# Reaction of (*R*)-(−)-2-Aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) with Guanosine

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**The reaction of a new antitumor platinum complex, (*R*)-(−)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (1) with guanosine at room temperature in an aqueous solution was followed by proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy and high performance liquid chromatography (HPLC) at intervals. Both techniques showed that a new compound was formed by displacement of the 1,1-cyclobutanedicarboxylate moiety of 1 with two guanosines, and its $^1$H-NMR spectrum and HPLC chromatogram were proved to be identical with those of [(*R*)-(−)-2-aminomethylpyrrolidine]bis($N_7$-guanosine)platinum(II) (2), which was obtained upon successive treatment of (*R*)-(−)-2-aminomethylpyrrolidinedichloroplatinum(II) (3) with $AgNO_3$ and 2 mol eq of guanosine in water. The binding sites of the platinum to the two guanosine moieties in 2 were confirmed by the pH dependence of the two G-$H_8$ signals.**

**Keywords** antitumor platinum complex; guanosine; 2-aminomethylpyrrolidine Pt(II) complex; 1,1-cyclobutanedicarboxylato Pt(II) complex; bis($N_7$-guanosine) Pt(II) complex

Since Rosenberg *et al.* discovered the antitumor activity of *cis*-diamminedichloroplatinum(II) (*cis*-DDP),[1] many antitumor platinum(II) complexes have been synthesized. However, a more effective organo-platinum complex with higher antitumor activity at a small dosage and with lower toxicity than *cis*-DDP has not been reported. Recently, (*R*)-(−)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) (**1**), having the unsymmetrical diamine [(*R*)-(−)-2-aminomethylpyrrolidine] as a carrier ligand (Chart 1), has been developed in our laboratories, and we found that its antitumor activity is almost the same as that of *cis*-DDP but its toxicity is much weaker.[2]

It has been established that *cis*-DDP exhibits its biological activity by binding to deoxyribonucleic acid (DNA) and inhibiting replication, and the preferred bifunctional, intrastrand cross-link between the *cis*-Pt($NH_3$)$_2$ moiety of DDP and two $N_7$ of adjacent guanines of DNA is supposed to play a crucial role in the antitumor action.[3-15] The mechanism of action of the new drug **1** may be reasonably presumed to be essentially the same as that of *cis*-DDP. As a part of a series of studies to obtain evidence supporting this concept, we examined the reaction of **1** with guanosine (Guo) in an aqueous solution in some detail by means of proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy and high performance liquid chromatography (HPLC).

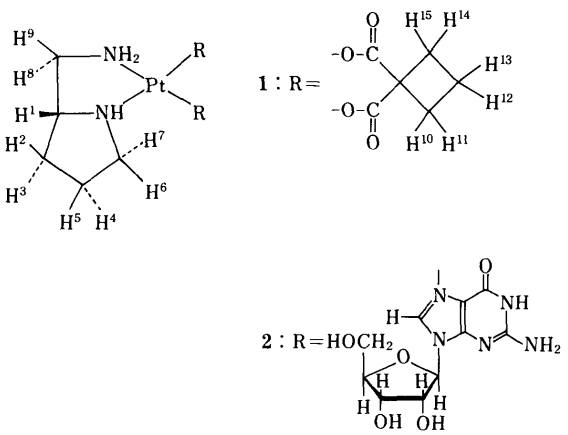


Chart 1

## Experimental

**Syntheses of Pt(II) Complexes** (*R*)-(−)-2-Aminomethylpyrrolidine (1,1-cyclobutanedicarboxylato)platinum(II) (**1**) was prepared according to the published method.[2]

(*R*)-(−)-2-Aminomethylpyrrolidinedichloroplatinum(II) (**3**): A solution of 0.7 g of (*R*)-(−)-2-aminomethylpyrrolidine dihydrochloride and 0.32 g of NaOH in 5 ml of water was added to a solution of 1.7 g of $K_2PtCl_4$ in 20 ml of water. The solution was stirred at room temperature for 1 d, and the deposited solid was filtered off, washed successively with water, methanol and acetone and dried under reduced pressure for several hours to give 1.14 g (76%) of **3** as slightly yellow crystals, mp 279—286 °C (dec.). *Anal.* Calcd for $C_5H_{12}Cl_2N_2Pt$: C, 16.40; H, 3.30; Cl, 19.37; N, 7.65; Pt, 53.28. Found: C, 16.24; H, 3.50; Cl, 19.14; N, 7.63; Pt, 53.77. $[\alpha]_D^{20}$ −20° ($c$=0.003, $H_2O$).

[(*R*)-(−)-2-Aminomethylpyrrolidine]bis($N_7$-guanosine)platinum(II) (**2**): A suspension of 1.14 g of **3** in 300 ml of water was treated with 1.06 g of $AgNO_3$, and the mixture was stirred at room temperature in the dark for 1 d. The precipitated AgCl was filtered off, and the filtrate was concentrated to 15 ml under reduced pressure below 40 °C. Then, 0.65 g of Guo was dissolved in 5 ml of this solution at 60 °C during 5 min, and the mixture was stirred at room temperature for 1 d, and concentrated under reduced pressure below 40 °C. The oily residue was triturated with acetone to give 1.07 g (98%) of **2** as a colorless powder, mp *ca.* 165 °C (dec.). *Anal.* Calcd for $C_{25}H_{38}N_{12}O_{10}Pt \cdot 2(NO_3) \cdot 2(H_2O)$: H/C, 0.141; N/C, 0.653; Pt/C, 0.650. Found: H/C, 0.143; N/C, 0.649; Pt/C, 0.644. $[\alpha]_D^{20}$ −29° ($c$=0.02, $H_2O$).

**Reaction of 1 with Guanosine in Aqueous Solution** A solution of 20 mg (67 μmol) of Guo in 20 ml of water was treated with 15 mg (33 μmol) of **1**, and the mixture was allowed to stand at 25 °C in a water bath. The reaction mixture was analyzed at intervals by HPLC and $^1$H-NMR spectroscopy. The $^1$H-NMR spectra were measured with samples prepared by adding 5 ml of $D_2O$ to 1 ml aliquots.

**Measurements** HPLC analyses were performed on a Shimadzu LC-6A liquid chromatograph with detection at 210 nm, on a Tosoh TSKgel G-Oligo-PW column (7.8 × 300 mm), using 0.1 M $Na_2SO_4$ aqueous solution as an eluant. The flow rate was 1 ml/min.

$^1$H-NMR spectra were recorded on a JEOL JNM-GX400 spectrometer operating in the Fourier-transform mode at 399.78 MHz with sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as an internal standard, and the chemical shifts are given in ppm units. Spectra of **1** and **2** were measured with samples prepared by dissolution in 99.8% $D_2O$ followed by lyophilization twice and finally dissolution in 99.95% $D_2O$. The assignments of chemical shifts were achieved by extensive decoupling experiments, two-dimensional correlation spectroscopy (2D-COSY) and nuclear Overhauser effect (NOE) analyses, and the coupling constants were determined by 2D-J resolution spectroscopy and computer simulation with a LAOCN3 type

program.

The pH of a solution of **2** in $D_2O$ was adjusted by adding NaOD or $D_2SO_4$, and the pH values were monitored with a microelectrode directly in an NMR tube.

## Results and Discussion

The $^1$H-NMR spectrum of **1** in $D_2O$ could be completely assigned as shown in Table I.[16]

To examine the stability of **1** in an aqueous solution, a solution of 0.1 g of **1** in 20 ml of water was stored at 25 °C for 30 d, and the sample recovered from this solution was checked by $^1$H-NMR spectroscopy and HPLC. No changes were observed in the $^1$H-NMR spectrum, and the HPLC showed only a single peak of **1** at $t_R = 17$ min ($t_R$: retention time). Thus it was proved that **1** is stable in water at ambient temperature.

The reaction of **1** with Guo (2 eq) in water at 25 °C was monitored by HPLC and $^1$H-NMR spectroscopy. On HPLC, the peak area of **1** at $t_R = 17$ min and that of Guo at $t_R = 28$ min decreased with time at approximately the same rate ($k = 2 \times 10^{-2}\,d^{-1}$: first-order rate constant). At the same time, two new peaks at $t_R = 27$ and 11 min appeared, and their peak areas increased in proportion to the decreases of peak areas of **1** and Guo. The peak at $t_R = 27$ min was identified as 1,1-cyclobutanedicarboxylic acid (CBDCA) from the retention time. The other peak at $t_R = 11$ min was considered to be due to a new product.

Figure 1 shows the $^1$H-NMR spectrum of the product mixture obtained from the reaction of **1** with 2 eq of Guo at 25 °C for 7 d. It contained one four-proton triplet centered at 2.35 ppm characteristic of H-2, H-2′, H-4 and H-4′ of CBDCA, indicating the liberation of CBDCA. At the same time, the decrease of the intensity of the H-8 proton signal of Guo (G-$H_8$) at 8 ppm was observed accompanied by the appearance of two new signals at 8.27 and 8.44 ppm, suggesting the new bindings of two Guo moieties. Integration analysis of these proton signals demonstrated that the new product had been formed by displacement of the leaving group of **1**, CBDCA, with two Guo moieties. Further, $^1$H-NMR spectrum and HPLC chromatogram revealed that only one product derived from 1 eq of **1** and 2 eq of Guo was always produced independently of the ratio of Guo to **1**; a product containing one Guo could not be detected at all. Binding of the second Guo, leading to **2** may be very rapid.

An attempt to isolate the product from the reaction of **1** with Guo was unsuccessful. However, [(*R*)-(−)-2-aminomethylpyrrolidine]bis($N_7$-guanosine)platinum(II) (**2**) was obtained as a colorless powder in high yield when **3** was treated successively with $AgNO_3$ and Guo in water, and its $^1$H-NMR spectrum and HPLC chromatogram were found to be identical with those of the product formed from **1** and Guo in water. The $^1$H-NMR data for **2** in $D_2O$ are given in Table II.

To determine the binding sites of the platinum to the two Guo moieties in **2**, the following examination was carried out. It is well known that protonation of heterocyclic nitrogens of Guo appreciably affects the chemical shift of G-$H_8$:[3-5,9,12,15] protonation at G-$N_1$ ($pK_a = 9.13$)[17] or at G-$N_7$ ($pK_a = 1.8$)[17] causes a downfield shift of the G-$H_8$ signal.[3,8,15] Since binding of platinum to a particular

Table I. $^1$H-NMR Data for **1** Measured at 25 °C in $D_2O$

| Proton No. | Chemical shift (ppm from DSS) | Coupling constant ($J$; Hz) |
|---|---|---|
| 1 | 3.25 | $J_{1,2}=8.4$, $J_{1,3}=6.8$ |
| 2 | 2.00 | $J_{2,3}=13.1$ |
| 3 | 1.77 | $J_{2,4}=4.5$, $J_{3,4}=9.2$ |
| 4 | 2.22 | $J_{4,5}=13.0$, $J_{6,7}=12.0$ |
| 5 | 1.88 | $J_{4,6}=6.5$, $J_{5,6}=7.3$ |
| 6 | 2.99 | $J_{4,7}=7.0$, $J_{5,7}=8.3$ |
| 7 | 3.05 | $J_{1,8}=6.7$, $J_{1,9}=5.0$ |
| 8 | 2.56 | $J_{8,9}=12.8$ |
| 9 | 2.72 | $J_{10,11}=11.8$ |
| 10 | 2.91 | $J_{12,15}=J_{13,14}=J_{12,11}=J_{13,10}=7.8$ |
| 11 | 2.96 | $J_{13,15}=J_{12,14}=J_{12,10}=J_{13,11}=8.2$ |
| 12, 13 | 1.90 | $J_{12,13}=0$ |
| 14 | 2.81 | $J_{14,15}=12.0$ |
| 15 | 2.79 | $J_{10,14}=J_{11,15}=1.4$ |

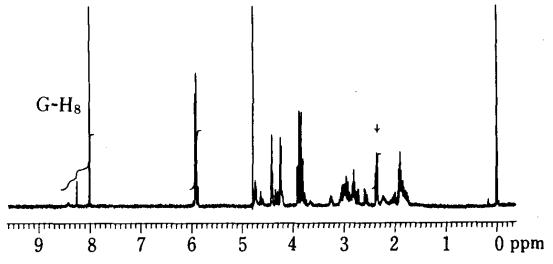


Fig. 1. $^1$H-NMR Spectrum of the Reaction Mixture of **1** with Guo

This spectrum was taken after storage of the sample at 25 °C for 7 d. The arrow indicates the 2.35 ppm signal of the liberated CBDCA.

Table II. $^1$H-NMR Data for **2** Measured at 25 °C in $D_2O$

| Proton No. | Chemical shift (ppm from DSS) | Coupling constant ($J$; Hz) |
|---|---|---|
| 1 | 3.64 | |
| 2 | 2.08 | |
| 3, 5 | 1.8 | |
| 4 | 2.1 | |
| 6, 8 | 2.9 | |
| 7, 9 | 3.0 | |
| G-$H_8$ | 8.44, 8.27 | |
| R-$H_1$ | 5.91, 5.88 | $J_{1,2}=J_{1',2'}=4.9$ |
| R-$H_2$ | 4.63, 4.59 | $J_{2,3}=J_{2',3'}=5.4$ |
| R-$H_3$ | 4.34, 4.30 | $J_{3,4}=J_{3',4'}=3.9$ |
| R-$H_4$ | 4.2, 4.2 | $J_{4,5a}=J_{4',5a'}=2.9$ |
| R-$H_{5a}$ | 3.86, 3.83 | $J_{4,5b}=J_{4',5b'}=3.9$ |
| R-$H_{5b}$ | 3.79, 3.76 | $J_{5a,5b}=J_{5a',5b'}=12.7$ |

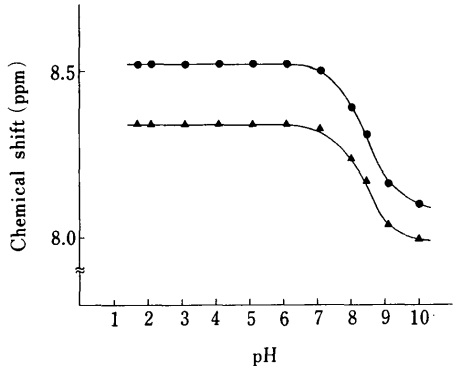


Fig. 2. pH *vs.* Chemical Shift Profile of the Two G-$H_8$ Signals of **2**

nitrogen atom would prevent the protonation, the effect of protonation of this nitrogen might not be observed. Therefore, analysis of $^1$H-NMR spectra obtained at various pH regions may enable localization of the binding site of the platinum. Figure 2 shows the pH dependence of the two G-$H_8$ signals in **2**. Both signals were shifted to higher field in the pH 8 to 10 region. This can be accounted for in terms of the deprotonation at each G-$N_1$. However, the downfield shift due to the protonation of G-$N_7$ at around pH 2 was not observed. Consequently, it can be concluded that the platinum in **2** is bound to two Guo moieties through the two N-7 atoms.

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