Cytotoxicity of Platinum(II) Dinuclear Complexes with 1-Alkylthymine Ligands against Mouse Sarcoma 180 Cells

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We synthesized five platinum(II) dinuclear complexes containing 1-alkylated thymines. Two of the 1-alkylated thymine, 1-MeThy and 1-EtThy, complexes afforded good crystals. The X-ray structures of these complexes were determined. The 1-MeThy complex has a head-to-head (H-H) arrangement, while the 1-EtThy complex has a head-to-tail (H-T) arrangement. The 1-MeThy complex (H-H) shows high electrophilicity against chloride anion (Cl^-) and high cytotoxicity against mouse sarcoma 180 (S-180) cells *in vitro.* The 1-EtThy complex (H-T) is inactive. The other 1-MeThy complex does not produce CDDP in the reaction with chloride ion and is inactive against the S-180 cell line. This complex is assumed to have an H-T arrangement. Similarly, two different 1-Pr^{n}Thy complexes, one with high electrophilicity and cytotoxicity and the other without, must have an H-H and H-T arrangement, respectively. For comparison, we investigated six complexes, 1-methyluracil (1-MeUra) (H-H) dimer, α-pyridone (H-T) dimer, α-pyridone blue tetramer (PPB), 1-methylcytosine (1-MeCyt) (H-T) dimer, acetate dimer, and 1-MeUra monomer complexes. The α-pyridone (H-T), PPB, 1-MeCyt (H-T) dimer, and 1-MeUra monomer complexes are inert to chloride ion and inactive against mouse sarcoma S-180. The 1-MeUra (H-H) dimer and acetate dimer complexes show high electrophilicity and high cytotoxicity. Cellular accumulation of the platinum complexes phenomenally shows that all are incorporated to cancer cells to a lesser extent than CDDP. The relationships between the accumulation, the electrophilicity, and the interaction of these complexes with proteins are discussed.

Introduction

The platinum complexes such as cisplatin (CDDP) and carboplatin are the subject of much attention because of their beneficial effects in the treatment of cancer.[1,2] Although CDDP exhibits strong activities against ovarian, gastric, and prostate cancers, it has serious problems of nephrotoxicity and emesis. To reduce these side effects, a great deal of effort has been focused on the preparation of new complexes as well as on methods of administration. One promising method is the oligomerization of complexes which are suggested earlier.[3] We synthesized platinum pyrimidine oligomers, from which so-called "blue" and "green" species were isolated as main products. Interestingly, the green complexes show significantly high antitumor activity while the blue ones are actually inactive. Despite the high activity, the structure of the green complex is still unknown, since many attempts to crystallize it have failed. In this work we focus on platinum dinuclear complexes, whose structures are simpler and thus can be determined. This approach might provide some useful information in the analysis of the green complexes.

Results and Discussion

X-ray Crystallographic Structure. Of the five platinum(II) dinuclear complexes synthesized in this work, two of them, **1** and **3**, gave good crystals suitable for X-ray structural analysis. The fundamental structure of 1-methylthymine dinuclear complex (H-H) was similar to that reported by Lippert *et al.*,[4] although there is a little difference between these crystal data (Figure 1a). The present crystal is monoclinic $P2_1/C$ with the crystal data a = 8.4121(6) Å, b = 17.6124(17) Å, c = 17.3648(14) Å, β = 114.4876(73)°, V = 2341.3103(3581) Å^3, Z = 4, D_c = 2.44 g cm^{-3} (R = 0.0904), while the reported crystal data are $P2_1/C$, a = 8.36 Å, b = 17.55 Å, c = 18.40 Å, β = 121.15°, V = 2310.4 Å^3, Z = 4, D_c = 2.47 g cm^{-3} (R = 0.094). Selected geometric features of **1** are summarized in Table 1. The Pt1−Pt2 distance (2.924 Å) is longer than the reported one (2.909 Å), and the tilt angle between the adjacent Pt coordination planes (31.3°) is also larger than the reported value (29.5°). The twisting angle about the Pt1−Pt2 axis is, however, very small in both complexes, which means that the coordinating NH_3 groups lie above each other. The structure of **3** (H-T form) was determined for the first time in this work (Figure 1b). The crystal data are as follows: triclinic, $P\bar{1}$, a = 9.9608(12) Å, b = 12.6015(13) Å, c = 13.0642(7) Å, α = 108.042(7)°, β = 96.546(8)°, γ = 111.490(7)°, V = 1402.0(2) Å^3, Z = 2, D_c = 2.126 g cm^{-3} (R = 0.0725). Selected geometric features of **3** are shown in Table 2. The Pt−Pt, Pt−N, and Pt−O distances of **3** are close to those of the Pt(II) H-T dimer with 1-methylthymine.[5]

Electrophilicity. Since the product (CDDP) was easily detected by HPLC, chloride anion (Cl^-) was chosen as a nucleophile to estimate the electrophilicity of the complexes. Figure 2 illustrates the time course of the formation of CDDP from the dimer complexes.

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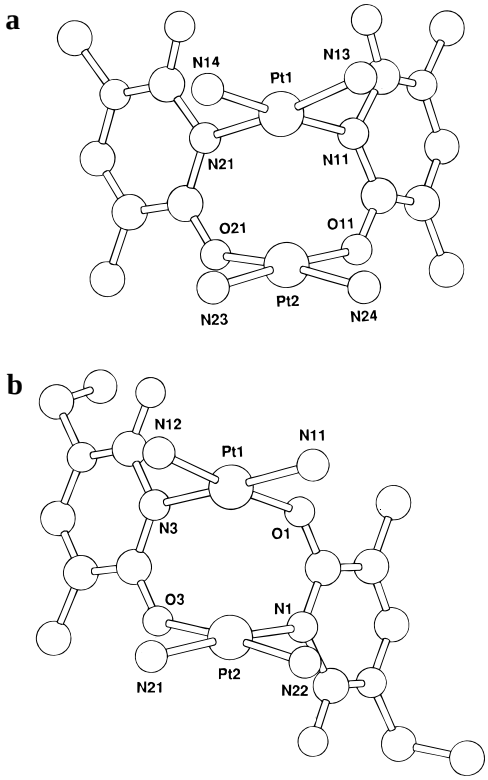


Figure 1. (a) Molecular cation **1**; (b) molecular cation **3**.

Table 1. Selected Geometric Features of Complex **1**

	distance, Å		angle, deg
Pt1−Pt2	2.924(1)	N11−Pt1−N21	92.42(80)
Pt1−N11	2.028(20)	N14−Pt1−N21	88.43(85)
Pt1−N21	2.041(20)	N11−Pt1−N13	88.95(83)
Pt1−N13	2.088(22)	N13−Pt1−N14	90.01(88)
Pt1−N14	2.028(22)	O11−Pt2−N24	90.94(79)
Pt2−O11	2.001(17)	N23−Pt2−O21	91.51(82)
Pt2−O21	2.002(17)	O11−Pt2−O21	88.33(67)
Pt2−N23	2.019(24)	N23−Pt2−N24	88.84(92)
Pt2−N24	2.043(22)		

Table 2. Selected Geometric Features of Complex **3**

	distance, Å		angle, deg
Pt1−Pt2	2.9441(6)	N3−Pt1−O1	91.0(3)
Pt1−O1	2.037(8)	N3−Pt2−N12	89.2(4)
Pt1−N3	2.044(8)	O1−Pt1−N11	87.2(4)
Pt1−N11	2.058(8)	N11−Pt1−N12	92.5(4)
Pt1−N12	2.057(10)	N1−Pt2−O3	90.6(3)
Pt2−O3	2.029(8)	N1−Pt2−N22	89.8(4)
Pt2−N1	2.041(8)	O3−Pt2−N21	87.9(4)
Pt2−N21	2.051(8)	N21−Pt2−N22	91.3(4)
Pt2−N22	2.060(9)		

Head-to-head forms of 1-MeThy dimer and 1-MeUra dimer complexes react with Cl^- very quickly. The observed rate constants (k_{obs}) are 0.23 h^{-1} (25 °C) and 0.79 h^{-1} (37 °C) for 1-MeThy dimer complex, and 0.097 h^{-1} (25 °C) and 0.58 h^{-1} (37 °C) for 1-MeUra dimer complex. That 1-MeThy H-H dimer has higher electrophilicity than 1-MeUra H-H dimer must be ascribed to the difference in the electronic state of these two complexes. PM3 calculation of these ligands shows that 1-MeThy anion has electron density (−0.4765) on the coordinating carbonyl oxygen lower than that on the 1-MeUra anion (−0.4795), which suggests that the Pt−O bond of 1-MeThy complex is weaker than that of 1-MeUra complex. Contrary to the pyrimidine type H-H dimer complexes, α-pyridone H-T dimer, PPB, and

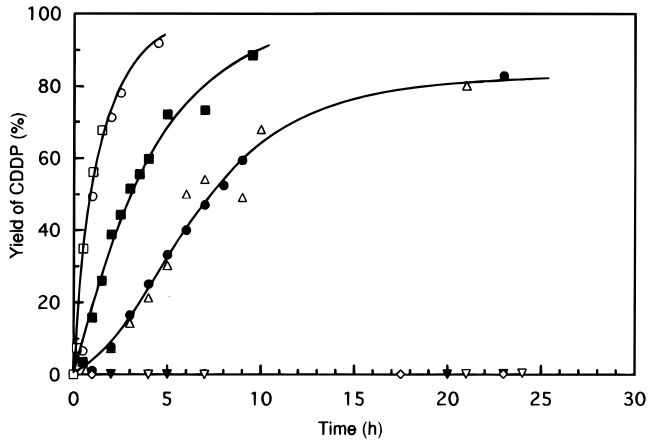


Figure 2. Time course of the formation of CDDP from **1** and various other Pt(II) complexes: **1** at 25 °C (■) and at 37 °C (□), 1-MeUra H-H dimer at 25 °C (●) and at 37 °C (○), 1-MeUra tetramer green at 25 °C (△), 1-MeCyt H-T dimer at 25 °C (◇), α-pyridone H-T dimer at 25 °C (▼), α-pyridone tetramer blue at 25 °C (▽).

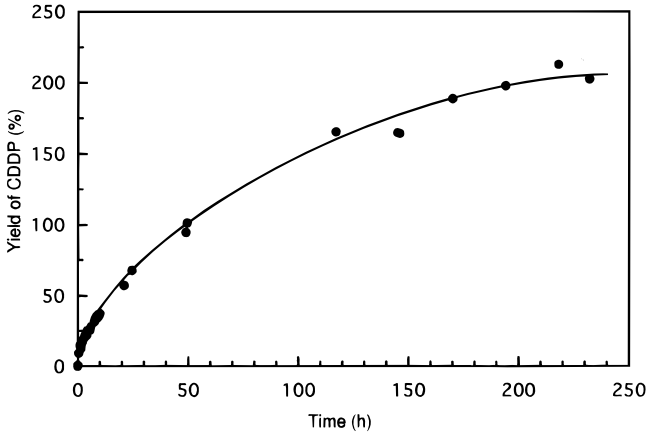


Figure 3. Time course of the formation of CDDP from acetate complex at 25 °C.

1-MeCyt H-T dimer did not produce CDDP. In α-pyridone complexes (H-T dimer and PPB), the Pt−O bonds are assumed to be too stable, as are the Pt−N bonds, to produce CDDP. In 1-MeCyt H-T complex the low reactivity is assumed to be due to high stability of both of the Pt−N bonds. In the pyrimidine H-H complexes, the Pt−O bonds are not so strong, and two Cl^- ions attack them to form CDDP. In contrast, the Pt−N bond is more stable, and therefore CDDP is not formed from the H-T form (data not shown), although the Pt−O bonds are cleaved by Cl^-. The reaction mechanisms of H-H and H-T complexes are shown in Scheme 1. The difference in the stability of Pt−O bonds between the pyrimidine complex and α-pyridone complex is believed to arise from the difference in the electronic states of these two types of ligands. PM3 calculation shows that α-pyridone anion has higher electron density (−0.5225) on the coordinating O atom than 1-MeThy anion (−0.4765) and 1-MeUra anion (−0.4795). This clearly indicates that the electrophilicity is in the order, 1-MeThy complex > 1-MeUra complex ≫ α-pyridone complex.

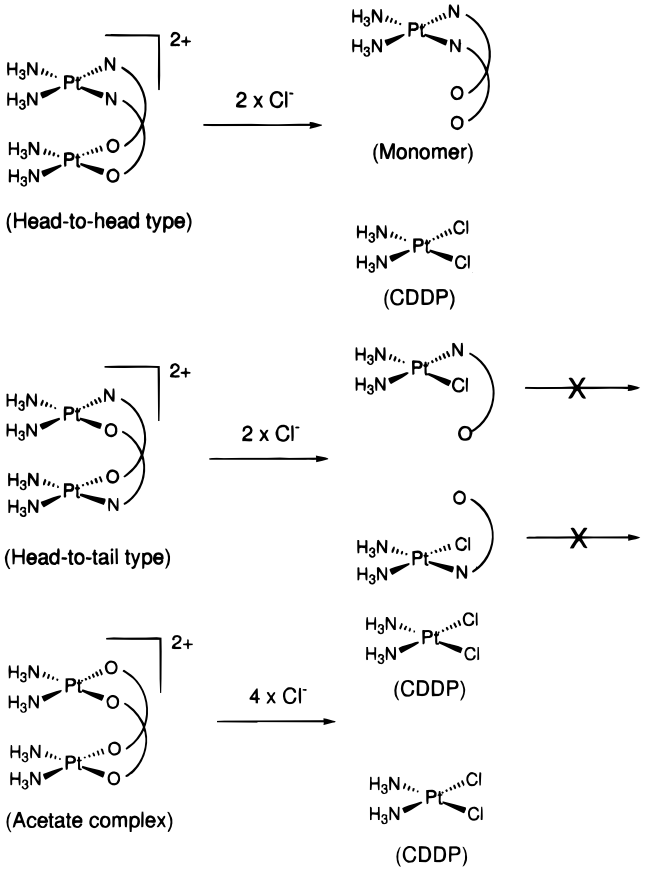
Acetate−Pt complex shows somewhat different behavior in the reaction with Cl^-. Figure 3 shows a time yield curve for CDDP formation from acetate dimer complex. Although the reaction rate is much slower (k_{obs} = 0.016 h^{-1} at 25 °C) than the pyrimidine complexes (k_{obs} = 0.097−0.23 h^{-1} at 25 °C), two CDDP

Table 3. Cytotoxicity of Platinum Complexes against S-180 Cell

Pt complex (ligand)	type	IC_{50} (μM)
CDDP	mononuclear	1.07
1-methyluracil	mononuclear	inactive
1-methyluracil	dinuclear/H-H	1.63
1-methylthymine	dinuclear/H-H	1.75
1-methylthymine	dinuclear/H-T	inactive
1-ethylthymine	dinuclear/H-T	inactive
1-propylthymine	dinuclear/H-H	2.21
1-propylthymine	dinuclear/H-T	inactive
1-methylcytosine	dinuclear/H-T	inactive
acetate	dinuclear	0.94
α-pyridone	dinuclear/H-T	inactive
PPB[a]	tetranuclear/H-H	inactive

[a] Platinum pyridone blue (PPB) dissociates into two head-to-head dimers in an aqueous solution.

Scheme 1. Proposed Reaction Mechanism of Pt Dinuclear Complexes



molecules were formed from one acetate complex (namely, final yield is 200 %), which means that Cl^- anions attack both Pt atoms of the acetate dimer complex. The possible reaction mechanism is shown in Scheme 1. It is thought that Pt−O bonds are more unstable than Pt−N bonds, and thus carboxylate ligands as well as carbonyl groups of uracil and thymine derivatives are labile against nucleophiles. The negative charge of carboxylate may account for the smaller reaction rate of the acetate−Pt complex than that of the pyrimidine−Pt complexes.

Antitumor Activity. Table 3 summarizes IC_{50} values against mouse sarcoma 180 (S-180) for the new complexes (five 1-alkylthymine complexes and acetate type complex) as well as CDDP, 1-MeUra dimer, 1-MeUra monomer, α-pyridone H-T dimer, and PPB complexes. The H-H dimers with pyrimidine ligands are

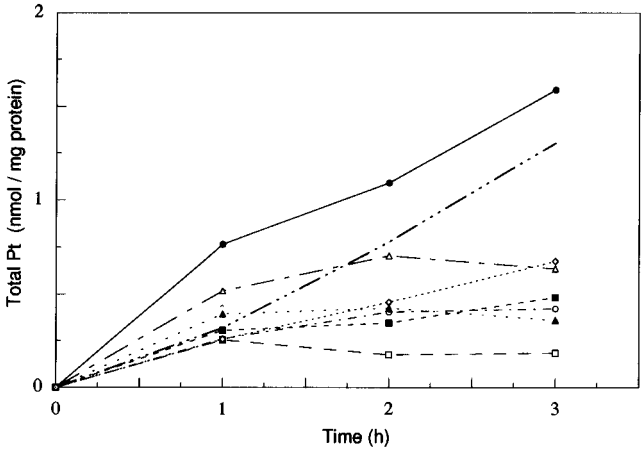


Figure 4. Cellular accumulation of platinum as a function of exposure time of Pt(II) complexes into S-180 cells: (●) CDDP, (■) **1**, (□) **2**, (○) 1-MeCyt H-T dimer, (△) α-pyridone H-T dimer, (▲) α-pyridone tetramer blue, (◇) acetate complex, (− ··· −) calculated curve for 1-MeThy H-H dimer.

active, although H-T species are all inactive. This is caused by the difference in the strength of the Pt−O and Pt−N bonds as indicated above. Therefore it is thought that in a saline solution H-H dimers are partly converted to CDDP and partly introduced into cells in their intact structures. It is believed that the H-H type complexes and CDDP, which are accumulated into cancer cells, are converted to an aqua complex before reacting to DNA. Since H-T complexes cannot form either an aqua complex or CDDP, however, they do not show cytotoxicity. There is an exception in PPB. Although PPB dissociates to the H-H dimer complexes, it is inactive. This is caused by the fact that the Pt−O bond is more stable than those of the other complexes, and therefore the α-pyriodone ligand is not substituted by a nucleophile. The high stability of the Pt−O bond of α-pyridone ligand complex was already pointed out based on the electron density calculated by PM3.

Cellular Platinum Accumulation. Figure 4 shows time dependence of the platinum amount accumulated into S-180 at 37 °C. As a whole, the dimer complexes appear less accumulated than CDDP. With respect to 1-MeThy complexes, the H-H complex appears more accumulated than the H-T complex. This difference is probably due to the CDDP formation from the H-H complex; namely, in the 1-MeThy H-H complex both the intact H-H dimer and the formed CDDP can be incorporated, whereas the H-T complex cannot give CDDP. In light of the fact that α-pyridone H-T dimer and PPB, which are stable in an aqueous solution, are also accumulated, it is reasonable that the 1-MeThy H-H dimer complex is accumulated in its intact form as well as in the form of CDDP. In Figure 4 is shown the curve calculated by eq 3 in the Appendix, which represents the total amount of Pt accumulated in both forms (1-MeThy H-H complex and CDDP). It is curious, however, that the calculated values are higher than the observed ones. One reason for this may be the complexation between the dimer complex and serum proteins like albumin. The interaction with the protein inhibits the incorporation of the intact Pt dimer complex into cancer cells, since the concentration of the free Pt dimer complex is reduced by the complexation. Furthermore, the complexation also hinders the formation of CDDP from the dimer complex, because the nucleo-

philic attack by Cl^- is prevented. In fact, this type of interaction is experimentally confirmed in 1-MeUra dimer complexes, which will be reported in detail elsewhere.

Experimental Section

Materials and Methods. *cis*-Diamminediiodoplatinum(II) (*cis*-$[PtI_2(NH_3)_2]$) was obtained from Nippon Engel Chemcat Co. 1-Methylthymine was obtained from Sigma Chemical Co. Iodoethane and 1-iodopropane were purchased from Tokyo Kasei Co. BCA* Protein Assay Reagent was purchased from Pierce. All other reagents were of the highest grade commercially available. The above-mentioned reagents were used without further purification.

The NMR spectra were measured on a JEOL EX270 spectrometer. Visible absorbance was obtained with Hitachi U-3200 or Shimadzu MPS-2000 spectrophotometers. Platinum content was determined with a Varian SpectrAA-400 Zeeman atomic absorption spectrometer. Cell number in the bioassay was counted by a Coulter Channelyzer 256 cell analyzer. Elemental analysis data were measured at the analytical center of National Institute of Materials and Chemical Research (AIST).

Synthetic Procedure. 1-Ethylthymine. A reaction mixture of thymine (1.26 g, 10.0 mmol), iodoethane (0.96 mL, 12.0 mmol), anhydrous K_2CO_3 (4.15 g, 30 mmol), and dry DMF (40 mL) was stirred for 4 h at 100 °C. K_2CO_3 was filtered off, and the solvent was evaporated to dryness, affording a white solid. After purification by flash chromatography (EtOAc/MeOH 1%), the white solid was recrystallized from EtOAc/MeOH to give white crystals (0.40 g, 30%): $R_f = 0.32$ (EtOAc/MeOH, 1%); ^{1}H NMR (DMSO-d_6) δ 1.15 (t, 3H, CH_3), 1.75 (s, 3H, CH_3), 3.64 (q, 2H, CH_2), 7.54 (s, 1H, C-H), 11.19 (s, 1H, N-H). Anal. ($C_7H_{10}N_2O_2$) C, H, N.

1-Propylthymine. 1-Propylthymine was synthesized in the same manner as 1-ethylthymine. The yield of white crystal was 0.60 g (35%): $R_f = 0.42$ (EtOAc/MeOH, 1%); ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, CH_3), 1.58 (m, 2H, CH_2), 1.75 (s, 3H, CH_3), 3.57 (t, 2H, CH_2), 7.52 (s, 1H, C-H), 11.31 (s, 1H, N-H). Anal. ($C_8H_{12}N_2O_2$) C, H, N.

Bis(*μ*-1-methylthyminato-*N3,O4*)bis(*cis*-diammineplatinum(II)) Dinitrate [$[Pt_2(C_6H_7N_2O_2)_2(NH_3)_4](NO_3)_2$, Head-to-Head, 1; $[Pt_2(C_6H_7N_2O_2)_2(NH_3)_4](NO_3)_2\cdot H_2O$, Head-to-Tail, 2]. The Pt(II) dimer complexes with 1-methylthymine ligand were synthesized in a similar manner as that of Lippert *et al.*[6] and Lock *et al.*[5] An aqueous mixture (60 mL) of *cis*-$[PtI_2(NH_3)_2]$ (3.38 g, 7.0 mmol) and $AgNO_3$ (2.37 g, 13.9 mmol) was stirred overnight at 60 °C in the dark, and the precipitated AgI was filtered off. To the light yellow filtrate was added 1-methylthymine (0.98 g, 7.0 mmol), and pH of the solution was adjusted to 6.5. The mixture was stirred at 37 °C for 2 weeks. The greenish yellow solution was cooled in an ice bath for 1.5 h, and then black precipitate was filtered off. After ethanol (100 mL) was added to the filtrate, the gray precipitate produced was removed. The filtrate was substantially reduced in volume in the air, and the gray precipitate was again filtered off. The final greenish yellow solution was allowed to stand at room temperature until a yellow precipitate was obtained. This precipitate was collected, washed with ethanol and acetone, and recrystallized from water at 4 °C. Yellow crystals were obtained (**1**). Anal. ($C_{12}H_{26}N_{10}O_{10}Pt_2$) C, H, N. From the above residual mixture was formed a yellow-green crystal at 4 °C (**2**). Anal. ($C_{12}H_{28}N_{10}O_{11}Pt_2$) C, H; N: calcd, 15.94; found, 15.27. The configuration of **1** was decided to be H-H by X-ray structural analysis. On the other hand, the configuration of **2** was estimated to be H-T, since **2** did not produce CDDP by the reaction with chloride anion (*cf.* Scheme 1).

Bis(*μ*-1-ethylthyminato-*N3,O4*)bis(*cis*-diammineplatinum(II)) Dinitrate [$[Pt_2(C_7H_9N_2O_2)_2(NH_3)_4](NO_3)_2\cdot 2H_2O$, Head-to-Tail, 3]. The Pt(II) dimer complex with 1-ethylthymine ligand was synthesized in a manner closely similar to that of **1** and **2**. *cis*-$[PtI_2(NH_3)_2]$ (0.78 g, 1.62 mmol) was stirred overnight with $AgNO_3$ (0.55 g, 3.21 mmol) at room temperature in the dark. Precipitated AgI was filtered off, giving a light yellow solution. 1-Ethylthymine was dissolved into the solution, and the pH was adjusted to 6.5. The reaction mixture was stirred at 40 °C until completeness. The reaction was followed by HPLC (ODS column, acetonitrile/1 mM HNO_3 55/45 v/v). The volume of the solution was reduced by evaporation and white-yellowish precipitate was removed. The residue was allowed to evaporate in air for 2 days and then cooled in a refigerator to 4 °C, affording yellow crystals. The product was recrystallized from water. Anal. ($C_{14}H_{34}N_{10}O_{12}Pt_2$) C, H, N: calcd, 15.15; found, 14.52. The configuration of **3** was decided to be H-H by X-ray structural analysis.

Bis(*μ*-1-propylthyminato-*N3,O4*)bis(*cis*-diammineplatinum(II)) Dinitrate [$[Pt_2(C_8H_{11}N_2O_2)_2(NH_3)_4](NO_3)_2$, Head-to-Head, 4; $[Pt_2(C_8H_{11}N_2O_2)_2(NH_3)_4](NO_3)_2\cdot 2H_2O$, Head-to-Tail, 5]. The Pt(II) dimer complexes with 1-propylthymine ligand were also synthesized in a similar manner to that of **1** and **2**. *cis*-$[PtI_2(NH_3)_2]$ (0.86 g, 1.77 mmol) was stirred overnight with $AgNO_3$ (0.60 g, 3.53 mmol) at room temperature in the dark. Precipitated AgI was filtered off, giving a light yellow solution. 1-Propylthymine was dissolved in the solution, and the pH was adjusted to 6.5. The reaction mixture was stirred at 50 °C until completeness. The reaction was followed by HPLC (ODS column, acetonitrile/1 mM HNO_3, 55/45 v/v). After filtration, the resultant clear brown solution was allowed to evaporate in air for a day. The white precipitate was filtered and recrystallized from water to give yellow needles (**4**). Anal. ($C_{16}H_{34}N_{10}O_{10}Pt_2$) H, N; C: calcd, 20.96; found, 18.77. The filtrate was evaporated in an open beaker for 2 days and then cooled in a refrigerator to 4 °C, affording green crystals (**5**). The products were recrystallized from water. The green crystals rapidly lost crystal water and turned into green powder. Anal. ($C_{16}H_{38}N_{10}O_{12}Pt_2$) C, H; N: calcd, 14.70; found, 14.27. The configurations of **4** and **5** were estimated to be H-H and H-T, respectively, from the reaction with chloride ion.

Bis(*μ*-acetato-*O,O′*)bis(*cis*-diammineplatinum(II)) Dinitrate [$[Pt_2(C_2H_3O_2)_2(NH_3)_4](NO_3)_2\cdot H_2O$, 6]. *cis*-$[PtI_2(NH_3)_2]$ (1.45 g, 3.0 mmol) was aquated by a reaction with $AgNO_3$ (1.01 g, 5.94 mmol) at room temperature in the dark. Precipitated AgI was filtered off, giving a light yellow solution. Acetic acid (0.172 mL, 3.0 mmol) was added, and the reaction mixture was stirred for 1 h at 70 °C. The solution was allowed to evaporate in an open beaker for 2 days and then cooled to 4 °C. The crystalline product was obtained from the solution as very small brown needles. Anal. ($C_4H_{20}N_6O_{11}Pt_2$) C, H; N: calcd, 11.70; found, 11.10. Although this crystalline product was not suitable for X-ray structural analysis, the complex is thought to be the platinum(II) dimer with bridging acetate as reported by Sakai *et al.*[7]

Other Complexes. All the other complexes were synthesized according to the literature.[6,8−11] The configuration of 1-MeUra Pt(II) dinuclear complex should be H-H, since it was prepared from the reaction between *cis*-$[Pt(NH_3)_2(H_2O)_2]$ and *cis*-$[Pt(1\text{-MeUra})_2(NH_3)_2]$ as reported.[6] The 1-MeCyt Pt(II) dinuclear complex was also synthesized in the same method as reported and thus should have an H-T arrangement.[10] The configurations of α-pyridone and PPB complexes were decided to be H-T and H-H, respectively, from the X-ray crystallographic data which agreed with those reported.[9,12]

Electrophilicity. Electrophilicity of the complexes was estimated by the reaction with chloride anion in saline (0.15 M NaCl) at 25 and 37 °C, where CDDP was formed from the dinuclear complexes. The amount of CDDP was determined by HPLC using an ODS column (Unisil Pack 250B type, 6.0 × 250 mm, GL Sciences Inc.) with water as an eluant.

Cellular Platinum Accumulation. The cellular platinum accumulation was examined on S-180 according to the method of Mistry *et al.*[13] The cells ($3-4 \times 10^6$) in exponential growth phase were exposed to the complex (100 μM) with various incubation times (1−3 h) at 37 °C in 5% CO_2. Immediately after the exposure the medium was aspirated, and the cells were washed twice with PBS. The cells were harvested in 0.5 mL of PBS and sonicated. For a protein determination, 50 μL of the cell sonicate was incubated with 200 μL of 1 N sodium hydroxide overnight at room temperature and subjected to the protein assay.[14] Cellular platinum concentrations were measured directly from the cell sonicate by Zeeman atomic

absorption spectroscopy. The platinum concentration in the samples was measured by the internal additions method using platinum standard solutions in 0.2% nitric acid. The amount of accumulated Pt was expressed as nmol/mg of protein.

Cytotoxicity. S-180 cells (1×10^5/well) were exposed to various concentrations (3.75−30 μM) of the complex for 2 days (37 °C, 5% CO_2). Culture medium was used as a negative control and MMC (mitomycin C) as a positive control. After the exposure the medium was removed and 200 μL of Trypsin-EDTA was added to each well. After the detachment of cells was confirmed, 700 μL of stop solution was added. The cell suspension was transferred to a tube containing 4 mL of saline for counting.

Conclusion

Five Pt(II) dimer complexes with 1-alkylthymines were synthesized, and their cytotoxicity was investigated. It is thought that some fraction of the H-H dimer complexes in the intact form affected cancer cells, while the other fraction is transformed to CDDP or to complexes with serum proteins.

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Appendix

The following scheme is assumed

$$\underset{(a_0 - z)}{\text{dimer}} + \underset{(c_0)}{2Cl^-} \xrightarrow{k_1} \underset{(z)}{\text{CDDP}} + \text{monomer}$$

$$\underset{(a_0 - z)}{\text{dimer}} \xrightarrow{k_d} \underset{(p)}{\text{Pt in cancer cell}}$$

$$\underset{(z)}{\text{CDDP}} \xrightarrow{k_c} \underset{(p)}{\text{Pt in cancer cell}}$$

where k_1, k_d, and k_c are the rate constants for CDDP formation, accumulation of dimer to cancer cells, and accumulation of CDDP to cancer cells, respectively, a_0 is the initial concentration of dimer, c_0 is the concentration of Cl^- which is considered to be constant (0.15 M), z is the concentration of CDDP at time t, and p is the total amount of Pt in cells at time t. It is assumed that the amount of Pt accumulated in cancer cells is much smaller than that of dimer and CDDP outside cells. Thus eqs 1 and 2 are obtained.

$$\frac{dp}{dt} = k_d(a_0 - z) + k_c z \quad (1)$$

$$\frac{dz}{dt} = k_1(a_0 - z) \quad (2)$$

Equations 1 and 2 lead to eq 3.

$$p = k_d a_0 \left\{ \frac{1 - \exp(-k_1 {c_0}^2 t)}{k_1 {c_0}^2} \right\} + k_c a_0 \left\{ t + \frac{\exp(-k_1 {c_0}^2) - 1}{k_1 {c_0}^2} \right\} \quad (3)$$

Putting the experimentally obtained values [$k_1 {c_0}^2 = 0.792$ (h^{-1}), $k_d a_0 = 0.20$ (nmol mg protein^{-1} h^{-1}), and $k_c a_0 = 0.58$ (nmol mg protein^{-1} h^{-1})] into eq 3, the curve shown in Figure 4 is obtained.

Supporting Information Available: Crystallographic properties, atomic coordinates, bond length and angles, and anisotropic thermal parameters for complexes **1** and **3** (12 pages). Ordering information is given on any current masthead page.

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