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A New Class of Nitrosoureas. I. Synthesis and Antitumor Activity of 1-(2-Chloroethyl)-3,3-disubstituted-1-nitrosoureas having a Hydroxyl Group at the β-Position of the Substituents

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1-(2-Chloroethyl)-3,3-disubstituted-1-nitrosoureas (**5a**—**m**), a new class of nitrosoureas, were synthesized and tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma.

The nitrosoureas (**5e**—**k**) having a hydroxyl group at the β-position of the substituents showed remarkable antitumor activities. In particular, 1-(2-chloroethyl)-3,3-bis(2-hydroxyethyl)-1-nitrosourea (**5k**) had excellent activities and showed 5 and 16 times greater therapeutic ratios than 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea against leukemia L1210 and Ehrlich ascites carcinoma, respectively. These nitrosoureas (**5e**—**k**) appear to be activated nonenzymatically by attack of the hydroxyl group on the carbonyl group to give the oxazolidinones (**6**) and chloroethyl diazohydroxide (**7**) without generation of the isocyanates (**8**).

Keywords——chloroethyl nitrosoureas; 3,3-disubstituted nitrosoureas; synthesis; antitumor activity; CCNU; leukemia L1210; Ehrlich ascites carcinoma; activation mechanism; oxazolidinones

Since the emergence of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) as useful antitumor agents in treating a variety of human malignancies, a large number of nitrosourea derivatives that are highly active in experimental tumor systems have been reported.[1] These nitrosoureas necessarily possess one hydrogen atom at their N-3 position and this seems likely to be important in conferring antitumor activity. Because nitrosoureas are chemically reactive compounds, they decompose nonenzymatically under physiological conditions to generate isocyanates and chloroethyl diazohydroxide and consequently exhibit antitumor activity. This decomposition is considered to be initiated by abstraction of the proton at the N-3 position as the first step.[2]

Meanwhile, nitrosoureas and related nitroso compounds are also known to be potent mutagens and carcinogens. It has been reported that both 1-butyl-1-nitrosourea (**1**)[3] and 1-butyl-3,3-dimethyl-1-nitrosourea (**2**)[4] showed strong leukemogenic activity in rats. We considered that the *n*-butylcarbonium ion generated from these compounds might cause leukemia in rats, though the carbonium ion would not be formed nonenzymatically under physiological conditions from the dimethyl compound (**2**) which is disubstituted at the N-3 position. We assumed that the dimethyl compound (**2**) would be activated *in vivo* by enzymatic demethylation to the N-nor compound which spontaneously decomposes to yield the *n*-butylcarbonium ion. Thus, it became of particular interest to prepare 3,3-disubstituted-1-(2-chloroethyl)-1-nitrosoureas as masked nitrosourea derivatives and to test their antitumor activity *in vivo*. Recently Brundrett *et al.*, synthesized a few derivatives of nitrosoureas disubstituted with methyl and alkyl groups at their N-3 position and found that they were stable in aqueous solution. These compounds were reported to be latent antitumor agents because they were inactive *in vitro* but cytotoxic *in vivo*.[5]

In this paper we report the synthesis and antitumor activities of a new class of 1-(2-chloroethyl)-3,3-disubstituted-1-nitrosoureas, especially those having a hydroxyl group at the β-

position of the substituents; these compounds are unstable in aqueous solution but are highly active against leukemia L1210.

Synthesis of Nitrosoureas and Discussion

Nitrosoureas (**5a**—**m**) disubstituted on the N-3 position were obtained by nitrosation of the corresponding ureas (**4a**—**m**) and are listed in Table I with some characteristic data. The nitrosoureas (**5a**—**m**) were usually obtained as yellow oils, though known nitrosoureas having a proton on the N-3 position have usually been reported to be yellow crystalline compounds. This difference might be due to the lack of an intramolecular hydrogen bond in the former, while the presence of the cyclic structure depicted in Fig. 1 is anticipated for the latter. The nitrosoureas (**5a**—**d**) having no hydroxyl group on the substituent were found to be very stable compared with known compounds such as BCNU and CCNU. This is apparently due to the absence of a proton on the N-3 position, which is considered to initiate the decomposition of nitrosoureas. On the contrary, the nitrosoureas (**5e**—**k**), which have a free hydroxyl group on the β-position of the substituents, were found to be very unstable. This is also apparent in the mass spectra of these compounds; the molecular ion peaks of **5a**—**d** can be clearly seen but those of **5e**—**k** are very weak or undetectable.

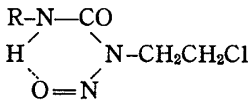


Fig. 1

We considered that the nitrosoureas (**5e**—**k**) would be activated nonenzymatically under physiological conditions by intramolecular attack of a hydroxyl group as depicted in Chart 2. In fact, the nitrosoureas (**5e**, **k**) readily decomposed in phosphate-buffered solution (pH 7.4) to give the oxazolidinones (**6e**, **k**) in nearly quantitative yields. This activation mechanism of **5e**, **k**, in which alkyl isocyanates (**8**) can never be produced, differs from that of known

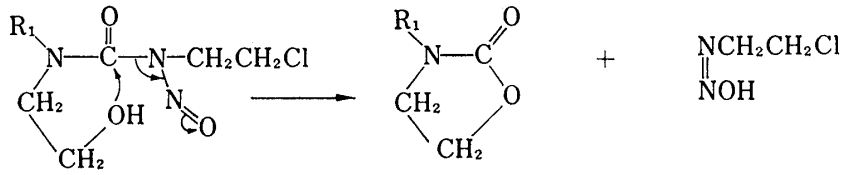
3 **4a—m** **5a—m**

Chart 1

TABLE I. The Properties of 1-(2-Chloroethyl)-3,3-disubstituted-1-nitrosoureas

No.	R_1	R_2	Form[a]	Yield (%)	MS M+	MS B+
5a	CH_3	CH_3	A	73	179, 181	72(M^+-107)
5b	CH_3	$CH_2CH_2CH_2CH_3$	A	85	221, 223	114(M^+-107)
5c	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	A	75	231, 233	124(M^+-107)
5d	$CH_2CH_2OCH_2CH_2$		A	76	221, 223	114(M^+-107)
5e	CH_3	CH_2CH_2OH	B	67	209[b]	102(M^+-107)
5f	CH_3	$CH_2CH(OH)CH_2OH$	B	58	Not detected	132(M^+-107)
5g	CH_3	$CH_2(CHOH)_4CH_2OH$	C	39		
5h	$CH_2CH_2CH_3$	$CH_2CH(OH)CH_2OH$	B	56	Not detected	160(M^+-107)
5i	$CH_2CH_2CH_2CH_3$	$CH_2(CHOH)_2CH_2OH$	B	48	Not detected	204(M^+-107)
5j	$CH_2CH_2CH_2CH_3$	$CH_2(CHOH)_4CH_2OH$	C	43		
5k	CH_2CH_2OH	CH_2CH_2OH	B	52	Not detected	132(M^+-107)
5l	CH_2CH_2OAc[c]	CH_2CH_2OAc	A	72	323, 325	174(M^+-149)
5m	CH_3	$CH_2(CHOAc)_4CH_2OAc$	C	38		

a) A, stable yellow oil; B, unstable yellow oil; C, pale yellow amorphous powder.
b) The intensity is very weak.
c) Ac, $COCH_3$.



5e : $R_1=CH_3$ **6e** : $R_1=CH_3$ **7**
5k : $R_1=CH_2CH_2OH$ **6k** : $R_1=CH_2CH_2OH$

Chart 2

BCNU : $R=CH_2CH_2Cl$ **8** **7**
CCNU : $R=cyclo\text{-}C_6H_{11}$

Chart 3

nitrosoureas such as BCNU and CCNU (Chart 3), though chloroethyl diazohydroxide (**7**), a necessary intermediate for antitumor activity, is generated in both mechanisms.

Antitumor Activities of the Nitrosoureas (5a—m) and Discussion

The nitrosoureas (**5a—m**) were tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma. In the *ip-ip* test system against Ehrlich ascites carcinoma (see Experimental section), it is well known that the growth of ascites tumor cells is localized and that direct action of the drug on the cells takes place in the peritoneal cavity, so that the result in this test system may be considered to be comparable with that of an *in vitro* test.

TABLE II. Antitumor Activities of 1-(2-Chloroethyl)-3,3-disubstituted-1-nitrosoureas

Compound	Anti-L1210 activity			Therapeutic ratio[c]	Anti-Ehrlich activity		
	ILS_{30}[a] (mg/kg×5)	OD[b] (mg/kg×5)	Maximum ILS (%)		MED[d] (mg/kg×5)	MTD[e] (mg/kg×5)	Therapeutic[f] ratio
CCNU	4.9	25	>757.1[g]	5.1	12.5	50	4
5a	2.4	12.5	>249.4	5.2	25	50	2
5b	18.9	100	420.8	5.3	50	100	2
5c	7.9	50	102.7	6.6	50	50	1
5d	10.5	50	>622.9	4.8	50	100	2
5e	3.8	25	140.3	6.6	6.25	50	8
5f	0.45	12.5	>432.9	27.8	0.39	25	64
5g	6.5	50	84.2	7.7	6.25	100	16
5h	1.6	12.5	>231.3	7.6	1.56	12.5	8
5i	1.1	25	>278.4	22.7	0.78	50	64
5j	1.5	25	143.2	16.7	0.78	50	64
5k	0.55	12.5	>688.5[g]	22.7	0.78	50	64
5l	0.56	3.12	>431.1	5.6	1.56	12.5	8
5m	2.8	6.25	62.0	2.2	1.56	12.5	8

a) Daily dose providing a 30% increase in life-span over the control. $ILS(\%)=(T/C-1)\times100$.
b) Optimal dose: the daily dose providing the maximum increase in life-span.
c) Therapeutic ratio=OD/ILS_{30}.
d) Minimum effective dose: the minimum dose which shows 100% inhibition of the growth of the tumor.
e) Maximum tolerated dose: the maximum dose which shows 100% inhibition of the growth of the tumor without causing the death of mice.
f) Therapeutic ratio=MTD/MED.
g) All treated mice survived more than sixty days.

The results are listed in Table II together with the comparative data for CCNU. The compounds (**5a—d**) scarcely showed antitumor activity against Ehrlich ascites carcinoma, while they showed considerable activity against leukemia L1210. This suggests that the compounds (**5a—d**) are inactive *in vitro* but are activated *in vivo* by enzymatic dealkylation in accordance with the reported observation of Brundrett *et al.* for trialkylated nitrosourea derivatives.[5] On the other hand, the compounds (**5e—k**) bearing a hydroxyl group on the β-position of the substituents showed remarkable activities against Ehrlich ascites carcinoma; their therapeutic ratios were 2 to 16 times greater than that of CCNU. As described above, the nitrosoureas (**5e—k**) appear to be activated nonenzymatically by attack of the hydroxyl group on the carbonyl group to give the oxazolidinones (**6**) and chloroethyl diazohydroxide (**7**) without generation of the isocyanates (**8**) (see Charts 2 and 3). It is considered that the production of the isocyanate (**8**) is one of the factors which determine the toxicity of nitrosourea derivatives and that nitrosoureas with higher carbamoylating activity in an *in vitro* assay have lower therapeutic ratios *in vivo*.[6] Consequently, the nitrosoureas (**5e—k**) would be expected to have some advantage as regards toxicity. In fact, many of the compounds (**5e—k**) have therapeutic ratios 3 to 5 times greater than that of CCNU as regards antitumor activity against leukemia L1210. In particular, compound **5k** has excellent activity against both leukemia L1210 and Ehrlich ascites carcinoma as compared with CCNU. Acetylation of the hydroxyl group in the substituents, however, resulted in an increase of toxicity (see **5l, m**), endorsing the apparent significance of a free hydroxyl group as an essential structural feature for strong antitumor activity in this class of compounds. Further work on the synthesis and antitumor activity of this new class of nitrosoureas with a sugar moiety as a substituent is in progress.

Experimental

Melting points were determined in a capillary tube in a liquid bath and are uncorrected. Infrared (IR) spectra were determined routinely in Nujol mull or as a liquid film. NMR spectra were recorded in a JEOL-PMX 60 spectrometer using tetramethylsilane as an internal standard in $CDCl_3$ or using sodium 3-(trimethylsilyl)propionic acid in D_2O. Mass spectra (MS) were obtained with a Hitachi RMU-6M spectrometer. The optical rotations were measured in a 0.5 dm tube with a Jasco DIP-180 polarimeter. The column chromatography was carried out on Merck silica gel 60. Elemental analyses were carried out with a Perkin-Elmer 240B elemental analyzer. Organic solutions were commonly dried over $MgSO_4$ and were concentrated by evaporation *in vacuo*.

1-(2-Chloroethyl)-3,3-dimethyl-1-nitrosourea (5a)——To a solution of 1.5 g of 1-(2-chloroethyl)-3,3-dimethylurea in 15 ml of acetic acid, 1 g of sodium nitrite was added portionwise during 1 h with stirring at room temperature. The reaction mixture was concentrated and the residue was extracted with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate, dried, and concentrated to give a yellow syrup, which was dissolved in small amount of chloroform and chromatographed on silica gel (chloroform–AcOEt=5: 1). Finally, 1.3 g (73%) of **5a** was obtained as a yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1700 (CO). MS *m/e*: 179, 181 (M^+, Calcd for $C_5H_{10}ClN_3O_2$), 72 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 3.16 (6H, s, NCH_3), 3.65 (2H, t, $J=6$ Hz, CH_2Cl), 4.16 (2H, t, $J=6$ Hz, $N(NO)CH_2$).

3-*n*-Butyl-1-(2-chloroethyl)-3-methylurea (4b)——A solution of 1.2 g of methyl-*n*-butylamine in 10 ml of ethyl ether was cooled to 0°C. To this solution, 1.6 g of 2-chloroethyl isocyanate was added dropwise and the mixture was stirred for 1 h at room temperature then concentrated. The residue was recrystallized from *n*-hexane to give 2.3 g (87%) of **4b** as colorless crystals. mp 34—36°C. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1640 (CO), 1530 (CHN). MS *m/e*: 192, 194 (M^+). NMR (in $CDCl_3$) δ: 0.8—1.7 (7H, m, $CH_2CH_2CH_3$), 2.90 (3H, s, NCH_3), 3.26 (2H, t, $J=6$ Hz, CH_2Cl), 3.60 (4H, m, NCH_2), 5.60 (1H, s, NH). *Anal.* Calcd for $C_8H_{17}ClN_2O$: C, 49.87; H, 8.89; Cl, 18.40; N, 14.54. Found: C, 49.95; H, 8.79; Cl, 18.31; N, 14.46.

3-*n*-Butyl-1-(2-chloroethyl)-3-methyl-1-nitrosourea (5b)——To a solution of 1.9 g of **4b** in 15 ml of acetic acid, 1.0 g of sodium nitrite was added portionwise during 1 h. The reaction mixture was worked up as in the case of the preparation of **5a** to give the nitrosourea (**5b**) (1.8 g, 85% yield) as a yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1700 (CO). MS *m/e*: 221, 223 (M^+, Calcd for $C_8H_{16}ClN_3O_2$), 114 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 0.90—2.00 (7H, m, $CH_2CH_2CH_3$), 3.15 (3H, s, NCH_3), 4.15 (2H, t, $J=6$ Hz, $N(NO)CH_2$).

3,3-Diallyl-1-(2-chloroethyl)urea (4c)——To a solution of 1.9 g of diallylamine in 20 ml of ethyl ether, 2.5 g of 2-chloroethyl isocyanate was added dropwise. The reaction mixture was worked up in the case of the preparation of **4b** to give the urea (**4c**) (3.0 g, 75% yeild) as colorless crystals. mp 41—42°C. IR ν_{max}^{Nujol}

cm^{-1}: 3330 (NH), 1630 (CO), 1525 (CNH). MS *m/e*: 202, 204 (M^+). *Anal.* Calcd for $C_9H_{15}ClN_2O$: C, 53.34; H, 7.45; Cl, 17.49; N, 13.82. Found: C, 53.42; H, 7.41; Cl, 17.55; N, 13.88.

3,3-Diallyl-1-(2-chloroethyl)-1-nitrosourea (5c)——The nitrosourea (**5c**) was similarly obtained from **4c** in 75% yield as a yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1700 (CO). MS *m/e*: 231, 233 (M^+, Calcd for $C_8H_{16}ClN_3O_2$), 124 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 3.57 (2H, t, $J=6$ Hz, CH_2Cl), 5.08—5.50 (4H, m, $CH_2=$), 5.62—6.25 (2H, m, –CH=).

4-[N-(2-Chloroethyl)carbamoyl]morpholine (4d)——The urea (**4d**) was similarly obtained in 82% yield as colorless crystals by the reaction of morpholine with 2-chloroethyl isocyanate. mp 146.5—147.5°C. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1620 (CO), 1540 (CNH). MS *m/e*: 192, 194 (M^+). *Anal.* Calcd for $C_7H_{13}ClN_2O_2$: C, 43.65; H, 6.80; Cl, 18.40; N, 14.54. Found: C, 43.71; H, 6.78; Cl, 18.32; N, 14.49.

4-[N-(2-Chloroethyl)-N-nitrosocarbamoyl]morpholine (5d)——The nitrosourea (**5d**) was obtained from **4d** in 76% yield as a yellow oil by the method described for the preparation of **5a**. IR $\nu_{max}^{liq.}$ cm^{-1}: 1700 (CO). MS *m/e*: 221, 223 (M^+, Calcd for $C_7H_{12}ClN_3O_3$), 114 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 4.15 (2H, t, $J=6$ Hz, $N(NO)CH_2$).

1-(2-Chloroethyl)-3-(2-hydroxyethyl)-3-methylurea (4e)——To a solution of 1.5 g of N-methyl ethanolamine in 15 ml of methanol, 2.3 g of 2-chloroethyl isocyanate was added dropwise at 5°C. The mixture was stirred for 1 h at room temperature then concentrated to give a colorless syrup. The residue was chromatographed on silica gel (chloroform–AcOEt–methanol=5: 1: 1), and 2.8 g (78%) of **4e** was obtained as a colorless oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3350 (broad, NH, OH), 1620 (CO), 1530 (CNH). MS *m/e*: 180, 182 (M^+, Calcd for $C_6H_{13}ClN_2O_2$), 113 (B^+, M^+-67).

1-(2-Chloroethyl)-3-(2-hydroxyethyl)-3-methyl-1-nitrosourea (5e)——A solution of 1.8 g of **4e** in 10 ml of formic acid was cooled to 5°C. To this solution, 2.1 g of sodium nitrite was added portionwise during 2 h. The reaction mixture was neutralized to pH 4—6 with aqueous sodium carbonate under cooling and extracted with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate, dried, and concentrated. The residue was chromatographed on silica gel (chloroform–methanol=10: 1), and 1.4 g (67%) of **5e** was obtained as a yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (OH), 1700 (CO). MS *m/e*: 209 (M^+, Calcd for $C_6H_{12}ClN_3O_3$: 209, 211), 102 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 2.85 (1H, s, OH), 3.23 (3H, s, NCH_3).

1-(2-Chloroethyl)-3-(2,3-dihydroxy-*n*-propyl)-3-methylurea (4f)——The urea (**4f**) was obtained in 56% yield as a colorless oil by the reaction of N-methyl-N-(2,3-dihydroxy-*n*-propyl)amine with 2-chloroethyl isocyanate as described for the preparation of **4e**. IR $\nu_{max}^{liq.}$ cm^{-1}: 3350 (OH), 3280 (NH), 1630 (CO), 1540 (CNH). MS *m/e*: 210 (M^+, weak, Calcd for $C_7H_{15}ClN_2O_3$: 210, 212), 143 (B^+, M^+-67).

1-(2-Chloroethyl)-3-(2,3-dihydroxy-*n*-propyl)-3-methyl-1-nitrosourea (5f)——The nitrosourea (**5f**) was obtained from **4f** in 58% yield as a yellow oil in the same manner as described for the preparation of **5e** (solvent used on chromatography: chloroform–AcOEt–methanol=3: 1: 1). IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (OH), 1690 (CO). MS *m/e*: M^+; not detected (Calcd for $C_7H_{14}ClN_3O_4$: 239, 241), 132 (B^+, M^+-107). NMR (in $CDCl_3$) δ: 3.20 (3H, s, NCH_3), 4.20 (2H, m, $N(NO)CH_2$), 4.78 (2H, s, OH).

1-[3-(2-Chloroethyl)-1-methylureido]-1-deoxy-D-glucitol (4g)——To a solution of 2.0 g of N-methylglucamine in 15 ml of methanol, 1.3 g of 2-chloroethyl isocyanate was added dropwise at 5°C. The mixture was stirred for 1 h at room temperature then concentrated. The residue was dissolved in a small amount of ethanol and ethyl acetate was added. Colorless crystals separated in 62% yield. mp 88—90°C. IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH), 3270 (NH), 1630 (CO), 1540 (CNH). *Anal.* Calcd for $C_{10}H_{21}ClN_2O_6$: C, 39.94; H, 7.04; Cl, 11.79; N, 9.32. Found: C, 40.08; H, 7.10; Cl, 11.65; N, 9.24.

1-[3-(2-Chloroethyl)-1-methyl-3-nitrosoureido]-1-deoxy-D-glucitol (5g)——A solution of 1.9 g of **4g** in 10 ml of formic acid was cooled to 5°C. To this solution, 1.0 g of sodium nitrite was added portionwise during 1.5 h under cooling. After 30 minutes, the mixture was lyophilized and the residue was dissolved in 20 ml of tetrahydrofuran. The solution was filtered. The filtrate was concentrated and the residue was chromatographed on silica gel (chloroform–AcOEt–methanol=2: 1: 1) to give 0.8 g (39%) of **5g** as a pale yellow caramel. $[\alpha]_D^{26}$ $-14.7°$ ($c=1.1$, methanol): IR ν_{max}^{Nujol} cm^{-1}: 3330 (OH), 1690 (CO), 1080. NMR (in D_2O) δ: 3.23 (3H, s, NCH_3), 4.20 (2H, t, $J=6$ Hz, $N(NO)CH_2$). *Anal.* Calcd for $C_{10}H_{20}ClN_3O_7$: C, 36.42; H, 6.07; Cl, 10.77; N, 12.75. Found: C, 37.03; H, 5.94; Cl, 10.32; N, 12.18.

1-(2-Chloroethyl)-3-(2,3-dihydroxy-*n*-propyl)-3-*n*-propylurea (4h)——To a solution of 5 g of *n*-propylamine in 50 ml of tetrahydrofuran, 3.3 g of α-monochlorohydrin was added dropwise. The mixture was allowed to stand at room temperature for 3 d then evaporated to dryness. The residue was dissolved in 50 ml of methanol and 3 g of triethylamine was added. To this solution, 4 g of 2-chloroethyl isocyanate was added. After 2 h, the mixture was concentrated and the residue was extracted with ethyl acetate. The organic layer was washed with sat. NaCl solution, dried, and concentrated, and the residue was chromatographed on silica gel (chloroform–AcOEt–methanol=3: 1: 1). The urea (**4h**) was obtained in 31% yield (from α-monochlorohydrin) as a colorless oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3300 (broad, NH, OH), 1620 (CO), 1530 (CNH), 1260, 1050. MS *m/e*: 238 (M^+, weak, Calcd for $C_9H_{19}ClN_2O_3$: 238, 240), 171 (B^+, M^+-67).

1-(2-Chloroethyl)-3-(2,3-dihydroxy-*n*-propyl)-1-nitroso-3-*n*-propylurea (5h)——Nitrosation of **4h** was carried out as described for the preparation of **5f**. The nitrosourea (**5h**) was obtained in 56% yield as a yellow oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3350 (OH), 1695 (CO). MS *m/e*: M^+, not detected (Calcd for $C_9H_{18}ClN_3O_4$: 267, 269), 160 (B^+, M^+-107). NMR (in $CDCl_3$): 0.87 (3H, t, CH_2CH_3), 1.63 (2H, m, CH_2CH_3).

1-[1-*n*-Butyl-3-(2-chloroethyl)ureido]-1-deoxy-erythritol (4i)——The urea (**4i**) was obtained in 52% yield as a colorless oil from 1-*n*-butylamino-1-deoxy-erythritol by the method described for the preparation of **4f**. IR $\nu_{max}^{liq.}$ cm^{-1}: 3330 (broad, NH, OH), 1620 (CO), 1540 (CNH), 1260, 1070. MS *m/e*: M$^+$, not detected (Calcd for $C_{11}H_{23}ClN_2O_4$: 282, 284), 215 (B$^+$, M$^+-67$). The starting material was prepared by catalytic hydrogenation and subsequent hydrolysis of 2,3-O-ethylidene-D-erythrose *n*-butylamine Schiff base obtained by the method of Ziderman.[7]

1-[1-*n*-Butyl-3-(2-chloroethyl)-3-nitrosoureido]-1-deoxy-erythritol (5i)——The nitrosourea (**5i**) was obtained from **4i** in 48% yield as a yellow oil in the same manner as described for the preparation of **5f**. $[\alpha]_D^{26}$ −23.5° (*c*=1.0, methanol). IR $\nu_{max}^{liq.}$ cm^{-1}: 3400 (OH), 1680 (CO). MS *m/e*: M$^+$, not detected (Calcd for $C_{11}H_{22}ClN_3O_5$: 311, 313), 204 (B$^+$, M$^+-107$). NMR (in $CDCl_3$) δ: 0.80—1.80 (7H, m, $CH_2CH_2CH_3$)

1-[1-*n*-Butyl-3-(2-chloroethyl)ureido]-1-deoxy-D-glucitol (4j)——The urea (**4j**) was obtained in 63% yield as a colorless oil from 1-*n*-butylamino-1-deoxy-D-glucitol in the same manner as described for the preparation of **4g**. IR $\nu_{max}^{liq.}$ cm^{-1}: 3350 (broad, OH, NH), 1620 (CO), 1540 (CNH), 1080. The starting material was obtained by catalytic hydrogenation of 1-*n*-butylamino-1-deoxy-D-glucose [8]

1-[1-*n*-Butyl-3-(2-chloroethyl)-3-nitrosoureido]-1-deoxy-D-glucitol (5j)——The nitrosourea (**5j**) was obtained from **4j** in 43% yield as a pale yellow amorphous powder by the method described for the preparation of **5g**. mp 65—67°C (dec.). $[\alpha]_D^{26}$ −17.8° (*c*=1.5, methanol). IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 1700 (CO), 1130, 1090. NMR (in D_2O) δ: 0.7—1.9 (7H, m, $CH_2CH_2CH_3$), 4.15 (2H, t, *J*=6 Hz, N(NO)CH_2). *Anal.* Calcd for $C_{13}H_{26}ClN_3O_7$: C, 41.99; H, 7.00; Cl, 9.56; N, 11.31. Found: C, 42.63; H, 6.92; Cl, 9.32; N, 10.94.

1-(2-Chloroethyl)-3,3-bis(2-hydroxyethyl)urea (4k)——The urea (**4k**) was obtained from diethanolamine in 71% yield as a colorless oil in the same manner as described for the preparation of **4e**. IR $\nu_{max}^{liq.}$ cm^{-1}: 3330 (broad, NH, OH), 1620 (CO), 1540 (CNH). MS *m/e*: 210 (M$^+$, weak, Calcd for $C_7H_{15}ClN_2O_3$: 210, 212), 143 (B$^+$, M$^+-67$).

1-(2-Chloroethyl)-3,3-bis(2-hydroxyethyl)-1-nitrosourea (5k)——The nitrosourea (**5k**) was obtained from **4k** in 52% yield as a yellow oil by the method described for the preparation of **5e**. IR $\nu_{max}^{liq.}$ cm^{-1}: 3370 (OH), 1690 (CO). MS *m/e*: M$^+$, not detected (Calcd for $C_7H_{14}ClN_3O_4$: 239, 241), 132 (B$^+$, M$^+-107$). NMR (in $CDCl_3$) δ: 4.10 (2H, t, *J*=6 Hz, N(NO)CH_2), 4.50 (2H, broad s, OH).

3,3-Bis(2-acetoxyethyl)-1-(2-chloroethyl)urea (4l)——A solution of 10 ml of pyridine, 5 ml of acetic anhydride, and 2.1 g of **4k** was allowed to stand at room temperature overnight then 5 ml of water was added to the solution. After 30 minutes, the mixture was poured into cold aqueous hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with aqueous sodium bicarbonate, dried, and concentrated. The residue was chromatographed on silica gel (chloroform–AcOEt–methanol=10: 10: 1) to afford 2.1 g (71% yield) of **4l** as a colorless oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3350 (NH), 1740 (CO), 1630 (CO), 1540 (CNH), 1240, 1050. MS *m/e*: 294, 296 (M$^+$, Calcd for $C_{11}H_{19}ClN_2O_5$).

3,3-Bis(2-acetoxyethyl)-1-(2-chloroethyl)-1-nitrosourea (5l)——The nitrosourea (**5l**) was obtained from **4l** in 72% yield as a yellow oil by the method described for the preparation of **5e**. IR $\nu_{max}^{liq.}$ cm^{-1}: 1740 (CO), 1700 (CO). MS *m/e*: 323, 325 (M$^+$, Calcd for $C_{11}H_{18}ClN_3O_6$), NMR (in $CDCl_3$) δ: 2.10 (6H, s, $COCH_3$), 3.60 (2H, t, CH_2Cl), 3.80 (4H, t, NCH_2), 4.15 (2H, t, N(NO)CH_2), 4.35 (4H, t, OCH_2).

2,3,4,5,6-Penta-O-acetyl-1-[3-(2-chloroethyl)-1-methylureido]-1-deoxy-D-glucitol (4m)——The urea (**4m**) was obtained from **4g** in 62% yield as a colorless caramel in the same manner as described for the preparation of **4l**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (CO), 1650 (CO), 1510 (CNH).

2,3,4,5,6-Penta-O-acetyl-1-[3-(2-chloroethyl)-1-methyl-3-nitrosoureido]-1-deoxy-D-glucitol (5m)——The nitrosourea (**5m**) was obtained from **4m** in 38% yield as a pale yellow amorphous powder in the same manner as described for the preparation of **5e**. mp 77—82°C (dec). $[\alpha]_D^{20}$ −1.1° (*c*=1.0, methanol). IR ν_{max}^{Nujol} cm^{-1}: 1750 (CO), 1695 (CO), 1225, 1215.

Decomposition of the Nitrosoureas (5e, k) in Phosphate-buffered Solution (pH 7.4)——The nitrosourea (**5e**) (1.0 g) was dissolved in 50 ml of 1 M phosphate-buffered solution (pH 7.4; prepared by dissolving 6.8 g of KH_2PO_4 and 1.6 g of NaOH in 50 ml of water) at 5°C and the mixture was stirred for 30 min. Then the solution was allowed to stand at room temperature for 3 h. The mixture was saturated with ammonium sulfate and extracted twice with ethyl acetate. The organic layer was washed with sat.NaCl solution, dried, and concentrated. The residual colorless oil, which gave only a single spot on thin-layer chromatography (TLC), was purified by short column chromatography using silica gel (chloroform–methanol=15: 1) to give 0.46 g (92%) of 3-methyl-oxazolidinone-2[9] (**6e**) as a colorless oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 1750 (CO), 1275, 1050. MS *m/e*: 101(M$^+$, Calcd for $C_4H_7NO_2$). NMR (in $CDCl_3$) δ: 2.87 (3H, s, NCH_3), 3.41—3.69 (2H, m, NCH_2), 4.18—4.45 (2H, m, OCH_2).

3-(2-Hydroxyethyl)-oxazolidinone-2[10] (**6k**) was similarly obtained from **5k** in 86% yield as a colorless oil. IR $\nu_{max}^{liq.}$ cm^{-1}: 3380 (OH), 1730 (CO), 1270, 1050. MS *m/e*: 131 (M$^+$, Calcd for $C_5H_9NO_3$). NMR (in d_6-DMSO) δ: 3.22 (2H, t, *J*=5 Hz, $C\underline{H}_2CH_2OH$), 3.64 (2H, t, *J*=5 Hz, $CH_2C\underline{H}_2OH$), 3.55 (2H, m, N–CH_2), 4.24 (2H, m, O–CH_2), 4.73 (1H, broad, OH).

Antitumor Activity Tests——The test for activity against leukemia L1210 was carried out by the following procedure. Leukemic cells (10^5) were inoculated intraperitoneally into a group of four or six mice (male BDF_1 mice). Intraperitoneal administration of nitrosoureas was begun 24 h after the inoculation and performed once daily for 5 d. The survival time (d) of the treated mice was observed and compared with

that of the control group. The test for activity against Ehrlich ascites carcinoma was done by the following procedure. The ascites cells (10^6) were inoculated intraperitoneally into a group of five mice (female ICR mice). Intraperitoneal administration of nitrosoureas was begun 24 h after the inoculation and performed once daily for 5 d. The volume of ascites fluid of the treated mice was measured after 7 d and compared with that of the control group.

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