Chem. Pharm. Bull. 29(11)3262—3273(1981)

A New Class of Nitrosoureas. II.[1] Synthesis and Antitumor Activity of 1-(2-Chloroethyl)-3,3-disubstituted-1-nitrosoureas having a Glucopyranosyl, Mannopyranosyl or Galactopyranosyl Moiety

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(Received May 20, 1981)

Many kinds of 1-(2-chloroethyl)-3-substituted-3-β-D-glycopyranosyl-1-nitrosoureas (V) were synthesized and tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma. The reaction of aldohexoses such as D-glucose, D-mannose, and D-galactose with primary amines followed by treatment with 2-chloroethyl isocyanate usually gave a mixture of structural isomers of glycosylureas (III'). Complete isomerization into thermodynamically stable β-D-glycopyranosylureas (III) was observed when the mixture of isomers was dissolved in formic acid. Glycopyranosylureas (III) were nitrosated with 5 equivalents of dinitrogen tetroxide followed by treatment with methanol to give the corresponding nitrosoureas (V) in good yields. Many of the nitrosoureas (V) were remarkably active against both leukemia L1210 and Ehrlich ascites carcinoma and showed greater therapeutic ratios than those of the positive controls, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, 3[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea, and 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea. In particular, some of the galactopyranosylnitrosoureas showed excellent antitumor activities and sixty-day survivors against leukemia L1210 were observed at dose levels of 25, 50 and 100 mg/kg of the compounds. These nitrosoureas (V) appear to be activated nonenzymatically by attack of the hydroxyl group of the sugar moiety on the carbonyl group to give the cyclic carbamate (VI) without generation of the isocyanate (XI).

Keywords——chloroethyl nitrosoureas; 3-subsutituted-3-β-D-glycopyranosylnitrosoureas; selective isomerization of glycosylureas; dinitrogen tetroxide; antitumor activities; leukemia L1210; Ehrlich asites carcinoma; CCNU; ACNU; GANU

Chloroethylnitrosoureas are an important class of antitumor agents with a broad spectrum of activity. However, the clinically effective agents of this class such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and 3[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU), *etc.*, invariably produce delayed and cumulative bone marrow depression in humans.[2] The clinical usefulness of these agents is thus limited significantly by this myelosuppressive toxicity.

Recently, as part of a search for compounds possessing strong antitumor activity with reduced myelotoxicity, several studies on water-soluble sugar derivatives of chloroethylnitrosoureas have been reported. Among such compounds, glycopyranosyl derivatives such as 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (DCNU),[3] 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU),[4] and 1-(2-chloroethyl)-3-(methyl-α-D-glucopyranos-6-yl)-1-nitrosourea (MCNU)[5] are currently under clinical trials.

In the preceding paper[1] we reported the synthesis of 1-(2-chloroethyl)-3,3-disubstituted-1-nitrosourea bearing no proton on their N-3 position. These nitrosoureas, especially those having a hydroxyl group on the β-position of their substituents, were found to exhibit remarkable antitumor activity. As a logical extension of the studies on this new class of nitrosoureas, aldohexoses were next chosen as substituents having a β-hydroxyl group. It was also hoped that a sugar moiety might act as a specific carrier transporting the drug into tumor cells. In this paper we report the synthesis and the remarkable antitumor activity of a new class of

3,3-disubstituted-nitrosourea derivatives bearing glucopyranosyl, mannopyranosyl, and galactopyranosyl moieties.

Synthesis of Nitrosoureas and Discussion

The aldohexose derivatives of nitrosoureas (V_{1-42}) which are disubstituted on their N-3 position were obtained by the sequence outlined in Chart 1.

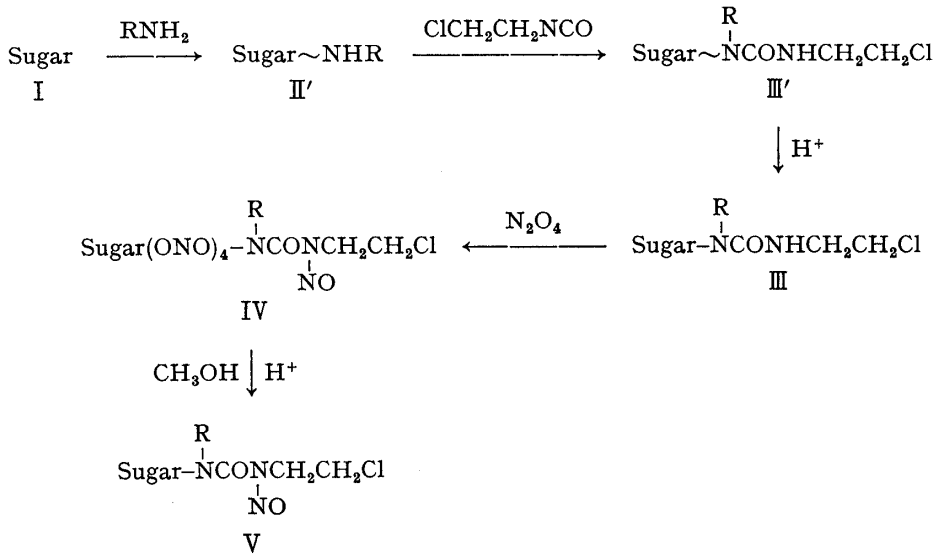
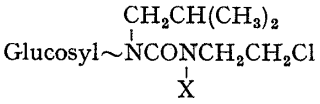


Chart 1

II′ and III′ represent mixtures of structural isomers

D-Glucose, D-mannose, and D-galactose were chosen as the sugar moiety (I) in the present study. Heating of I with primary amines in methanol at 65—70°C generally gave a mixture of the structural isomers of N-substituted-glycosylamines (II′), though N-alkylglycopyranosylamines are reported to be easily obtained by the reaction of aldohexoses with primary and secondary aliphatic amines.[6] The reaction of this mixture (II′) with 2-chloroethyl isocyanate usually gave the ureas (III′) as a mixture of structural isomers with respect to the sugar moiety. For instance, the reaction of D-glucose with isobutylamine followed by treatment with 2-chloroethyl isocyanate gave a mixture of the ureas (III′), and thin-layer chromatography (TLC) showed two spots in a ratio of one to ten (*Rf*: 0.55 and 0.40). This mixture was chromatographed on silica gel to give the two compounds (III_{6a} and III_{6b}), which were considered to be structural isomers with respect to the sugar moiety on the basis of their physical and spectroscopic data (see Table I). The major product (III_{6b}) was determined to be the β anomer of 1-(2-chloroethyl)-3-isobutyl-3-D-glucopyranosylurea from its nuclear magnetic resonance (NMR) spectrum, in which a doublet ascribable to the anomeric proton of a β anomer of glucopyranoside was detected at δ 4.60 (J=8.0 Hz). Although the structure of the minor product (III_{6a}) was not ascertained, it appears to be the α anomer of 1-(2-chloroethyl)-3-isobutyl-3-D-glucopyranosylurea or the β anomer of 1-(2-chloroethyl)-3-isobutyl-3-D-glucofuranosylurea judging from its NMR spectrum, which showed a doublet at δ 4.85 (J=3.2 Hz). Complete isomerization of this minor product (III_{6a}) to the β anomer of a pyranoid structure (III_{6b}) was observed when the former was dissolved in an acidic medium such as acetic acid or formic acid. Thus, when III_{6a} (*Rf*: 0.55, $[\alpha]_D$=−4.0°) was dissolved in a small amount of formic acid at room temperature, the spot on TLC changed completely to that of III_{6b}. III_{6b} was isolated from this solution as an amorphous powder (*Rf*: 0.40, $[\alpha]_D$=+16.4°) in nearly quantitative yield. The properties of these isomeric glycosylureas are listed in Table I together with those of the corresponding nitrosourea derivatives. The details of the structure of the isomeric

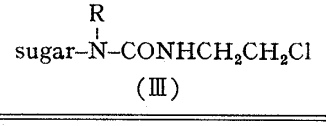
TABLE I. Properties of Structural Isomers of Some Glucosylureas and Nitrosoureas



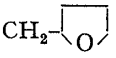
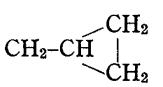
Compound No.	Glucosyl	X	*Rf* Values[a]	$[\alpha]_D^{20}$ ($c=1.0$, MeOH)	IR (ν_{max}^{Nujol} cm^{-1})	NMR of C_1–H[b] (δ)
III_{6a}	D-Glucosyl	H	0.55	−4.0°	3330, 1630 1530, 1070	4.85 (d, $J=3.2$ Hz)
III_{6b}	β-D-Glucopyranosyl	H	0.40	+16.4°	3340, 1635 1530, 1070	4.60 (d, $J=8.0$ Hz)
V_{6a}	D-Glucosyl	NO	0.75	−22.8°	3350, 1690 1070	5.16 (d, $J=1.4$ Hz)
V_{6b}	β-D-Glucopyranosyl	NO	0.63	−12.1°	3350, 1695 1080	4.64 (d, $J=7.4$ Hz)

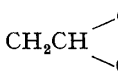
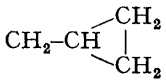
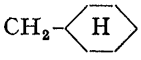
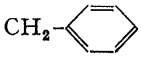
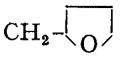
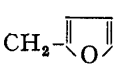
a) Silica gel, G $CHCl_3$–AcOEt–MeOH (2:1:1).
b) Measured in d_6-DMSO–D_2O.

TABLE II. Properties of β-D-Glycopyranosylureas



(III)

III No.	Sugar	R	Acid Treatment[f]	Yield (%)	IR ν_{max}^{Nujol} (cm^{-1})	NMR (in D_2O) δ
1b	Gluco[a]	CH_3	No	68	3300, 1630, 1530, 1070, 1030	3.10 (3H, s)
2b	Gluco	CH_2CH_3	No	77	3350, 1640, 1535, 1080, 1040	1.25 (3H, t)
3b	Gluco	$CH_2CH_2CH_3$	No	75	3300, 1630, 1530, 1080, 1040	0.93 (3H, t) 1.35—2.0 (2H, m)
4b	Gluco	$CH(CH_3)_2$	Yes	80	3350, 1640, 1530, 1070, 1030	1.38 (6H, d)
5b	Gluco	$CH_2CH_2CH_2CH_3$	No	85	3300, 1640, 1530, 1070, 1030	0.75—1.70 (7H, m)
6b	Gluco	$CH_2CH(CH_3)_2$	Yes	75	3350, 1635, 1535, 1070, 1030	0.90 (6H, d) 1.7—2.3 (1H, m)
7b	Gluco	$CH_2(CH_2)_3CH_3$	Yes	77	3350, 1640, 1540, 1070, 1030[b]	0.7—2.0 (9H, m)
8b	Gluco	$CH_2(CH_2)_4CH_3$	Yes	80	3350, 1640, 1520, 1080, 1040[b]	
9b	Gluco	$CH_2(CH_2)_{10}CH_3$	Yes	85	3350, 1640, 1530, 1080, 1030[b]	
10b	Gluco	$CH_2(CH_2)_{14}CH_3$	Yes	77	3350, 1640, 1530, 1080, 1040[b]	
11b	Gluco	$CH_2(CH_2)_{16}CH_3$	Yes	83	3350, 1645, 1530, 1080, 1040[b]	
12b	Gluco	$CH_2(CH_2)_2CH_2OH$	Yes	75	3340, 1630, 1530, 1070, 1020	1.43—1.90 (4H, m)
13b	Gluco	$CH_2CH_2CH_2OCH_3$	Yes	78	3350, 1640, 1530, 1110, 1070, 1020	1.7—2.2 (2H, m) 3.30 (3H, s)
14b	Gluco		Yes	72	3350, 1640, 1540, 1070	1.5—2.16 (4H, m)
15b	Manno[c]	$CH_2CH_2CH_2CH_3$	No	85	3350, 1640, 1540, 1070, 1030	0.7—1.9 (7H, m)
16b	Manno		No	78	3350, 1630, 1530, 1060	0.2—0.72 (4H, m) 0.96—1.26 (1H, m)
17b	Manno	$CH_2CH_2OCH_3$	No	83	3320, 1630, 1550, 1110, 1060	3.40 (3H, s)

III No.	Sugar	R	Acid Treatment[f]	Yield (%)	IR ν_{max}^{Nujol} (cm^{-1})	NMR (in D_2O) δ
18b	Galacto[d]	$CH_2CH_2CH_3$	No	86	3400, 1635, 1530, 1070, 1040	0.95 (3H, t) 1.7—2.1 (2H ,m)
19b	Galacto	$CH(CH_3)_2$	Yes	79	3350, 1640, 1530, 1050	1.38 (6H, d)
20b	Galacto	$CH_2CH_2CH_2CH_3$	No	82	3350, 1640, 1540, 1070, 1030	0.8—1.9 (7H, m)
21b	Galacto	$CH_2CH(CH_3)_2$	Yes	76	3350, 1640, 1540, 1070	0.93 (6H, d) 1.75—2.20 (1H, m)
22b	Galacto	$CH_2(CH_2)_3CH_3$	Yes	78	3350, 1640, 1535, 1060[b]	0.75—2.0 (9H, m)
23b	Galacto	$CH_2CH_2CH(CH_3)_2$	Yes	75	3350, 1640, 1530, 1060[b]	0.87 (6H, d) 1.2—2.0 (3H, m)
24b	Galacto	$CH_2C(CH_3)_3$	Yes	68	3350, 1640, 1540, 1070[b]	1.90 (9H, s)
25b	Galacto	$CH_2CH{=}CH_2$	No	78	3400, 1640, 1535, 1070	
26b	Galacto	$CH_2C{\equiv}CH$	Yes	74	3350, 1640, 1535, 1050	2.80 (1H, s)
27b	Galacto	$CH_2CH_2C{\equiv}N$	No	67	3350, 1640, 1530, 1070	2.97 (2H, t)
28b	Galacto	$CH_2CH_2OCH_3$	No	81	3350, 1630, 1545, 1110, 1060	3.35 (3H, s)
29b	Galacto	$CH_2CH_2CH_2OCH_3$	Yes	83	3350, 1630, 1540, 1050	1.75—2.15 (2H, m) 3.35 (3H, s)
30b	Galacto	$CH(CH_3)CH_2OCH_3$	Yes	68	3360, 1635, 1540, 1080, 1040	
31b	Galacto	$CH_2CH(OCH_2CH_3)_2$	Yes	65	3370, 1635, 1545, 1110, 1070	1.06 (3H, t)
32b	Galacto		Yes	67	3350, 1635, 1540, 1090, 1050	0.95 (3H, t) 1.2—1.8 (2H, m)
33b	Galacto	$CH_2CH_2COOCH_3$	Yes	64	3350, 1725, 1635, 1535, 1050	2.73 (2H, t) 3.73 (3H, s)
34b	Galacto		No	70	3350, 1640, 1545, 1060	0.35—0.75 (4H, m) 1.1—1.5 (1H, m)
35b	Galacto		Yes	66	3360, 1630, 1540, 1060	1.0—2.0 (m)
36b	Galacto		Yes	72	3350, 1640, 1545, 1070	0.6—2.0 (m)
37b	Galacto		Yes	75	3350, 1640, 1540, 1080	4.73 (2H, s) 7.30 (5H, s)
38b	Galacto		Yes	71	3370, 1635, 1540, 1070, 1040	1.8—2.3 (4H, m)
39b	Galacto		Yes	76	3350, 1640, 1530, 1060	4.60 (2H, m) 6.45 (2H, m) 7.45 (1H, m)
40b	Gluco $(OAc)_4$[e]	CH_3	No	63	1750, 1650, 1530, 1230, 1060[b]	
41b	Gluco $(OAc)_4$[e]	$CH_2CH_2CH_2CH_3$	No	68	1750, 1650, 1540, 1230, 1050[b]	
42b	Gluco $(OAc)_4$[e]	CH_2CH_2OAc	No	60	1750, 1660, 1530, 1240, 1040[b]	

a) Gluco represents β-D-glucopyranosyl moiety.
b) Measured in $CHCl_3$ solution.
c) Manno represents β-D-mannopyranosyl moiety.
d) Galacto represents β-D-galactopyranosyl moiety.
e) Represents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl moiety.
f) Selective isomerization to the β isomer was done by dissolving the isomeric mixture in formic acid (see "Experimental" section).

glycosylureas and their isomerization by acid will be reported elsewhere. Thus, a number of the β anomers of glycopyranosylurea (III) listed in Table II could easily be obtained from the mixture of the structural isomers (III') by this acid treatment.

Nitrosation of the ureas (III) presented some difficulty. Treatment of the ureas (III) with sodium nitrite in acetic or formic acid resulted in formation of the equilibrium mixture (1:1) of the nitrosourea (V) and III, though the nitrosation of 1-(2-chloroethyl)-3-β-D-glucopyranosylurea (possessing a proton on the N-3 position) proceeded completely to give the corresponding nitrosourea (GANU) under the same conditions.[7] Reaction of the N-substituted glycosylamine (II') with o-nitrophenyl N-(2-chloroethyl)-N-nitrosocarbamate (VII) also failed to give V, though primary amino sugars such as N-unsubstituted-glucosylamine, methyl 6-amino-6-deoxy-α-D-glucopyranoside, and D-glucosamine were reported to react with VII to give the corresponding nitrosourea in good yields.[8]

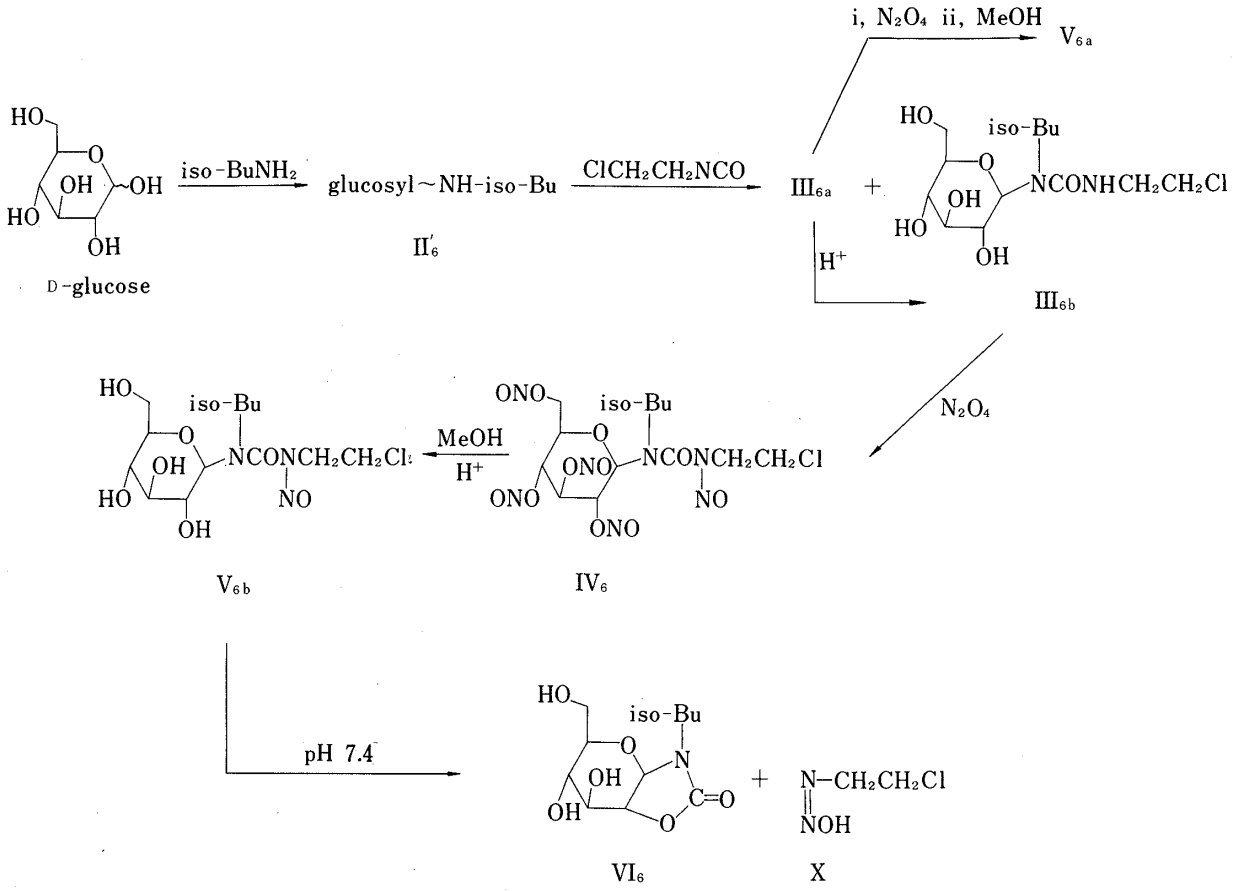


Chart 2

Finally, nitrosation of the ureas (III) was achieved successfully by the use of dinitrogen tetroxide in the presence of an acid acceptor in an inert solvent at low temperature. The nitrosation of III required five equivalents of dinitrogen tetroxide, indicating that the four hydroxyl groups of the glycopyranosyl moiety are nitrosated first, followed by nitrosation of one ureido group. The nitrous esters of the hydroxyl groups could be decomposed without affecting the N-nitroso group by addition of methanol under acidic conditions at low temperature. Thus, in a typical procedure, five equivalents of dinitrogen tetroxide gas was introduced into a mixture of the urea (III_{6b}) and anhydrous sodium acetate in tetrahydrofuran at -5°C to form the nitrous ester (IV_6) quantitatively. Methanol was added at the same temperature

and the mixture was extracted with ethyl acetate after addition of sodium acetate and a small amount of water. The nitrosourea (V_{6b}) was obtained in 73% yield after chromatography on silica gel. Its spectroscopic data were compatible with the structure (V_{6b}), and listed in Table I.

Thus, various 1-(2-chloroethyl)-3-substituted-3-β-D-glycopyranosyl-1-nitrosoureas (V) were easily obtained in this way. They are generally unstable yellow caramels, and are listed in Table III together with some characteristic data.

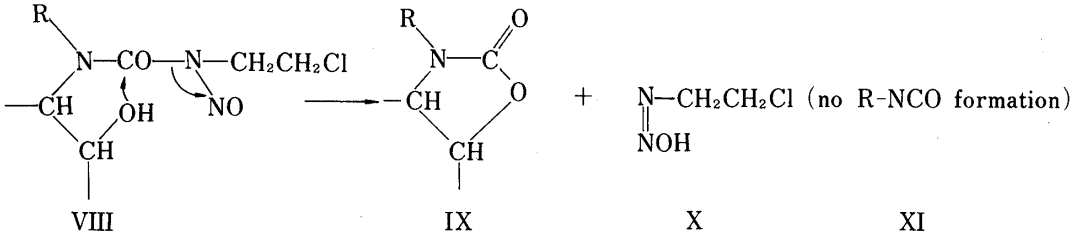


Chart 3

In the preceding paper we demonstrated that 1-(2-chloroethyl)-3,3-disubstituted-1-nitrosoureas (VIII) having a hydroxyl group on the β-position of their substituents are activated nonenzymatically by attack of the hydroxyl group on the carbonyl group to give the oxazolidinones (IX) and chloroethyl diazohydroxide (X) or a related compound without generation of the isocyanate (XI) (see Chart 3). This activation mechanism differs distinctly from those of known nitrosoureas such as BCNU and CCNU, which are activated by losing the proton on the N-3 position to give X and XI.

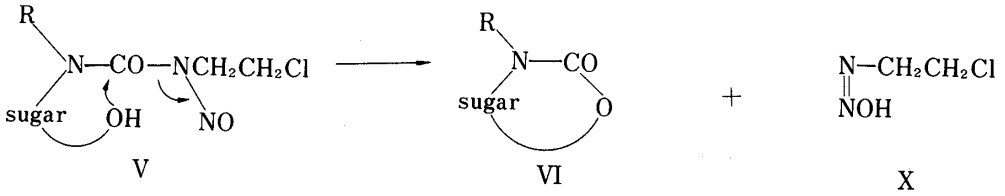
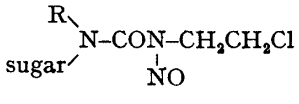


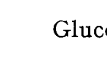
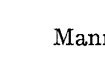
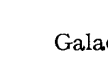
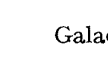
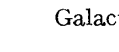
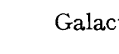
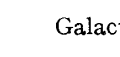
Chart 4

TABLE III. Properties of β-D-Glycopyranosylnitrosoureas



V

V No.	Sugar	R	Yield (%)	$[\alpha]_D^{a)}$	IR $\nu_{max}^{CHCl_3}$ (cm^{-1})	NMR (in D_2O) δ
1b	Gluco[b)]	CH_3	50	−22.9°	3350, 1690, 1070[c)]	3.15 (3H, s), 4.20 (2H, t)[i)]
2b	Gluco	CH_2CH_3	76	+16.0°	3370, 1700, 1090[c)]	1.26 (3H, t), 4.20 (2H, t)[i)]
3b	Gluco	$CH_2CH_2CH_3$	72	+5.0°	3300, 1700, 1070[c)]	0.90 (3H, t), 1.6—2.0 (2H, m), 4.20 (2H, t)[i)]
4b	Gluco	$CH(CH_3)_2$	64	+21.0°	3400, 1690, 1070[c)]	1.35 (6H, d), 4.20 (2H, t)[i)]
5b	Gluco	$CH_2CH_2CH_2CH_3$	70	+8.0°	3350, 1700, 1080	0.7—1.8 (7H, m), 4.15 (2H, t)[i)]
6b	Gluco	$CH_2CH(CH_3)_2$	73	−12.1°	3400, 1700, 1080	0.90 (6H, d), 1.8—2.3 (1H, t), 4.15 (2H, t)[i)]
7b	Gluco	$CH_2(CH_2)_3CH_3$	80	+3.3°	3400, 1690, 1080	0.7—2.0 (9H, m), 4.15 (2H, t)[i)]

V No.	Sugar	R	Yield (%)	$[\alpha]_D^{a)}$	IR $\nu_{max}^{CHCl_3}$ (cm^{-1})	NMR (in D_2O) δ
8b	Gluco	$CH_2(CH_2)_4CH_3$	56	+4.4°	3400, 1700, 1080	
9b	Gluco	$CH_2(CH_2)_{10}CH_3$	65	+4.1°	3400, 1690, 1070	
10b	Gluco	$CH_2(CH_2)_{14}CH_3$	68	+2.0°	3370, 1690, 1080	
11b	Gluco	$CH_2(CH_2)_{16}CH_3$	72	+2.7°	3350, 1690, 1080	
12b	Gluco	$CH_2(CH_2)_2CH_2OH$	67	+5.5°	3360, 1695, 1070[c)]	1.41—1.9 (4H, m), 4.20 (2H, t)[i)]
13b	Gluco	$CH_2CH_2CH_2OCH_3$	74	+9.8°	3400, 1695, 1070	1.75—2.3 (2H, m), 3.35(3H, s), 4.20 (2H, t)[i)]
14b	Gluco		71	+21.6°	3400, 1695, 1070	1.56—2.1 (4H, m), 4.20 (2H, t)[i)]
15b	Manno[d)]	$CH_2CH_2CH_2CH_3$	79	+33.1°	3350, 1690, 1080	0.8—2.0 (7H, m), 4.20 (2H, t)[i)]
16b	Manno		74	+26.3°	3400, 1690, 1080	0.20—0.68 (4H, m), 1.04—1.36 (1H, m)
17b	Manno	$CH_2CH_2OCH_3$	66	+20.0°	3370, 1695, 1090	3.3 (3H, s)
18b	Galacto[e)]	$CH_2CH_2CH_3$	65	+18.0°	3380, 1690, 1080[c)]	0.90 (3H, t), 1.6—2.0 (2H, m)
19b	Galacto	$CH(CH_3)_2$	63	+21.1°	3400, 1690, 1070	1.4 (6H, d), 4.16 (2H, t)[i)]
20b	Galacto	$CH_2CH_2CH_2CH_3$	77	+16.4°	3400, 1700, 1080	0.8—1.9 (7H, m), 4.20 (2H, t)[i)]
21b	Galacto	$CH_2CH(CH_3)_2$	69	−3.6°	3380, 1695, 1090	0.95 (6H, d), 1.8—2.25 (1H, m), 4.20 (2H, t)[i)]
22b	Galacto	$CH_2(CH_2)_3CH_3$	71	+11.4°	3400, 1690, 1090	0.7—2.0 (9H, m), 4.15 (2H, t)[i)]
23b	Galacto	$CH_2CH_2CH(CH_3)_2$	64	−3.2°	3380, 1690, 1090	0.89 (6H, d), 1.2—1.9 (3H, m)
24b	Galacto	$CH_2C(CH_3)_3$	72	+48.7°	3400, 1705, 1075	0.90 (9H, s)
25b	Galacto	$CH_2CH=CH_2$	65	−13.1°	3400, 1700, 1090	
26b	Galacto	$CH_2C\equiv CH$	65	−9.2°	3370, 1690, 1080[c)]	2.75 (1H, m)
27b	Galacto	$CH_2CH_2C\equiv N$	52	−37.3°	3460, 2260, 1690, 1080	2.97 (2H, t)
28b	Galacto	$CH_2CH_2OCH_3$	73	+9.2°	3350, 1700, 1080	3.26 (3H, s), 4.82 (1H, d, anomeric proton)[f)]
29b	Galacto	$CH_2CH_2CH_2OCH_3$	70	+15.5°	3380, 1700, 1080	1.8—2.25 (2H, m), 3.35 (3H, s), 4.20 (2H, t)[i)]
30b	Galacto	$CH(CH_3)CH_2OCH_3$	69	+12.9°	3400, 1690, 1090[c)]	1.38 (3H, d), 3.33 (3H, s)
31b	Galacto	$CH_2CH(OCH_2CH_3)_2$	62	−3.4°	3400, 1700, 1120, 1070	1.08 (6H, t), 4.80 (1H, d, anomeric proton)[f)]
32b	Galacto		51	+10.0°	3370, 1695, 1080[c)]	0.96 (3H, t) 1.2—1.67 (2H, m)
33b	Galacto	$CH_2CH_2COOCH_3$	65	+13.7°	3400, 1710, 1070[c)]	2.86 (2H, t), 3.73 (3H, s) 4.20 (2H, t)[i)]
34b	Galacto		71	+18.6°	3380, 1710, 1090	0.3—1.7 (4H, m) 1.0—1.4 (1H, m)
35b	Galacto		50	+21.6°	3400, 1690, 1060	0.9—2.2 (m)
36b	Galacto		70	−11.5°	3400, 1690, 1080[c)]	0.6—2.0 (m)
37b	Galacto		68	−22.6°	3380, 1695, 1080[c)]	4.64 (2H, s), 7.29 (5H, s)

V No.	Sugar	R	Yield (%)	$[\alpha]_D^{a)}$	IR $\lambda_{max}^{CHCl_3}$ (cm^{-1})	NMR (in D_2O) δ
38b	Galacto	CH_2-[O]	71	+16.6°	3380, 1700, 1060	1.75—2.25 (4H, m)
39b	Galacto	CH_2-[O]	70	−2.5°	3380, 1690, 1070	6.4 (2H, m), 7.4 (1H, m)
40b	Gluco $(OAc)_4$[h)]	CH_3	82	+2.9°	1750, 1710, 1230, 1060	1.98, 2.02, 2.05, 2.08 (12H, OAc), 3.07 (3H, s), 3.55 (2 H, t) 4.20 (2H, t)[g,i)]
41b	Gluco $(OAc)_4$[h)]	$CH_2CH_2CH_3$	85	+12.0°	1750, 1700, 1230, 1050	0.7—1.85 (7H, m) 1.95, 1.97, 2.00, 2.05 (12H, OAc)[g)]
42b	Gluco $(OAc)_4$[h)]	CH_2CH_2OAc	78	+4.3°	1750, 1700, 1230, 1040	1.97, 2.00, 2.05 (15H, OAc) 3.55 (2H, t)[g)]

a) Measured in methanol at 15—25°C.
b) Gluco represents β-D-glucopyranosyl moiety.
c) Measured in Nujol mull.
d) Manno represents β-D-mannopyranosyl moiety.
e) Galacto represents β-D-galactopyranosyl moiety.
f) Measured in d_6-DMSO–D_2O.
g) Measured in $CDCl_3$.
h) Represents 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl moiety.
i) Signals due to $N(NO)C\underline{H}_2CH_2Cl$.

Glycopyranosylnitrosoureas (V) are expected to be activated to X in a manner analogous to VIII (see Chart 4), because V possesses a β-hydroxyl group at the C-2 position of the glycopyranosyl moiety. In fact, V_{6b} readily decomposed in phosphate-buffered solution (pH 7.4) to give colorless crystals (VI_6) in high yield. VI_6 gave the characteristic IR absorption band at 1770 cm^{-1} due to a five-membered cyclic carbamate, and the NMR spectrum showed signals due to an isobutyl group at δ 0.85 and 1.7—2.04 and the anomeric proton of a β anomer at δ 4.56 (d, J=8.0 Hz). The mass spectrum exhibited the parent ion at *m/e* 261. The structure of VI_6 was thus determined to be 1-isobutylamino-1-deoxy-β-D-glucopyranose 1,2-carbamate from these spectroscopic data together with elemental analysis. The synthesis of 1-(2-chloroethyl)-3-isobutyl-3-β-D-glycopyranosyl-1-nitrosourea (V_{6b}) from D-glucose and isobutylamine is shown in Chart 2 as a representative of the syntheses of various β-D-glycopyranosyl-nitrosoureas (V).

Antitumor Activities of the Nitrosoureas (V_{1b-42b}) and Discussion

The glycopyranosylnitrosoureas (V_{1b-42b}) were tested for antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma by the methods described in the preceding paper.[1)] The results are summarized in Table IV together with the comparative data for positive controls, CCNU, ACNU, and GANU.

TABLE IV. Antitumor Activities of 1-(2-Chloroethyl)-3-substituted-3-β-D-glycopyranosyl-1-nitrosoureas (V)

V No.	Anti-L1210 activity[a)]				Anti-Ehrlich activity[b)]		
	ILS_{30}[c)] (mg/kg/d×5)	OD[d)]	ILS_{max} (%)	Therapeutic[e)] ratio	MED[f)] (mg/kg/d×5)	MTD[g)]	Therapeutic[h)] ratio
CCNU	4.9	25	>757.1[i)]	5.1	12.5	50	4
ACNU	2.9	25	>757.1[i)]	9.3	3.12	25	8
GANU	0.8	6.25	>198.6[i)]	7.8	0.39	12.5	32
1b	4.3	50	>689.5[i)]	11.5	6.25	100	16
2b	2.8	50	>721.9[i)]	17.9	1.56	100	64
3b	1.25	25	>710.8[i)]	20.0	1.56	50	32

V No.	Anti-L1210 activity[a]				Anti-Ehrlich activity[b]		
	ILS_{30}[c] (mg/kg/d×5)	OD[d] (mg/kg/d×5)	ILS_{max} (%)	Therapeutic[e] ratio	MED[f] (mg/kg/d×5)	MTD[g] (mg/kg/d×5)	Therapeutic[h] ratio
4b	0.7	25	>328.6	35.7	1.56	100	64
5b	1.5	25	>689.5[i]	16.7	0.78	100	128
6b	0.7	25	>669.2[i]	35.7	0.78	50	64
7b	7.3	25	60.3	8.3	1.56	50	32
8b	17.0	50	62.2	2.9	1.56	50	32
9b	62.0	100	73.0	1.6	12.5	100	8
10b	32.0	100	101.1	3.1	0.78	100	128
11b	2.7	100	100.0	37.0	1.56	100	64
12b	12.0	50	123.3	4.2	12.5	50	4
13b	1.7	50	>721.5[i]	29.4	3.12	100	32
14b	1.7	50	>757.1[i]	29.4	3.12	50	16
15b	12	200	>262.2	17.4	6.25	400	64
16b	17	100	90.0	5.9	12.5	200	16
17b	5.6	200	>528.6	35.7	6.25	400	64
18b	3.5	50	>363.9	14.3	1.56	100	64
19b	2.1	50	>669.2[i]	23.8	1.56	100	64
20b	2.1	100	>589.7	47.6	0.78	200	256
21b	1.0	50	>710.8[i]	50.0	0.78	100	128
22b	20	200	>460.9	10.0	1.56	200	128
23b	22	200	>425.0	9.1	1.56	200	128
24b	4.0	100	>710.8[i]	25.0	1.56	200	128
25b	1.4	50	>669.2[i]	35.7	1.56	100	64
26b	2.0	50	>669.2[i]	25.0	3.12	100	32
27b	11	400	109.5	36.4	12.5	400	32
28b	3.3	50	>700.0[i]	15.2	3.12	100	32
29b	2.6	100	>700.0[i]	38.5	3.12	100	32
30b	1.4	50	>721.9[i]	35.7	1.56	100	64
31b	7.0	50	60.0	7.1	3.12	50	16
32b	9.5	200	>231.3	21.1	12.5	400	32
33b	0.8	25	>757.1[i]	29.8	6.25	50	8
34b	1.8	50	>700.0[i]	27.8	0.78	100	128
35b	3.0	100	>622.9[i]	33.3	1.56	100	64
36b	4.0	50	>224.4	12.5	0.78	100	128
37b	4.2	100	>700.0[i]	23.8	1.56	100	64
38b	1.7	100	>689.5[i]	58.8	1.56	100	64
39b	3.3	50	>650.0[i]	15.2	1.56	100	64
40b	77	400	79.1	5.2	25	400	16
41b	60	200	83.5	3.3	12.5	400	32
42b	16	50	59.2	3.1	3.12	100	32

a) Leukemic cells (10^5) were inoculated intraperitoneally into BDF_1 mice and intraperitoneal administration was begun 24 h after the inoculation and performed once daily for 5 days.
b) The ascites cells (10^6) were inoculated intraperitoneally into ICR mice and intraperitoneal administration was begun 24 h after the inoculation and performed once daily for 5 days.
c) Daily dose providing 30% increase in life-span over the control. $ILS(\%)=(T/C\text{-}1)\times 100$.
d) Optimal dose: the daily dose providing maximum increase in life-span.
e) Theraperutic ratio=OD/ILS_{30}
f) Minimum effective dose: the minimum dose which shows 100% inhibition of the growth of the tumor.
g) Maximum tolerated dose: the maximum dose which shows 100% inhibition of the growth of the tumor without causing the death of the mice.
h) Therapeutic ratio=MTD/MED.
i) All treated mice survived more than sixty days.

A large number of the nitrosoureas prepared in the present study were remarkably active against both leukemia L1210 and Ehrlich ascites carcinoma and showed greater therapeutic ratios than those of the three positive controls. Sixty-day survivors against leukemia L1210 (as an indicator of complete cure) were found at the optimal dose for many of these nitrosoureas.

Meanwhile, we hoped that the sugar moiety of these nitrosoureas would act as a specific carrier to transport the drug into tumor cells. In fact, as can be seen in Table IV, a galacto-

pyranosyl moiety appears to be the most favorable sugar moiety among aldohexoses. The other substituent (R) on the N-3 position also plays an important role in the antitumor activities of these nitrosoureas. Optimal activity is found in the compounds bearing an alkyl or alkenyl group with three or four carbon atoms (for example, see V_{21b} and V_{25b}). A striking fall in activity is observed in the compounds (V_{7b-11b}) with an alkyl group larger than this optimal length. Alkyl groups with an ether linkage are also effective substituents, as indicated by the excellent antitumor activities of the methoxypropyl and tetrahydrofurfuryl derivatives (for example, see V_{13b} and V_{38b}). As a consequence of combining the effects of a suitable sugar moiety and R group, the galactopyranosylnitrosoureas (V_{21b}, V_{29b}, and V_{38b}) showed excellent antitumor activities and had 4—11 times greater therapeutic ratios than those of the active controls, CCNU, ACNU, and GANU, against leukemia L1210. Moreover, all leukemic mice survived more than sixty days at dose levels of 25, 50, and 100 mg/kg of these three compounds, except for one death out of six mice occasioned by the administration of 100 mg/kg of V_{21b}. On the other hand, sixty-day survivors were observed for CCNU and ACNU only at a dose of 25 mg/kg. Acetylation of the hydroxyl group in the sugar moiety resulted in a marked decrease of antitumor activity against leukemia L1210 (see $V_{40b-42b}$), endorsing the apparent significance of a free hydroxyl group in the sugar moiety for high antitumor activity in this class of compounds.

Further studies on the synthesis and antitumor activity of this new class of nitrosoureas bearing other sugar moieties are in progress.

Experimental

Infrared (IR) spectra were determined with a Hitachi IR-215 spectrometer in Nujol mull or in chloroform solution. NMR spectra were recorded on a JEOL PMX-60 spectrometer using tetramethylsilane as an internal standard in d_6-DMSO–D_2O and in $CDCl_3$, or sodium 3-(trimethylsilyl)-propionic acid in D_2O. The optical rotations were measured in a 0.5 dm tube with a Jasco DIP-180 polarimeter. Column chromatography was carried out by the use of Merck silica gel 60. TLC was performed on Merck TLC plate silica gel 60 F254 and 30% sulfuric acid was used as the spray reagent.

General Procedure for the Preparation of Glycosylamine (II'_{1-39})——A mixture of sugar (0.01 mol) and amine (0.012—0.015 mol) in 10 ml of methanol was stirred and heated at 65°C for 20—40 min. The reaction mixture was concentrated under reduced pressure and ethanol was added to the residue. The mixture was again concentrated and the residue was washed with ether. In general the mixture of structural isomers of glycosylamine was obtained as an amorphous powder or caramel in nearly quantitative yield and was used in the next step without any purification.

General Procedure for the Preparation of 1-(2-Chloroethyl)-3-substituted-3-β-D-glycopyranosylureas (III_{1b-39b})——Glycosylamine (0.01 mol) was dissolved in 30 ml of methanol. A solution of 2-chloroethyl isocyanate (0.012—0.015 mol) in 5 ml of tetrahydrofuran was added dropwise to the above solution at 5°C and the whole was stirred for 1.5 h at room temperature. After the reaction, the mixture was concentrated under reduced pressure and the residue was washed with ether. When this crude product showed essentially only a single spot ascribable to β-D-glycopyranosylurea on TLC, it was chromatographed on silica gel (solvent: $CHCl_3$–AcOEt–MeOH) to give pure β-D-glycopyranosylurea. On the other hand, when the crude product showed two or three spots on TLC due to the presence of structural isomers of glycosylureas, it was subjected to acid treatment (see below) and then chromatographed on silica gel. The ureas obtained were almost colorless amorphous powders or colorless caramels and are listed in Table II together with the yields and some characteristic data.

The Acid Treatment of the Isomeric Mixture of Glycosylureas——The mixture of ureas (0.01 mol) was dissolved in 10 ml of formic acid and the solution was allowed to stand at room temperature for 10 min. TLC of the solution gave only one spot ascribable to β-D-glycopyranosylurea. To the solution, 100 ml of the mixture of *n*-hexane–ethyl ether (1 : 1) was added, and the mixture was stirred. The oil that separated was collected and washed with ethyl ether. The crude product was chromatographed on silica gel.

The Nitrosation of 1-(2-Chloroethyl)-3-*n*-butyl-3-β-D-glucopyranosylurea (III_{5b}) with Sodium Nitrite——Sodium nitrite 2.1 g (0.03 mol) was added portionwise to a solution of III_{5b} (3.4 g, 0.01 mol) in 15 ml of formic acid at 5°C over a period of 1.5 h under stirring. TLC of the reaction mixture showed two spots due to the corresponding nitrosourea (V_{5b}) and the starting material (III_{5b}) in an equal ratio. The reaction mixture was lyophilized and the residue was chromatographed on silica gel to give V_{5b} in 28% yield as a yellow caramel.

The Reaction of N-*n*-Butyl-glycopyranosylamine (II'_5) with *o*-Nitrophenyl N-(2-chloroethyl)-N-nitrosocarbamate (VII)——A solution of VII 4.1 g (0.015 mol) in 15 ml of tetrahydrofuran was added to a solution

of II′$_5$ 2.4 g (0.01 mol) in 15 ml of methanol at 20°C over a period of 10 min with stirring, and the mixture was allowed to stand for 5 h. TLC of the mixture gave no spot due to the nitrosourea (V_{5b}).

General Procedure for the Preparation of 1-(2-Chloroethyl)-3-substituted-3-β-D-glycopyranosyl-1-nitrosourea (V_{1b-24b})——The glycopyranosylurea (III) (0.01 mol) was dissolved in 40 ml of tetrahydrofuran and then anhydrous sodium acetate (0.05 mol) was added. Dinitrogen tetroxide (0.055 mol) was introduced into the mixture at −5°C for 10 min under vigorous stirring. After 10 min, 7 ml of methanol was added to the mixture. The whole was stirred at the same temperature for 10 min, then cold ethyl acetate (40 ml), anhydrous sodium acetate (0.03 mol), and 8 ml of water at −5°C were added with stirring. The mixture was stirred vigorously for 10 min and its pH was confirmed to be about 5. After filtration the organic layer was collected, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (solvent: AcOEt–Benzene–MeOH). The nitrosoureas (V_{1b-42b}) thus obtained were usually unstable yellow caramels and are listed in Table III together with the yields and some characteristic data. The following compounds had melting (decomposition) points. mp (dec.) °C: V_{1b}, 69; V_{12b}, 65; V_{20b}, 44—46.5; V_{21b}, 48—53; V_{30b}, 56; V_{32b}, 62; V_{40b}, 60—63.

Preparation of (2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)ureas ($III_{40b-42b}$)——Acetylation of the ureas was done as follows. A mixture of the urea (0.01 mol), acetic anhydride (15 ml) and pyridine (30 ml) was stirred for 5 h at room temperature, then it was concentrated under reduced pressure. Ethyl acetate (100 ml) was added to the residue. The solution was washed with cold aqueous hydrochloric acid, water, aqueous sodium bicarbonate, and aqueous sodium chloride successively. The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel. The yields and the IR data of these ureas are listed in Table II.

Decomposition of the Glycopyranosylnitrosourea (V_{6b}) in Phosphate-buffered Solution (pH 7.4)——The nitrosourea (V_{6b}) (1.0 g) was dissolved in 30 ml of 1 M phosphate-buffered solution (pH 7.4) at 5°C and the mixture was stirred for 30 min. Then the solution was allowed to stand at room temperature for 8 h. The mixture was saturated with ammonium sulfate and extracted twice with a mixture of ethyl acetate and tetrahydrofuran (1: 4). The organic layer was dried over $MgSO_4$ and concentrated. The residual colorless caramel, which gave only a single spot on TLC, was purified by short column chromatography on silica gel to give 1-isobutylamino-1-deoxy-β-D-glucopyranoside 1,2-carbamate (VI_6) in 78% yield as colorless crystals. mp 144—145°C (from ethanol–ethyl acetate). $[\alpha]_D^{27}$ +39.2° (c=1.1, methanol). IR_{max}^{Nujol} cm^{-1}: 3380, 3350, 3280, 1770, 1140, 1090, 1020. MS m/e: 262 (M^++1), 261 (M^+), 260 (M^+−1), 218 (M^+−43), 174 (M^+−87), 142 (M^+−119). NMR (in d_6-DMSO–D_2O) δ: 0.85 (6H, d, J=6 Hz, $-CH\langle^{CH_3}_{CH_3}$), 1.70—2.04 (1H, m, $-CH\langle$), 4.56 (1H, d, J=8.0 Hz, H-1). *Anal.* Calcd for $C_{11}H_{19}NO_6$: C, 50.56; H, 7.33; N, 5.36. Found: C, 50.55; H, 7.21; N, 5.56.

Preparation of Active Controls, CCNU, ACNU, and GANU——These active controls were synthesized in our laboratory according to the cited methods. CCNU: mp 87.5—88.5°C (dec.) (lit[9] 90°C (dec.)). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1710, 1540, 1500. MS m/e: 235, 233 (M^+), 126 (B^+). NMR (in $CDCl_3$) δ: 3.52 (2H, t, N(NO)$CH_2\underline{C}H_2Cl$), 4.22 (2H, t, N(NO)$\underline{C}H_2CH_2Cl$), 6.90 (1H, br s, NH). *Anal.* Calcd for $C_9H_{16}N_3ClO_2$: C, 46.25; H, 6.85; N, 17.99; Cl, 15.20. Found: C, 46.25; H, 6.88; N, 18.18; Cl, 15.20. ACNU (HCl salt): mp 171°C (dec.) (lit.[10] 170°C (dec.)). IR $_{max}^{Nujol}$ cm^{-1}: 3430, 3280, 3120, 1705, 1645, 1605, 1515. MS m/e: 274, 272 (M^+ of free base), 122 (B^+). NMR (in D_2O) δ: 2.63 (3H, s, CH_3), 3.65 (2H, t, N(NO)$CH_2\underline{C}H_2Cl$), 4.22 (2H, t, N(NO)$\underline{C}H_2CH_2Cl$), 4.60 (2H, s, $NH\underline{C}H_2$), 8.19 (1H, s, ring proton). *Anal.* Calcd for $C_9H_{13}N_6ClO_2\cdot HCl$: C, 34.96; H, 4.56; N, 23.00; Cl, 27.19. Found: C, 35.05; H, 4.63; N, 23.21; Cl, 27.02. GANU: mp 83—85°C (dec.) (lit.[7] 85°C (dec.)). $[\alpha]_D^{20}$ −3° (c=1.0, methanol). IR $_{max}^{Nujol}$ cm^{-1}: 3300, 1720, 1530. NMR (in D_2O) δ: 4.00 (2H, t, N(NO)$C\underline{H}_2CH_2Cl$). *Anal.* Calcd for $C_9H_{16}N_3ClO_7$: C, 34.46; H, 5.14; N, 13.40; Cl, 11.30. Found: C, 34.40; H, 5.18; N, 13.20; Cl, 11.19.

Acknowledgement The authors thank Dr. S. Saito, Director of this laboratory, and Drs. M. Takeda and N. Itoh for their encouragement and helpful discussions. Thanks are also due to the staff of the Analytical Center of this company for spectral measurements and elemental analyses.

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