Platinum(II) Complexes with Diglycine: X-ray Crystal Structure, ^{15}N NMR Spectra, and Growth-Inhibitory Activity against Mouse Meth A Solid Tumor in Vivo

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Two new dipeptide complexes of the form H[Pt(digly)Cl] **(2)** (H_2digly = glycylglycine) and H[Pt(Hdigly)Cl_2] **(4)** were newly prepared, and K[Pt(Hdigly)Cl_2] **(3)** was isolated. Complex **1**, K[Pt(digly)Cl], crystallizes in the monoclinic space group *C*2/*c* with unit cell dimensions $a = 25.77(1)$ Å, $b = 4.09(2)$ Å, $c = 16.432(9)$ Å, $\beta = 103.74(4)°$, and $Z = 8$. Complex **3** crystallizes in the monoclinic space group $P2_1/c$ with unit cell dimensions $a = 8.892(5)$ Å, $b = 11.387(4)$ Å, $c = 9.974(4)$ Å, $\beta = 105.45(4)°$, $Z = 4$. Complex **4** crystallizes in the monoclinic space group $P2_1/c$ with unit cell dimensions $a = 9.311(6)$ Å, $b = 7.737(8)$, $c = 15.627(4)$ Å, $\beta = 105.92(3)°$, $Z = 4$. Complex **4** has the rare iminol type H_2digly coordinating to Pt. The ^{15}N chemical shifts and the coupling constants of the deprotonated coordinated amide N were obtained for the first time for these complexes. These amide peaks showed almost no coordination shift compared with the large coordination shift of the amine nitrogen. The coupling constants between Pt and deprotonated nitrogen for K[Pt(Hdipep)Cl_2] were larger than those for K[Pt(dipep)Cl]. The growth inhibition assays of K[Pt(digly)Cl], K[Pt(Hdigly)Cl_2], and *cis*-diamminedichloroplatinum(II) (cisplatin) against methylcholanthrene-induced Meth A fibrosarcoma (Meth A) solid tumor transplanted in BALB/c mice were measured. In mice, 35.9% of slight growth inhibition was observed in the group administered with K[Pt(digly)Cl] (dose of 26 mg/kg/day), and 40.6% in the group administered with K[Pt(Hdigly)Cl_2] (dose of 52 mg/kg/day), and 45.3% cisplatin (dose of 10 mg/kg/day). The side effects related to the decrease in body weight are milder than that of cisplatin. Their toxicity against normal mouse bone marrow cells was measured. All of them exhibited toxicity against bone marrow cells, but K[Pt(digly)Cl] and K[Pt(Hdigly)Cl_2] had only $^1/_{10}$ the toxicity of cisplatin.

Introduction

Cisplatin is one of the most commonly used anticancer drugs and is active against testicular, ovarian, head, and neck cancers but has a limited spectrum of activity.[1−7] However, it is associated with side effects such as nephrotoxicity, nausea, and vomiting. Many platinum complexes have been prepared in the hope of developing new derivatives with more potent activity and less toxicity. The second-generation compounds carboplatin and iproplatin are less toxic but show a limited spectrum of activity.[3] It is well-known that only neutral complexes have shown appreciable antitumor activity and most of the charged complexes tested have been inactive and nontoxic or highly toxic in general.[8] However, Matsunami et al. reported the antitumor active "platinum blues", which are tetranuclear cations.[9] Most of the antitumor active platinum complexes examined have amines or diamines as nonlabile groups, and much effort has been devoted to finding new ligands of appropriate lability instead of chloride ion. The antitumor activity of only a few peptide platinum(II) complexes has been reported, in which di- or tripeptides coordinated to platinum.[10,11] Compared with the number of platinum(II) complexes with amines or diamines, the number of platinum(II) complexes with di- or tripeptides which have been isolated is very small. The major difficulty in studying platinum complexes containing peptides is their kinetic inertness, that is to say, peptide complexes of platinum are nonlabile species.[12] Reactions of platinum(II) with oligopeptides have produced several species in all cases, and most of them have been studied only in solution.[13−15] In a few cases analytical data and/or X-ray crystal data[16,17] have been reported.

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It is important to isolate the peptide platinum complexes and to know their chemical and chemotherapeutic properties. Deprotonation of the peptide amide NH is indirectly measured by titration and elemental analysis, except for one report which showed the deprotonation of the peptide amide NH by using X-ray and neutron diffraction of the crystal structure of $[Pt(glymet)Cl]^-$, where H_2glymet indicates glycylmethionine, showing that the amine nitrogen, the peptide nitrogen, and the thioether sulfur are coordinated to the metal.[17] ^{15}N NMR spectra provided a lot of information about Pt and Pd chemistry, and Appleton et al. and other groups reported ^{15}N NMR spectra of these complexes containing ^{15}N ammine,[18−28] amine,[29,30] and peptide.[11,14−16] Spectrophotometric data suggest that iminol nitrogen and amide oxygen with a proton might exist at a low pH.[31,32] We are interested in the relationship between the deprotonation of amide N and the ^{15}N NMR spectral data for the platinum(II) complexes. We have previously reported the results of ^{13}C and ^{195}Pt NMR spectra and FABMS of the anion peptide platinum(II) complexes containing $xgly^{2-}$ or $glyx^{2-}$, where x is a glycine, alanine, valine, or leucine moiety, and the antitumor activity of the platinum(II) complexes with $glyala^{2-}$ or $alagly^{2-}$.[11] On the basis of the spectral data, the proposed structure of the dipeptide complexes is $[Pt(dipep)Cl]^-$, in which a tridentate dipeptide dianion ($dipep^{2-}$) is coordinated to platinum through N(amine), N^-(deprotonated amide), and O^-(carboxylate). The complex K[Pt(glyala)Cl] showed better antitumor activity for Meth A than did the complex K[Pt(alagly)Cl], indicating that the amine N terminal of the peptide needs to be simple for good antitumor activity. Our previous report suggested that the Pt complexes containing $digly^{2-}$ might lead to better antitumor activity than the complexes containing $glyala^{2-}$ and $alagly^{2-}$. Because the K[Pt(digly)Cl] complex reported previously was slightly light sensitive and always contained a small amount of green complex, we could not test its antitumor activity for the Meth A fibrosarcoma solid tumor. We report here the preparation and X-ray structure determination of the platinum(II) complexes with the tridentate $digly^{2-}$ or the bidentate $Hdigly^-$, the ^{15}N chemical shifts of N-coordinated peptide, and the antitumor activity of their complexes in vitro and in vivo.

Experimental Section

Starting Materials. Dipeptides except enriched dipeptides were purchased from Tokyo Kasei, and K_2PtCl_4 was purchased from Kojima Kagaku. All of the other reagents were of analytical grade. Enriched dipeptides were synthesized in our laboratory as described earlier.[11]

Preparation of the Complexes. K[Pt(digly)Cl] (1). The complex was prepared by using a modified procedure for the synthesis of (dipeptide)platinum(II) complexes as reported previously.[11] A solution of $K_2[PtCl_4]$ (2.05 g, 5 mmol) and diglycine (0.675 g, 5 mmol) in 100 mL water was heated at 70 °C and stirred for 8 h. During the heating, $KHCO_3$ solution (10 mmol) was added to keep the pH of the solution at 5.5. The solution was concentrated to 5 mL under vacuum. A pale yellow precipitate appeared. The crude product was dissolved in water (5 mL), and then powdered KCl (1 g) was added to precipitate **(1)** (yield 900 mg, 40%). Crystals suitable for X-ray structure analysis were recrystallized from water containing KCl. The material is soluble in methanol and water. Anal. Calcd for K[Pt(digly)Cl], $KPtClC_4H_6N_2O_3$: C, 12.02; H, 1.51; N, 7.01. Found: C, 12.04; H, 1.52; N, 7.02. ^1H NMR: $\delta = 3.60$ (s, 2H, CH_2) and 4.01 (s, 2H, CH_2). ^{13}C NMR: $\delta = 51.68$ (CH_2), 53.57 (CH_2), 183.00 (C═O), and 193.62 (COO). ^{195}Pt NMR: $\delta = -1887$.

H[Pt(digly)Cl] (2). Complex **1** (200 mg, 0.5 mmol) was dissolved in 2 mL water, and 0.6 mL of 3 M HCl was added to the solution in an ice bath with stirring for 2−3 min. The precipitate was quickly filtered off, washed twice with 1 mL of ice, and dried over P_2O_5 in a desiccator. Yellow crystals of **2** deposited (yield 150 mg, 83%: insoluble in methanol and H_2O, soluble in HCl. Anal. Calcd for H[Pt(digly)Cl], $PtClC_4H_7N_2O_3$: C, 13.28; H, 1.95; N, 7.75. Found: C, 13.19; H, 1.99; N, 7.70.

K[Pt(Hdigly)Cl_2] (3). Complex **3** was prepared by two methods. (a) Complex **1** (200 mg, 0.5 mmol) was dissolved in 1 mL of water, and 0.8 mL of 1 M HCl was added to the solution. The mixture was heated at 70 °C for 30 min. Then 0.3 g of powdered KCl was added to it. The reaction mixture was left at room temperature for several days. The precipitate was quickly filtered off, washed with 1 mL of ice water and a small portion of ethanol and ether, and dried over P_2O_5 in a desiccator. Yellow crystals of **3** were deposited (yield 100 mg, 45%).[11] (b) Complex **2** (200 mg, 0.55 mmol) was dissolved in 1 mL of water, and 3 mmol of KCl (0.300 g) was added to the solution. The mixture was heated at 60 °C for 10 min. The reaction mixture was left at room temperature for 1 day. Yellow crystals of **3** were deposited. The precipitate was quickly filtered off, washed with 1 mL of ice-cooled water and a small portion of ethanol and ether, and dried over P_2O_5 in a desiccator (yield 180 mg, 75%). Anal. Calcd for K[Pt(Hdigly)Cl_2], $KPtCl_2C_4H_7N_2O_3$: C, 11.01; H, 1.62; N, 6.42. Found: C, 10.89; H, 1.59; N, 6.32. ^1H NMR: $\delta = 3.54$ (t, 2H, CH_2, ^2J(HH) = 6.1 Hz), 3.99 (s, 2H, CH_2), and 5.26 (b, 2H, NH_2). ^{13}C NMR: $\delta =$ 49.74 (CH_2), 51.41 (CH_2), 178.20 (C═O), and 187.62 (COO). ^{195}Pt NMR: $\delta = -2152$.

H[Pt(Hdigly)Cl_2] (4). Complex **2** (150 mg, 0.42 mmol) was suspended in 1 mL of 2 M HCl and heated at 60 °C for 5 min. The reaction mixture was left at room temperature for 1 day. Yellow crystals of **4** were deposited, washed with 1 mL of ice water, 1 mL of ethanol, and 1 mL of ether, and dried under reduced pressure (yield 130 mg, 78%). Anal. Calcd for $H[Pt(Hdigly)Cl_2]\cdot 2H_2O$, $KPtCl_2C_4H_7N_2O_3$: C, 11.01; H, 2.78; N, 6.45. Found: C, 11.08; H, 2.69; N, 6.46. ^1H NMR: $\delta = 3.54$ (t, 2H, CH_2, ^2J(HH) = 6.1 Hz), 3.99 (s, 2H, CH_2), and 5.26 (b, 2H, NH_2). ^{13}C NMR: $\delta = 49.74$ (CH_2), 51.41 (CH_2), 178.20 (C═O), and 187.62 (COO). ^{195}Pt NMR: $\delta = -2152$

^{15}N-Enriched K[Pt(glyala)Cl] (5) and K[Pt(alagly)Cl] (6) were prepared by same method as in the previous paper.[11]

^{15}N-Enriched K[Pt(Hglyala)Cl_2] and K[Pt(Halagly)Cl_2] were obtained from the reaction mixture of **5** or **6** using Sephadex G10 gel chromatography (first eluate (E1), oligomeric complex; second eluate (E2), **5** or **6**; and last eluate, E3). E3 eluates were the desired complexes **7** and **8**. Anal. Calcd for $K[Pt(Hglyala)Cl_2]\cdot 1.5H_2O$, $KPtCl_2C_5H_9N_2O_3\cdot 1.5H_2O$: C, 12.58; H, 2.52; N, 5.87. Found: C, 13.13; H, 2.46; N,

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Table 1. Crystal Data for K[Pt(digly)Cl], K[Pt(Hdigly)Cl_2], and H[Pt(Hdigly)Cl_2]·$2H_2O$

	K[Pt(digly)Cl]	K[Pt(Hdigly)Cl_2]	H[Pt(Hdigly)Cl_2]·$2H_2O$
empirical formula	$PtKClC_4H_6N_2O_3$	$PtKCl_2C_4H_7N_2O_3$	$PtCl_2C_4H_{12}N_2O_5$
formula weight	399.74	436.21	434.15
crystal color, habit	yellow, prismatic	yellow, prismatic	yellow, prismatic
crystal dimens, mm	0.25 × 0.15 × 0.10	0.25 × 0.15 × 0.20	0.10 × 0.25 × 0.25
cryst syst	monoclinic	monoclinic	monoclinic
space group	*C*2/*c* (No. 15)	$P2_1/c$ (No. 14)	$P2_1/c$ (No. 14)
a, Å	25.77(1)	8.892(5)	9.311(6)
b, Å	4.09(2)	11.387(4)	7.737(8)
c, Å	16.432(9)	9.974(4)	15.627(4)
β, deg	103.74(4)	105.45(4)	105.92(3)
V, Å^3	1682(7)	973.4(8)	1082(1)
Z	8	4	4
D_{calc}, g/cm^3	3.157	2.976	2.663
T, K	296	296	296
λ(Mo Kα), Å	0.710 69	0.710 69	0.71069
μ, cm^{-1}	173.96	153.08	134.00
F(000)	1456	800	808
$2\theta_{max}$, deg	55.0	55.0	55.0
no. of rflns measd	2255	2507	2839
no. of unique rflens/R_{int}	2204/0.056	2362/0.024	2682/0.059
no. of observations ($I > 3.00\sigma(I)$)	0.033	1896	1937
no. of variables	0.031	119	128
max peak in final diff. map, e Å^{-3}	2.77	2.30	1.86
min peak in final diff. map, e Å^{-3}	−3.55	−2.93	−7.38
R		0.033	0.053
R_w		0.031	0.067
goodness of fit indicator		2.63	2.79

6.13. Calcd for K[Pt(Halagly)Cl_2]·H_2O, $KPtCl_2C_5H_9N_2O_3$·H_2O: C, 12.83; H, 2.37; N, 5.98. Found: C, 13.12; H, 2.42; N, 5.86.

Measurement. NMR Spectra and Elemental Analyses. The NMR spectra were recorded on a JEOL JNM-EX270 NMR spectrometer (^{15}N (27.25 MHz), ^{195}Pt (57.82 MHz), and ^{13}C (67.80MHz)). 98% ^{15}N-enriched samples were used in the ^{15}N NMR measurement. All of the ^{15}N, ^{195}Pt, and, ^{13}C NMR spectra were proton-decoupled in D_2O. ^{15}N NMR spectra were externally referenced to the saturated $^{15}NH_4Cl$ solution (δ_{NH4^+} is 27.34 ppm relative to δ_{NH3} (=0 ppm)), and ^{195}Pt spectra were externally referenced to K_2PtCl_4 (from Na_2PtCl_6 in D_2O,δ_{Pt} for K_2PtCl_4 −1622 ppm). ^{13}C spectra were referenced to sodium 3-(trimethylsilyl)propionate (TSP). Elemental analyses (C, H, N) were carried out by YANACO MT-3.

Data Collection. Crystal data and details on data collection and refinement for three complexes are given in Table 1. Complexes **1**, **3**, and **4** were recrystallized from water. Cell constants were determined on a Rigaku AFC5S four-circle automated diffractometer from the setting angles of 20−25 reflections. The crystal parameters along with details of data collection are summarized in Table 1. Data collection was carried out on a Rigaku AFC5S difractometer. Intensities were measured by the $2\theta-\omega$ scan method using Mo Kα radiation (λ = 0.710 69 Å). A total of 2255 independent intensities ($2\theta < 50°$) was measured for **1**, 2507 ($2\theta < 50°$) for **3**, and 2839 ($2\theta < 50°$) for **4**. Of these, there are respectively 1527, 1896, and 1937 unique reflections with $I > 3.0\sigma(I)$ which were used in the solutions and refinements of the structures. Intensities were corrected for Lorentz and polarization effects and absorption. Atomic scattering factors and anomalous dispersion effects were taken from the usual tabulation. All calculations were performed on the teXsan crystallographic software package of Molecular Structure Corporation.

Determination of the Structures. The structures were solved by heavy-atom Patterson methods. The platinum atom was located in the initial *E* map, and subsequent Fourier syntheses gave the positions of other non-hydrogen atoms. Hydrogen atoms were calculated at the ideal positions and were not refined. The non-hydrogen atoms were refined with anisotropic thermal parameters by using full-matrix least-squares methods. The final refinement converged to $R = 0.033$ and $R_w = 0.031$ for **1**, 0.033 and 0.031 for **3**, and 0.053 and 0.067 for **4**, respectively. Final difference Fourier syntheses showed peaks at heights up to 1.94−3.55 e Å^{-3} for **1**, 2.30−2.93 e Å^{-3} for **3**, and 1.86−7.38 e Å^{-3} for **4**.

In Vivo Antitumor Assay. Experimental details of (a) in vivo antitumor assay of Pt complexes against Meth A solid tumor, (b) direct cytotoxicities against Meth A cells, and (c) toxicities against normal bone marrow cells appear in the Supporting Information.

Table 2. Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters for K[Pt(digly)Cl]

atom	*x*	*y*	*z*	B_{eq}
Pt1	0.09758(2)	0.0677(1)	0.04188(3)	1.506(8)
K1	0.17668(9)	0.3128(7)	−0.0962(1)	2.20(5)
Cl	0.0507(1)	0.1510(8)	−0.0973(2)	2.45(7)
O1	0.1538(3)	0.193(2)	0.2915(4)	2.7(2)
O2	0.1576(3)	−0.193(2)	0.0134(4)	1.9(2)
O3	0.2311(3)	−0.468(2)	0.0731(4)	2.9(2)
N1	0.0491(3)	0.326(2)	0.0967(5)	1.9(2)
N2	0.1407(3)	0.014(2)	0.1562(5)	1.8(2)
C1	0.0692(4)	0.321(3)	0.1903(7)	2.6(3)
C2	0.1248(5)	0.172(3)	0.2183(7)	2.5(3)
C3	0.1918(4)	−0.152(3)	0.1615(7)	2.2(3)
C4	0.1948(4)	−0.287(3)	0.0785(6)	1.9(2)

Results and Discussion

The atomic coordinates, equivalent isotropic temperature factors, selected bond distances, and bond angles for complexes **1**, **3**, and **4** are listed in Tables 2, 3, 4, 5, 6, and 7, respectively.

K[Pt(digly)Cl] (1). The structure of **1** is shown in Figure 1. The complex anion adopts a square-planar four-coordinate geometry with the amine (N1), amide (N2), and carboxylate oxygen (O2) of the tridentate diglycine dianion (digly^{2-}) and chloride (Cl1). The geometry of the complex anion is in agreement with the structure for **1** previously proposed, on the basis of NMR spectra and FABMS.[11] The platinum atom is in an approximately square-planar environment but is displaced by 0.051 Å from the least-squares plane which contains Cl1, N1, N2, and O2 (mean deviation 0.012 Å). The two five-membered chelate rings are puckered and have a twist conformation, and the dihedral angle between the plane Pt1, N2, C2 and the plane Pt1, N2, C3 is 5.2°, indicating that the N2 nitrogen atom is almost in the sp^2 hybridization. The Pt1−N2 (amide) bond (1.954(7) Å) is shorter than the Pt1−N1 (amine) bond (2.008(9) Å) as in [Pt(glymet)Cl]$^-$.[17]

K[Pt(Hdigly)Cl_2] (3). The structure of **3** is shown in Figure 2. The complex anion adopts a four-coordinate geometry with

Table 3. Selected Bond Lengths (Å) and Angles (deg) for K[Pt(digly)Cl]

Bond Distances (Å)			
Pt1−Cl1	2.343(3)	Pt1−O2	2.022(7)
Pt1−N1	2.007(9)	Pt1−N2	1.954(7)
O1−C2	1.26(1)	O2−C4	1.31(1)
O3−C4	1.21(1)	N1−C1	1.50(1)
N2−C2	1.35(1)	N2−C3	1.46(1)
C1−C2	1.52(2)	C3−C4	1.49(1)
Bond Angles (deg)			
Cl1−Pt1−O2	95.5(2)	Cl−Pt1−N1	97.4(2)
Cl1−Pt1−N2	176.2(3)	O2−Pt1−N1	166.8(3)
O2−Pt1−N2	82.6(3)	N1−Pt1−N2	84.3(3)
Pt1−O2−C4	114.6(6)	Pt1−N1−C1	110.5(6)
Pt1−N2−C2	117.8(7)	Pt1−N2−C3	114.0(6)
C2−N2−C3	127.2(9)	N1−C1−C2	112.8(9)
O1−C2−N2	122(1)	O1−C2−C1	124(1)
N2−C2−C1	113.2(10)	N2−C3−C4	111.0(8)
O2−C4−O3	123.1(10)	O2−C4−C3	116.7(9)
O3−C4−C3	120.2(9)		

Table 4. Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters for K[Pt(Hdigly)Cl_2]

atom	*x*	*y*	*z*	B_{eq}
Pt1	0.02602(4)	0.33547(3)	0.60616(3)	1.397(7)
K1	0.6583(3)	0.3874(2)	0.8809(2)	2.51(5)
Cl1	0.0274(3)	0.1469(2)	0.5207(3)	2.62(5)
Cl2	−0.2326(3)	0.3202(2)	0.6107(2)	2.41(5)
O1	0.4444(7)	0.5046(5)	0.6766(6)	2.1(1)
O2	0.4521(8)	0.2037(5)	0.7268(6)	2.2(1)
O3	0.4327(8)	0.1146(5)	0.5237(6)	2.4(1)
N1	0.0398(8)	0.4981(6)	0.6894(7)	1.8(2)
N2	0.2422(8)	0.3728(6)	0.5974(7)	1.5(1)
C1	0.206(1)	0.5317(8)	0.7434(9)	2.3(2)
C2	0.306(1)	0.4688(7)	0.6676(9)	1.5(2)
C3	0.333(1)	0.3094(7)	0.5204(8)	1.5(2)
C4	0.408(1)	0.1990(7)	0.5883(9)	1.8(2)

Table 5. Selected Bond Lengths (Å) and Angles (deg) for K[Pt(Hdigly)Cl_2]

Bond Distances (Å)			
Pt1−Cl1	2.311(2)	Pt1−Cl2	2.319(3)
Pt1−N1	2.019(7)	Pt1−N2	1.993(7)
O1−C2	1.276(10)	O2−C4	1.332(10)
O3−C4	1.209(9)	N1−C1	1.48(1)
N2−C2	1.34(1)	N2−C3	1.449(9)
C1−C2	1.49(1)	C3−C4	1.50(1)
Bond Angles (deg)			
Cl1−Pt1−Cl2	92.03(8)	Cl1−Pt1−N1	176.0(2)
Cl1−Pt1−N2	94.7(2)	Cl2−Pt1−N1	90.7(2)
Cl2−Pt1−N2	171.9(2)	N1−Pt1−N2	82.4(3)
Pt1−N1−C1	109.4(5)	Pt1−N2−C2	115.5(5)
Pt1−N2−C3	126.8(5)	C2−N2−C3	117.7(7)
N1−C1−C2	111.3(7)	O1−C2−N2	124.3(8)
O1−C2−C1	120.2(8)	N2−C2−C1	115.4(7)
N2−C3−C4	115.0(6)	O2−C4−O3	122.6(8)
O2−C4−C3	114.1(7)	O3−C4−C3	123.3(8)

amine (N1) and amide (N2) of bidentate diglycine anion ($Hdigly^-$) and two chlorides (Cl1 and Cl2). The platinum atom is in a slightly distorted square-planar environment, and the dihedral angle between the plane which contains Cl1, Pt1, and Cl2 atoms and that of N1, Pt1, and N2 is 5.2°. The five-membered chelate ring formed by Pt1, N1, C1, C2, and N2 has an envelope conformation in which C1 and C2 atoms are displaced by 0.492 and 0.240 Å, respectively, on the same side of the plane defined by Pt1, N1, and N2. The dihedral angle between the plane Pt1, N2, C2 and the plane Pt1, N2, C3 is 1.2°, indicating that the amide N2 nitrogen atom is in the sp^2 hybridization even in the complex with a bidentate dipeptide. The Pt1−N2 (amide) bond (1.993(7) Å) is somewhat shorter than the Pt1−N1 (amine) bond (2.019(7)Å). Both complexes [Pt(digly)Cl]$^-$ and [Pt(Hdigly)Cl_2]$^-$ have shorter Pt−N (amide) bonds than the Pt−N (amine) bonds, suggesting that the N(amide) atom is a better donor for platinum(II) than N(amine).

Table 6. Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters for H[Pt(Hdigly)Cl_2]·2H_2O

atom	*x*	*y*	*z*	B_{eq}
Pt1	0.03744(6)	0.20690(8)	0.03874(3)	1.86(1)
Cl1	0.0424(5)	0.1082(7)	0.1800(2)	3.39(9)
Cl2	0.2943(4)	0.1986(7)	0.0719(3)	3.50(9)
O1	−0.380(1)	0.333(2)	−0.1113(7)	2.7(2)
O2	−0.272(1)	0.486(2)	0.1013(7)	3.1(3)
O3	−0.362(1)	0.282(2)	0.1737(7)	2.6(2)
O4	−0.303(1)	0.677(2)	−0.3173(8)	3.6(3)
O5	−0.505(1)	0.998(2)	0.2427(6)	2.5(2)
N1	0.021(2)	0.295(2)	−0.0868(8)	2.6(3)
N2	−0.183(1)	0.237(2)	−0.0020(7)	2.1(3)
C1	-0.134(2)	0.361(2)	-0.1317(9)	2.4(3)
C2	-0.237(2)	0.307(2)	-0.0790(9)	1.9(3)
C3	-0.287(2)	0.190(2)	0.0498(8)	1.9(3)
C4	-0.303(2)	0.338(2)	0.1103(9)	1.8(3)

Table 7. Selected Bond Lengths (Å) and Angles (deg) for [Pt(Hdigly)Cl_2]·2H_2O

Bond Distances (Å)			
Pt1−Cl1	2.324(4)	Pt1−Cl2	2.305(4)
Pt1−N1	2.04(1)	Pt1−N2	1.99(1)
O1−C2	1.31(2)	O2−C4	1.20(2)
O3−C4	1.33(2)	N1−C1	1.51(2)
N2−C2	1.29(2)	N2−C3	1.47(2)
C1−C2	1.48(2)	C3−C4	1.52(2)
Bond Angles (deg)			
Cl1−Pt1−Cl2	91.6(2)	Cl1−Pt1−N1	176.9(4)
Cl1−Pt1−N2	95.3(3)	Cl2−Pt1−N1	91.5(4)
Cl2−Pt1−N2	172.4(4)	N1−Pt1−N2	81.6(5)
Pt1−N1−C1	111.8(9)	Pt1−N2−C2	116.7(9)
Pt1−N2−C3	125.0(9)	C2−N2−C3	118(1)
N1−C1−C2	108(1)	O1−C2)N2	121(1)
O1−C2−C1	119(1)	N2−C2−C1	119(1)
N2−C3−C4	110(1)	O2−C4−O3	124(1)
O2−C4−C3	125(1)	O3−C4−C3	110(1)

H[Pt(Hdigly)Cl_2] (4). The structure of **4** is shown in Figure 3. The complex anion adopts a four-coordinate geometry with amine (N1) and amide (N2) of bidentate diglycine anion ($Hdigly^-$) and two chlorides (Cl1 and Cl2). The platinum atom is in an approximately square-planar environment. The five-membered chelate ring formed by Pt1, N1, C1, C2, and N2 has an envelope conformation in which C1 and C2 atoms are displaced by −0.242 and −0.112 Å, respectively, on the same side from the plane defined by Pt1, N1, and N2. The dihedral angle between the plane Pt1, N2, C2 and the plane Pt1, N2, C3 is 0.9°. This indicates that the N2 nitrogen atom is also in the sp^2 hybridization, suggesting that the added proton (H7) is attached to amide O (O1) rather than amide N (N2). The iminol structure is consistent with the bond lengths of the peptide moiety, i.e., the C2−N2(amide) bond (1.29(2) Å) for complex **4** is shorter than that for complex **3** (1.34(1) Å). The O1−C2-(amide) bond (1.31(2) Å) for complex **4** is slightly longer than that for complex **3** (1.276(10) Å). It has been well established from the study of numerous peptide complexes that protonation occurs first at the peptide oxygen, because of its greater basicity, rather than at the peptide nitrogen.[33,34] Our crystal data might be the first report of the protonation of the Pt complex with a deprotonated peptide. This result is consistent with the site of protonation being acetyl oxygen rather than amide nitrogen for [Pt(NH_3)$_2$(Hacgly-*N,O*)]$^+$, where Hacgly stands for acetylgly-

(33) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 185.
(34) Freeman, H. C. *Adv. Protein Chem.* **1967**, *22*, 257.

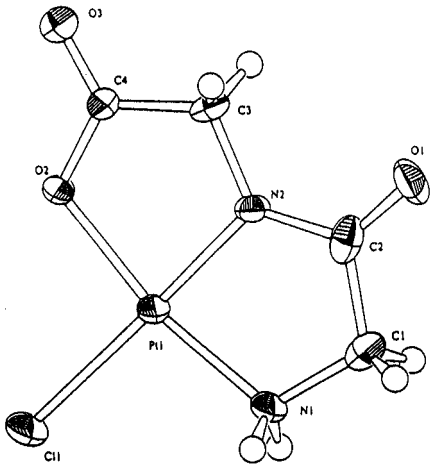


Figure 1. ORTEP drawing of the anion of K[Pt(digly)Cl] with 50% probability ellipsoids giving atomic numbering.

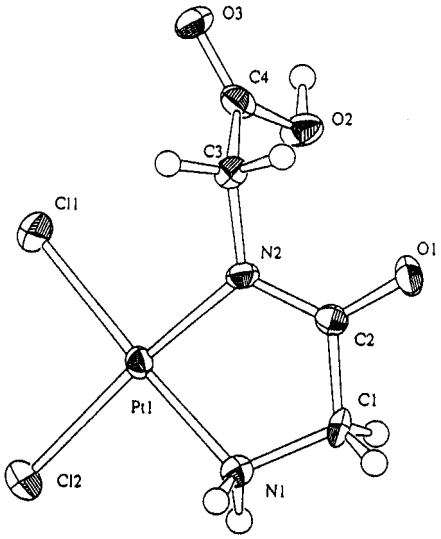


Figure 2. ORTEP drawing of the anion of K[Pt(Hdigly)Cl_2] with 50% probability ellipsoids giving atomic numbering.

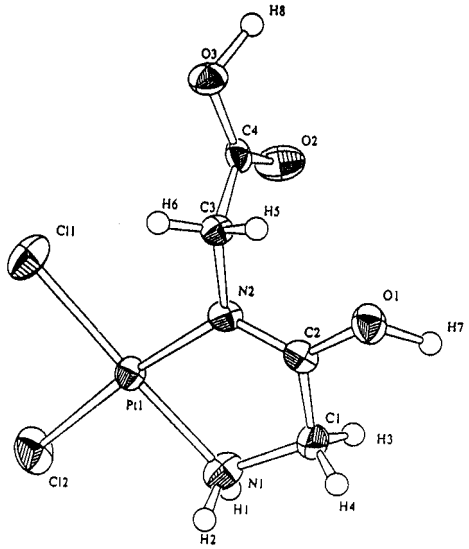
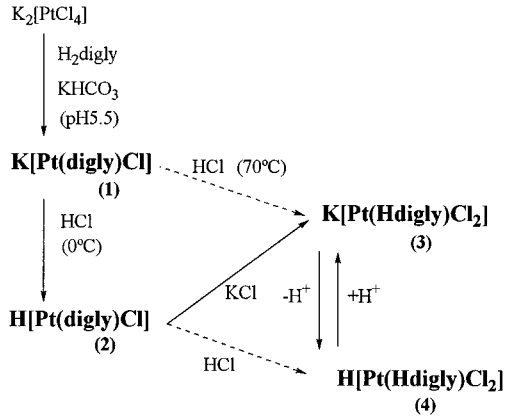


Figure 3. ORTEP drawing of the anion of H[Pt(Hdigly)Cl_2] with 50% probability ellipsoids giving atomic numbering.

cine, based on the NMR spectra.[26,31,32] Fairlie et al. reported amido−iminol tautomerism for the (diethylenetriamine)platinum(II) complexes with carboxylic acid amides or urea.[35,36]

Scheme 1



When dienPt coordinates, only N-coordination stabilizes both amide (NH_2COR) and iminol (NH═C(OH)R) tautomers, but this stabilization depends on R; the structure of [Pt(dien)NH_2-$CONMe_2$] and the structure of [Pt(dien)NH═C(OH)CH_3)] in solution are based on the NMR spectra.

Reaction of Four Complexes with HCl or OH^-. The relationships of the four Pt(II) complexes containing $digly^{2-}$, $Hdigy^-$, and H_2digly are summarized in Scheme 1. On the addition of HCl to a solution of **1** at 0 °C a precipitate of **2** resulted, and on the addition of HCl to a solution of **1** at 70 °C for 30 min a solution of **3** resulted. The addition of HCl to a solution of **3** resulted in **4** after 5 min at 60 °C. The structure of complex **2** is considered to be similar to that of **4** in that the protonation occurred at the amide oxygen. The reactions of complexes **1** and **3** in aqueous solution were monitored using ^{195}Pt NMR spectra (Figure 4). The interconversion between complexes **1** and **3** is reversible without decomposition. The reaction of [Pt(Hdigly)Cl_2]$^-$ with OH^- at room temperature produced [Pt(digly)Cl]$^-$ **(1)** quantitatively. The pruduct **1** is stable at pH 12 for several hours, at pH 6.6 for several days, and for a half-hour at pH 1.8 (Figures 4a−c), but it reacts readily with HCl at 70 °C to produce only **3** (Figure 4d). The chemical shift in Figure 4c differs by 15 ppm from that in Figure 4a. On the other hand, complex **3** showed weak acidity, was less stable in neutral aqueous solution, and produced a small amount of **1** as time passed (Figure 4e). However, complex **3** was stable in aqueous solution containing chloride ion. As is shown in Figure 4f, addition of HCl to **3** resulted in a downfield shift of 31 ppm of the ^{195}Pt NMR signal. These ^{195}Pt NMR chemical shifts are listed in Table 8. As is shown in Scheme 1, two $digly^{2-}$ complexes and two $Hdigly^-$ complexes were prepared and isolated in the solid state. These lower field shifts as pH decreases indicate the chemical exchange between two species,

$$[\text{Pt(digly)Cl}]^- + \text{H}^+ \leftrightarrow \text{H[Pt(digly)Cl]}$$

$$[\text{Pt(Hdigly)Cl}_2]^- + \text{H}^+ \leftrightarrow \text{H[Pt(Hdigly)Cl}_2]$$

along with the slow conversion between the complex [Pt(digly)Cl]$^-$ and [Pt(Hdigly)Cl_2]$^-$. Because H[Pt(digly)Cl] is slightly soluble in H_2O, the ^{195}Pt chemical shift of **2** could not be obtained. On the other hand H[Pt(Hdigly)Cl_2] **4** is soluble to such an extent that ^{195}Pt NMR spectra can be obtained in acidic solution. We assigned the peak at −2152 ppm to **3**, and the peak at −2113 ppm to **4**, where the proton was attached to the peptide O atom.

(35) Fairlie, D. P.; Woon, T. C.; Wickramasinghe, W. A.; Willis, A. C. *Inorg. Chem.* **1994**, *33*, 6425.
(36) Watson, A. A.; Fairlie, D. P. *Inorg. Chem.* **1995**, *34*, 3087.

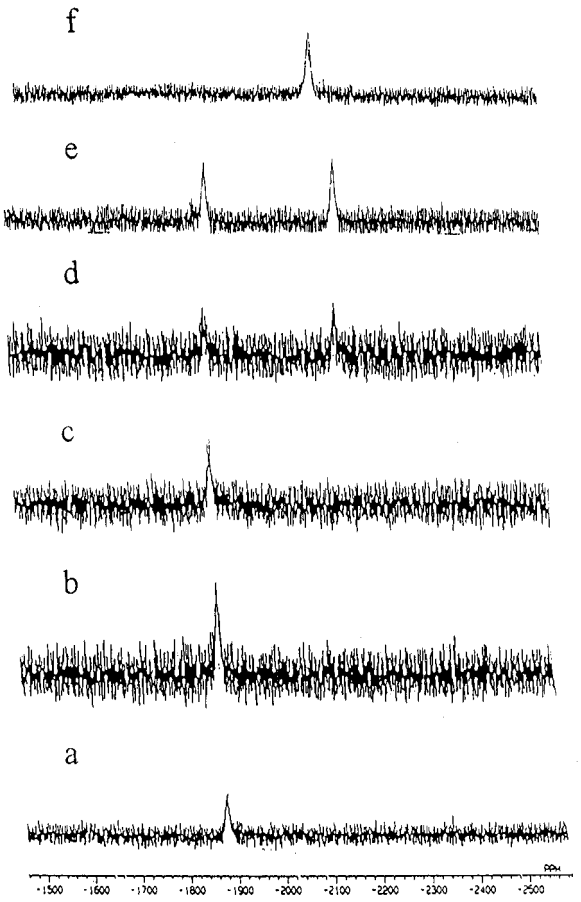


Figure 4. The pH dependence of the ^{195}Pt NMR spectra of K[Pt(digly)Cl] and K[Pt(Hdigly)Cl_2]: (a) pH 12.3, (b) pH 6.6, (c) pH 1.8, and (d) pH 1.0 for K[Pt(digly)Cl]; (e) pH 7.0 and (f) 1 M HCl solution for K[Pt(Hdigly)Cl_2].

Table 8. NMR Data of the Peptide Platinum (II) Complexes

	δ_N[a]		J_{Pt-N}, Hz		
	amine	amide	amine	amide	δ_{Pt}
digly	28.6 (34)	117.5 (106)			
K[Pt(digly)Cl]	−49.6 (−40)	107.4 (100)	351[b]	492[b]	−1887[b]
[Pt(Hdigly)Cl]	(−24)	(126)			
K[Pt(Hdigly)Cl_2]	−27.1 (−23)	123.4 (107)	*c*	*c*	−2152
H[Pt(Hdigly)Cl_2]	(−30)	(138)			−2113
glyala	31.6 (29)	128.1 (125)			
K[Pt(glyala)Cl]	−48.3 (−49)	125.6 (114)	346[b]	507[b]	−1918[b]
K[Pt(Hglyala)Cl_2]	−24.2 (−33)	129.8 (122)	335	558	−2180
alagly	45.3 (46)	113.9 (117)			
K[Pt(alagly)Cl]	−28.5 (−31)	106.7 (99)	373[b]	498[b]	−1946[b]
K[Pt(Halagly)Cl_2]	−6.7 (−11)	123.4 (120)	326	564	−2206

[a] CPMAS data in parentheses. [b] The previous data. [c] Two J_{PtN} for K[Pt(Hdigly)Cl_2] did not appear clearly.

^{15}N Chemical Shifts and the Coupling Constants of K[Pt(dipep)Cl] and K[Pt(Hdipep)Cl_2]. Though there are many ^{15}N NMR spectra of ammines coordinated to platinum,[17−28] only a few reports exist for coordinated amine,[29] azo,[30] and amide.[32] The ^{15}N chemical shifts for these platinum complexes containing dipeptide are listed in Table 8. One doublet around a high singlet peak appeared at 107.4 ppm for peptide ^{15}N and the other −49.6 ppm for amine ^{15}N (^{15}N, $I = {}^1/_2$; ^{195}Pt, $I = {}^1/_2$, 33.6%). The large coupling constant between peptide N and Pt (J_{PtN} = 492 Hz) compared to that for the coordinated amine N (J_{PtN} = 351 Hz) shows that peptide N is coordinated to platinum(II). A large shift on coordination was observed for the amine N of K[Pt(digly)Cl] (78.2 ppm to higher field). When a ^{15}N atom ligates to platinum(II), a large upfield shift occurrs by 60−80 ppm for ammine and amine.[17−29] The coordination shifts of amine for [Pt(digly)Cl]$^-$ obtained here are comparable to those of $^{15}NH_3$ for [Pt(NH_3)$_2$(Ngly-*N*,*O*)] (50−65 ppm). On the other hand, only small coordination shifts of the amide N for [Pt(digly)Cl]$^-$ were observed, by 10 ppm to a higher field. As far as we know, the deprotonated ^{15}N chemical shift of the amide coordinated to platinum has not been reported. The small coordination shifts for the amide ^{15}N were also observed for the complexes containing glyala^{2-} and alagly^{2-}. Such small coordination shifts of the ^{15}N atom after coordination of nitrogen to platinum are surprising. Two reasons can be offered: (a) the amide N changed to N^- anion or (b) the substitution of a platinum for a hydrogen did not change the environment of the sp^2 hybrid N atom. Case a is excluded because protonation occurs on the amide O atom rather than on the amide N when HCl is added to the solution of **3**, producing **4**. Case b seems reasonable because the X-ray data suggest that the amide N atom is in the sp^2 hybridization; moreover the coordination of amide N did not produced an iminium complex but a deprotonated complex; that is to say, metalation occurred, which does not change the environment of the sp^2 hybrid N atom. As is shown in Table 8, the ^{15}N chemical shifts of amine for **3** and its analogues show relatively lower field shifts (≅20 ppm) than those for **1** and its analogues, for example, $\delta_{N(amine)} = -27.1$ ppm for **3** and $\delta_{N(amine)} = -49.6$ ppm for **1**. Appleton et al. reported the correlation of the symmetry of the complex for [{Pt(NH_3)$_2$}$_2$(digly)]$^{2+}$ using the X-ray crystal data and the ^{15}N NMR spectra of the complex with ^{15}N ammine and N terminal ^{15}N-enriched digly^{2-}.[16] Of the four ^{15}N ammine peaks, two showed lower shielding and are assigned to ammine trans to coordinated nitrogen, while for other two, the higher shielding is attributed to ammine trans to oxygen. The coordination shift difference between **1** and **2** may be attributed to this effect, because the atom trans to the coordinated amine for **1** is an oxygen atom and the atom trans to the coordinated amine for **3** is a chlorine atom. On the other hand, the ^{15}N chemical shift of amide for **3** was relatively lower (10 ppm) than that for K[Pt(dipep)Cl], and that for free ligand is between them. Because the atoms trans to coordinated amide N for [Pt(dipep)Cl]$^-$ and for [Pt(Hdipep)Cl_2]$^-$ are the same, that is, a chlorine atom, this lower shielding is not considered to be due to the atom trans to the amide nitrogen. The X-ray data showed that the dihedral angle between the plane Pt1, N2, C2 and the plane Pt1, N2, C3 is 5.0° for **1** and 1.4° for **3**, and the bond angle C2, N2, C3 for **1** is 127.2°, and for **3**, 117.7°. The amide N of complex **3** might have less strain energy in the sp^2 hybrid N atom than that of **1**, having a larger extent of sp^2 hybridization and resonating at a lower field than that for **1**, $\delta_{N(amide)}$ = 123.4 ppm for **3** and $\delta_{N(amide)}$ = 107.4 ppm for **1**. This argument can be applied to the difference in the^{15} N NMR coordination shifts of coordinated amine.

Table 9. Antitumor Activity of K[Pt(digly)Cl] and K[Pt(Hdigly)Cl_2] against Meth A Solid Tumor Implanted in BALB/c Mice

	dose, mg/kg	inhibition ratio, (%)
Cisplatin	10	45.3
K[Pt(digly)Cl]	13	12.5
	26	35.9
	52	29.6
K[Pt(Hdigly)Cl_2]	13	10.7
	26	16.3
	52	40.6

Growth-Inhibitory Activity of 1 and 3 against Meth A Solid Tumor by Intravenous Administration. Table 9 shows

the results of the growth inhibition assay of cisplatin and the two complexes against Meth A solid tumor transplanted in BALB/c mice. The data are the average of three experiments. Complex **1** showed slight growth inhibition in a dose of 26 mg/kg (35.9%), but twice this dose did not increase the growth inhibition ratio (29.6%). On the other hand, complex **3** showed significant growth-inhibitory activity of 40.6% at a dose of 52 mg/kg. The mice administered with cisplatin (10 mg/kg) and **3** (52 mg/kg) appeared to be unhealthy compared with those with **1** (26 mg/kg).

Supporting Information Available: Tables giving crystallographic details, non-hydrogen atom thermal parameters, non-hydrogen interatomic distances, non-hydrogen interbond angles, hydrogen atom parameters, least-squares planes for K[Pt(digly)Cl], K[Pt(Hdigly)Cl_2], and H[Pt(Hdigly)Cl_2], the experimental details of (a) in vivo antitumor assay of Pt complexes against Meth A solid tumor, (b) direct cytotoxicities against Meth A cells, and (c) toxicities against normal bone marrow cells, and results of b and c (8 pages). Ordering information is given on any current masthead page.

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