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Unprecedented Sugar-Dependent In Vivo Antitumor Activity of Carbohydrate-Pendant *cis*-Diamminedichloroplatinum(II) Complexes

Yuji Mikata,[a,*] Yoshie Shinohara,[b] Kazumi Yoneda,[c] Yuka Nakamura,[b] Izabela Brudziñska,[c] Tomoaki Tanase,[b] Takashi Kitayama,[d] Rie Takagi,[d] Tadashi Okamoto,[d] Isamu Kinoshita,[e] Matsumi Doe,[e] Chris Orvig[f] and Shigenobu Yano[c,*]

[a]*KYOUSEI Science Center, Nara Women's University, Nara 630-8506, Japan*
[b]*Department of Chemistry, Nara Women's University, Nara 630-8506, Japan*
[c]*Division of Material Science, Nara Women's University, Nara 630-8506, Japan*
[d]*Department of Agricultural Chemistry, Kinki University, Nara 631-8505, Japan*
[e]*Department of Chemistry, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan*
[f]*Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver BC, Canada V6T 1Z1*

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Abstract—Eight carbohydrate-pendant platinum(II) complexes have been synthesized from carbohydrate–diamine conjugates. D-Glucose, D-mannose, D-galactose, D-xylose, and L-glucose are attached to the dichloroplatinum(II) moiety by 1,3- or 1,2-diaminopropane chelates through with an *O*-glycoside bond. All the carbohydrate moieties reduced the toxicity inherent with platinum(II) complexes.

Carbohydrates are an important element in many natural antibiotics, including bleomycin, neocarzinostatin chromophore, and other DNA-targeting drugs.[1] The role of the carbohydrate moiety includes enhancement of DNA sequence specificity and cell recognition.[2] Other properties augmented by carbohydrates are membrane permeability, water solubility, and chirality of chromophores or DNA damaging molecules. These characteristics suggest that introduction of carbohydrate moieties into synthetic drugs should generate hybrid molecules of considerable interest. Examples of such compounds include porphyrin derivatives as chromophores for photodynamic therapy[3] and other glycoconjugated drug candidates.[4]

Cisplatin [*cis*-diamminedichloroplatinum(II), *cis*-DDP] is an effective anticancer drug.[5] It binds to DNA after loss of its chloride leaving groups by aquation and/or hydrolysis. Cisplatin induces significant bends into the DNA structure upon binding to the N7 atoms of guanine bases of the d(GpG) sequence. The recognition of these bent structures by high-mobility group (HMG) proteins is believed to play an important role in the anticancer activity of cisplatin.[5,6] Because the importance of the interactions between carbohydrates and proteins is well known, the construction of carbohydrate–cisplatin conjugates[7,8] is of significant interest. Recently, we established a general and convenient synthesis of carbohydrate–diamine hybrid molecules utilizing peracetylated glycopyranoses.[9] Here we report a systematic synthesis of carbohydrate-pendant cisplatin derivatives and their in vivo antitumor activity.

Eight carbohydrate-pendant platinum(II) complexes (Fig. 1) have been synthesized from K_2PtCl_4 and the corresponding carbohydrate–diamine conjugate[9] in aqueous solution (4–55% yield). D-Glucose, D-mannose, D-galactose, D-xylose, and L-glucose are attached

*Corresponding authors. Tel.: +81-742-20-3095; fax: +81-742-20-3095; e-mail: mikata@cc.nara-wu.ac.jp (Y. Mikata); yano@cc.nara-wu.ac.jp (S. Yano).

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to the dichloroplatinum(II) moiety by 1,3- or 1,2-diaminopropane chelates through with an *O*-glycoside bond. This series of compounds includes the α and β anomers for the D-galactose derivative (**3** and **4**), the D and L isomers for the glucose derivative with a chiral 1,2-diaminopropane linker (**6** and **7**), and both six- and five-membered chelate ring size isomers with D-glucose (**1** and **6**) and D-xylose (**5** and **8**). All of the compounds had NMR, CD, MS, and elemental analyses consistent with their proposed structures.[10]

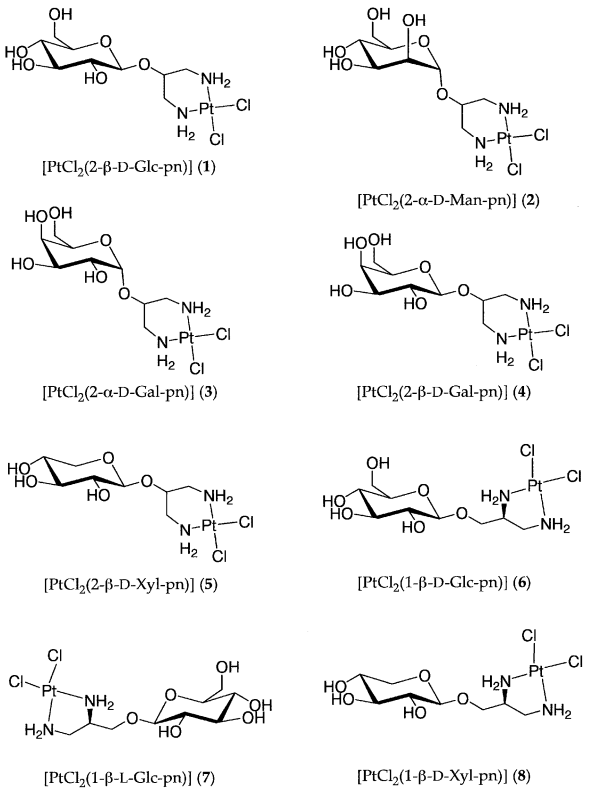

Figure 1. Structures of platinum(II) complexes **1**–**8**.

Wang and coworkers have determined the crystal structure of compound **1**.[7c] In the present study we investigated the solution structure of all the platinum complexes by NMR in D_2O. A portion of the ^1H NMR spectrum for **6** is shown in Figure 2. The diamine moiety of the carbohydrate–diamine conjugate afforded a well-defined coordination environment for the platinum atom. The structural similarity in both the solid and solution states was confirmed for compound **1**. ^1H–^1H coupling constants reveal that the carbohydrate moiety is in the axial position of the six-membered chelate ring for 1,3-diaminopropane derivatives **1**–**5** ([$PtCl_2$(2-sugar-pn]), but in the equatorial position for the five-membered ring of 1,2-diaminopropane derivatives **6**–**8** ([$PtCl_2$(1-sugar-pn)]) (Fig. 3).[11]

Since compound **1** has promising anticancer activity against several cancer cell lines,[7c] in vivo studies of this type of platinum(II) complex having different carbohydrate groups is of significant interest. The anticancer activity of these platinum(II) complexes was investigated against P388 cells implanted in mice. Cancer cells

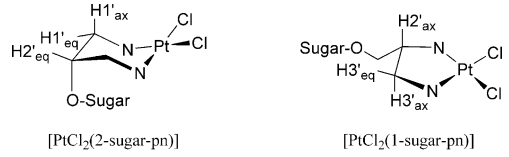

Figure 3. Chelate ring conformations in D_2O as derived from ^1H NMR spectroscopy.

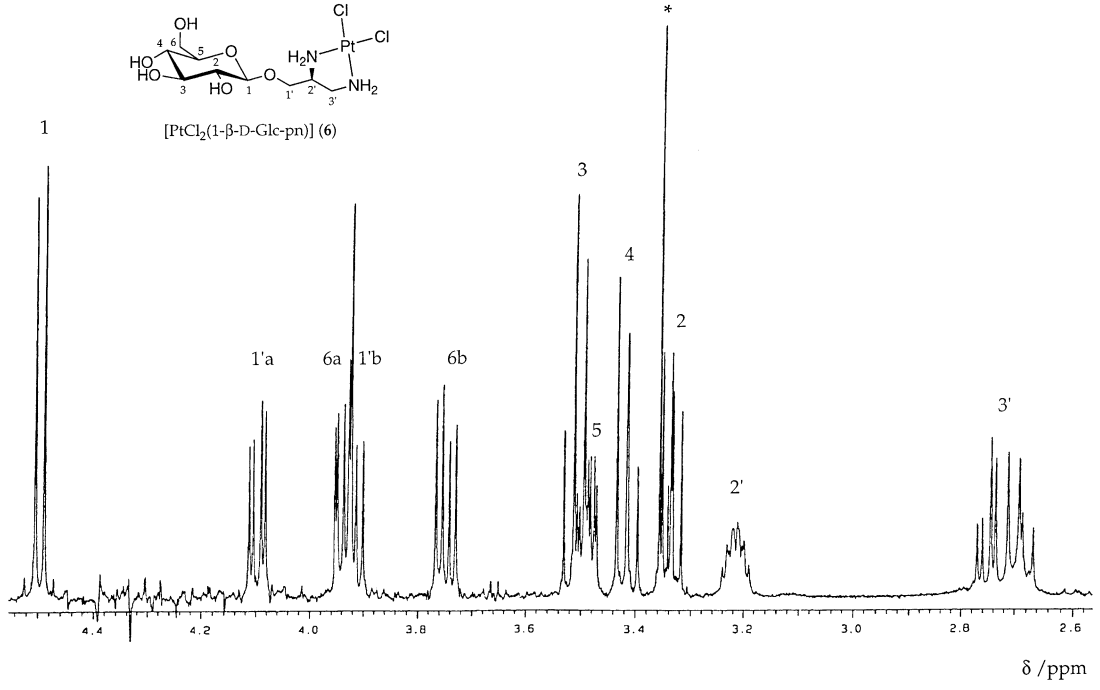

Figure 2. Partial 500 MHz ^1H NMR spectrum for **6** in D_2O. Asterisk indicates a signal from a small amount of methanol.

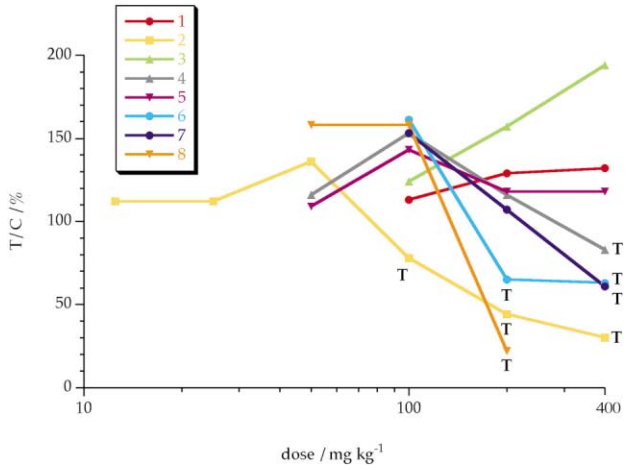

Figure 4. Antitumor activity of carbohydrate-pendant platinum(II) complexes **1**–**8**. T indicates more than half of the mice died within day 5.

(10^6 of P388 cell) were transplanted intraperitoneally (ip) into CDF1 mice (6 mice/group), followed by ip administration of the drugs to the mice on days 1 and 5. The mean survival time of the treated group (*T*) was compared with that of the untreated control group (*C*) (Fig. 4). A *T*/*C* value of 194% was obtained for the α-D-galactose derivative **3** at 400-mg/kg dose, whereas the β-D-galactose derivative **4** showed low toxicity at the same dose. Significant toxicity was observed for the mannose derivative **2**, even at a dose of 100 mg/kg. Because the LD_{50} of cisplatin is approximately 10 mg/kg,[7a] all the carbohydrate moieties reduced the toxicity inherent with platinum(II) complexes significantly. The linker structure also affects the antitumor activity of **1** versus **6**, or **5** versus **8**; however, D- and L-glucose derivatives (**6**, **7**) showed similar dose/activity profiles.

Selective uptake in cancer cells or specific interaction between drug-damaged DNA and recognition protein may cause the difference in antitumor activity of these complexes. Another possible explanation is the prodrug hypothesis, according to which all carbohydrate-pendant platinum(II) complexes have poor cell-membrane permeation properties. Selective hydrolysis of the carbohydrate moiety in the blood stream to generate the active drug may explain the sugar-dependent antitumor activity. This hypothesis may correspond to the reducing effect of cytotoxicity of the platinum complexes by sugar moiety.

These data suggest that the appropriate carbohydrate and/or linker increases the clinical activity of carbohydrate-pendant platinum(II) complexes. The synthesis of new platinum complexes bearing other carbohydrate moieties, as well as further study to elucidate the detailed mechanism of the carbohydrate effect is now in progress in our laboratories.

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10. Elemental analyses: Anal. calcd (found) for **1**·H_2O $PtCl_2C_9H_{20}N_2O_6$·H_2O: C, 20.16 (20.10); H, 4.13 (4.21); N, 5.22 (5.12)%. Anal. calcd (found) for **2** $PtCl_2C_9H_{20}N_2O_6$: C, 20.86 (20.37); H, 3.89 (3.64); N, 5.41 (5.37); Cl, 13.68 (13.87)%. Anal. calcd (found) for **3** $PtCl_2C_9H_{20}N_2O_6$: C, 20.86 (20.34); H, 3.89 (3.73); N, 5.41 (5.15)%. Anal. calcd (found) for **4**·H_2O $PtCl_2C_9H_{20}N_2O_6$·H_2O: C, 20.16 (20.48); H, 4.13 (3.66); N, 5.22 (4.90); Cl, 13.22 (13.60)%. Anal. calcd (found) for **5** $PtCl_2C_8H_{18}N_2O_5$: C, 19.68 (19.45); H, 3.72 (3.85); N, 5.74 (5.62)%. Anal. calcd (found) for **6**·H_2O $PtCl_2C_9H_{20}N_2O_6$·H_2O: C, 20.16 (20.13); H, 4.13 (4.17); N, 5.22 (5.23)%. Anal. calcd (found) for **7**·H_2O $PtCl_2C_9H_{20}N_2O_6$·H_2O: C, 20.16 (20.15); H, 4.13 (3.87); N, 5.22 (5.23); Cl, 13.22 (13.42)%. Anal. calcd (found) for **8**·H_2O $PtCl_2C_8H_{18}N_2O_5$·H_2O: C, 18.98 (18.94); H, 3.98 (3.82); N, 5.53 (5.62); Cl, 14.01 (14.17)%.
11. The absence of a large coupling ($J > 7$ Hz) between H2′ and H1′ (or H3′) in the six-membered chelate complex ([$PtCl_2$ (2-sugar-pn)]) precludes an axial conformation for H2′.