The Intramolecular Ligand-Exchange Reaction of (*SP*-4-2)-Dichlorobis-(2-hydroxyethylamine)platinum(II) and (*OC*-6-22)-Tetrachlorobis-(2-hydroxyethylamine)platinum(IV), a ^{1}H and $^{15}N,^{1}H$-HMQC NMR Study

Markus Galanski,[a] Wolfgang Zimmermann,[a] Christian Baumgartner,[a] and Bernhard Klaus Keppler*[a]

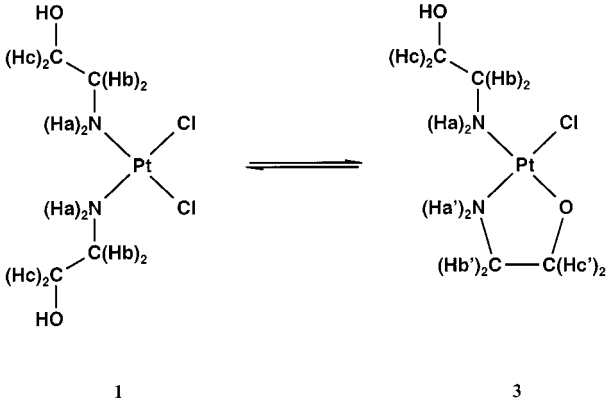
Keywords: Platinum / Ligand exchange / X-ray diffraction / NMR spectroscopy / N ligands

The bis(ethanolamine)platinum complexes (*SP*-4-2)-dichlorobis(2-hydroxyethylamine)platinum(II) (**1**) and (*OC*-6-22)-tetrachlororbis(2-hydroxyethylamine)platinum(IV) (**2**) have been synthesized and their chemistry in aqueous solution has been investigated due to the fact that **1** forms very stable monoadducts with 5′-GMP. In water **1** and **2** are converted into (*SP*-4-3)-chloro(2-ethanolatoamine-κ^2N,O)(2-hydroxyethylamine)platinum(II) (**3**) and (*OC*-6-31)-trichloro(2-ethanolatoamine-κ^2N,O)(2-hydroxyethylamine)platinum(IV) (**4**) with a chelating ethanolatoamine ligand by a intramolecular ligand exchange reaction, which was confirmed by ^{1}H and 2D $^{15}N,^{1}H$-HMQC NMR experiments and the crystal structure determination of **4**.

Introduction

Cisplatin,[1,2] *cis*-$[PtCl_2(NH_3)_2]$, is commonly used for the treatment of testicular and ovarian cancer as well as cervical, bladder and head and neck tumors.[3] The application of cisplatin in therapy is limited by the dose-dependent nephrotoxicity, among a variety of other side effects.[4] There are different strategies for the development of new platinum-based anticancer compounds. Great efforts are undertaken to reduce toxicity and side effects of the platinum compounds, to increase their activity spectrum and to circumvent acquired resistance of cells to cisplatin.[5−8]

A key objective of our research in this subject is to achieve a selective accumulation of antitumor platinum complexes in specific tissues of the organism and to investigate the mode of action of tumor-inhibiting platinum compounds. Therefore, the bis(ethanolamine) platinum(II) and (IV) complexes (*SP*-4-2)-dichlorobis(2-hydroxyethylamine)-platinum(II) (**1**, Scheme 1) and (*OC*-6-22)-tetrachlorobis(2-hydroxyethylamine)platinum(IV) (**2**, Scheme 2) have been synthesised. These compounds can, on the one hand, be used for further derivatisation at the OH group with carrier molecules due to the concept of drug targeting,[9] and on the other hand the bis(ethanolamine) complexes themselves are very interesting because their hydroxy groups can act as acceptors or donors for hydrogen bonds, which could play an important role in the binding of platinum complexes to DNA, the major target of platinum-based chemotherapy.[10,11] Furthermore, it has been shown previously that the formation of bisadducts between **1** and 5′-GMP is very slow and that **1** forms extremely stable 5′-GMP monoadducts, which can be detected in solution by capillary electrophoresis over a period of more than 10 days in the presence of 5′-GMP (1:2).[12]



Scheme 1. Equilibrium of complexes **1** and **3** in water

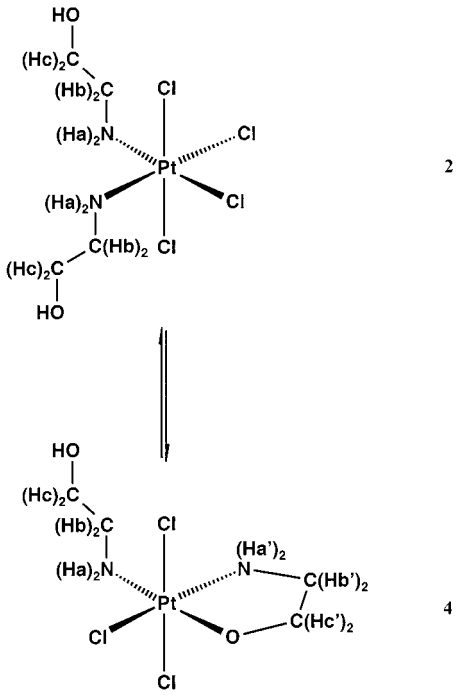
This unexpected observation has led us to focus on the chemistry of **1** and its kinetically more inert tetrachloro analogue **2** in aqueous solution.

Results and Discussion

Whenever (*SP*-4-2)-dichlorobis(2-hydroxyethylamine)-platinum(II) or (*OC*-6-22)-tetrachlorobis(2-hydroxyethylamine)platinum(IV) are dissolved in water intramolecular ligand exchange reactions can be observed: One of the chloro ligands is released and a ethanolatoamine chelate is formed resulting in the complexes (*SP*-4-3)-chloro(2-ethanolatoamine-κ^2N,O)(2-hydroxyethylamine)platinum(II) (**3**) and (*OC*-6-31)-trichloro(2-ethanolatoamine-κ^2N,O)(2-hydroxyethylamine)platinum(IV) (**4**) (Scheme 1 and 2).

[a] Institute of Inorganic Chemistry, Vienna University,
Waehringer Straße 42, 1090 Vienna, Austria
Fax: (internat.) + 43-1/4277-52680
E-mail: Keppler@ap.univie.ac.at

  1434−1948/01/0505−1145 $ 17.50+.50/0



Scheme 2. Equilibrium of complexes **2** and **4** in water

Intramolecular Ligand Exchange Reaction of Compound 1

When dissolving **1** in aqueous solution one should expect three ^{1}H resonances, two multiplets for the CH_2 protons and a broad multiplet for the NH_2 group. However, five minutes after dissolution of **1** in water four additional ^{1}H signals of methylene protons and two additional NH_2 resonances can already be detected; these increase with time (Figure 1). After one day 75% of the bis(ethanolamine) complex **1** and 25% of the ethanolatoamine chelate **3** are present in solution.

The mixture of **1** and **3** shows two sets of three multiplets in the region of the methylene protons, whereas three broad signals for the NH_2 protons can be found. The three methylene protons in the neighborhood of the coordinated amine resonate in the region δ = 2.9 to 2.4. They are broad and not clearly resolved due to coupling with the amine protons and the quadrupolar moment of the ^{14}N nucleus. The before-mentioned signals are separated from the three CH_2 triplets of the methylene protons in the neighborhood of the oxygen atom, which can be found between δ = 3.8 and 3.4.

The amine protons of **1** and **3** are detected between δ = 4.8 and 5.6 and can be assigned to the three different NH_2 groups by 2D ^{15}N,^{1}H-HMQC spectroscopy (Figure 2). The NH_2 protons of complex **1** (trans to Cl) resonate at δ = 4.92 with a ^{15}N chemical shift of δ = −50.02. The cross signal of the coordinated amine ligand of **3** within the chelate ring was found at δ = 5.49/−31.46 whereas the signal of the NH_2 group *trans* to oxygen was detected at δ = 5.11 and −69.98, respectively. With this information the signal assignment of the CH_2 protons to the different ligands [complex **1**: δ = 3.75 (H_c), 2.78 (H_b); complex **2**: δ = 3.71 (H_c), 3.49 ($H_{c'}$), 2.63 (H_b), 2.49 ($H_{b'}$)] was performed by ^{1}H,^{1}H-DQF shift correlation spectroscopy.

As expected, the intramolecular ligand exchange reaction can be suppressed by dissolving complex **1** in DCl solution. Under these conditions only two ^{1}H signals in the CH_2 region at δ = 3.63 (H_c) and 2.95 (H_b) are observed which indicates that the equilibrium is shifted towards complex **1**.

Intramolecular Ligand Exchange Reaction of Compound 2

In case of the kinetically more inert tetrachloroplatinum-(IV) complex **2** the intramolecular ligand exchange reaction can also be observed in water but it is slowed down in com-

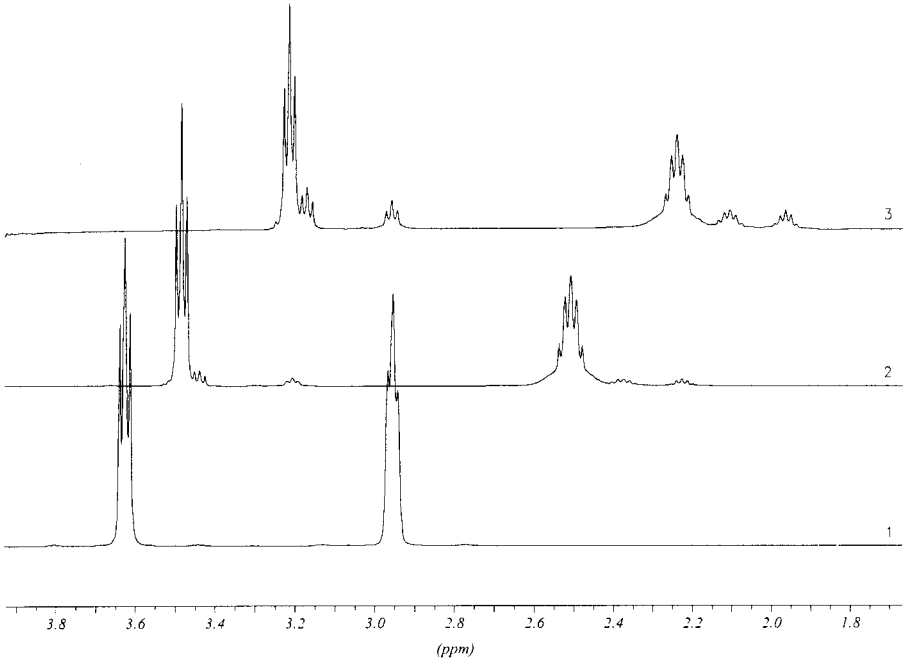


Figure 1. ^{1}H NMR spectra of **1** in DCl solution (1) and after one (2) and 24 hours (3) in H_2O/D_2O (9:1)

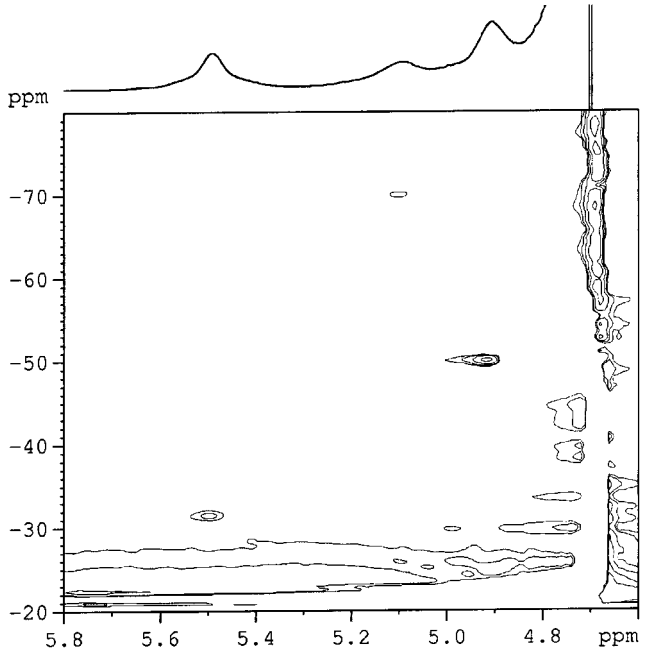


Figure 2. $^{15}N,^{1}H$-HMQC NMR spectrum of **1** after three hours

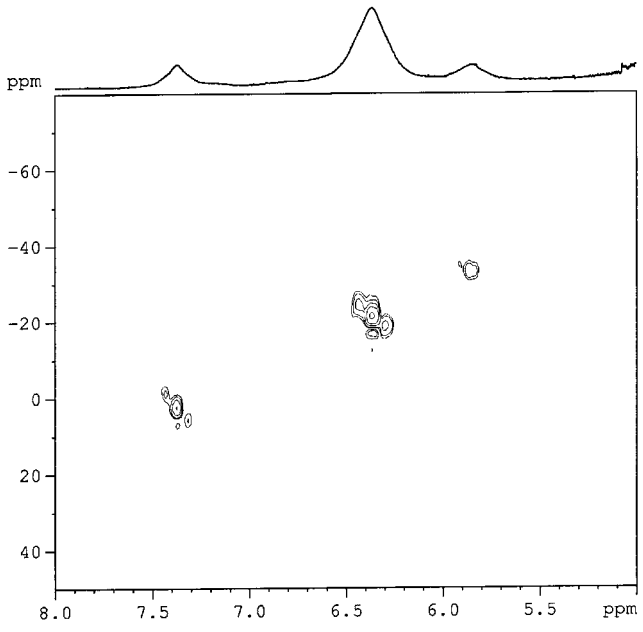


Figure 3. $^{15}N,^{1}H$-HMQC NMR spectrum of **2** after seven days

parison to the platinum(II) analogue **1**. After 12 days a nearly equimolar mixture of **2** and **4** (Scheme 2) is present in solution, whereas after 23 days 20% of **2** and 80% of **4** can be detected. Analogous to the mixture of compounds **1** and **3** there are two sets of CH_2 protons found in the ^{1}H NMR spectrum: three triplets for the methylene protons in the neighborhood of oxygen [**2**: $\delta = 3.70$ (H_c); **4**: 3.65 (H_c), 3.19 ($H_{c'}$)] and two broad multiplets at $\delta = 2.98$ (H_b of **2** and **4**) and 2.60 ($H_{b'}$) for the CH_2 protons in the neighborhood of the coordinated amine.

The NH_2 protons of compound **2** (*trans* to Cl) resonate at $\delta = 6.36$ with a ^{15}N chemical shift of $\delta = -21.78$ (Figure 3). The protons of the coordinated amine of **4** *trans* to the chloro ligand are found at $\delta = 7.31$; the ^{15}N chemical shift is observed at $\delta = 2.52$. The $^{1}H,^{15}N$ cross peak of the amine ligand *trans* to oxygen was detected at $\delta = 5.87$ and -33.02, respectively.

The assignment of the signals was done in this case by 2D $^{15}N,^{1}H$-HMQC, 2D $^{1}H,^{1}H$-COSY and 2D HMQC-TOCSY where the latter experiment combines both the heteronuclear and the homonuclear shift correlation in one experiment.

It was possible to isolate crystals of complex **4** after dissolution of **2** in water for nine days.

The crystal structure of (*OC*-6-31)-trichloro(2-ethanolatoamine-κ^2N,O)(2-hydroxyethylamine)platinum(IV) is shown in Figure 4. The atoms around the platinum center are arranged octahedrally. The two N atoms as well as Cl3 and O1 show a square-planar arrangement around the platinum atom. The angular sum of the equatorial atoms is 360.1° [N1−Pt−N2 = 91.9(2)°, N1−Pt−O1 = 85.3(2)°, N2−Pt−Cl3 = 93.22(17)° and O1−Pt−Cl3 = 89.59(18)°]. The Pt−N [Pt−N1 = 2.059(6) Å, Pt−N2 = 2.066(6) Å] and Pt−Cl [Pt−Cl1 = 2.319(18) Å, Pt−Cl2 = 2.315(19) Å, Pt−Cl3 = 2.331(18) Å] distances are similar to those in (*OC*-6-22)-tetrachlorobis(2-hydroxyethylamine)-platinum(IV).[13] The Cl1 and Cl2 atoms are axial to the equatorial plane and the Cl1−Pt−Cl2 axis is nearly linear [Cl1−Pt−Cl2 = 177.46(7)°]. Four intermolecular hydrogen bonds of each complex are found [N1−H1A···O2 = 2.955 Å (N1−O2); N1−H1B···O1 = 2.895 Å (N1−O1); N2−H2A···O2 2.772 Å (N2−O2); N2−H2B···O1 = 2.791 Å (N2−O1)].

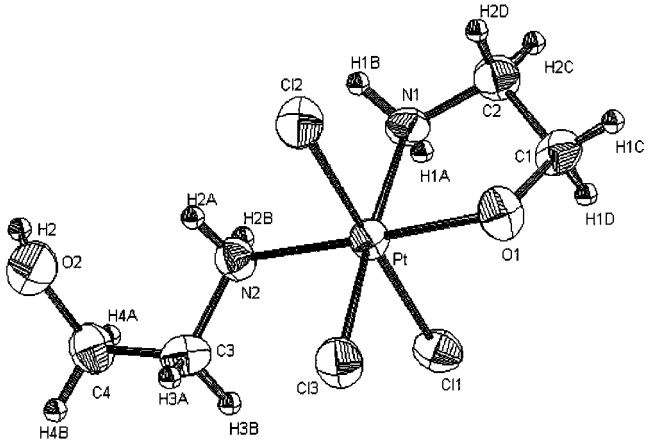


Figure 4. Molecular structure of **4** in the crystal

^{1}H, 2D-$^{1}H,^{1}H$ and 2D $^{15}N,^{1}H$ NMR studies were also carried out with the crystals of compound **4**. In aqueous solution, four ^{1}H signals are observed for the methylene protons at $\delta = 3.66$ (H_c), 3.19 ($H_{c'}$), 2.97 (H_b) and 2.61

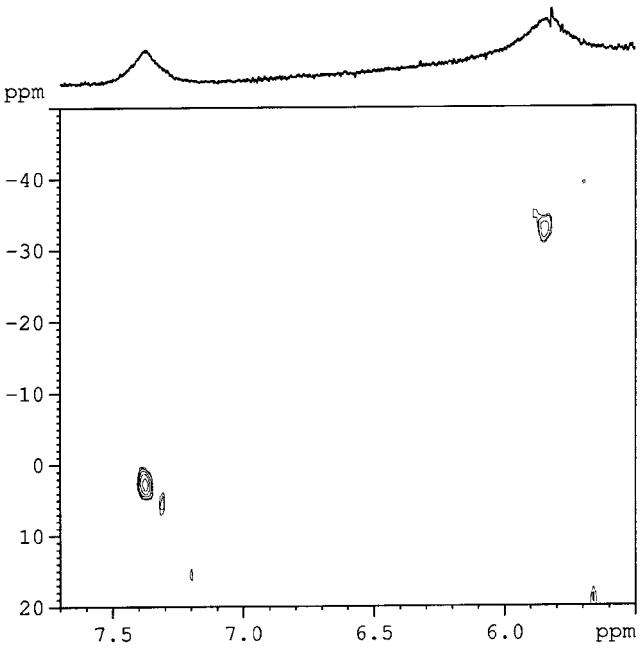


Figure 5. $^{15}N,^{1}H$-HMQC NMR spectrum of the crystals of compound **4**

($H_{b'}$), whereas the protons of the coordinated amine resonate at δ = 7.38 and 5.83. In the $^{15}N,^{1}H$ correlated spectrum of **4** two $^{1}H,^{15}N$ cross peaks (δ = 7.38/2.57 and 5.83/−32.29) are found (Figure 5) in accordance with the signals of compound **2** in solution.

Conclusion

In conclusion, it has been shown that the bis(ethanolamine)platinum complexes **1** and **2** undergo intramolecular ligand exchange reactions in water resulting in platinum compounds **3** and **4** with a chelating ethanolatoamine ligand. This conversion was confirmed by ^{1}H and $^{15}N,^{1}H$-HMQC spectroscopy. The structure of compound **4** in the crystal was also determined. These results clearly indicate that the formation of monoadducts between **1** and 5′-GMP which are very stable over a period of more than 10 days can be explained by the presence of the ethanolatoamine chelate **3** in solution.

Experimental Section

General Remarks: ^{1}H, $^{1}H,^{1}H$-DQF-COSY, $^{15}N,^{1}H$-HMQC and $^{15}N,^{1}H$-HMQC-TOCSY spectra were recorded in H_2O/D_2O (9:1) or DCl at 301 K in a gradient-enhanced mode using a Bruker Avance DPX 400 instrument (UltraShield™ Magnet) and standard pulse programmes at 400.13 (^{1}H) and 40.55 MHz (^{15}N). Chemical shifts were measured relative to the solvent or to external aqueous $^{15}NH_4Cl$ (δ = 0). The X-ray data were collected on a Nonius Kappa CCD diffractometer at room temperature. Elemental analyses were performed by the microanalytical laboratory at the University of Vienna.

(*SP*-4-2)-Dichlorobis(2-hydroxyethylamine)platinum(II) (1) and (*SP*-4-3)-chloro(2-ethanolatoamine-κ²*N,O*)(2-hydroxyethylamine)-platinum(II) (3): The preparation of **1** has been described previously.[13] Compound **1** was dissolved in H_2O/D_2O or D_2O/DCl for NMR spectroscopic studies. Dissolution of **1** in DCl (0.4 mL D_2O and 0.2 mL DCl [20% in D_2O]):
1: ^{1}H NMR (D_2O/DCl): δ = 3.63 [t, ($^{3}J(H_c,H_b)$ = 5.3 Hz), 4 H, H_c], 2.95 [t, ($^{3}J(H_b,H_c)$ = 5.0 Hz), 4 H, H_b].

Upon dissolving **1** in water two products are observed (75% of **1** and 25% of **3** after 24 hours):
1: ^{1}H NMR (H_2O/D_2O, 9:1): δ = 4.92 [m, 4 H, H_a], 3.75 [t, $^{3}J(H_c,H_b)$ = 5.1 Hz, 4 H, H_c], 2.78 [m, $^{3}J(H_b,H_c)$ = 5.1 Hz, 4 H, H_b]. − ^{15}N NMR (H_2O/D_2O, 9:1): δ = −50.02 (N_a).

3: ^{1}H NMR (H_2O/D_2O, 9:1): δ = 5.49 [m, 2 H, $H_{a'}$], 5.11 [m, 2 H, H_a], 3.71 [t, $^{3}J(H_c,H_b)$ = 4.9 Hz, 2 H, H_c], 3.49 [t, $^{3}J(H_{c'},H_{b'})$ = 5.2 Hz, 2 H, $H_{c'}$], 2.63 [m, $^{3}J(H_b,H_c)$ = 4.9 Hz, 2 H, H_b], 2.49 [m, $^{3}J(H_{b'},H_{c'})$ = 5.2 Hz, 2 H, $H_{b'}$]. − ^{15}N NMR (H_2O/D_2O, 9:1): δ = −69.98 (N_a), −31.46 ($N_{a'}$).

(*OC*-6-22)-Tetrachlorobis(2-hydroxyethylamine)platinum(IV) (2) and (*OC*-6−31)-trichloro(2-ethanolatoamine-κ²-*N,O*)(2-hydroxyethylamine)platinum(IV) (4): The preparation of **2** has been described previously.[13] For the preparation of **4** compound **2** (21 mg) was dissolved in 3 mL of water at 50 °C. The solution was stored in an open flask for nine days and orange crystals of **4** were obtained. − $C_4H_{13}Cl_3N_2O_2Pt$ (422.60): calcd. C 11.37, H 3.10, Cl 25.17, N 6.63, Pt 46.16; found C 11.44, H 3.07, Cl 25.03, N 6.44, Pt 45.86. By dissolving **2** in water two products are observed (50% of **2** and **4** after 12 days):
2: ^{1}H NMR (H_2O/D_2O, 9:1): δ = 6.36 (m, 4 H, H_a), 3.70 [t, $^{3}J(H_c,H_b)$ = 5.23 Hz, 4 H, H_c], 2.98 (m, 4 H, H_b). − ^{15}N NMR (H_2O/D_2O, 9:1): δ = −21.78 [^{1}J(N,Pt) = 228 Hz, ^{2}J(H,Pt) = 60 Hz, N_a].

4: ^{1}H NMR (H_2O/D_2O, 9:1): δ = 7.31 (m, 2 H, $H_{a'}$), 5.87 (m, 2 H, H_a), 3.65 [t, $^{3}J(H_c,H_b)$ = 5.2 Hz, 2 H, H_c], 3.19 [t, $^{3}J(H_{c'},H_{b'})$ = 5.5 Hz, 2 H, $H_{c'}$], 2.98 (m, 2 H, H_b), 2.60 (m, 2 H, $H_{b'}$). − ^{15}N NMR (H_2O/D_2O, 9:1): δ = 2.52 [^{1}J(N,Pt) = 282 Hz, ^{2}J(H,Pt) = 48 Hz, $N_{a'}$], −33.02 (N_a).

Upon dissolving **4** in water only one product is observed and the spectrum is equivalent to that of **4** in the mixture of **2** and **4**.

4: ^{1}H NMR (H_2O/D_2O, 9:1): δ = 7.38 (m, 2 H, $H_{a'}$), 5.83 (m, 2 H, H_a), 3.66 [t, $^{3}J(H_c,H_b)$ = 5.3 Hz, 2 H, H_c], 3.19 [t, $^{3}J(H_{c'},H_{b'})$ = 5.5 Hz, 2 H, $H_{c'}$], 2.97 (m, 2 H, H_b), 2.61 (m, 2 H, $H_{b'}$). − ^{15}N NMR (H_2O/D_2O, 9:1): δ = 2.57 [^{1}J(N,Pt) = 251 Hz, ^{2}J(H,Pt) = 52 Hz, $N_{a'}$], −32.29 (N_a).

X-ray Crystallographic Study:[14] The single crystal data were collected on a Nonius Kappa CCD diffractometer at room temperature. The measured intensities were corrected for Lorentz and polarisation effects. The crystal structure was determined by direct methods (SHELXS-97, Sheldrick, 1997a)[15] and subsequent Fourier and difference-Fourier syntheses. Final structure parameters of all three substances were obtained by full-matrix least squares techniques on F^2 (SHELXL-97, Sheldrick, 1997b).[16] Further X-ray structure analysis data are given in Table 1.

Table 1. X-ray crystallographic data of **4**

Molecular formula	$C_4H_{13}Cl_3N_2O_2Pt$
Mol. wt. ($g \cdot mol^{-1}$)	422.6
Space group	*P*4(1)
a (Å)	7.2020(10)
b (Å)	7.2020(10)
c (Å)	20.270(4)
α,β,γ	90
V ($Å^3$)	1051.4(3)
Formula units per cell	4
Density ($g \cdot cm^{-3}$)	2.670
Radiation (Mo-K_α) (Å)	0.71073
$2\theta_{max}$ (°)	56.45
Unique data set	2515
Independent data, [$F_0 > 4\sigma(F_0)$]	2326
Variables	111
Largest difference peak and hole ($eÅ^{-3}$)	2.04/−0.93
*R*1 [for $F_0 > 4\sigma(F_0)$]	0.0294
*wR*2 [for all F_0^2]	0.0691
GOF	1.044
Exposure time/frame (s)	300

Acknowledgments

The support of the FWF (Fonds zur Förderung der wissenschaftlichen Forschung) is gratefully acknowledged. The authors gratefully thank Mrs. R. Strobl for her help in preparing the manuscript.

[1] B. Rosenberg, *Interdiscip. Sci. Rev.* **1978**, *3*, 134.
[2] *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug* (Ed.: B. Lippert), Weinheim, Wiley-VCH, **1999**.
[3] M. C. Christian, *Semin. Oncol.* **1992**, *19*, 720.
[4] D. D. Von Hoff, R. Schilsky, C. M. Reichert, R. L. Reeddick, R. C. Young, F. M. Muggia, *Cancer Treat. Rep.* **1979**, *63* 1527.
[5] R. B. Weiss, M. C. Christian, *Drugs* **1993**, *46*, 360.
[6] L. R. Kelland, S. J. Clarke, M. J. McKeage, *Platinum Metals Rev.* **1992**, *36*, 178.
[7] L. R. Kelland, *Crit. Rev. Oncol. Hematol.* **1993**, *15*, 191.
[8] E. Wong, C. M. Giandomenico, *Chem. Rev.* **1999**, *99*, 2451
[9] W. Zimmermann, M. Galanski, B. K. Keppler, in *Relevance of Tumor Models for Anticancer Drug Development* (Eds.: H. H. Fiebig, A. M. Burger), *Contrib. Oncol. Basel, Karger* **1999**, *54*, 447.
[10] J. Reedijk, *Inorg. Chim. Acta* **1992**, *198/200*, 873.
[11] E. R. Jamieson, S. J. Lippard, *Chem. Rev.* **1999**, *99*, 2467.
[12] A. Zenker, M. Galanski, T. L. Bereuter, B. K. Keppler, W. Lindner, *J. Biol. Inorg. Chem.* in press.
[13] W. Zimmermann, M. Galanski, G. Giesster, B. K. Keppler, *Inorg. Chim. Acta* **1999**, *292*, 127.
[14] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-132673. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk.
[15] G. M. Sheldrick, *SHELXS-97*, University of Göttingen, **1997**.
[16] G. M. Sheldrick, *SHELXL-97*, University of Göttingen, **1997**.

Received July 26, 2000
[I00293]