnetic field strengths. However, they were observed using 80–100 MHz instruments with H2 and H5 affected differently, depending on the Pt binding site(s).

Reactions of [M(dien)(H_2O)]$^{2+}$ with *N*-Acetylhistidine. The pK_a constants of achisH$_4^+$ are 3.00 (pK_{a1}, carboxyl group) and

(30) Lallemand, J.-Y.; Soulié, J.; Chottard, J.-C. *J. Chem. Soc., Chem. Commun.* **1980**, 436.

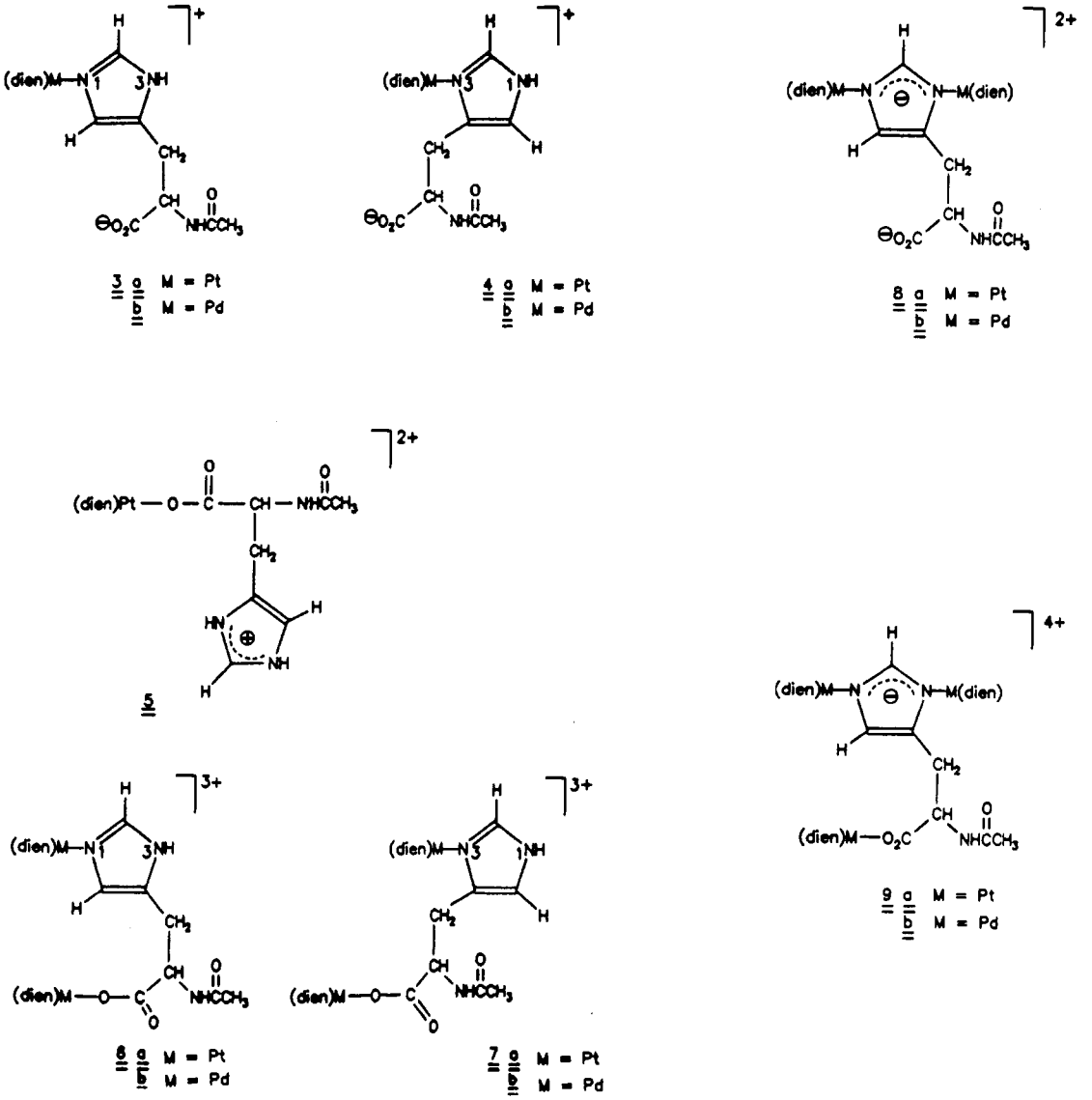

Figure 2. Products obtained from reaction of (dien)M^{II} with *N*-acetylhistidine.

6.99 (pK_{a2}, first imidazole proton).[31] When dissolved in water, *N*-acetylhistidine exists in the form shown in Figure 1, with both imidazole nitrogens protonated. It is therefore necessary to add base before significant quantities of achisH_2^-, with the imidazole ring neutral, are present.

NaOD solution was added to a D_2O solution of *N*-acetylhistidine (initial pD 5.0) to increase the pD to 7.3. Upon addition of solid [Pt(dien)(ONO_2)]NO_3, the pD immediately dropped to 6.5. The ^1H NMR spectrum run shortly afterward showed peaks due to free ligand in addition to two species with imidazole-bound ligand, [Pt(dien)(achisH_2-*N1*)]$^+$ (**3a**) and [Pt(dien)-(achisH_2-*N3*)]$^+$ (**4a**) (Figure 2). The assignment of peaks to particular isomers, as listed in Table I, was made on the basis that the isomer in which both imidazole protons showed relatively large ^{195}Pt coupling constants (H2, 20 Hz; H5, 18 Hz) is **3a** with the Pt bound to N1, while the isomer with a relatively large coupling to H2 only (19 Hz) and a smaller coupling to H5 (9 Hz) is **4a**, with the metal bound to N3.[21-23] The ratio of N3:N1-bound complex (**4a**:**3a**) from peak heights was approximately 2:1.

Because one isomer was present in excess over the other, it was possible to assign the methine and acetyl methyl proton signals to each isomer, as listed in Table I. It was then apparent that coordination through N1 had a relatively minor effect (shift to higher shielding) on the methine proton, but coordination through N3 caused a considerable (ca. 0.7 ppm) shift to lower shielding, usually downfield from the HDO peak at 4.8 ppm. This relatively large shift can be rationalized on the basis that metal coordination at N3 would be expected to perturb the populations of rotamers about the CH–CH_2 bond, which are affected by N3–amide interactions, while coordination at N1, relatively remote from this bond, will have little effect on these populations. Analogous shifts to lower shielding occur for the methine proton in acetylhistidine complexes with Pd(dien) and for M(dien) complexes with histidine and glycylhistidine bound via N3 (see below and Table I).

If the reaction between [Pt(dien)$(H_2O)]^{2+}$ and *N*-acetylhistidine was carried out at 25 °C, without added base, the ^1H NMR spectrum showed that a new species was formed, with imidazole and acetyl methyl resonances only slightly shifted from those of the free ligand. Neither of the imidazole proton peaks showed any coupling to ^{195}Pt at 100 MHz. The ^{195}Pt NMR spectrum showed a peak at −2504 ppm, in the same vicinity as that from [Pt(dien)$(H_2O)]^{2+}$, −2547 ppm. These data are consistent with the formulation of the complex present as [Pt-(dien)(achisH_3-*O*)]$^{3+}$ (**5**), in which the ligand is bound through carboxylate oxygen (Figure 2).

The formation of **5** under these conditions is analogous to the formation of *cis*-[Pt$(NH_3)_2$(achisH_3-*O*)$(H_2O)]^{2+}$ as the initial product from reaction of *cis*-[Pt$(NH_3)_2(H_2O)_2]^{2+}$ and *N*-acetyl-

(31) Tanokura, M. *Biochim. Biophys. Acta* **1983**, *742*, 576.

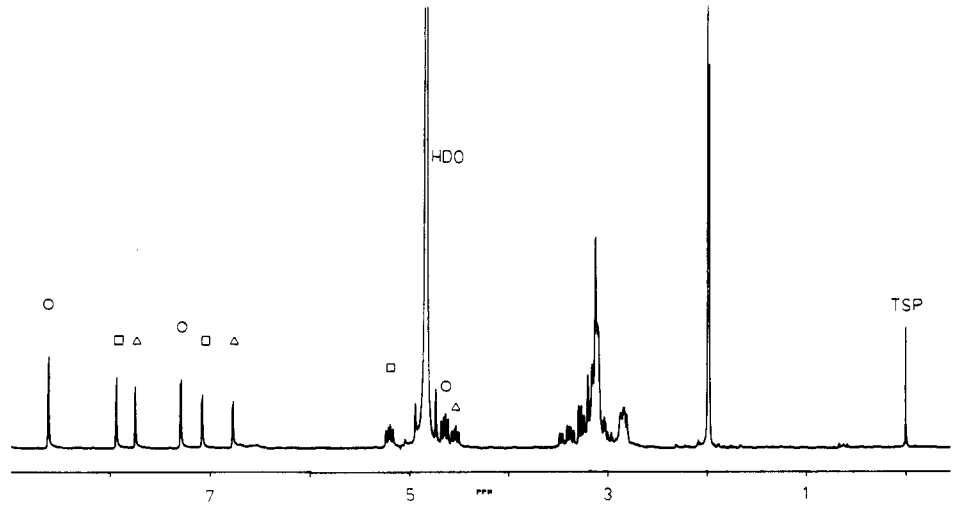

Figure 3. ^{1}H NMR spectrum (D_2O, pD 3.5, no base added) of [Pd(dien)(D_2O)] $(NO_3)_2$ with excess *N*-acetylhistidine. Resonances are indicated as follows: ○, free ligand; △, N1 linkage isomer; □, N3 linkage isomer.

histidine at pH <6.[10] If alkali was added to a solution containing **5** and excess *N*-acetylhistidine, or if the weakly acidic solution (initial pH 4.5, dropping to ca. 3.5 during reaction) was heated for an hour at 60 °C, the ^{1}H NMR spectrum of the resulting solution showed that a mixture of the isomers with imidazole-bound ligand, **4a** and **3a**, was formed, with **4a** predominant, as before.

When a solution containing $[Pt(dien)(H_2O)]^{2+}$, achisH_3, and **5** "in equilibrium" was allowed to stand at 25 °C for several days, ^{1}H NMR spectra showed that **4a** and **3a** slowly formed under these conditions also. The free carboxylate groups of these complexes, however, were able to compete with that of *N*-acetylhistidine itself so that the binuclear species **6a** and **7a** (Figure 2) were formed, giving rise to new peaks in the ^{1}H NMR spectrum. Signals of these species were usually only slightly shifted from those of **3a** and **4a** or, under some pD conditions, were coincident with the latter (Table I).[32] The exception was the methine signal of **7a**, which occurred still more to lower shielding than in the parent **4a**. The shifts of the H5 protons were more sensitive to the coordination of the second Pt than those of the H2 protons, which are more remote from the carboxylate group.

Peaks from **6a** and **7a** were also present, relatively weak, in spectra from reaction of $[Pt(dien)(H_2O)]^{2+}$ with acetylhistidine in small excess at pD 6–8. They were always absent in the final spectra when a large excess (>2:1) of acetylhistidine was applied.

When excess $[Pt(dien)(H_2O)]^{2+}$ was allowed to react with *N*-acetylhistidine at pD 8.5–9 at 25 °C, the only complexes present in significant amounts after 1 h were **4a** and **3a** (approximately 3:1) with small amounts of **6a** and **7a**. After 24 h at 25 °C, only a little reaction occurred to give a new complex formulated as the binuclear complex **8a**, with imidazole bridging between two Pt atoms (Figure 2). This reaction proceeded much faster when the solution was heated at 60 °C, although after 1 h with a total Pt(dien):achisH_2^- ratio of 2.5, the concentrations of **8a** and **4a** were comparable. In the course of this reaction, however, the ratio of **4a** and **3a** increased to 4.3:1, indicating that the N3-bound isomer **4a** reacts more slowly than the N1-bound isomer **3a** to give **8a**. Bridging of metal ions by imidazole bridges is not uncommon.[33,34]

From the results described above, it is clear that complexes with imidazole-bound ligands are thermodynamically most stable, and compound **5** with an O-bound ligand represents a kinetically-preferred initial product. The proportions of N1- and N3-platinated isomers obtained in these reactions also presumably arise primarily from kinetic factors. With the more labile Pd(II) analogues, kinetic factors are expected to be less important, and the product distribution after a short time should reflect the thermodynamic stability order of the various compounds. It was therefore not surprising to us that reaction of $[Pd(dien)(H_2O)]^{2+}$ with excess achisH_3 in D_2O, without any added alkali, gave immediately a solution whose ^{1}H NMR spectrum corresponded to a mixture of N1- and N3-imidazole-bound complexes, **3b** and **4b** (Figure 3). With Pd, of course, there are no coupling constants to assist assignments of binding sites, but there are obvious correspondences between the spectra of Pt and Pd compounds which can be used in assigning resonances (Table I).

Table III. Proportions of N3- to N1-Bonded Isomers of $[Pd(dien)(achisH_2\text{-}N_1)]^{2+}$ (**4b**, **3b**) as a Function of the pD of the Solution

pD	**4b**	pD	**4b**:**3b** ratio
2	0.7	8.5	0.97
3.5	1.10	10	0.8
6.5	1.10	13	0.2
7.5	0.97		

The relative proportions of **3b** and **4b** depended on the pD (Table III). If no acid or base was added to the reaction mixture (pD of final solution was approximately 3.5), there were almost equal amounts of the two isomers **3b** and **4b**, with a slight preference for the N3-bound isomer **4b**. If DNO_3 was added (final pD 2), there was a slight preference for the N1-bound isomer. Addition of NaOD caused the ratio of N3- to N1-bound isomer to reach a maximum near pD 7. At higher pD values, the proportion of N3-bound isomer decreased again.

At higher pD values, even with excess *N*-acetylhistidine present, there were peaks, of intensity comparable with those of **3b** and **4b**, to be assigned to the imidazole-bridged complex **8b** (Table I). At a particular pD value, the proportion of this complex present increased as the ratio of Pd to ligand increased. With Pd:achisH_2^- > 2 and at pD >7, this was the only detectable species present containing *N*-acetylhistidine.

With Pd:achisH_2 > 2, at lower pD values, the spectra became more complex. We will not describe them in detail, but at pD 4.5 they showed that the three species **3b**, **4b**, and **8b** were present in equilibrium, together with derivatives in which a second Pd(dien)$^{2+}$ unit was coordinated to the free carboxylate group (**6b**, **7b**, and **9b**). Reaction between $[Pd(dien)(H_2O)]^{2+}$ and the carboxylate groups occurred at an intermediate rate on the NMR time scale, so that there was broadening of peaks in the spectra run at 25 °C.

(32) Peaks from **3a** and **4a** shifted slightly over the pD range 3–5, corresponding to deprotonation of the carboxyl group, while those from **6a** and **7a** did not shift.

(33) Hawkins, C. J.; Horn, E.; Martin, J.; Palmer, J. A. L.; Snow, M. R. *Aust. J. Chem.* **1986**, *39*, 1213.
(34) Morris, P. J.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1971**, *33*, 2913.

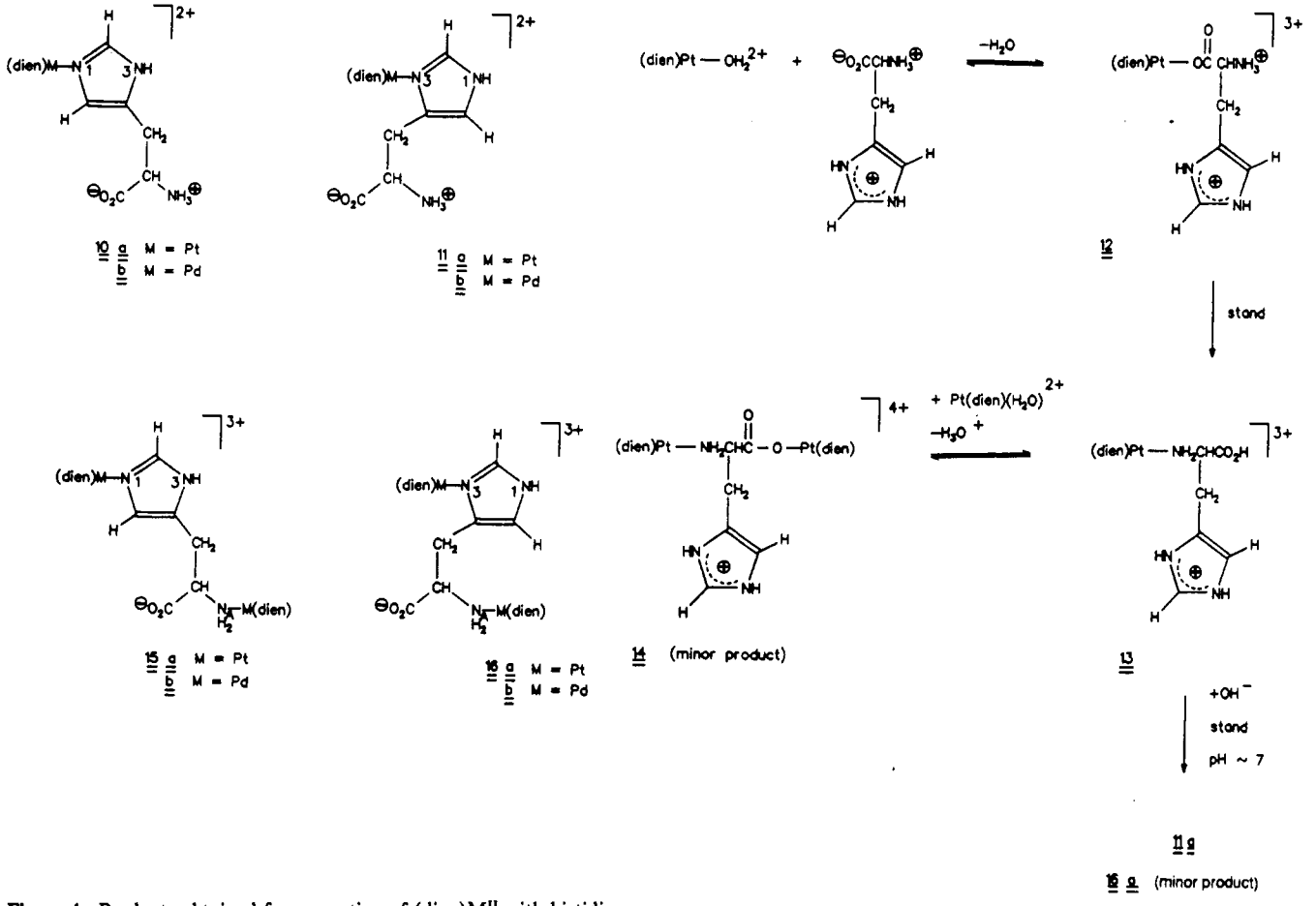

Figure 4. Products obtained from reaction of (dien)M^{II} with histidine.

Reactions of $[M(dien)(H_2O)]^{2+}$ with Histidine. For $hisH_4^{2+}$, the acid dissociation constants correspond to $pK_{a1} = 1.78$ (carboxyl), $pK_{a2} = 6.03$ (first imidazole proton), and $pK_{a3} = 9.04$ (amine group).[29] The pH of a solution of histidine in water is near 7, with the imidazole ring singly protonated on the nitrogen atoms. Reaction of $[Pt(dien)(H_2O)]^{2+}$ with excess histidine, with addition of acid or base, gave a solution whose ^1H NMR spectrum showed the two imidazole-bound isomers **10a** and **11a** as the only Pt-containing species (Figure 4). Again, the assignments were made on the basis of ^{195}Pt–^1H coupling constants observable in spectra run at low magnetic fields. Once again, there was a shift of lower shielding of the methine resonance for the N3-bound isomer **11a**. The ratio of N3- to N1-bound isomers (**11a**:**10a**) was 1.5.

The 42.8-MHz ^{195}Pt NMR spectrum of this solution showed a single, broad peak only, at −2880 ppm; separate peaks for the two isomers were not resolved. The shift to higher shielding when water in $[Pt(dien)(H_2O)]^{2+}$ (−2547 ppm) was replaced by a N-donor ligand is as expected.[35,36]

When DNO_3 was added to the his solution before addition of $[Pt(dien)(ONO_2)]NO_3$, the reaction took a different course, as outlined in Figure 4. With the initial pD adjusted to 3.5, both imidazole N atoms were protonated, and spectra recorded soon after addition of Pt showed a set of ^1H peaks only slightly shifted from those of his in the same solution. They were assigned to $[Pt(dien)(hisH_3\text{-}O)]^{3+}$ (**12**), as in the cases discussed above. Unlike **5**, whose acetylated N_A atom is unable to coordinate unidentate, **12** underwent a facile isomerization reaction to $[Pt(dien)(hisH_3\text{-}N_A)]^{3+}$ (**13**) on standing. The reaction was virtually complete overnight. During the reaction, the pD dropped to 2. The isomerization reaction had, as expected, only a small effect on the chemical shifts of the imidazole protons, but the methine proton signal shifted to higher shielding (Table I). The ^{195}Pt NMR spectrum of this solution had a peak at −2954 ppm, still in the region corresponding to a Pt(dien)(N donor) complex, but in a position different from those of $[Pt(dien)(hisH_2\text{-}N_I)^{2+}$ complexes (−2880 ppm).

When his was present in excess (>2-fold), the reaction occurred cleanly, with the spectrum after 24 h showing peaks from **13** and free $hisH_3^+$ only. When the initial Pt:his ratio was close to unity, additional weak peaks were observed, which were assigned (Table I) to **14**, in which a second $Pt(dien)^{2+}$ moiety has coordinated to the free carboxyl group of **13**.

Heating a solution of **13** with excess his at pD 2 did not affect the ^1H NMR spectrum significantly. However, when NaOD was added and the pD adjusted to 7, the main peaks on standing corresponded to one isomer with the his bound through an imidazole N atom, N3 (**11a**). There was only a trace of the N1-bound isomer, **10a**. Minor peaks in the spectrum (15% of the intensity of those from **11a**) were assigned to **15**, in which a Pt is bound to each of N3 and N_A. This product formed, albeit as a minor component of the mixture, even with the overall his:Pt ratio >2. Not surprisingly, the ^{195}Pt NMR spectrum showed a peak at −2874 ppm, similar to that from the mixture of **10a** and **11a** discussed above.

Heating this solution at 60 °C after DNO_3 had been added to decrease the pD to 3.5 did not cause any significant changes in the ^1H NMR spectrum. In particular, there was no reversion to the N_A-bonded complex, **13**.

To check whether the rearrangement from **13** to **11a** was entirely intramolecular (i.e., not involving free his in solution), a solution of **13** was allowed to stand at pD 8.8 with a 2.3-fold excess of *N*-acetylhistidine. Under these conditions, the imidazole ring of *N*-acetylhistidine would be neutral, with one N_I atom protonated,

(35) Appleton, T. G.; Hall, J. R.; Ralph, S. F. *Inorg. Chem.* **1985**, *24*, 4685.
(36) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247.

so that it should compete with his if the reaction were intermolecular. The ^{1}H NMR spectrum of the resultant solution showed that the major products of the reaction were the imidazole-bound isomers **11a** and **10a**, with smaller amounts (approximately 33%) of the *N*-acetylhistidine complexes **3a** and **4a**. We interpret this result as indicating that **13** can react by either an intra- or intermolecular reaction to form a complex with histidine imidazole-bound, but in the absence of a large excess of added ligand, the intramolecular reaction is likely to be dominant.

Reaction of $[Pd(dien)(H_2O)]^{2+}$ with excess his in D_2O, pD 6.8, gave predominantly the imidazole bound complexes **10b** and **11b**, as deduced from comparison with the Pt analogues (Table I). The ratio of N3- to N1-bonded complexes (**11b**:**10b**) was 0.56. Weaker peaks were also present in the aromatic region, which tentatively were assigned to complexes with $Pd(dien)^{2+}$ entities bound to N_A as well as N_I, **15b** and **16b** (Table I). Spectra became quite complex when either acid or alkali was added, and were not examined in detail.

Reaction of $[Pt(dien)(H_2O)]^{2+}$ with Glycylhistidine. The reaction of $[Pt(dien)(H_2O)]^{2+}$ with excess glycylhistidine without addition of any acid or alkali (pD approximately 7) gave a solution of N3- and N1-bound isomers. ^{195}Pt–^{1}H coupling constants (even deduced from 200-MHz spectra) were used, as usual, to make the assignments given in Table I. The ratio of N3- to N1-coordinated isomers was 0.66.

Reactions of $[Pt(trpy)Cl]^+$ with *N*-Acetylhistidine and with Histidine. In view of the results obtained with $[Pt(dien)(H_2O)]^{2+}$, there did not appear any reasons why mixtures of N1- and N3-bonded linkage isomers should not also be obtained when [Pt(trpy)Cl]Cl was allowed to react with his derivatives. We therefore decided to reexamine the reactions of this compound with *N*-acetylhistidine and histidine. We mixed $[Pt(trpy)Cl]^+$ with achisH_3 in slight excess in D_2O and heated the mixture at 50 °C. Spectra of solutions heated for 10 min, 1 h, and several hours were identical.

It was clear from the persistent orange-red color of the solution and from ^{1}H NMR spectra, which showed aromatic peaks from the initial $[Pt(trpy)Cl]^+$, that the reaction with *N*-acetylhistidine did not go to completion without the addition of sufficient base to get above pD 5. In the absence of base, an equilibrium was reached between $[Pt(trpy)Cl]^+$ and $[Pt(trpy)(achisH_2\text{-}N_I)]^+$. When base was added to fully remove one imidazole proton (cf. $pK_a = 6.99$), the solution color faded to yellow, and the ^{1}H NMR spectrum showed no peaks remaining from the chloro complex.

A disadvantage of using terpyridyl complexes to study the coordination modes of his derivates is, of course, the complexity of the spectrum from the aromatic protons of the trpy ligand, which can make it difficult to observe the imidazole protons. However, in this instance, once $[Pt(trpy)Cl]^+$ was no longer present, the region from 7 to 7.8 ppm was free from trpy resonances, allowing three distinct H5 signals to be clearly seen—that from free *N*-acetylhistidine plus two signals that could be plausibly assigned to imidazole-bound linkage isomers, at ca. 7.48 and 7.35 ppm (intensity ratio 2.8:1). Careful examination of the more "crowded" region near 8.5 ppm allowed the signal from H2 of the major isomer to be identified (8.53 ppm), but not the corresponding signal from the minor tautomer. The COSY spectrum (Figure 5) confirmed this interpretation, even though the one-dimensional 200-MHz spectrum did not permit an unambiguous assignment of the H2 resonance of the major isomer either.

Kostic et al.[11] reported ^{1}H NMR data for $[Pt(trpy)(achisH_2)]^+$. The peaks listed correspond to those that we have assigned to the major isomer.

The aliphatic region of the ^{1}H NMR spectrum provided useful information that two linkage isomers of $[Pt(trpy)(achis\text{-}H_2\text{-}N_I)]^+$ were present. There were small splittings only of the methine proton signals, but there were two distinct peaks, in addition to that from free *N*-acetylhistidine, from the acetyl methyl protons, at 1.79 and 2.05 ppm (intensity ratio 2.4:1). The remote possibility was considered that one of these peaks might be due to acetate ions generated by hydrolysis of *N*-acetyl groups. The peak from a small amount of sodium acetate added to the solution did not coincide with any of the peaks already present, however.

The increased overlap of peaks in the aromatic region in spectra run at lower magnetic field strengths makes it difficult to measure coupling constants to ^{195}Pt, and anisotropic shielding effects of the trpy ligand make it dangerous compared with (dien)Pt complexes to make assignments from relative chemical shifts, so that definite assignments of particular peaks to N1 and N3 complexes cannot be made. If, however, the H5 resonance to lower shielding does correspond to the N3-bound isomer, this would be the more abundant isomer, as with *N*-acetylhistidine complexes of $Pt(dien)^{2+}$.

Reaction of [Pt(trpy)Cl]Cl with his occurred immediately at 25 °C, as indicated by the fading of the red color of the solution, and the disappearance of $[Pt(trpy)Cl]^+$ aromatic resonances from the ^{1}H NMR spectrum of a D_2O solution. As with the *N*-acetylhistidine complex, there were peaks visible in 200- and 300-MHz spectra which could be assigned to H5 protons of two linkage isomers of $[Pt(trpy)(hisH_2\text{-}N_I)]^{2+}$, at 7.65 and 7.24 ppm (intensity ratio 1.6), but only the H2 signal of the more abundant isomer could be identified at 8.54 ppm. Kostic et al.[11] did not *report* ^{1}H NMR data for a histidine complex.

The methine protons gave a signal with a complex envelope. From the 300-MHz spectrum, it was evident that this was due to the presence of three distinct methine multiplets, one from free his and two others. Once again, definite assignments of peaks to particular isomers cannot be made, but if the H5 peak to lower shielding corresponds to the N3-bound isomer, this is the more abundant.

Reaction of *cis*-$[(NH_3)_2Pt(1\text{-}MeU)(H_2O)]^+$ with *N*-Acetylhistidine. Reaction of *cis*-$[(NH_3)_2Pt(1\text{-}MeU)(H_2O)](NO_3)$ with 2 equiv of *N*-acetylhistidine at pH 7 (readjusted with base) and subsequent preparative separation of the mixture by HPLC (H_2O/MeOH) gave N1- and N3-imidazole isomers together with *cis*-$[(NH_3)_2Pt(1\text{-}MeU)_2Pt(NH_3)_2](NO_3)_2$ (head–tail)[28] and unreacted ligand.

The two isomers, *cis*-$(NH_3)_2Pt(1\text{-}MeU)(achisH_2\text{-}N1)$ (**16**) and *cis*-$(NH_3)_2Pt(1\text{-}MeU)(achisH_2\text{-}N3)$ (**17**) were readily distinguished by their differences in ^{195}Pt–^{1}H coupling constants to the aromatic imidazole protons (Figure 6): in the spectrum of the N1 bound isomer, **16**, both imidazole protons show comparable ^3J coupling constants, 21.0 Hz (H2) and 21.3 Hz (H5). In contrast, the aromatic imidazole protons of isomer **17** display clearly different couplings, 20.1 Hz with H2, and 7.9 Hz with H5. In both complexes, the usual ^4J coupling between ^{195}Pt and H5 of the 1-MeU ligands (15 Hz)[37] is observed.

pD-dependent ^{1}H NMR spectra for the two isomers indicated acid/base equilibria of the types

$$cis\text{-}[(NH_3)_2Pt(1\text{-}MeUH)(achisH_3\text{-}N_I)]^{2+} \overset{pK_{a1}}{\rightleftharpoons} cis\text{-}[(NH_3)_2Pt(1\text{-}MeU)(achisH_3\text{-}N_I)]^{+} \overset{pK_{a2}}{\rightleftharpoons} cis\text{-}(NH_3)_2Pt(1\text{-}MeU)(achisH_2\text{-}N_I) \overset{pK_{a3}}{\rightleftharpoons} cis\text{-}[(NH_3)_2Pt(1\text{-}MeU)(achisH\text{-}N_I)]^{-}$$

with estimated pK_a values of $pK_{a1} \approx 0.5$ (**16**, **17**), $pK_{a2} \approx 3.2$ (**16**), 3.6 (**17**), and $pK_{a3} \approx 11$ (**16**), 11.5 (**12**). The pK_{a1} is in the typical range for mono(uracil) compounds,[38] and pK_{a3} shows an increase

(37) Lippert, B. In *Platinum, Gold, and Other Metal Chemotherapeutic Agents*; Lippard, S. J., Ed.; ACS Symposium Series 209; American Chemical Society: Washington, DC, 1983; pp 147–170.
(38) Schöllhorn, H.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1989**, *111*, 7213.

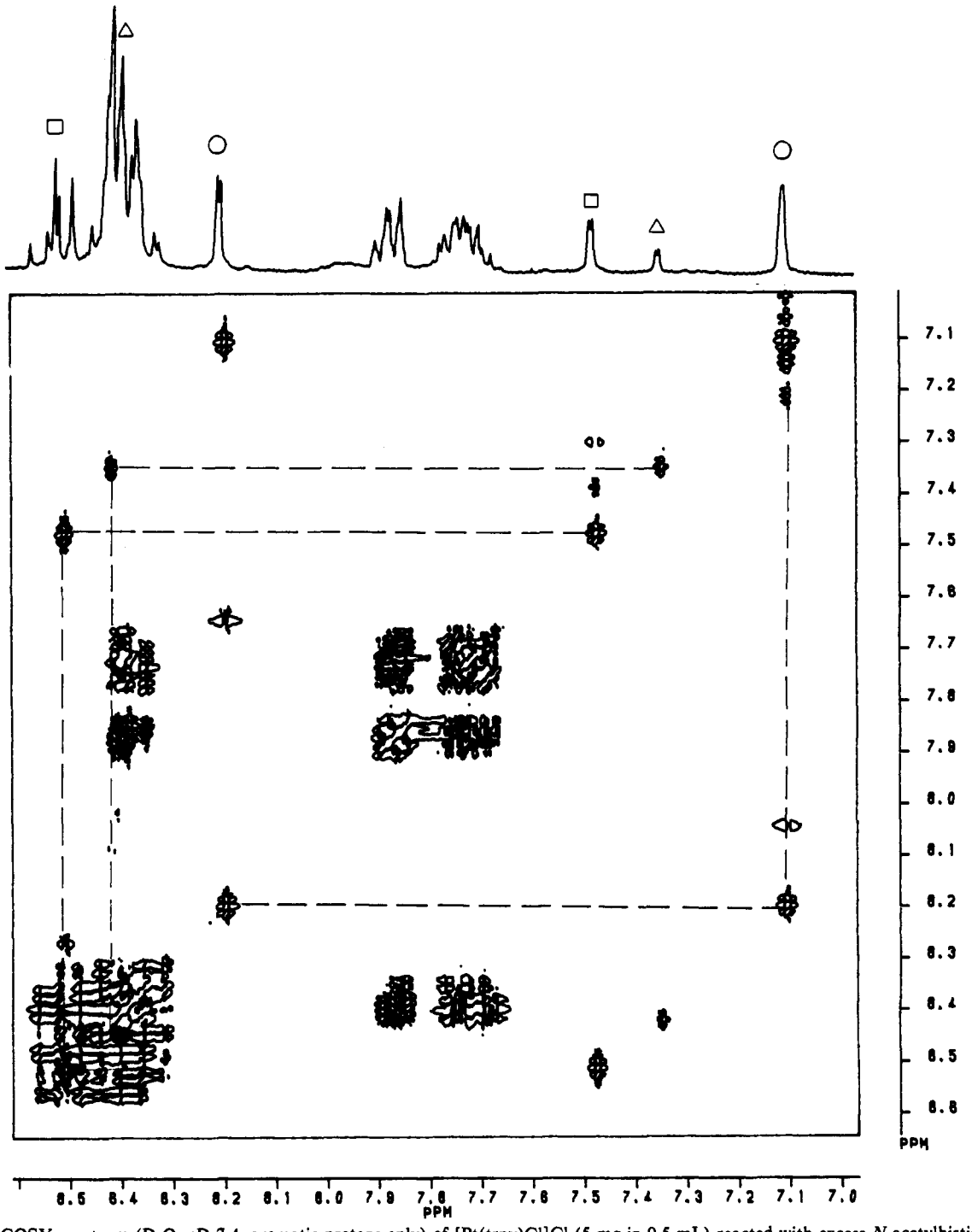

Figure 5. COSY spectrum (D_2O, pD 7.4, aromatic protons only) of [Pt(trpy)Cl]Cl (5 mg in 0.5 mL) reacted with excess *N*-acetylhistidine (5 mg) for 1 h at 60 °C. Histidine resonances are indicated as follows: ○, free ligand; △, minor linkage isomer; □, major linkage isomer.

in imidazole NH acidity of approximately 3 log units on Pt binding to the neutral ring, which is similar to the effect of Pd^{II} in [Pd(en)(L-his)]$^+$.[39]

While UV spectroscopy did not prove helpful in differentiating the two isomers, Raman spectra (solid state) of isomers **16** and **17** did reveal some differences. In particular the most intense imidazole ring vibration, which was observed at 1250 cm^{-1} in the case of **11** and at 1240 cm^{-1} with **12** might be a useful marker, as confirmed by the position of this band in *trans*-[$(NH_2CH_3)_2$Pt(1-MeC)(achisH_2-*N1*)]$^+$ (**18**). However, the relatively minor difference in the two tautomers makes a differentiation of related tautomer complexes less reliable than between the unmetalated tautomers.[3c,40]

Reaction of *trans*-[$(NH_2CH_3)_2$Pt(1-MeC)(H_2O)]$^{2+}$ with *N*-Acetylhistidine. The product with N1-bound achisH_2 was isolated on a preparative scale and unambiguously identified by elemental analysis and ^1H NMR spectroscopy. In the 80-MHz spectrum, *trans*-[$(NH_2CH_3)_2$Pt(1-MeC)(achisH_2-*N1*)]$^+$ (**18**) displays well-resolved ^{195}Pt satellites for both H2 and H8 imidazole protons (21.5 Hz each) as well as for H5 of 1-MeC ($^4J \approx 15$ Hz). The methyl resonances of CH_3NH_2 initially display a triplet structure due to coupling with NH_2, but simplify to a singlet (with ^3J coupling to ^{195}Pt of 42.7 Hz) as the isotopic exchange $NH_2 \rightarrow ND_2$ progresses.[29]

(39) Sundberg, R. J.; Martin, R. B. *Chem. Rev.* **1974**, *74*, 471.

(40) Ashikawa, I.; Itoh, K. *Chem. Lett.* **1978**, 681.

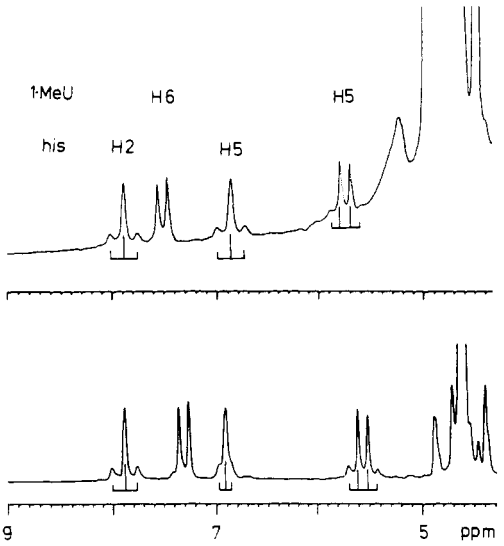

Figure 6. ^{1}H NMR resonances (D_2O, pD 6.8, 80-MHz, lowfield region only) of linkage isomers of *cis*-$(NH_3)_2$Pt(1-MeU)(achisH_2-*N*$_1$): N1 isomer (top) and N3 isomer (bottom).

pD-dependent ^{1}H NMR spectra of **18**, and for pK_{a1} potentiometric titration as well, were indicative of the following equilibria, with pK_{a1} = 3.4, pK_{a2} = 10.3, and pK_{a3} = 13.0.

$$\textit{trans}\text{-}[(NH_2CH_3)_2Pt(1\text{-}MeC)(achisH_3\text{-}\textit{N1})]^{2+} \overset{pK_{a1}}{\rightleftharpoons}$$

$$\textit{trans}\text{-}[(NH_2CH_3)_2Pt(1\text{-}MeC)(achisH_2\text{-}\textit{N1})]^{+} \overset{pK_{a2}}{\rightleftharpoons}$$

$$\textit{trans}\text{-}[(NH_2CH_3)_2Pt(1\text{-}MeC)(achisH\text{-}\textit{N1})] \overset{pK_{a3}}{\rightleftharpoons}$$

$$\textit{trans}\text{-}[(NH_2CH_3)_2Pt(1\text{-}MeC^-)(achisH\text{-}\textit{N1})]^-$$

Reaction of *cis*-$[(NH_3)_2$Pt(1-MeC)$(H_2O)]^{2+}$ with *N*-Acetylhistidine. Reaction carried out with no base added (2 equiv of ligand per Pt, 37 °C, final pD ≈ 4) leads to rapid formation of a compound assigned to *cis*-$[(NH_3)_2$Pt(1-MeC)(achisH_3-*O*$)]^{2+}$ (**19**). Aromatic achis resonances in **19** are slightly shifted upfield compared to the free ligand (Table II). Addition of excess NaCl leads to formation of free ligand and *cis*-$[(NH_3)_2$Pt(1-MeC)-Cl]Cl, consistent with the assignment. Without NaCl and upon extended reaction times, new resonances appear upfield from those of the free ligand and **19**, which are interpreted as being due to the two isomers *cis*-$[(NH_3)_2$Pt(1-MeC)(achisH_2-*N1*$)]^+$ (**20**) and *cis*-$[(NH_3)_2$Pt(1-MeC)(achisH_2-*N3*$)]^+$ (**21**). They are present in a 1:1 ratio. A comparison of the chemical shifts of the aromatic imidazole protons in **20** and **21** with those observed in the free ligand and the *trans*-Pt^{II} complex **18** is in agreement with expectations, viz., larger upfield shifts of H2 and H5 in the N1-isomer as compared to the N3-isomer as a consequence of intracomplex base stacking between the nucleobase and the imidazole ring of the amino acid (Table II).[41]

When the reaction mixture is kept at pH 7, both isomers **20** and **21** are present in higher amounts, but surprisingly the O-bound compound **19** still represents the main product (4 d at 37 °C). Relative intensities **19**:**20**:**21** are 2:1:1.

Discussion

Linkage Isomerism of Imidazole-Bound Histidines. For all the systems discussed in this paper, both N1- and N3-bound isomers were observed. Clearly, whenever the reactions of a square-planar complex with a histidyl residue in a peptide or protein are under consideration, the possibility of such isomerism should be taken into account. In a large protein molecule, the preferred coordination site, N1 or N3, may often depend on details of the conformation of the molecule and may be difficult to predict without an intimate knowledge of the protein structure. This may not be very important when the reacting metal complex is truly monofunctional, as with $[M(dien)(H_2O)]^{2+}$ or $[Pt(trpy)Cl]^+$. The use of $[Pt(trpy)Cl]^+$ as a probe for accessible histidine coordination sites[11,12] remains valid, with the added insight that either N1 or N3 may be accessible for the probe to function. If, however, the reacting metal complex were a species such as *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$, an N3-bound isomer would be capable of forming a six-membered N_A,N3-chelate ring with the adjacent N_A atom.[10] If binding occurred at N1, such a chelate ring cannot form, although Pt could form a cross-link to another part of the protein molecule or another molecule altogether.

For the relatively labile $Pd(dien)^{2+}$ complexes, the different species will be expected to be in equilibrium. The relative proportions of N1- and N3-bound products will depend on the equilibrium constant for reaction of the particular N_I atom with H^+, as well as the equilibrium constant of N_I with $[Pd(dien)(H_2O)]^{2+}$. Tanokura[31] has estimated that the mole fraction of the N1-protonated tautomer **1** of achisH_2^- is 0.62. Since the proportions of N3- and N1-bound $Pd(dien)^{2+}$, **4b**:**3b**, are close to 1:1 over the pD range where achisH_2^- exists as the predominant form of the ligand, the ratio of the equilibrium constants K_{N1}:K_{N3}[42] is given by the ratio of mole fractions of **1** and **2**, 0.62:(1 − 0.62) = 1.6.

In this system, the higher reactivity of deprotonated N1 is just balanced by the higher concentration of deprotonated N3. At lower values of pD, where achisH_3 predominates, or at very high pD, where concentrations of the fully deprotonated imidazole become significant, the slightly different basicities of N1 and N3 become less important in determining the proportions of linkage isomers formed, and then the N1-bound tautomer predominates (Table III), possibly because it is somewhat less crowded sterically.

With histidine the ratio of isomers with N3- and N1-bound $Pd(dien)^{2+}$, **11b**:**10b**, was 0.56, while the mole fraction of tautomer **1** in hisH_2 is 0.80.[31] A calculation similar to that above gives

$$K_{N1}/K_{N3} = 0.80/(1 - 0.80)0.56 = 7.1$$

That is, reaction at deprotonated N1 is much more preferred thermodynamically relative to N3 in histidine than in *N*-acetylhistidine. The difference between the two ligands may be a consequence of the presence of a positively charged $N_AH_3^+$ group in histidine. Coordination of the positively charged $Pd(dien)^{2+}$ entity at N3 brings these groups closer together than coordination at N1.

We have argued that the distribution of imidazole-bound isomers of $Pt(dien)^{2+}$ complexes reflects primarily kinetic rather than thermodynamic factors. This contention is supported by the observation reported above, that a solution of the single isomer $[Pt(dien)(hisH_2\text{-}N3)]^{2+}$ (**11a**), heated at pD 7 or at pD 3.5, does not produce an equilibrium mixture of the isomers **10a** and **11a** or the same distribution of isomers as was obtained from reaction of $[Pt(dien)(H_2O)]^{2+}$ with histidine near pD 7. Instead, the concentration of the N1 isomer **10a** remained negligible.

The ratio of the isomers of $[Pt(dien)(achisH_2\text{-}N_1)]^+$ produced at pD 6.5, **4a** (N3):**3a** (N1), was 2. With Tanokura's estimate of mole fractions of tautomers of achisH_2^-, the ratio of rate constants in this system is k_{N1}/k_{N3} = 0.8. For histidine, the ratio of N3- to N1-bound isomers was 1.5, which leads to a calculated value of k_{N1}/k_{N3} = 2.7.

(41) This basic difference between complexes of *cis*- and *trans*-$(NH_3)_2$Pt(II) is well documented. See, e.g.: Beyerle-Pfnür, R.; Brown, B.; Faggiani, R.; Lippert, B.; Lock, C. J. L. *Inorg. Chem.* **1985**, *24*, 4001.

(42) K_{N1} defined by achisH_2 + $[Pd(dien)(H_2O)]^{2+}$ ⇌ **3b** etc. The coordinated water molecule in $[Pd(dien)(H_2O)]^{2+}$ will be partially deprotonated in weakly acidic and neutral medium. The ratio of K_{N1}/K_{N3} therefore refers to equilibrium constants at a particular pH.

Kinetic factors clearly favor Pt binding to N1 over N3 less than the thermodynamic factors for the palladium complexes.

Carboxylate-Bound Histidine Ligands. Complexes in which the ligand was bound through the carboxylate oxygen were never observed in the $Pd(dien)^{2+}$ system, where complexes were in equilibrium. They are clearly thermodynamically unstable relative to the imidazole-bound complexes. Nevertheless, both $[Pt(dien)(achisH_3\text{-}O)]^{2+}$ (**5**) and *cis*-$[Pt(NH_3)_2(1\text{-}MeC)(achisH_3\text{-}O)]^{2+}$ (**19**) have considerable kinetic stability. Although coordination of an acetylated N atom, when part of a chelate ring, is now well established,[10,43] unidentate coordination of such an atom is not known. An intramolecular O to N linkage isomerization of the kind discussed below for histidine is therefore not possible for O-bound *N*-acetylhistidine, and it will react to give an imidazole-bound complex by reaction of a free acetylhistidine either directly with the Pt–carboxylate bond or with the $Pt\text{–}OH_2$ bond in an aqua complex in equilibrium with the former. Whether a carboxylate-bound complex is observed under particular reaction condition will therefore depend on (i) the relative rates of reaction of carboxylate and imidazole groups with the Pt starting compound (with a given system, a more acidic solution favors O coordination), (ii) the rate of reaction of the O-bound complex, once formed, with the imidazole ring, and (iii) the equilibrium constant for formation of the carboxylate-bound complex from the starting complex.

The carboxylate-bound complex with histidine (**12**) is much less kinetically stable than the *N*-acetylhistidine analogue **5**. This is so because of the possibility of unidentate coordination through N_A, so that a facile O to N_A linkage isomerism occurs, even in strongly acidic solution. The reaction is analogous to the *intra*molecular reaction[44]

$$[(NH_3)_3Pt\text{–}O\text{–}\overset{\overset{O}{\|}}{C}\text{–}CH_2NH_3]^{2+} \longrightarrow [(NH_3)_3Pt\text{–}NH_2\text{–}CH_2COOH]^{2+}$$

It was suggested[43] that this reaction proceeded via a five-membered "pseudo-chelate" ring in which both O and N were bound to Pt. The conversion of $[Pt(dien)(hisH_3\text{-}O)]^{3+}$ (**12**) to $[Pt(dien)(hisH_3\text{-}N_A)]^{3+}$ (**13**) is remarkable when it is considered that the complexes with imidazole bound ligands (**10a**, **11a**) are thermodynamically more stable than **13** and that the imidazole N-atoms are more readily deprotonated. Specific formation of **13** presumably occurs because a five-membered "pseudo-chelate" may form. A corresponding "pseudo-chelate" is not possible with N1, and one with N3 would involve an unfavorable seven-membered ring. The specific reactions of $[Pt(dien)(hisH\text{-}N_A)]^{+}$ to give just one imidazole-bound isomer, $[Pt(dien)(hisH_2\text{-}N3)]^{2+}$ (**11a**), at pH 7 is also remarkable, when it is taken into account that direct reaction of histidine with $(dien)Pt^{II}$ near pH 7 produced significant amounts of the N1-bound isomer **10a** as well as **11a**. From the results of the competition reaction with free acetylhistidine, we propose that the dominant reaction path, in the absence of a large excess of free ligand, is again intramolecular. It could conceivably proceed via a six-membered "pseudo-chelate" ring with both N_A and N3 simultaneously bound to Pt. Such a ring could not form with N1, and the N1-bound isomer would be formed only by an intermolecular reaction.

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