counter (Coulter Electronics Ltd., Harpenden, Herts, England). The ID_{50} (50% inhibitory dose) was defined as the concentration of compound that reduced the number of living cells by 50%.

Preparation of L1210/0 Cell Extracts. One-milliliter cell pellets were first washed with cold PBS (phosphate-buffered saline), and then 3 mL of suspension buffer (10 mM potassium phosphate buffer, pH 7.5, containing 0.01 M β-mercaptoethanol and 0.1 M KCl) was added. The cell suspension was sonicated two times for 10 s and cleared by centrifugation at 25000*g* for 30 min. The 30–70% $(NH_4)_2SO_4$ precipitate of the cell homogenate was resuspended in 1 mL of suspension buffer, dialyzed against the same buffer for 2–3 h at 4 °C, and stored in aliquots at −20 °C.

Thymidylate Synthetase Assay. The cell extracts were assayed for dTMP synthetase activity in a standard reaction mixture containing 0.26 mM tetrahydrofolate, 5.0 mM formaldehyde, 15 mM β-mercaptoethanol, 0.1 M NaF, 45 μM (0.025 μCi) [5-^{3}H]dUMP, and an appropriate amount of inhibitor (FdUrd or any of its derivatives) in a total volume of 30 μL of 0.05 M potassium phosphate buffer, pH 7.5. The reaction was initiated by addition of 10 μL of cell extract. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was terminated by the addition of 160 μL of a charcoal suspension (at 100 mg/mL in 2% trichloroacetic acid). After centrifugation for 10 min at 1000*g*, 100 μL of supernatant was assayed for radioactivity in a toluene-based scintillant.

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Synthesis and Antitumor Activity of *cis*-Dichloroplatinum(II)–N-Aminated Nucleoside Complexes†

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N-Aminated nucleoside complexes of *cis*-dichloroplatinum(II) were synthesized, and their antitumor activities against L1210 cells in mice and in vitro were studied. While the native nucleosides failed to show any antitumor activity, the complexes exhibited high antitumor activity and had no nephrotoxicity in mice. Studies on their mode of action in vitro indicated that the ligands played characteristic roles in the appearance of antitumor activity; ribonucleoside complexes caused the inhibition of RNA synthesis, and deoxyribo- or arabinonucleoside complexes caused the inhibition of DNA synthesis.

cis-Diamminedichloroplatinum(II) (*cis*-DDP) is an inorganic platinum compound that has been shown to possess antitumor activity in a variety of animal tumor models.[2,3] Clinical studies with *cis*-DDP have shown it to be effective in the treatment of human solid tumors.[4,5] Furthermore, various platinum(II) complexes, such as that of 1,2-diaminocyclohexane, which has an altered structure in the ligand moiety, have been synthesized and tested on some tumor systems in mice.[6]

In our previous papers, we have described how SeG–Pt(II) and TG–Pt(II) complexes show antitumor activity against L1210 cells in mice and how that activity was mainly dependent upon the ligand SeG or TG.[7,8] We have also reported that SeG–Pt(II) had a direct cytotoxicity against L5178Y cells in vitro.[9] However, very few papers have supported the concept that the ligand plays a characteristic role in the antitumor action of the *cis*-DDP complexes.

The present study was conducted to synthesize the *cis*-dichloroplatinum(II)–N-aminated nucleoside complexes 3-aminocytidine–dichloroplatinum(II) (**8**), 3-amino-2′-deoxycytidine–dichloroplatinum(II) (**9**), 3-amino-1-β-D-arabinofuranosylcytosine–dichloroplatinum(II) (**10**), 3-aminotubercidin–dichloroplatinum(II) (**13**), and 5-amino-1-β-D-ribofuranosylimidazol-4-carboxamide–dichloroplatinum(II) (**15**) and to examine their antitumor effects against L1210 cells in mice and in vitro. In addition, the nephrotoxicity of these complexes on mice was examined and compared to that of *cis*-DDP.

Results and Discussion

Synthesis and Structure Elucidation of the Complexes. N-Amination reaction of nucleosides was carried out by using 2,4-dinitrophenoxyamine (**1**) as an aminating agent, which is directed toward the most basic endo nitrogen in a nucleoside. The reaction site in a nucleoside has been well established as position 3 in cytidine and position 1 in adenosine.[10]

It has been reported that 3-aminocytidine hydrochloride (**5**) reacts with orthoesters, such as ethyl orthoformate or ethyl orthoacetate, to give fused heterocyclic bases.[11] Also, 3-aminouridine reacts with acetic anhydride to afford the *N*-acetyl derivative.[11] Therefore, it is reasonable to assume

† A part of this work was presented at the 10th Symposium on Nucleic Acid Chemistry, Kyoto, Japan, Nov 24–26, 1982. For the abstract, see ref 1. The following abbreviations are used: *cis*-DDP, *cis*-dichlorodiammineplatinum(II); SeG–Pt(II), selenoguanine–platinum(II) complex; TG–Pt(II), thioguanine–platinum(II) complex; SeG, selenoguanine; TG, thioguanine.

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Table I. Elemental Analyses of N-Aminated Nucleoside-$PtCl_2$ Complexes

compd	formula	elemental analysis									
		found					calcd				
		C	H	N	Pt	Cl	C	H	N	Pt	Cl
8	$C_9H_{14}N_4O_5PtCl_2 \cdot {}^1/_2H_2O$	20.63	2.82	10.57	37.56	13.80	20.90	2.92	10.83	37.72	13.71
9	$C_9H_{14}N_4O_4PtCl_2 \cdot H_2O$	20.88	2.97	10.60	37.92	13.64	21.12	3.16	10.98	38.24	13.90
10	$C_9H_{14}N_3O_5PtCl_2 \cdot H_2O$	20.34	2.75	10.54	37.41	13.12	20.53	3.06	10.65	37.07	13.48
13	$C_{11}H_{15}N_5O_4PtCl_2 \cdot H_2O$	23.41	3.33	12.37	34.65	12.34	23.37	3.03	12.39	34.51	12.54
15	$C_9H_{14}N_4O_5PtCl_2 \cdot 2H_2O$	19.13	3.45	10.37	34.56	12.48	19.29	3.24	10.00	34.82	12.66

Table II. UV and IR Data of N-Aminated Nucleoside-$PtCl_2$ Complexes and Related Compounds

compd	yield, %	UV λ_{max}, nm			IR[a]
		pH 1	H_2O	pH 13	
5	90	278	278	unstable	3290-3410 (br, s) (OH, NH_2), 3060-3100 (br, s) (CH, NH), 1712 (s) (C=O), 1660 (s) (C=N), 1625 (s), 1340 (s), 1280 (s), 755 (m)
6	87	278	278	unstable	3310-3370 (s) (OH, NH_2), 3080 (CH, NH), 1725 (vs) (C=O) 1640 (vs) (C=N), 1602 (s), 1292 (s), 1193 (m), 1075 (m), 760 (w)
7	93	277	277	unstable	3120-3370 (s) (OH, NH_2), 3090-3140 (br, s) (CH, NH), 1725 (vs) (C=O), 1650 (vs) (C=N), 1600 (s), 1420 (br, w), 1290 (s), 1110 (s), 1010 (m), 750 (m)
8	92	244, 306	245, 307	unstable	3400 (br, s) (OH, NH_2), 1700 (s) (C=O), 1673 (vs) (C=N), 1280 (m), 1050 (m), 740 (w)
9	84	243, 305	243, 308	unstable	3400 (br, s) (OH, NH_2), 1710 (vs) (C=O), 1645 (vs) (C=N), 1280 (m), 1055 (m), 740 (w)
10	85	243, 305	242, 306	unstable	3400 (br, s) (OH, NH_2), 1700 (s) (C=O), 1540 (vs) (C=N), 1285 (m), 1050 (m), 740 (w)
12	95	271	271	unstable	3200-3400 (br, s) (OH, NH_2), 3080-3200 (br, s) (CH, NH), 1680 (vs) (C=N), 1645 (m) (C=C), 1510 (m), 1450 (w), 1260 (w), 1050 (s), 740 (m)
13	82	223 (sh), 280	282	unstable	3400 (br, s) (OH, NH_2), 1640 (vs) (C=N), 1500 (m) (C=C), 1055 (m), 720 (w)
14		247, 268	267	267	3300-3400 (br, s) (OH, NH_2), 3100 (m) (CH), 1640 (vs) (C=O), 1570 (s) (C=N), 1340 (m), 1120 (s), 1080 (m), 870 (w), 730 (w)
15	98	252, 330 (sh)	252, 330 (sh)	252, 330 (sh)	3350 (br, s) (OH, NH_2), 1630 (vs) (C=O), 1570 (m) (C=C), 1075 (m), 760 (w)

[a] Absorption strength of the IR bands are indicated in parentheses as follows: br, broad; vs, very strong; s, strong; m, medium; w, weak; sh, shoulder.

that the amino and imino groups in the N-aminated nucleosides, which are located at the ortho positions on the heteroaromatic rings, would react with the tetrachloroplatinate to give the desired *cis*-dichloroplatinum(II)–nucleoside complexes. Three 3-aminocytosine nucleosides (**5**–**7**) and 3-aminotubercidin (**12**)[10] were reacted with tetrachloroplatinate to afford the less soluble chelated compounds.

The complexes were synthesized according to the usual method[12] in which the corresponding N-aminated nucleoside hydrochloride was reacted with an equimolar amount of potassium tetrachloroplatinate in aqueous solution at 40–50 °C for 2 h to yield yellow or gray precipitates. The reaction schemes are shown in Scheme I. The structure of each product was determined by means of UV, IR, NMR, and elemental analysis.

The elemental analysis data showed a one to one molar ratio of platinum to nucleoside, as shown in Table I.

The UV spectra of the complexes (**8**–**10**) showed absorption maxima at 243 and 305 nm in 0.1 N HCl and at 243 and 307 nm in aqueous solution. The parent 3-aminocytosine derivatives (**5**–**7**), on the other hand, showed absorption maxima at 278 nm in both 0.1 N HCl and neutral solutions. These observed red shifts (~30 nm) are due to the formation of the complexes. It is known that interactions of naturally occurring nucleoside complexes with platinum cause red shifts. The site of these interactions is position 3 of the cytosine ring and position 1 of the adenine ring.[13] Their UV spectra were identical at both pH 1 and 9, even though the N^4-imino nitrogen became less basic after complexation. For **13**, the UV absorption maxima differed only slightly at acidic and neutral pH (at pH 1, λ_{max} 280 nm; at pH 7, λ_{max} 282 nm) but did show a red shift of 12 nm when compared to the parent **12**. All of these complexes were unstable in alkaline solution and gave shorter UV absorption maxima, which could be explainable by cleavage of the C_2–N_3 bond, depending upon the length of exposure time to base. This phenomenon is observed for any of the parent N-aminated nucleosides that have a quaternary structure at position 3 of the pyrimidine ring as previously reported.[11,14] The lability in alkaline solution indicates that the skeletal structure of the ligand is retained after complex formation with the platinum. The UV data are summarized in Table II.

In the IR spectra, the ν (C=N) vibration was moved to lower frequencies with the chelation to the platinum when compared to the parent N-aminated nucleoside hydro-

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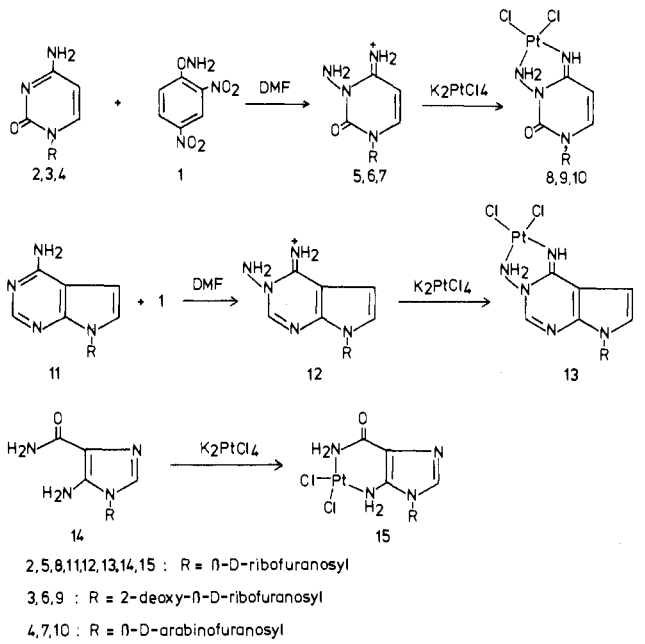
Scheme I



chlorides (**5**–**7**). The ν (N–H) vibration at 3300 cm^{-1} was shifted to 3400 cm^{-1}, and the 3100-cm^{-1} band of the immonium ion was decreased in intensity.

The ν (C=O) vibration at 1660 cm^{-1} in free cytidine is shifted to 1717 cm^{-1} in the complex.[15] These data indicate that the N-3 position of cytidine is crucial for complex formation with platinum. However, in the present study, the N-3 position of the cytidine nucleosides and of tubercidin was substituted with an amino group, which formed a more basic *exo*-imino group. The pK_a value of the 3-methylcytidine appears at 8.7, which corresponds to the deprotonation of the *exo*-immonium moiety.[16] Here the ν (C=O) vibration is moved to lower frequencies upon complexation with platinum when compared to that of the parent *N*-amino nucleoside hydrochloride. The shift in the opposite direction to that seen in the free cytidine and the shift of the ν (C=N) and ν (N–H) vibrations mentioned above indicate that the complex achieves a more neutral form than the parent N-aminated nucleoside during chelation. All these data suggest that the nitrogen atoms chelate with the platinum. The IR data are summarized in Table II.

The ^1H NMR spectra were measured in D_2O solution, and the chemical-shift data are shown in Table III. The H(1′) and H(5) resonances of the pyrimidine complexes are shifted slightly upfield with respect to the parent *N*-amino nucleoside hydrochloride. However, the H(6) resonance in all three cytosine–platinum(II) complexes is shifted about 0.5-ppm upfield. The protons in the sugar moeity do not change their chemical shifts. Chu et al.[17] have reported that a very small downfield shift is observed for a bis platinum complex of cytidine measured at pH 5.4. They also noted that the H(5) and the H(6) peaks shift upfield with the formation of a platinum complex with uridine. In these experiments, the parent nucleosides have a positive charge on the pyrimidine ring, and the upfield shifts are possible due to the reduction of that charge during complexation.

Table III. ^1H NMR Chemical Shifts of N-Aminated Nucleoside-$PtCl_2$ Complexes and Related Compounds[a]

compd	position 1′-H	5-H	6-H
5	6.35	6.77	8.56
6	6.69	6.75	8.50
7	6.68	6.78	8.50
8	6.33	6.61	8.07
9	6.70	6.59	8.03
10	6.61	6.73	8.00
2	6.32	6.46	8.27
2·HCl	6.20	6.68	8.41

compd	position 1′-H	2-H	5-H	6-H
12	6.68	7.70	7.34	8.04
13	6.68	8.21	7.65	8.06

[a] The chemical-shift values are shown in δ units from external Me_4Si capillary, and the data are not calibrated.

Similar shifts were observed for **13** in the ^1H NMR spectra. H(1′) and H(6) were unchanged in chemical shift, while H(2) was shifted upfield by 0.68 ppm and H(5) was shifted downfield. The upfield shift for H(2) is due to a decrease in the positive charge on the pyrimidine ring, and the downfield shift for H(5) is due to a deshielding effect by the platinum atom adjacent to the H(5).

The coupling constants (J_{5-6}) in **8**–**10** and **13** were unchanged by chelation with the platinum. No coupling of the ^{195}Pt to either the H(2), the H(5), or the H(6) of the complexes could be detected. The reason for this is not clear, but it is reasonable to assume that either the angle or the bond length between the ^{195}Pt and the H(2), the H(5), or the H(6) is not suitable for coupling. The ^1H NMR spectra are consistent with the UV, IR, and elemental analysis data.

The platinum coordination site in the cytidine has been reported to be the free base form of N-3.[18] However, in a 3-aminated nucleoside–platinum complex, the metal could be bound to the *N*-amino and the 4-imino groups to form a fused five-membered ring.

It is known that for antitumor activity, a complex requires the following: (1) the complex must be neutral; (2) the central metal atom should be platinum with an oxidation state of 2+ or 4+; (3) the complex should contain a pair of cis leaving groups of intermediate lability; and 4) the complex should contain a relatively inert carrier ligand.[19] Based on all of the spectral data and bioassay results, these complexes satisfy the above requirements.

Bioassay Results. The bioassay results indicate that all four of the newly synthesized *cis*-dichloroplatinum(II)–nucleoside complexes possess high antitumor activity against L1210 cells in vivo in a wide dose range from 10 to 200 mg/kg, as shown in Table IV. Compound **13** showed the widest effective dose range, while **10** (200 mg/kg) exhibited the highest T/C value, followed by **9** and **8**. It should be emphasized that none of the uncomplexed ligands, **5**, **6**, **7**, or **12**, possess antitumor activity at the corresponding dose. Therefore, the antitumor activity is due to the complex itself. While the complexes of the cytosine derivatives, containing the same active *cis*-dichloroplatinum function and the same base, differ only in

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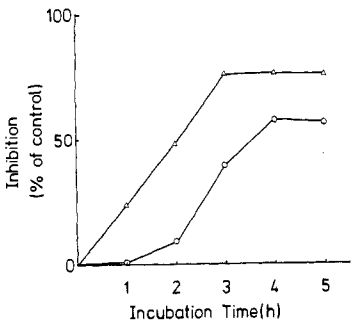

Figure 1. Effect of 200 μg/mL of **9** on the incorporation of isotopically labeled thymidine and uridine into the acid-insoluble fraction of L1210 cells. Both the complex and the [^{14}C]thymidine- and [^{3}H]uridine-containing media were applied to cells at time zero. The open triangle and open circle represent inhibition against control cells of [^{14}C]thymidine and [^{3}H]uridine, respectively.

their sugar moieties, their bioassay results were markedly different. This suggests that the sugar moieties of ribose, deoxyribose, and arabinose play an important role in the expression of the antitumor activity.

When an equimolar mixture of *cis*-DDP and N-aminated nucleoside was administered intraperitoneally, it caused only toxicity. This eliminates the mechanistic possibility that the complexes exhibit activity only after decomposition into their parent forms. The in vitro mode of action implicating the critical role of the sugar portion to exhibit activity will be discussed later. Compounds containing ribose, **8** and **13**, were less active than those containing deoxyribose, **9**, or arabinose, **10**. However, the toxicity of the ribose complexes was less than that of the deoxyribose or the arabinose complexes.

Only **14** showed no activity in doses of 25–200 mg/kg and exhibited a minimum effective value (T/C = 125%) at 800 mg/kg. This is due to the poor basicity of the ligand, since one of the nitrogens in the complex is an amide.

In Vitro Studies. The direct cytocidal effects of the complexes were studied using L1210 cells in vitro. The complexes showed much higher cytocidal effects than the parent N-aminated nucleosides (Table V). These results also showed that the *cis*-dichloroplatinum function is necessary for activity. The IC_{50} values of the complexes were higher than that of *cis*-DDP by factors of 10 to 10^3. Although the cytocidal effect of **12** is a similar order to the corresponding platinum complex, the compound did not exhibit any antitumor activity in vivo and had no toxicity against the host animals in doses of 114 mg/kg (Table IV). The order of cytotoxicity in vitro is **10** > **13** > **9** > **8**. This does not correspond to the order of their antitumor potency in vivo.

In Vitro Studies on the Mode of Action. To examine if the complexes inhibit only DNA synthesis to express their antitumor activity in vivo as *cis*-DDP does, we carried out the incorporation of isotopically labeled precursors ([^{14}C]thymidine, [^{3}H]uridine, or [^{14}C]leucine) into the acid-insoluble fraction via the standard methods in vitro using L1210 cells. The complexes did not inhibit the incorporation of [^{14}C]leucine into the acid-insoluble fraction under the conditions used; the complexes did not inhibit protein synthesis. The results are similar to those previously observed for *cis*-DDP and its analogues.[20]

Compounds **9** and **10** did inhibit [^{14}C]thymidine incorporation into the acid-insoluble fraction, as shown in Figures 1 and 2. These results agree with those for *cis*-DDP, which only inhibits DNA synthesis in vivo and in vitro.[21,22] Compounds **8** and **13**, however, showed a much

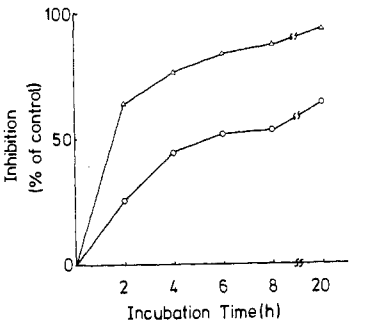

Figure 2. Effect of 200 μg/mL of **10** on the incorporation of isotopically labeled thymidine and uridine into the acid-insoluble fraction of L1210 cells; see legend of Figure 1.

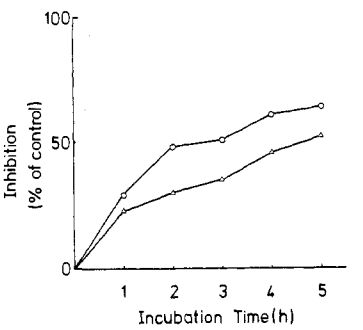

Figure 3. Effect of 400 μg/mL of **8** on the incorporation of isotopically labeled thymidine and uridine into the acid-insoluble fraction of L1210 cells; see legend of Figure 1.

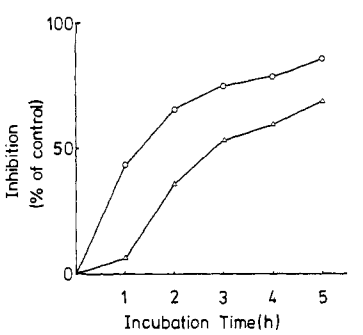

Figure 4. Effect of 200 μg/mL of **13** on the incorporation of isotopically labeled thymidine and uridine into the acid-insoluble fraction of L1210 cells; see legend of Figure 1.

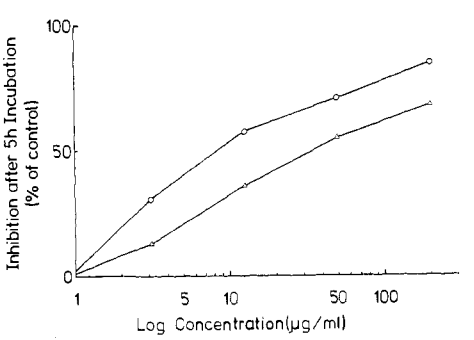

Figure 5. Concentration dependency of **13** on the incorporation of isotopically labeled thymidine and uridine into the acid-insoluble fraction of L1210 cells after 5-h incubation. The symbols used are the same as those used in Figure 4.

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Table IV. Effect of N-Aminated Nucleoside-$PtCl_2$ Complexes and Related Nucleosides on the Survival Time of Mice Bearing L1210 Cells

compd[a]	dose mg/kg × 2 days	dose mmol/kg × 2 days	median survival time, days	T/C,[b] %
8[c]	10	0.019	9.5	118.8
	25	0.048	10.0	125.0
	50	0.092	10.0	125.0
	100	0.191	10.5	131.3
	200	0.382	11.0	137.5
	300	1.572	11.0	137.5
9[c]	10	0.020	10.0	125.0
	25	0.049	10.5	131.3
	50	0.098	13.0	162.5
	100	0.198	14.5	181.3
	200	0.394	8.5	106.3
	300	0.591	toxic	
10[c]	10	0.019	10.0	125.0
	25	0.048	11.0	137.5
	50	0.095	12.0	150.0
	100	0.191	14.0	175.0
	200	0.382	15.5	193.5
	300	0.573	toxic	
13[c]	10	0.018	9.0	112.5
	25	0.049	10.0	125.0
	50	0.091	10.5	131.3
	100	0.183	11.5	143.8
	200	0.366	11.5	143.8
	300	0.548	12.0	150.0
	600	1.097	12.5	156.3
	900	1.645	12.5	156.3
15[c]	25	0.048	9.0	100.0
	50	0.095	9.0	100.0
	100	0.191	10.0	111.1
	200	0.382	11.0	122.2
	800	1.527	10.0	125.0
12	2	0.0063	11.0	110.0
	114	0.359	9.5	95.0
7	30	0.102	9.5	95.0
	112	0.380	10.0	100.0
6 + *cis*-DDP	55	0.197	toxic	
	59	0.197		
5 + *cis*-DDP	112	0.380	toxic	
	115	0.383		

[a] The sample was injected on days 1 and 5. [b] Ratio of the median survival time of the treated (T) vs. control (C). [c] Nikkol (7 times by weight) was used as a vehicle for a higher dose than 100 mg/kg.

Table V. IC_{50} Values of N-Aminated Nucleoside-$PtCl_2$ Complexes and Related Nucleosides on L1210 Cell Growth in Vitro[a]

compd[b]	IC_{50}[c] μg/mL	IC_{50}[c] M
8	64	1.22×10^{-4}
9	22	4.33×10^{-5}
10	1.9	3.63×10^{-6}
13	4.9	8.96×10^{-6}
5	400	1.36×10^{-3}
6	2000	7.18×10^{-3}
7	45	1.53×10^{-3}
12	2.7	8.50×10^{-6}
11	0.018	6.77×10^{-8}
cis-DDP	0.19	6.33×10^{-7}

[a] The cells (1×10^5) were cultivated in RPMI 1640 medium containing 10% fetal calf serum and 50 μg/mL of kanamycin at 37 °C for 48 h. [b] The sample was used as the medium solution. [c] The values were determined from the log probit dose-response curve.

greater inhibition of [^{3}H]uridine incorporation than for [^{14}C]thymidine (Figures 3 and 4). Figure 5 shows a typical example of the dose-dependent inhibition of both [^{3}H]-uridine and [^{14}C]thymidine incorporation into the acid-insoluble fraction after 5 h incubation with various concentrations of **13**. These results show that these two complexes inhibit [^{3}H]uridine incorporation into the acid-insoluble fraction at all incubation intervals, and in any dosage. This is very unusual, as the inhibition is probably caused by the *cis*-dichloroplatinum(II) with a suitable amine ligand. Although both **8** and **13** have this structure, those complexes having ribose inhibit RNA synthesis, while those with deoxyribose inhibit only DNA synthesis. This may not correlate completely with their antitumor activity in vivo. However, the order of the activity in vivo seems to be partially reflected in these in vitro results for their mode of action. The complexes that inhibited DNA synthesis showed higher activity in vivo than those complexes that inhibited RNA synthesis. Also, those that inhibited RNA synthesis showed a wider dose range.

This study clearly demonstrates that the sugar moiety in the complexes plays a critical role in their mode of action: nucleoside complexes having ribose inhibit RNA synthesis, and nucleoside complexes having deoxyribose or arabinose inhibit DNA synthesis.

Nephrotoxicity Studies in Mice. Since administration of *cis*-DDP often leads to irreversible and occasionally fatal nephrotoxicity, there has been considerable interest in the study of second-generation platinum compounds that retain an antitumor activity but do not have significant renal toxicity.[23] The nephrotoxicity of the four nucleoside–*cis*-dichloroplatinum(II) complexes was studied relative to *cis*-DDP as a function of body weight (bw), kidney weight (kw), and blood urea nitrogen levels (BUN), using ICR mice. The complexes were administered intraperitoneally in an equimolar dose to the LD_{50} for *cis*-DDP (13 mg/kg)[24] and showed a marked nephrotoxicity in all markers (Tables VI and VII). The complexes showed no differences in bw, kw, or BUN values when compared to the control group, except for kw on day 3 with **7** and for bw on day 9 with **8**. However, these differences were much smaller than those for *cis*-DDP in comparison to the control group. None of these complexes gave an increase in the BUN levels. The nephrotoxicity in mice of these complexes was much lower than that of *cis*-DDP, and this may be reflected in their therapeutic index in vivo.

Experimental Section

Chemicals. Tubercidin was the kind gift of Kaken Co. (Tokyo), and AICAR was the kind gift of Ajinomoto Co. (Tokyo). Cytidine (**2**), 2′-deoxycytidine (**3**), and 1-β-D-arabinofuranosylcytosine (**4**) were purchased from Yamasa Co. (Tokyo). Potassium tetrachloroplatinate and other reagents were purchased from Kojima Chemical Co. (Tokyo) and Tokyo Kasei Co. (Tokyo) and used without further purification. The radioisotopic labels of thymidine, uridine, and leucine were purchased from New England Nuclear (MA). Thin-layer chromatography (TLC) was carried out with Avicel SF cellulose precoated glass plates from Funakoshi Co. (Tokyo). The following solvent systems were used: A, 1-butanol–acetic acid–water (5:2:3); B, 1% $(NH_4)_2SO_4$–2-propanol (1:1); C, 2-propanol–NH_4OH–water (7:2:1) in ascending technique. All melting points were determined with a Yanagimoto Micro melting point apparatus (Tokyo) and are uncorrected. Infrared spectra were measured with a JASCO-IRA-1 spectrophotometer using KBr disks. Ultraviolet spectra were measured with a Shimadzu UV-210A spectrophotometer, and the ^{1}H NMR spectra

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Table VI. Effect of N-Aminated Nucleoside-$PtCl_2$ Complexes on Body Weights and Kidney Weights in ICR Mice following a Single Intraperitoneal Administration[a]

	day 3[c]		day 6		day 9	
compd[b]	bw	kidney wt[d]	bw	kidney wt[d]	bw	kidney wt[d]
8	35.6 ± 6.6	545 ± 52[e]	39.9 ± 4.7	637 ± 124	36.5 ± 4.5	571 ± 104
9	38.3 ± 3.6	626 ± 91	39.3 ± 3.4	654 ± 107	35.5 ± 2.7[e]	634 ± 85
10	36.9 ± 4.0	582 ± 62	39.1 ± 3.4	629 ± 63	37.4 ± 3.8	641 ± 68
13	38.4 ± 2.7	587 ± 47	39.4 ± 2.1	686 ± 79	40.0 ± 2.6	750 ± 80
cis-DDP	34.0 ± 4.0[e]	599 ± 78[e]	31.0 ± 3.0[e]	555 ± 87[e]	all death	
control	39.4 ± 1.4	650 ± 58	39.4 ± 1.4	673 ± 69	39.4 ± 1.4	702 ± 123

[a] Number of mice = 6 (25–30 weeks old weighing 39 ± 4 g), unless otherwise noted. [b] Dose of the complexes were equivalent molar dose of *cis*-DDP (13 mg/kg). [c] Number of mice = 5. [d] Paired weights; values are means plus or minus standard deviation. [e] Mean is significantly different from control group mean; $p \leq 0.05$.

Table VII. Blood Urea Nitrogen Values in ICR Mice after Administration of the Complexes[a]

compd	day 3[b]	day 6[c]	day 9[c]
8	22.0 ± 4.1[d]	26.3 ± 6.3	28.7 ± 4.3
9	27.2 ± 1.6	28.0 ± 6.6	26.0 ± 1.3
10	24.5 ± 2.9	20.7 ± 1.4	25.7 ± 5.4
13	24.0 ± 3.0	22.8 ± 3.3	26.3 ± 3.2
cis-DDP	37.0 ± 4.2[e]	45.3 ± 5.6[e]	all death
control	26.0 ± 2.2	21.3 ± 3.1	23.2 ± 4.5

[a] The complexes were administered intraperitoneally in the equivalent molar dose of the LD_{50} of *cis*-DDP (13 mg/kg). [b] Number of mice = 5. [c] The mice used were 6 mice for each group, and their age was 25–30 weeks old. [d] The values are the means plus or minus standard deviation in milligrams per 100 mL of blood. [e] Mean is significantly different from control group mean; $p \leq 0.01$.

were taken with JEOL PS-FT-100 and Brucker CXP-300 spectrometers in D_2O or Me_2SO-d_6 solutions. The elemental analyses were performed by Toray Research Center Inc. (Tokyo).

Synthesis of N-Aminated Nucleosides. 3-Aminocytidine hydrochloride (**5**) and 3-aminotubercidin (**12**) were synthesized by the method previously reported.[10]

3-Amino-2′-deoxycytidine hydrochloride (**6**) and 3-amino-1-β-D-arabinofuranosylcytosine hydrochloride (**7**) were synthesized by a method similar to that described previously. The free nucleosides were reacted with 1.5 molar equiv of 1 in DMF at room temperature for 12 h. Compound **6** was recrystallized from ethanol–ether to give fine white needles (decomposes without melting, 164–170 °C). Compound **7** was recrystallized from 80% ethanol to give white needles (decomposes without melting, 184–192 °C).

General Procedure for the Synthesis of *cis*-Dichloroplatinum(II)–Nucleoside Complexes. N-Aminated nucleoside hydrochloride (4 mmol) was dissolved in 5 mL of water, and 10 mL of an aqueous solution of K_2PtCl_4 (4 mmol) was added. The reaction mixture was warmed to 40 °C in a water bath for 2–4 h to yield yellowish or grayish-yellow precipitates. During the reaction the pH of the solution was adjusted to 4.5–5.5 by the addition of 4 M NaOH. The precipitates were filtered and washed with small volumes of chilled water, ethanol, and ether and dried over P_2O_5 at 110 °C for 8 h. The products were recrystallized from ethanol–water (2:8) to give amorphous solids. Percent yields, UV, IR, ^1H NMR and elemental analyses are summarized in Tables I–III.

Assay of Antitumor Activity. (BALB/c × DBA/2)F_1 mice, weighing 20 g, were used for the antitumor assays. L1210 cells (10^4) were transplanted intraperitoneally. The test samples were dissolved in saline solution, injection volume 0.2 mL, and were intraperitoneally injected on days 1 and 5 after tumor inoculation. In the cases with doses over 100 mg/kg, the test samples were homogenized with Nikkol (7 times by weight) as previously reported.[25] Antitumor activity was determined by comparison of the median survival time of the treated group to that of the control group.

In Vitro Culture of L1210 Cells. L1210 cells (10^5), in 1 mL of medium containing various concentrations of the complex, were incubated in a CO_2 incubator at 37 °C for 48 h. Their viability, estimated by staining with 0.17% Trypan blue, was compared to that of control cells incubated in the identical medium without the drugs. Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum was used for the culture medium. The platinum complexes, after heating at 110 °C for 8 h, were used as solutions in the medium and taken in specific volumes to give the desired concentration. The assays were carried out using at least six different concentrations to allow logarithmic dose–response curves to be drawn.

Determination of DNA, RNA, and Protein Synthesis in Vitro. [^{14}C]Thymidine (0.33 μCi/mL; specific activity 55.7 mCi/mmol), [^3H]uridine (0.56 μCi/mL; specific activity 5.1 Ci/mmol), and [^{14}C]leucine (0.25 μCi/mL; specific activity 342 mCi/mmol) were used as precursors. Biosynthesis was determined by the incorporation of ^{14}C or ^3H into the cold trichloroacetic acid insoluble fraction of the cells after the incubation of 5×10^5 cells/mL for 1 to 5 h in the presence of varying concentrations of the complexes. Radioactivity in the fractions was measured as follows: the reaction mixture was chilled in an ice–water bath, and the cells in 0.15 mL of medium were collected on a glass-fiber disk. They were washed twice with 10 mL of ice-cold 10% trichloroacetic acid and then with ethanol and then dried in an oven at 100 °C for 30 min. Radioactivity in the disks was determined with a Beckman LS9000 liquid scintillation counter using toluene–PPO–Me-POPOP counting solution.

Nephrotoxicity in Mice. ICR mice weighing 39 ± 4 g were used for the nephrotoxicity. The test samples were administered intraperitoneally in doses of 0.043 mmol/kg. Six mice for each group were used. On days 3, 6, and 9, the treated and the control mice were used to determine individual bw. Blood samples were collected from the heart under ether anesthesia. The blood samples were stored frozen, and the BUN values were determined at a later time by a modified uriase indophenol method using a Beckman BUN analyzer. A pair of kidneys were taken and, after immediate weighing, were stored on ice.

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