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Synthesis of Purine Nucleoside Nitrosoureas and Their Antitumor Activities

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Guanine and xanthine nucleoside derivatives (**3**, **4** and **6**) bearing nitrosourea functional groups were synthesized from guanine nucleoside ureas (**2**) obtained by the reaction of 2′-deoxy-2′-aminoguanosine (**1**) with isocyanates and their antitumor activity against sarcoma-180 solid tumor and leukemia L-1210 were determined. Among the compounds tested, 2′-deoxy-2′-[3-(2-chloroethyl)-3-nitrosoureido]-xanthosine (**4b**) found to have the most potent activity. Moreover, very slight decrease in white blood cells of mice bearing sarcoma-180 solid tumor was observed after administration of **4b**.

Keywords——nitrosourea; aminonucleoside derivative; guanine derivative; xanthine derivative; nitrosation; antitumor activity

Nitrosourea compounds such as 1,3-bis-chloroethyl-1-nitrosourea (BCNU) and 1-chloroethyl-3-cyclohexyl-1-nitrosourea (CCNU) are highly potential experimental and clinical antitumor agents.[2] Their high lipophilicity enables these agents cross cell membranes and so-called blood-brain-barrier readily. Thus, they have been used effectively in the treatment of brain tumors.[3] Their lipophilicity, however, besides their inherent cytotoxity, often give rise to undesirable side effects. This limits their use at doses below those which seem necessary for eradicating cancer cells. In fact, myelosuppression has been reported to be the limiting toxicity in clinical use of BCNU[2b] and CCNU.[4] Therefore, water-soluble nitrosourea compounds having reduced toxicity were searched and compounds such as chlorozotocin (DCNU),[5] 3-(tetraacetyl-D-glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea (GCNU)[6] and 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU)[7] were synthesized.

We attempted to synthesize the new type of water-soluble nitrosourea compounds having the nucleoside moieties. Nucleoside bearing nitrosourea functional group is scarcely found in the literature except recent report on thymidine nitrosourea[8] which prompted us to report our result. We adopted 2′-deoxy-2′-aminoguanosine (2AG) as a starting material which was isolated from the fermentation medium in our laboratory and reported to have anti-

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bacterial and antitumor activities.[9] This paper deals with the synthesis of those nitrosourea compounds and their antitumor activities.

GCNU and DCNU have glucopyranose skeleton and ACNU has pyrimidine one, while our synthesized compounds have nucleoside skeleton as carriers of nitrosourea moieties. It is expected that these compounds may excert selective activity and reduced toxicity owing to their assumed affinity to the nucleus of the cells, and that they may resume the activity of parent molecule, 2AG itself, as an antimetabolite depending on the mode of decomposition of them *in vivo*.[10]

Results and Discussion

Synthesis of Nitrosourea Compounds

The outline of synthetic scheme is given in Chart 1. 2AG (**1**) was treated with methyl isocyanate in dimethylsulfoxide (DMSO) at room temperature to give urea (**2a**) in 71% yield.

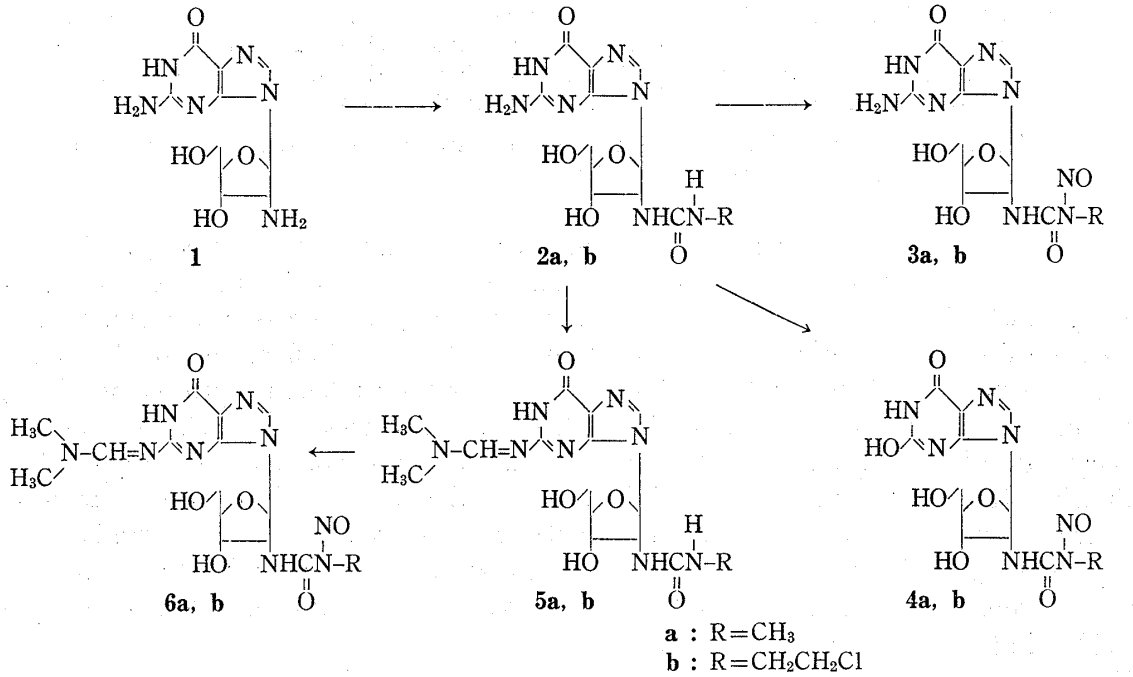

Chart 1

Similarly, compound **1** was treated with chloroethyl isocyanate in N,N-dimethylformamide (DMF) at 0° to give urea (**2b**), contaminated with a more polar unassigned product. Purification was carried out using Diaion HP-10 resin to give nonhygroscopic white powder in 64% yield. In these reactions, no protection of **1** was needed, and only amino group of the 2′ position of the ribose was participated in the reactions.

Nitrosation of **2a** with 3 equivalents of 1 N hydrochloric acid solution and 4 equivalents of sodium nitrite at 0° gave **3a** in 79% yield, while 4 equivalents of 1 N hydrochloric acid solution and 3 equivalents of sodium nitrite at 0° afforded the diazotized xanthine derivative **4a** in 69% yield. These results seemed to be derived from the different solubilities of the product **3a** in these reaction medium. That is, **3a** is crystallized out before diazotization in the former medium, while it stays in solution long enough to be diazotized in the latter

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medium. An alternative method to prepare **4a** was successfully carried out by treating **2a** with 2.4 equivalents of sodium nitrite in excess 99% formic acid at 0°. Nitrosation of chloroethylurea derivative **2b** with aqueous hydrochloric acid and sodium nitrite was failed because of sparing solubility of the compound. Therefore, **3b** was prepared by treating with one equivalent of sodium nitrite in 99% formic acid at 0° in 55% yield, contaminated with about 20% amounts of **4b**. **4b** was prepared similarly in 45% yield except that 2.4 equivalents of sodium nitrite was employed.

In all conditions above, nitrosation occurs selectively at the urea nitrogen attached to methyl or chloroethyl group. This was proved by the following nuclear magnetic resonance (NMR) spectral data. The NH proton of the compound **3** or **4** was observed as the doublet owing to the coupling with the adjacent methine proton of the ribose. Furthermore, the protons of the chloroethyl moiety of the compound **3b** or **4b** were observed as the coupling pattern of A_2B_2 symmetry as reported by Johnston and co-workers.[11]

Compounds **2a** and **2b** were converted to the corresponding dimethylaminomethyleneamino derivatives, **5a** and **5b**, by treating with N,N-dimethylformamide dimethylacetal in DMF[12] in 92 and 72% yield, respectively. These urea derivatives were then treated with 2 equivalents of sodium nitrite in 99% formic acid at 0° to give **6a** and **6b** in 71 and 35% yield respectively. These compounds had as expected several tens times increased solubility in water than the corresponding compounds, **3** and **4**, and dimethylaminomethyleneamino moieties of them are expected to degradate gradually to amino group in physiological conditions.

Antitumor Activities and Evaluation

Antitumor activities of the compounds against leukemia L-1210 and sarcoma-180 solid tumor were shown in Table I. As shown in Table I, all nitrosourea derivatives except **3a**

Table I. Antitumor Activities of Nitrosourea Compounds

Compd.	Vehicle	LD_{50}[a] (mg/kg)	Dose[b] (mg/kg)	Antitumor activities: Sarcoma-180 (T/C)[c]	Antitumor activities: L-1210[d] (ILS%)
3a	CMC[e]	60	30	0.64 (+1.2)[f]	−11
3b	CMC	60	40	0.42 (+3.0)	46
4a	CMC	>1000	1000	0.15 (+0.9)	25
4b	CMC	68	45	0.20 (+3.0)	59
6a	CMC	>1000	1000	0.49 (+3.0)	22
6b	CMC	500	333	0.18 (−3.1)	—
2AG	Saline	2000	1000	0.64 +(1.8)	4

a) LD_{50} of the test compounds by a single intraperitoneal administration in normal dd mice were calculated by the method of Behrens-Kärber.[13]
b) Administered intraperitoneally once 24 hr after the tumor implantation.
c) T/C was calculated by the mean tumor volume of the treated group divided by that of control group.
d) Percent of increase in line span.
e) 0.3% carboxymethyl cellulose.
f) Changes of body weight (g) from 1st to 7th day after implantation. Control group: +3.6 (g).

were active against both tumor systems. Compounds having chloroethyl group showed higher activities than those having methyl group. Moreover, it is interesting to note that compounds having xanthine base showed higher activities than those having guanine base. It is not clear whether these results come from the difference of permeability or affinity to the target between the two or the difference of activities of 2AG and 2′-deoxy-2′-amino-

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xanthosine as latent antimetabolites or so on. Although compound **6b** showed a considerably potent activity against sarcoma-180, it seemed to have severe toxicity as indicated by the decreased body weight of the mice. Finally, compound **4b** appeared to be the most effective against the both tumors among tested compounds.

Therefore, the effect of **4b** on white blood cell (WBC) counts was measured using mice bearing sarcoma-180 solid tumor in comparison with that of 1-chloroethyl-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU). The test compounds were given by a single administration close to the maximum tolerated dose. As shown in Table II, a number of white blood cells of mice decreased to 41—23% of the control at the 4th day after administration of MeCCNU, while only very slight decrease was observed after administration of **4b**. Thus, **4b** may be considered to have a low bone-marrow toxicity. It is interesting to find that nucleoside nitrosourea compounds also have antitumor activity and yet apparently have low bone-marrow toxicity.

Table II. Effects of **4b** and MeCCNU on White Blood Cell Counts of dd Mice Bearing Sarcoma-180

Compd.	Dose (mg/kg)	Schedule	WBC counts ($\times 10^2$/mm^3) Mean±SD	Lethality (Day-7)
P.S.S.[a]			130.6±33.6	0/5
4b	60	*i.p.*×1[b]	112.2±21.4	0/5
	40	*i.p.*×1	120.2±27.4	0/5
MeCCNU	30	*i.p.*×1	30.0±13.3	1/5
	20	*i.p.*×1	53.2±18.0	0/5

a) 0.5 ml of physiological saline solution were administered intraperitoneally once 24 hr after implantation.
b) Administered intraperitoneally once 24 hr after implantation.

Experimental

Melting points were determined using a Yanagimoto melting points apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Hitachi EPS-3 spectrophotometer. Infrared (IR) spectra were obtained using a Hitachi 215 spectrophotometer. NMR spectra were determined on a JEOL JNM-PS100 spectrometer using tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was performed in Eastman Kodak 13181 silica gel plate (plate A), Merk Art 5719 No. 6060 (plate B) and Avicel SF cellulose plate (plate C) in solvent system: solv. A, *n*-butanol–acetic acid–water (4: 1: 2, v/v); solv. B, *n*-butanol saturated with water.

2′-Deoxy-2′-(3-methylureido)-guanosine (2a)——To a stirred solution of 2AG (13.3 g, 47.2 mmol) in DMSO (280 ml) was added methyl isocyanate (4.1 g, 71.7 mmol) at 12°. Stirring was continued for 30 min at same temperature and for 2 hr at room temperature. The solvent was removed by evaporation *in vacuo*. The resulting oil was treated with acetone to obtain a precipitate which was collected by filtration, washed with water, recrystallized from water and dried to give 11.4 g (71.2%) of white fine needles: The compound decomposed coloring in brown from 268° to above 300°. UV $\lambda_{max}^{pH7.0}$ phosphate buffer nm: 254, $\lambda_{max}^{0.1N\ HCl}$ nm: 258, $\lambda_{max}^{0.1N\ NaOH}$ nm: 258—268. IR ν_{max}^{KBr} cm^{-1}: 1640 (urea). NMR (DMSO-d_6) δ: 3.96, 4.14 (2H, m, H-3′ and H-4′), 4.82 (1H, m, H-2′), 5.16 (1H, t, J=5.0 Hz, OH-5′), 5.66 (1H, d, J=8.0 Hz, H-1′), 5.84 (1H, d, J=5.0 Hz, OH-3′), 6.44 (2H, br, NH_2-2), 7.94 (1H, s, H-8), 10.48 (1H, br, NH-1). TLC (solv. A, plate C): *Rf* 0.42.

2′-Deoxy-2′-[3-(2-chloroethyl)ureido]-guanosine (2b)——To a stirred solution of 2AG (11.9 g, 42.2 mmol) in DMF was added chloroethyl isocyanate (3.81 ml, 44.2 mmol) at 0°. Stirring was continued for one hour at 0°. To the reaction mixture was added 2 l of ethyl ether at 0° and the resulting hygroscopic precipitate was collected by filtration. The white precipitate dissolved in 800 ml of water was then applied to a column (6×50 cm) of Diaion HP-10 resin (Mitsubishi Kasei Co., Ltd.) and washed thoroughly with 3 l of water, and the adsorbed product was eluted with 50% aqueous methanol. The solvent was evaporated *in vacuo* below 25° to obtain white solid which was dried *in vacuo* over P_2O_5 to give 10.5 g (63.8%) of amorphous white powder: UV $\lambda_{max}^{pH7.0\ phosphate\ buffer}$ nm: 254, $\lambda_{max}^{0.1N\ HCl}$ nm: 257, $\lambda_{max}^{0.1N\ NaOH}$ nm: 260—268. IR ν_{max}^{KBr} cm^{-1}: 1670 (urea). NMR (DMSO-d_6) δ: 3.92, 4.11 (2H, br, H-3′ and H-4′) 4.76 (1H, m, H-2′), 5.08 (1H, br, OH-5′), 5.68 (1H, d, J=8.8 Hz, H-1′), 5.83 (1H, br.d, OH-3′), 6.25 (1H, d, J=8.8 Hz, –NHCONHCH$_2$CH$_2$Cl), 6.43

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(2H, br, NH_2-2), 6.53 (1H, t, J=5.4 Hz, -NHCONHCH$_2$CH$_2$Cl), 7.87 (1H, s, H-8), 10.64 (1H, br, NH-1). TLC (solv. B, plate B): *Rf* 0.65.

2′-Deoxy-2′-(3-methyl-3-nitrosoureido)-guanosine (3a)——To a stirred solution of urea (**2a**) (3.6 g, 10.6 mmol) in 30 ml of 1 N HCl was added a solution of sodium nitrite (2.93 g, 42.5 mmol) in 15 ml of water at 0°, and stirring was continued for 30 min at 0°. A pale yellow solid precipitated out was collected by filtration, washed with cold water (two 15 ml portions) and dried *in vacuo* to give 3.1 g (79.3%) of amorphous pale yellow powder. UV $\lambda_{max}^{7.0\ phosphate\ buffer}$ nm: 254, $\lambda_{max}^{0.1N\ HCl}$ nm: 258, $\lambda_{max}^{0.1N\ NaOH}$ nm: 258—268. IR ν_{max}^{KBr} cm^{-1}: 1720 (nitroso urea). NMR (DMSO-d_6) δ: 3.04 (3H, s, N-CH$_3$), 3.68 (2H, br, CH$_2$-5′), 4.04, 4.36 (2H, br, H-3′ and H-4′), 5.11 (1H, m, H-2′; after addition of D$_2$O, dd, $J_{1',2'}$=8.0 Hz, $J_{2',3'}$=6.5 Hz), 5.24 (1H, t, J=5.0 Hz, OH-5′), 5.94 (1H, d, J=4.0 Hz, OH-3′), 6.01 (1H, d, J=8.0 Hz, H-1′), 6.44 (2H, br, NH_2-2), 7.92 (1H, s, H-8), 8.26 (1H, d, J=8.0 Hz, -NHCON-NO), 10.72 (1H, br, NH-1). TLC (solv. A, plate A): *Rf* 0.71.

2′-Deoxy-2′-[3-(2-chloroethyl)-3-nitrosoureido]-guanosine (3b)——To a stirred solution of urea (**2b**) (3.5 g, 9.0 mmol) in 60 ml of 99% formic acid was added sodium nitrite (0.65 g, 9.5 mmol) at 0°, and stirring was continued for 45 min at 0°. Then 250 ml of cold water was added to the reaction mixture and stirring was further continued for 30 min. The reaction mixture was applied to a column (3.5×45 cm) of Diaion HP-10 resin and washed thoroughly with water until the pH of effluent was neutral. Then the adsorbed product was eluted with 50% aqueous methanol. The solvent was evaporated *in vacuo* below 30° to obtain a pale yellow glass which was dried *in vacuo* over P_2O_5 to yield 2.05 g (54.5%) of amorphous pale yellow powder. This powder appeared to be contaminated with about 20% amounts of **4b** from the data of NMR spectra. NMR (DMSO-d_6) δ: 3.52, 4.03 (4H, pseudo two t, J=6.0 Hz, CH$_2$CH$_2$Cl), 6.00 (1H, d, J=8.0 Hz, H-1′), 6.47 (2H, br, NH_2-2), 7.99 (1H, s, H-8), 8.26 (1H, d, J=7.6 Hz, -NHCON-NO), 10.74 (1H, br, NH-1).

2′-Deoxy-2′-(3-methyl-3-nitrosoureido)-xanthosine (4a)——The urea (**2a**) (3.24 g, 9.6 mmol) was treated with excess sodium nitrite (1.5 g, 21.7 mmol) as above and the reaction mixture was worked up in the same manner to afford 2.44 g (69.2%) of amorphous pale yellow powder. UV $\lambda_{max}^{pH7.0\ phosphate\ buffer}$ nm: 250, 277, $\lambda_{max}^{0.1N\ HCl}$ nm: 235, 262, $\lambda_{max}^{0.1N\ NaOH}$ nm: 250, 278. IR ν_{max}^{KBr} cm^{-1}: 1730 (nitroso urea). NMR (DMSO-d_6) δ: 3.05 (3H, s, -NCH$_3$), 6.15 (1H, d, J=8.3 Hz, H-1′), 7.87 (1H, s, H-8), 8.39 (1H, d, J=7.8 Hz, -NHCON-NO), 10.87 (1H, s, NH-1 or NH-3), 11.77 (1H, br, NH-3 or NH-1). TLC (solv. A, plate A): *Rf* 0.70.

2′-Deoxy-2′-[3-(2-chloroethyl)-3-nitrosoureido]-xanthosine (4b)——The urea (**2b**) (3.5 g, 9.0 mmol) was treated with excess sodium nitrite (1.5 g, 21.7 mmol) as above and the reaction mixture was worked up in the same manner to afford 1.7 g (45.1%) of amorphous pale yellow powder. UV $\lambda_{max}^{pH7.0\ phosphate\ buffer}$ nm: 248, 277, $\lambda_{max}^{0.1N\ HCl}$ nm: 235, 262, $\lambda_{max}^{0.1N\ NaOH}$ nm: 250, 278. IR ν_{max}^{KBr} cm^{-1}: 1730 (nitroso urea). NMR (DMSO-d_6) δ: 3.52, 4.03 (4H, pseudo two t, J=6.0 Hz, -CH$_2$CH$_2$Cl), 3.76 (2H, br, H-5′), 4.17, 4.36 (2H, br, H-3′ and H-4′), 6.11 (1H, d, J=8.3 Hz, H-1′), 7.86 (1H, s, H-8), 8.31 (1H, d, J=8.1 Hz, -NHCON-NO), 10.85 (1H, s, NH-1 or NH-3), 11.48 (1H, br, NH-3 or NH-1). TLC (solv. B, plate B): *Rf* 0.52.

N^2-Dimethylaminomethylene-2′-deoxy-2′-(3-methylureido)-guanosine (5a)——To a stirred solution of urea (**2a**) (9.5 g, 28.0 mmol) in 120 ml of DMF was added, N,N-dimethylformamide dimethylacetal (16.7 g, 140 mmol) at room temperature, and stirring was continued for 16 hr. After evaporation of the solvent, the residue was dissolved in 50 ml of ethanol. To the solution was added slowly one liter of ethyl ether with vigorous stirring. The white precipitate that formed was collected by filtration and dried *in vacuo* to give 10.2 g (92.4%) of amorphous white powder which was used for the next step without further purification. Analytical samples were obtained by recrystallization from DMSO and water (3:2, v/v). mp 248—249° (dec. colored in brown above 245°). UV $\lambda_{max}^{H_2O}$ nm: 233, 298. IR ν_{max}^{KBr} cm^{-1}: 1635 (urea), 1650 (>N-CH=N-). NMR (DMSO-d_6) δ: 3.04, 3.16 [6H, two s, (CH$_3$)$_2$N-], 3.98, 4.16 (2H, br, H-3′ and H-4′), 4.84 (1H, m, H-2′; after addition of D$_2$O, dd, $J_{1',2'}$=8.0 Hz, $J_{2',3'}$=5.0 Hz), 5.14 (1H, t, J=6.0 Hz, OH-5′), 5.74 (1H, d, J=8.0 Hz, H-1′), 5.85 (1H, d, J=4.0 Hz, OH-3′), 6.04, 6.12 (2H, br, -NHCONH-), 8.00 (1H, s, H-8), 8.56 (1H, s, >N-CH=N-), 11.28 (1H, br, NH-1). TLC (solv. B, plate B): *Rf* 0.16.

N^2-Dimethylaminomethylene-2′-deoxy-2′-[3-(2-chloroethyl)-ureido]-guanosine (5b)——To a stirred solution of urea (**2b**) (12.6 g, 32.5 mmol) in 150 ml of DMF was added N,N-dimethylformamide dimethylacetal (17.9 g, 150 mmol) at 5°, and stirring was continued at the same temperature for 16 hr. After evaporation of the solvent below 30°, the residue was dissolved in water. Then the aqueous solution was applied to a column (8×45 cm) of Diaion HP-10 resin and washed thoroughly with water, and the adsorbed product was eluted gradiently with 10% to 50% aqueous methanol. The solvent was evaporated *in vacuo* below 30° to obtain white solid which was dried *in vacuo* over P_2O_5 to give 12.6 g (72.1%) of amorphous white powder. UV $\lambda_{max}^{H_2O}$ nm: 237, 291. IR ν_{max}^{KBr} cm^{-1}: 1640 (>N-CH=N-). NMR (DMSO-d_6) δ: 3.07, 3.20 [6H, two s, (CH$_3$)$_2$N-], 5.76 (1H, d, J=8.0 Hz, H-1′), 6.26—6.67 (2H, m, -NHCONH-), 8.02 (1H, s, H-8), 8.56 (1H, s, >N-CH=N-). TLC (solv. B, plate A): *Rf* 0.39.

N^2-Dimethylaminomethylene-2′-deoxy-2′-(3-methyl-3-nitrosoureido)-guanosine (6a)——To a stirred solution of urea (**5a**) (5.0 g, 12.7 mmol) in 80 ml of 99% formic acid was added sodium nitrite (1.8 g, 26.1 mmol) at 0°, and stirring was continued for one hour at the same temperature. Then, 200 ml of cold water was added to the reaction mixture and stirring was further continued for additional one hour at 0°. The reaction mixture was applied to a column (3.5×55 cm) of Diaion HP-10 resin and washed thoroughly with water until the pH of the effluent was neutral. Then the adsorbed product was eluted with 50% aqueous methanol. The solvent was evaporated *in vacuo* below 30° to obtain a pale yellow glass which was dried

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in vacuo over P_2O_5 to give 3.8 g (70.8%) of amorphous pale yellow powder. UV $\lambda_{max}^{H_2O}$ nm: 234, 294. IR ν_{max}^{KBr} cm^{-1}: 1640 (>N–CH=N–), 1725 (nitroso urea). NMR (DMSO-d_6) δ: 3.04 (3H, s, N–CH_3), 3.06, 3.18 [6H, two s, $(CH_3)_2$N–], 6.18 (1H, d, J=8.0 Hz, H-1′), 8.06 (1H, s, H-8), 8.57 (1H, s, >N–CH=N–), 11.18 (1H, br, NH-1). TLC (solv. B, plate B): *Rf* 0.34.

N^2-Dimethylaminomethylene-2′-deoxy-2′-[3-(2-chloroethyl)-3-nitrosoureido]-guanosine (6b)——The urea (**5b**) (4.0 g, 9 mmol) was treated with sodium nitrite (1.24 g, 18.0 mmol) as above and the reaction mixture was applied to a column (6×50 cm) of Diaion HP-10 resin and washed thoroughly with water until the pH of the effluent was neutral. Then the adsorbed product was eluted gradiently with 20% to 70% aqueous methanol. The solvent was evaporated *in vacuo* below 30° to obtain a pale yellow glass which was dried *in vacuo* over P_2O_5 to give 1.5 g (35.2%) of amorphous pale yellow powder. UV $\lambda_{max}^{H_2O}$ nm: 233, 298. IR ν_{max}^{KBr} cm^{-1}: 1640 (>N–CH=N–), 1735 (nitroso urea). NMR (DMSO-d_6) δ: 3.05, 3.16 [6H, two s, $(CH_3)_2$N–], 3.52, 4.02 (4H, pseudo two t, J=6.0 Hz, $-CH_2CH_2Cl$), 5.00—5.32 [2H, m, H-2′ and OH-5′; 5.12 after addition of D_2O (1H, dd, $J_{1',2'}$=8.0 Hz, $J_{2',3'}$=6.0 Hz)], 5.98 (1H, br.d, OH-3′), 6.12 (1H, d, J=8.0 Hz, H-1′), 8.04 (1H, s, H-8), 8.34 (1H, d, J=8.0 Hz, NHCO–N–NO), 8.50 (1H, s, >N–CH=N–), 11.28 (1H, br, NH–1). TLC (chloroform–methanol 4:1 v/v, plate B): *Rf* 0.49.

Tumor System and Evaluation of Antitumor Activity——Male dd mice and male CDF_1 mice weighing 19+1 g and 22±2 g, respectively, were used. Experiments were carried out with 5 mice in each group. In order to investigate the antitumor activity and the effect on WBC counts, 5×10^6 cells of Sarcoma-180 (ascitic tumor) were implanted subcutaneously into the right axillary region of male dd mice. The test compounds were administered intraperitoneally once 24 hr after implantation. Antitumor activity was evaluated by comparing the mean tumor volume of the treated group (T) with that of the control group (C), *i.e.*, T/C, 7 days after tumor implantation. WBC of mice were counted 4 days after administration. For testing the antitumor activity against Leukemia L-1210, 10^5 cells were implanted intraperitoneally into male CDF_1 mice. The test compounds were administered intraperitoneally once 24 hr after implantation. Antitumor activity was evaluated by comparing the mean survival days of the treated groups with that of control group, *i.e.*, percent increase in life span (ILS).

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