were examined by thin-layer chromatography on silica gel as described earlier.[51]

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Synthesis and Activities of Antitumor Agents

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N-(2-Chloroethyl)-*N*-nitrosocarbamoyl derivatives of glycosylamines have been prepared. Six *N*-(2-chloroethyl)-*N*-nitrosoureas, including three disaccharide derivatives, were submitted to a determination of antitumor activity. All the compounds tested exhibited strong antitumor activity against leukemia L1210 in mice.

Streptozotocin (NSC 85998) is a unique antitumor antibiotic produced by a fermentation of *Streptomyces achromogenes* var 128,[2-4] and its structure has been established as 2-(3-methyl-3-nitrosoureido)-2-deoxy-D-glucopyranose.[5,6] By synthetic studies of its methyl glycoside[7] and analogues,[8-11] it was found that the *N*-methyl-*N*-nitrosoureido group was an essential functional group for antitumor activity.

Prior to this finding, the research group at the Southern Research Institute had demonstrated that *N*-methyl-*N*-nitrosoureas were effective against leukemia L1210.[12] Soon after, it was discovered that replacements of the methyl group on the nitrosated nitrogen atom of these agents by a 2-chloroethyl group achieved an improved antitumor activity against leukemia L1210,[13,14] and three *N*-(2-chloroethyl)-*N*-nitrosoureas [*N*,*N*′-bis(2-chloroethyl)-(BCNU: NSC 409962),[13] *N*-(2-chloroethyl)-*N*′-cyclohexyl-(CCNU: NSC 79037),[13] and *N*-(2-chloroethyl)-*N*′-(*trans*-4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU: NSC 95441)][14] have been prepared and used for clinical trials.

When *N*′-substituted *N*-(2-chloroethyl)-*N*-nitrosoureas decompose in a buffer solution, a (2-chloroethyl)diazo hydroxide is probably formed, which alkylates biological materials.[15,16,30] On the other hand, isocyanates that are generated on the decomposition of the nitrosoureas also

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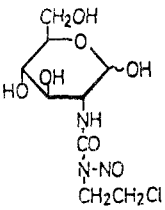
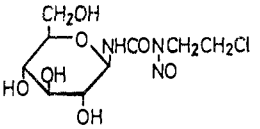
react with biological materials, giving carbamoylation products and causing other physiological effects.[17-19]

Since chlorozotocin[20-23] (NSC 178248: 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose) and 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU: NSC 254157)[24-26] exhibited a remarkable anti-



Chlorozotocin

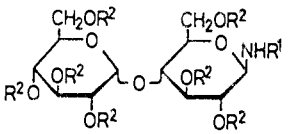


GANU

tumor activity against leukemia L1210 and a low bone-marrow toxicity, the substituents on the N-3 of 1-nitrosoureas must play an important role in causing a side effect of the agents. Therefore, *N*-(2-chloroethyl)-*N*-nitrosoureas which bear other carbohydrates rather than a D-glucopyranose group on the N-3 must be interesting compounds from a toxicological standpoint, and three *N*-(2-chloroethyl)-*N*-nitrosocarbamoyl derivatives of β-D-galactopyranosylamine, β-D-mannopyranosylamine, and β-D-xylopyranosylamine have been prepared.[24]

In continuation of our studies, we have prepared three new *N*-(2-chloroethyl)-*N*-nitrosoureas: 1-(2-chloroethyl)-3-(β-maltosyl)-1-nitrosourea (**4**), 1-(2-chloroethyl)-3-(β-lactosyl)-1-nitrosourea (**10**) and 3-(β-cellobiosyl)-1-(2-chloroethyl)-1-nitrosourea (**15**). In the present article, we report antitumor activities of the six nitrosoureas listed above against experimental leukemia L1210 in mice.

Chemistry. When hepta-*O*-acetyl-β-maltosylamine[27] (**1**) was treated with 2-chloroethyl isocyanate in dioxane,

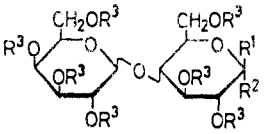


1, R^1=H, R^2=Ac
2, R^1=CONHCH$_2$CH$_2$Cl, R^2=Ac
3, R^1=CONHCH$_2$CH$_2$Cl, R^2=H
4, R^1=CON(NO)CH$_2$CH$_2$Cl, R^2=H
5, R^1=CON(NO)CH$_2$CH$_2$Cl, R^2=Ac

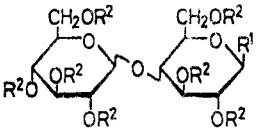
1-(2-chloroethyl)-3-(hepta-*O*-acetyl-β-maltosyl)urea (**2**) was obtained. O-Deacetylation of **2** in methanolic ammonia afforded crystalline 1-(2-chloroethyl)-3-(β-maltosyl)urea (**3**). Nitrosation of **3** was performed with nitrogen trioxide in an acetone solution, and **4** was obtained as crystals. Acetylation of **4** or nitrosation of **2** yielded 1-(2-chloroethyl)-3-(hepta-*O*-acetyl-β-maltosyl)-1-nitrosourea (**5**).

Starting from hepta-*O*-acetyl-α-lactosyl bromide[28] (**6**), hepta-*O*-acetyl-β-lactosyl azide (**7**) was prepared. Catalytic hydrogenation of **7** in the presence of Raney nickel, followed by carbamoylation with 2-chloroethyl isocyanate, gave crystalline 1-(2-chloroethyl)-3-(hepta-*O*-acetyl-β-lactosyl)urea (**8**). O-Deacetylation of **8** afforded 1-(2-chloroethyl)-3-(β-lactosyl)urea (**9**). Nitrosation of **9** with nitrogen trioxide gave **10** in a crystalline state. Acetylation of **10** or nitrosation of **8** gave 1-(2-chloroethyl)-3-(hepta-*O*-acetyl-β-lactosyl)-1-nitrosourea (**11**).

Catalytic hydrogenation of hepta-*O*-acetyl-β-cellobiosyl azide[27] (**12**) gave hepta-*O*-acetyl-β-cellobiosylamine, which



6, R^1=H, R^2=Br, R^3=Ac
7, R^1=N_3, R^2=H, R^3=Ac
8, R^1=NHCONHCH$_2$CH$_2$Cl, R^2=H, R^3=Ac
9, R^1=NHCONHCH$_2$CH$_2$Cl, R^2=R^3=H
10, R^1=NHCON(NO)CH$_2$CH$_2$Cl, R^2=R^3=H
11, R^1=NHCON(NO)CH$_2$CH$_2$Cl, R^2=H, R^3=Ac



12, R^1=N_3, R^2=H
13, R^1=NHCONHCH$_2$CH$_2$Cl, R^2=Ac
14, R^1=NHCONHCH$_2$CH$_2$Cl, R^2=H
15, R^1=NHCON(NO)CH$_2$CH$_2$Cl, R^2=H
16, R^1=NHCON(NO)CH$_2$CH$_2$Cl, R^2=Ac

was further worked up as described above, giving **15** and its heptaacetate **16**.

The nitrosation used in the present experiments with nitrogen trioxide[29] in an acetone solution gave a satisfactory result for nitrosation of a proper position without any detectable cleavage of a glycosidic linkage. The attached position of the nitroso group was demonstrated by the ^{1}H NMR spectrum as follows, being exemplified in the case of **15**. The proton on a nitrogen atom attached to a glycosyl residue revealed its signal at δ 10.24 as a doublet, and by adding deuterium oxide the triplet signal of the proton (H-1) at δ 5.87 was transformed into a doublet. These facts indicated that the nitrogen atom (N-1) attached to a 2-chloroethyl group was nitrosated.

Biological Activity. The six *N*-(2-chloroethyl)-*N*-nitrosoureas have been submitted to a determination of antitumor activities against leukemia L1210. Effects of the compounds on the lymphoid leukemia L1210 transplanted in mice are shown in Table I, including that of GANU[25] as a control agent.

The administration of the D-galactopyranosyl derivative produced 405.4% ILS at an optimal effective dose of 8 mg/kg each day, and it also produced the same ILS value at 4 mg/kg each day. The D-mannopyranosyl derivative produced 103.3% ILS at 8 mg/kg each day, and the activity was somewhat lower than the other derivatives. No animal survived for 60 days. The D-xylopyranosyl derivative gave 445.5% ILS at doses of 8 and 4 mg/kg each day. All animals treated with these two doses survived for 60 days. The D-xylopyranosyl derivative has a greater range of therapeutic doses than other compounds.

Compound **4** produced 403.4% ILS at the optimal effective dose of 20 mg/kg each day, and its toxicity seemed to be lower than those of the monosaccharide derivatives. Pathoanatomically, in animals treated with compound **4** at 40 mg/kg each day, the livers were congestive and the spleens were atrophic. The 60-day surviving animals treated with the dose of 20 mg/kg each day showed the atrophy in the liver. Compound **10** produced 384.5% ILS at the optimal effective dose of 10 mg/kg each day. Compound **15** produced 483.3% ILS at the optimal effective dose, 8 mg/kg each day, and 60% of the dosed

Table I. Antitumor Effect of *N*-(2-Chloroethyl)-*N*-nitrosourea Derivatives of Carbohydrates on L1210[a]

compd	dosage[b] [(mg/kg)/day]	schedule[c]	mean survival days, T/C	ILS,[d] %	60-day survivors, T/C
β-D-galactopyranosyl	8	qd 1 – 3	47.0/9.3	405.4	2/3
β-D-mannopyranosyl	8	qd 1 – 3	18.3/9.0	103.3	0/3
β-D-xylopyranosyl	8	qd 1 – 3	60.0/11.0	445.5	3/3
β-maltosyl (4)	20	qd 1 – 3	45.3/9.0	403.3	2/3
β-lactosyl (10)	10	qd 1 – 3	47.0/9.7	384.5	2/3
β-cellobiosyl (15)	8	qd 1 – 3	42.0/7.2	483.3	3/5
GANU	8	qd 1 – 3	60.0/9.8	512.2	5/5

[a] Male BDF_1 hybrid mice were inoculated intraperitoneally with 10^6 cells of lymphoid leukemia L1210. Compounds were dissolved in distilled water and were administered intraperitoneally at a volume of 0.1 mL/animal once a day for 3 days from 24 h (day 1) after the tumor implantation. [b] The shown dosages were optimal effective ones. [c] Intraperitoneal injection was begun 24 h after the inoculation and performed once a day for 3 days. [d] Percentage increase in life span of treated animals compared with control tumor bearers [100(T/C – 1)]. Most of the animals were surviving for 60 days; therefore, we calculated percent ILS including 60-day survivors.

animals survived for 60 days.

GANU showed a high efficacy on the leukemia L1210 by oral administration, and it had low bone-marrow toxicity in rats.[26] Henceforward, these six compounds also will be examined on the alteration of antitumor activity by the various administration schedules and on the toxicities.

Experimental Section

Melting points were determined in a capillary tube in a liquid bath and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were measured on potassium bromide disks with a JASCO IR-E spectrometer. ^1H NMR spectra were determined at 60 MHz on a Varian T-60 spectrometer with reference to tetramethylsilane as an internal standard. Solutions were evaporated under reduced pressure at 25–30 °C. Thin-layer chromatography was carried on 5 × 10 cm glass plates with silica gel HF (E. Merck). Analytical results indicated by element symbols were within ±0.4% of the theoretical values.

Hepta-*O*-acetyl-β-maltosylamine (1) was prepared by the method of Bertho.[27]

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-maltosyl)urea (2). To a solution of 1 (2.5 g) in dioxane (40 mL), 2-chloroethyl isocyanate (0.7 mL) was added under ice cooling with stirring. After 2 h, the solution was concentrated. The residue was dissolved in acetone, and to the solution was added isopropyl ether to give 1.6 g (56%) of **2** as amorphous solid: mp 72 °C; $[\alpha]^{21}_D$ +74° (*c* 0.5, chloroform); ^1H NMR ($CDCl_3$) δ 2.00 (s, 9, 3 OAc), 2.03 (s, 3, OAc), 2.05 (s, 3, OAc), 2.07 (s, 3, OAc), 2.12 (s, 3, OAc), 3.57 (br s, 4, $HNCH_2CH_2Cl$). Anal. ($C_{29}H_{41}N_2ClO_{18}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(β-maltosyl)urea (3). Compound **2** (1.4 g) was dissolved in methanolic ammonia (50 mL). After 5 h at ambient temperature, the solution was concentrated. The residue was recrystallized from ethanol–isopropyl alcohol to give 650 mg (76%) of **3**: mp 108–110 °C dec; $[\alpha]^{23}_D$ +68° (*c* 0.5, water); IR (KBr) 1650 (C=O), 1565 cm^{-1} (CNH); ^1H NMR (Me_2SO-d_6) δ 6.23 (m, 1, $HNCH_2CH_2Cl$), 6.57 (d, 1, NHCO). Anal. ($C_{15}H_{27}N_2ClO_{11}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(β-maltosyl)-1-nitrosourea (4). To a solution of **3** (314 mg) in acetone (1.5 mL), nitrogen trioxide was bubbled under ice cooling. After 15 min, TLC in 2:1 (v/v) chloroform–methanol showed a disappearance of the starting material **3** and an appearance of a new product at R_f 0.12. The solution was diluted with hexane and the supernatant liquid was decanted. The residual solution was washed with isopropyl ether repeatedly to give a pale-yellow solid. The product was recrystallized from methanol–isopropyl alcohol to give 250 mg (75%) of **4**: mp 96 °C dec; $[\alpha]^{23}_D$ +60° (*c* 0.5, water); IR (KBr) 1730 (C=O), 1540 (CNH), 1505 cm^{-1} (NN=O); ^1H NMR (pyridine-d_5) δ 3.47 (t, 2, CH_2CH_2Cl), 9.97 (d, 1, *J* = 8 Hz, NHCO). Anal. ($C_{15}H_{26}N_3ClO_{12}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-maltosyl)-1-nitrosourea (5). Compound **4** (100 mg) was acetylated with acetic anhydride (1 mL) in pyridine (1 mL) overnight at ambient temperature. A product was recrystallized from ethanol to give 107 mg (66%) of **5**: mp 95–96 °C dec; $[\alpha]^{24}_D$ +63.8° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 1.99 (s, 6, 2 OAc), 2.02 (s, 6, 2 OAc), 2.05 (s, 3, OAc), 2.09 (s, 3, OAc), 2.12 (s, 3, OAc), 3.50 (t, 2, *J* = 6 Hz, CH_2CH_2Cl), 7.64 (d, 1, *J* = 8 Hz, NHCO). Anal. ($C_{29}H_{40}N_3ClO_{19}$) C, H, N, Cl.

Hepta-*O*-acetyl-α-lactosyl bromide (6) was prepared by the method of Stevens and Blumbergs.[28]

Hepta-*O*-acetyl-β-lactosyl Azide (7). A mixture of **6** (18 g) and sodium azide (8.2 g) in DMF (120 mL) was heated at 100 °C for 2 h. The reaction mixture was concentrated and the residue was dissolved in chloroform (150 mL). The solution was washed with water repeatedly. After drying over sodium sulfate, the solution was evaporated. The residue was quenched into ice-cold water (300 mL) to give 13.8 g of a crude product, which was used for a successive reaction without further purification.

A part of the product (1.0 g) was purified on a silica gel column using 1:100 (v/v) acetone–chloroform to give 642 mg of analytically pure **7** as a glass: $[\alpha]^{24}_D$ –19.6° (*c* 1.0, chloroform); IR (KBr) 2130 cm^{-1} (N_3). Anal. ($C_{26}H_{35}N_3O_{17}$) C, H, N.

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-lactosyl)urea (8). Compound **7** (12.0 g) was hydrogenated in dioxane (40 mL) in the presence of Raney nickel under hydrogen atmosphere (3.4 kg/cm^2) for 2 h. After the catalyst was filtered off, the filtrate was concentrated to give an amine as a syrup (12.5 g).

To a solution of the amine (9 g) in dioxane (45 mL) was added 2-chloroethyl isocyanate (2.5 mL) under ice cooling with stirring. After 3 h, the solution was concentrated and the residue was recrystallized from acetone–isopropyl ether to give 8.3 g (86%) of **8**: mp 127–130 °C; $[\alpha]^{24}_D$ –7.0° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 1.95 (s, 3, OAc), 2.03 (s, 12, 4 OAc), 2.08 (s, 3, OAc), 2.13 (s, 3, OAc), 3.57 (br s, 4, CH_2CH_2Cl). Anal. ($C_{29}H_{41}N_2ClO_{18}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(β-lactosyl)urea (9). Compound **8** (8.3 g) was deacetylated in methanolic ammonia (160 mL) at ambient temperature for 2 h. The solution was concentrated to dryness and the residue was washed with methanol. The product was recrystallized from hot water to give 3.8 g (70%) of **9**: mp 123–124 °C dec; $[\alpha]^{20}_D$ –3.3° (*c* 1.0, water); IR (KBr) 1625 (C=O), 1570 cm^{-1} (CNH); ^1H NMR (Me_2SO-d_6) δ 6.23 (m, 1, $HNCH_2CH_2Cl$), 6.67 (d, 1, NHCO). Anal. ($C_{15}H_{27}N_2ClO_{11}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(β-lactosyl)-1-nitrosourea (10). Nitrogen trioxide was bubbled into a solution of **9** (306 mg) in acetone (1.5 mL). After 10 min, TLC showed the disappearance of the starting material and the appearance of a new compound at R_f 0.18 in 2:1 (v/v) chloroform–methanol. The solution was worked up as in the preparation of **4**. A crude product was recrystallized from ethanol to give 291 mg (89%) of **10**: mp 131 °C dec; $[\alpha]^{23}_D$ +4.0 (*c* 0.5, water); IR (KBr) 1725 (C=O), 1530 (CNH), 1495 cm^{-1} (NN=O); ^1H NMR (pyridine-d_5) δ 3.30 (t, 2, *J* = 6 Hz, CH_2CH_2Cl), 10.27 (d, 1, *J* = 8 Hz, NHCO). Anal. ($C_{15}H_{26}N_3ClO_{12}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-lactosyl)-1-nitrosourea (11). Compound **10** (150 mg) was acetylated with acetic anhydride in pyridine. A crude product was recrystallized from isopropyl alcohol to give 181 mg (75%) of **11**: mp 107–109 °C dec; $[\alpha]^{24}_D$ –2.3° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 1.96 (s, 3, OAc), 2.04 (s, 12, 4 OAc), 2.09 (s, 3, OAc), 2.14 (s, 3, OAc), 3.47 (t, 2, *J* = 6 Hz, CH_2CH_2Cl), 7.62 (d, 1, *J* = 8 Hz, NHCO).

Anal. ($C_{29}H_{40}N_3ClO_{19}$) C, H, N, Cl.

Hepta-*O*-acetyl-β-cellobiosyl azide (12) was prepared by the method of Bertho.[27]

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-cellobiosyl)urea (13). Compound 12 (2.97 g) was hydrogenated in ethyl acetate (20 mL) in the presence of platinum oxide (280 mg) under hydrogen atmosphere (3.4 kg/cm^2) for 24 h. The catalyst was filtered off and to the filtrate was added 2-chloroethyl isocyanate (0.8 mL) under ice cooling with stirring. After 24 h in a refrigerator, the solution was concentrated. The residue was recrystallized from ethanol to give 2.65 g (81%) of 13: mp 206–207 °C dec; $[\alpha]^{20}_D$ −8.5° (c 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3, OAc), 2.02 (s, 6, 2 OAc), 2.05 (s, 6, 2 OAc), 2.08 (s, 3, OAc), 2.12 (s, 3, OAc), 3.56 (br s, 4, $HNCH_2CH_2Cl$). Anal. ($C_{29}H_{41}N_2ClO_{18}$) C, H, N, Cl.

3-(β-Cellobiosyl)-1-(2-chloroethyl)urea (14). Compound 13 (1.48 g) was deacetylated in 0.14 M methanolic sodium methoxide (17.5 mL). After 30 min, the solution was neutralized with Amberlite IR-120 (H^+) resin and subsequently concentrated. The residue was recrystallized from methanol to give 665 mg (75%) of 14: mp 143–149 °C dec; $[\alpha]^{20}_D$ −16° (c 1.0, water); IR (KBr) 1650 (C=O), 1560 cm^{-1} (CNH); ^{1}H NMR (Me_2SO-d_6) δ 6.17 (m, 1, $HNCH_2CH_2Cl$), 6.58 (d, 1, NHCO). Anal. ($C_{15}H_{27}N_2ClO_{11}$) C, H, N, Cl.

3-(β-Cellobiosyl)-1-(2-chloroethyl)-1-nitrosourea (15). Compound 14 (113 mg) was suspended in acetone (1.5 mL) and to the suspension, nitrogen trioxide was bubbled under ice cooling with stirring. After 10 min, the solution was diluted with hexane to give a syrupy precipitate. The precipitate was obtained by decantation and washed with isopropyl ether. The product was dissolved in ethanol, and the ethanolic solution was stored in a refrigerator to give 73 mg (61%) of **15** as pale-yellow needles: mp 159–160 °C dec; $[\alpha]^{20}_D$ −12.6° (c 1.04, water); IR (KBr) 1725 (C=O), 1540 (CNH), 1490 cm^{-1} (NN=O); ^{1}H NMR (pyridine-d_5) δ 3.46 (t, 2, J = 6 Hz, CH_2CH_2Cl), 5.05 (d, 1, J = 8 Hz, H-1'), 5.87 (t, 1, J = 8 Hz, H-1), 10.24 (d, 1, J = 8 Hz, NHCO). Anal. ($C_{15}H_{26}N_3ClO_{12}$) C, H, N, Cl.

1-(2-Chloroethyl)-3-(hepta-*O*-acetyl-β-cellobiosyl)-1-nitrosourea (16). Compound 15 (31 mg) was acetylated with acetic anhydride (3 mL) in pyridine (5 mL). The solution was concentrated to give a crystalline residue. The crystals were washed with ethanol to give 26 mg (52%) of 16: mp 179–180 °C dec; $[\alpha]^{26}_D$ −13.3° (c 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3, OAc), 1.99 (s, 6, 2 OAc), 2.02 (s, 6, 2 OAc), 2.07 (s, 3, OAc), 2.11 (s, 3, OAc), 3.46 (t, 2, J = 6 Hz, CH_2CH_2Cl), 7.67 (d, 1, J = 8 Hz, NHCO). Anal. ($C_{29}H_{40}N_3ClO_{19}$) C, H, N, Cl.

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References and Notes

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