A New Class of Nitrosoureas. 4.[1] Synthesis and Antitumor Activity of Disaccharide Derivatives of 3,3-Disubstituted 1-(2-Chloroethyl)-1-nitrosoureas

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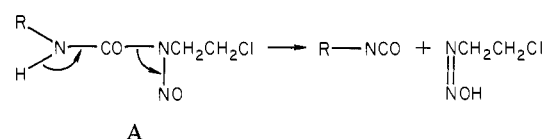
A series of 33 N-(2-chloroethyl)-N-nitrosocarbamoyl derivatives of N-substituted glycosylamines has been prepared and tested for antitumor activities. The compounds were obtained by reaction of glycosylamines with isocyanate, followed by nitrosation with N_2O_4. Structure–activity relationships of these trisubstituted nitrosoureas were investigated by varing the N-substituents and disaccharide groups and by comparing them with the corresponding disubstituted analogues. A large number of the nitrosoureas bearing a maltosyl group exhibited strong antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma, and 60-day survivors against leukemia L1210 were found at the optimal dose for these derivatives. In contrast, the lactosyl and the melibiosyl derivatives were almost inactive. The most interesting compound in this series, the 3-isobutyl-3-maltosyl derivative (37), was tested against leukemia L1210 by single and multiple treatment. Its therapeutic ratio (96.3) obtained by multiple treatment is 3 times larger than that (31.5) obtained by single treatment, suggesting a possible clinical utility of 37 by multiple treatment. The favorable effect of a maltosyl moiety in this class of compounds is discussed.

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.[2] Among these derivatives, water-soluble 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU) has already been on the market, and some nitrosourea derivatives, such as 1-(2-chloroethyl)-3-(2,6-dioxo-1-piperidyl)-1-nitrosourea (PCNU),[3] 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (DCNU),[4] 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU),[5] and 1-(2-chloroethyl)-3-(methyl-α-D-glucopyranos-6-yl)-1-nitrosourea (MCNU),[6] etc., are currently under clinical trials.
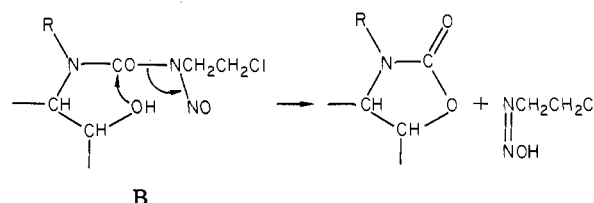
These nitrosoureas (A) necessarily possess one hydrogen atom at the N-3 position, and this seems likely to be important in conferring antitumor activity. They decompose under physiological conditions to generate isocyanates and chloroethyl diazohydroxide and consequently exhibit antitumor activity. This decomposition is considered to be initiated by abstraction of the proton at the N-3 position as the first step[7] (Scheme I).

Our interest in the antitumor activity of 3,3-disubstituted 1-(2-chloroethyl)-1-nitrosoureas as masked nitrosourea derivatives[8] has led to the synthesis of the nitrosoureas (B) having an hydroxyl group at the β position of their substituents. This new class of nitrosoureas having no proton at their N-3 position differed from known nitrosoureas in its activation mechanism and exhibited re-
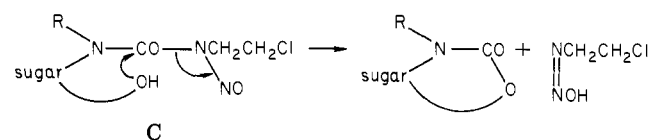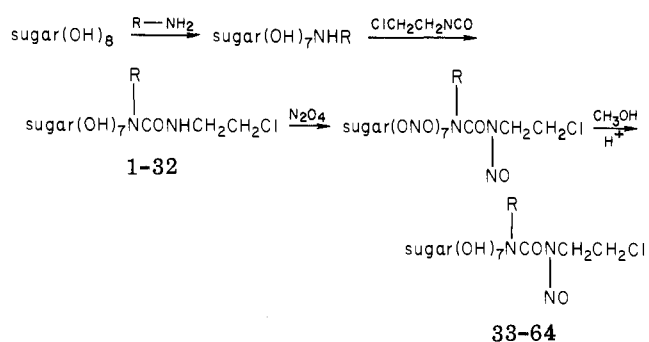
Scheme I

A

Scheme II

B

Scheme III

C

Scheme IV

sugar(OH)$_8$ $\xrightarrow{R-NH_2}$ sugar(OH)$_7$NHR $\xrightarrow{ClCH_2CH_2NCO}$

sugar(OH)$_7$NCONHCH$_2$CH$_2$Cl $\xrightarrow{N_2O_4}$ sugar(ONO)$_7$NCONCH$_2$CH$_2$Cl $\xrightarrow[H^+]{CH_3OH}$

1–32

sugar(OH)$_7$NCONCH$_2$CH$_2$Cl

33–64

markable antitumor activity.[9] They were activated by attack of the hydroxyl group on the carbonyl group to give oxazolidinones and chloroethyl diazohydroxide without generation of isocyanates (Scheme II).

This observation was followed by the finding that the 3,3-disubstituted nitrosoureas (C) bearing aldohexose[10] and aldopentose[1] moieties are activated by essentially the same
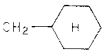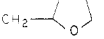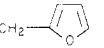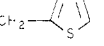
(1) For part 3 of this series, see Morikawa, T.; Ozeki, M.; Umino, N.; Kawamori, M.; Arai, Y.; Tsujihara, K. *Chem. Pharm. Bull.*, in press.

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(3) Phase I study of PCNU: Gralla, R. J.; Young, C. W.; Tan, C. T.; Sykes, M. P. *Proc. Am. Assoc. Cancer Res.* **1980**, *21*, 71 Meet., 185.

(4) Phase II study of DCNU: For example, see Casper E. S.; Gralla, R. J. *Cancer Treat. Rep.* **1979**, *63*, 549.

(5) Phase I study of GANU: Kanko, T.; Saito, T. *Gan To Kagaku Ryoho* **1981**, *8*, 557.

(6) Sekido, S.; Ninomiya, K.; Iwasaki, M. *Cancer Treat. Rep.* **1979**, *63*, 961.

(7) For example, see Brundrett, R. B.; Cowens, J. W.; Colvin, M.; Jardine, I. *J. Med. Chem.* **1976**, *19*, 958.

(8) The trisubstituted nitrosoureas, such as 1-(2-chloroethyl)-3,3-dimethyl-1-nitrosourea, have been reported to be latent antitumor agents: see Brundrett, R. B.; Cowens, J. M.; Colvin, M. *Proc. Am. Assoc. Cancer Res. ASCO* **1976**, *17*, 102.

(9) For part 1 of this series, see Tsujihara, K.; Ozeki, M.; Morikawa, T.; Arai, Y. *Chem. Pharm. Bull.* **1981**, *29*, 2509.

(10) For part 2 of this series, see Tsujihara, K.; Ozeki, M.; Morikawa, T.; Taga, N.; Miyazaki, M.; Kawamori, M.; Arai, Y. *Chem. Pharm. Bull.*, **1981**, *29*, 3262.

Table I. Properties of Urea Derivatives of Disaccharides

$$\text{sugar—N—CONHCH}_2\text{CH}_2\text{Cl}$$
with R on N

no.	sugar	R	yield, %	IR (Nujol) ν_{max}, cm^{-1}	NMR (D$_2$O), δ
1	malto[a]	CH$_3$	68	3350, 1640, 1535, 1070, 1030	3.15 (3 H, s)
2	malto	CH$_2$CH$_2$CH$_3$	73	3350, 1640, 1535, 1070, 1040	0.90 (3 H, t), 1.4-1.9 (2 H, m)
3	malto	CH(CH$_3$)$_2$	70	3350, 1620, 1540, 1070, 1040	1.38 (6 H, d)
4	malto	CH$_2$CH$_2$CH$_2$CH$_3$	75	3350, 1640, 1540, 1070, 1030	0.7-2.0 (7 H, m)
5	malto	CH$_2$CH(CH$_3$)$_2$	77	3350, 1635, 1540, 1080, 1030	0.91 (6 H, d), 1.8-2.3 (1 H, m)
6	malto	CH(CH$_3$)CH$_2$CH$_3$	62	3350, 1640, 1540, 1070, 1030	0.91 (3 H, t), 1.28 (3 H, d), 1.5-1.9 (2 H, m)
7	malto	CH$_2$(CH$_2$)$_3$CH$_3$	72	3350, 1640, 1540, 1070	0.7-1.0 (3 H, m), 1.0-2.0 (6 H, m)
8	malto	CH$_2$(CH$_2$)$_4$CH$_3$	75	3350, 1640, 1540, 1070, 1030	0.7-2.1 (11 H, m)
9	malto	CH$_2$(CH$_2$)$_{14}$CH$_3$	70	3350, 1630, 1535, 1070	
10	malto	CH$_2$CH=CH$_2$	72	3350, 1645, 1540, 1070, 1030	
11	malto	CH$_2$CH=CHCH$_3$	74	3350, 1640, 1530, 1070, 1030	1.75 (3 H, d)
12	malto	CH$_2$CH$_2$CH=CH$_2$	74	3350, 1635, 1530, 1070, 1030	2.4-2.6 (2 H, m)
13	malto	CH$_2$C(=CH$_2$)CH$_3$	70	3350, 1640, 1535, 1070, 1030	1.78 (3 H, s)
14	malto	CH$_2$CH$_2$OCH$_3$	75	3350, 1630, 1540, 1070, 1025	3.35 (3 H, s)
15	malto	CH$_2$CH$_2$OCH$_2$CH$_3$	76	3340, 1640, 1555, 1070, 1025	1.20 (3 H, t)
16	malto	CH$_2$CH$_2$CH$_2$OCH$_3$	74	3350, 1635, 1540, 1070, 1030	1.75-2.15 (2 H, m), 3.30 (3 H, s)
17	malto	CH$_2$CH(CH$_3$)OCH$_3$	72	3380, 1640, 1550, 1070, 1030	2.20 (3 H, d), 3.42 (3 H, s)
18	malto	CH(CH$_3$)CH$_2$OCH$_3$	68	3350, 1630, 1530, 1065, 1020	
19	malto	CH$_2$-C$_3$H$_5$	70	3350, 1630, 1540, 1070, 1040	0.30-0.70 (4 H, m), 1.25 (1 H, t)
20	malto	CH$_2$—⟨cyclohexyl, H⟩	66	3350, 1630, 1540, 1070, 1030	0.7-2.15 (m, ring protons)
21	malto	CH$_2$—⟨O⟩	68	3370, 1640, 1540, 1070, 1040	1.75-2.25 (m, ring protons)
22	malto	CH$_2$-C$_6$H$_5$	70	3320, 1640, 1535, 1080, 1030	7.3-7.55 (4 H, m)
23	malto	p-CH$_2$-C$_6$H$_4$-Cl	71	3350, 1640, 1535, 1080, 1030	7.2-7.5 (4 H, m)
24	malto	p-CH$_2$-C$_6$H$_4$-CH$_3$	73	3350, 1640, 1535, 1070, 1030	2.3 (3 H, s), 7.20 (4 H, q)
25	malto	p-CH$_2$-C$_6$H$_4$-OCH$_3$	75	3320, 1640, 1530, 1080, 1030	3.80 (3 H, s), 7.20 (4 H, q)
26	malto	CH$_2$—⟨furyl, O⟩	70	3350, 1640, 1530, 1070, 1030	6.46 (2 H, m), 7.53 (1 H, m)
27	malto	CH$_2$—⟨thienyl, S⟩	67	3340, 1620, 1530, 1070, 1020	6.9-7.2 (2 H, m), 7.3-7.5 (1 H, m)
28	malto	CH(CH$_3$)-C$_6$H$_5$	65	3350, 1630, 1525, 1070, 1030	1.3-1.8 (3 H, m), 7.3 (5 H, s)
29	malto	CH$_2$CH$_2$-C$_6$H$_5$	70	3330, 1640, 1530, 1070, 1020	7.3 (5 H, br s)
30	lacto[b]	CH$_2$CH$_2$CH$_2$CH$_3$	72	3350, 1640, 1540, 1080, 1040	0.7-1.9 (7 H, m)
31	lacto	CH$_2$CH(CH$_3$)$_2$	75	3350, 1630, 1525, 1070, 1040	0.9 (6 H, d), 1.8-2.2 (1 H, m)
32	melibio[c]	CH$_2$CH(CH$_3$)$_2$	73	3340, 1640, 1530, 1070, 1030	0.9 (6 H, d), 1.8-2.2 (1 H, m)

[a] Malto represents a β-maltosyl moiety (see Table IV). [b] Lacto represents a β-lactosyl moiety (see Table IV). [c] Melibio represents a β-melibiosyl moiety (see Table IV).

mechanism (Scheme III) and exhibit excellent antitumor activity as compared with known nitrosoureas, such as CCNU, ACNU, and GANU.

Since the sugar moieties in these derivatives played an important role on the SAR (structure–activity relationship) and appeared to act as a specific carrier to transport these compounds into tumor cells,[1,10] the study was extended to the synthesis of the corresponding disaccharide derivatives. Described herein are the synthesis and the antitumor activity of the nitrosoureas (33–65) which are disubstitued at their N-3 position by disaccharide groups and various alkyl groups. Several of these compounds exhibited potent antitumor activity with extremely large therapeutic ratios.
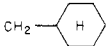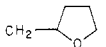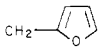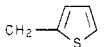
Chemistry. The synthesis of the 3,3-disubstituted nitrosoureas (33–65) followed the procedure employed in the monosaccharide series[1,10] (Scheme IV).

The reaction of disaccharides (maltose, lactose, and melibiose) with various primary amines, followed by treatment with 2-chloroethyl isocyanate, gave the corresponding ureas (1–32) in high yields after chromatography on silica gel. The ureas were usually obtained as unstable amorphous powders and are listed in Table I with some characteristic data. Nitrosation of the ureas was achieved by the use of 8 equiv of dinitrogen tetroxide in the presence of an acid acceptor at low temperature. This indicates that

the seven hydroxyl groups in the sugar moiety are nitrosated first, followed by nitrosation of the ureido group.[10] The nitrous acid esters of the hydroxyl groups could be decomposed without affecting the N-nitroso group by addition of methanol under acidic conditions. After purification by chromatography on silica gel, the nitrosoureas (33–64) were obtained in good yields as unstable light yellow amorphous powders and are listed in Table II together with some characteristic data.

The structure of the maltosyl derivative (37) bearing an isobutyl group is discussed in detail as a representative of these nitrosourea derivatives. Compound 37 showed an IR absorption at 1695 cm^{-1} due to a nitrosoureido group; its NMR spectra, in addition to isobutyl and chloroethyl group proton absorption, exhibited the axial and equatorial anomeric protons of two glucose moieties as two doublets at δ 4.70 (J = 7 Hz) and 5.03 (J = 3 Hz), respectively. Acetylation of 37 gave the crystalline heptaacetate (65) whose mass spectra showed a molecular ion peak at m/e 825. Like the previous cases,[1,8,10] on treatment with phosphate-buffered solution (pH 7.4), 37 readily decomposed to give a high yield of the crystalline compound 66, whose structure was established to be 1-(isobutyl-amino)-1-deoxy-β-maltose-1,2-carbamate by its spectroscopic data by X-ray study[11] (Scheme V). These results

Table II. Properties of Nitrosourea Derivatives of Disaccharides[a]

$$\begin{array}{c} R \\ | \\ \text{sugar}-N-CONCH_2CH_2Cl \\ | \\ NO \end{array}$$

no.	sugar	R	yield, %	mp, °C dec	$[\alpha]_D$,[b] deg	NMR (D_2O), δ
33	malto[c]	CH_3	62	66-70	+42.9	3.12 (3 H, s), 4.15 (2 H, t)[g]
34	malto	$CH_2CH_2CH_3$	70	59-62	+62.9	0.91 (3 H, t), 1.4-1.9 (2 H, m), 4.20 (2 H, t)[g]
35	malto	$CH(CH_3)_2$	65	66-71	+70.5	1.36 (6 H, d), 4.15 (2 H, t)[g]
36	malto	$CH_2CH_2CH_2CH_3$	73	76-80	+61.5	0.7-1.9 (7 H, m), 4.25 (2 H, t)[g]
37	malto	$CH_2CH(CH_3)_2$	76	95-97	+47.0	0.90 (6 H, d), 1.8-2.25 (1 H, m), 4.20 (2 H, t)[g]
38	malto	$CH(CH_3)CH_2CH_3$	72	78	+67.9	0.90 (3 H, t), 1.30 (3 H, d), 1.5-1.9 (2 H, m)
39	malto	$CH_2(CH_2)_3CH_3$	72	71-75	+58.4	0.7-1.0 (3 H, m), 1.0-2.0 (6 H, m), 4.15 (2 H, t)[g]
40	malto	$CH_2(CH_2)_4CH_3$	68	70-72	+60.3	0.7-2.1 (11 H, m)
41	malto	$CH_2(CH_2)_{14}CH_3$	63	caramel	+43.4	
42	malto	$CH_2CH=CH_2$	70	67	+41.3	
43	malto	$CH_2CH=CHCH_3$	72	73-76	+43.0	1.65 (3 H, d)
44	malto	$CH_2CH_2CH=CH_2$	73	74	+52.9	2.38-2.6 (2 H, m), 4.18 (2 H, t)
45	malto	$CH_2C(=CH_2)CH_3$	72	76-80	+58.1	1.80 (3 H, s)
46	malto	$CH_2CH_2OCH_3$	72	52	+54.2	3.38 (3 H, s)
47	malto	$CH_2CH_2OCH_2CH_3$	70	58	+52.0	1.21 (3 H, t)
48	malto	$CH_2CH_2CH_2OCH_3$	68	70-74	+62.4	1.85-2.25 (2 H, m), 3.30 (3 H, s)
49	malto	$CH_2CH(OCH_3)CH_3$	70	51	+55.3	2.20 (3 H, d), 3.36 (3 H, s)
50	malto	$CH(CH_3)CH_2OCH_3$	68	69	+59.8	1.4 (3 H, d), 3.33 (3 H, s)
51	malto	$CH_2-C_3H_5$	72	57	+58.5	0.30-0.70 (4 H, m), 1.20 (1 H, t)
52	malto	CH_2—(cyclohexyl, H)	70	68	+51.6	0.7-2.1 (m, ring protons)
53	malto	CH_2—(tetrahydrofuranyl, O)	76	69-71	+52.5	1.7-2.2 (4 H, m), 4.20 (2 H, t)
54	malto	$CH_2-C_6H_5$	70	70-74	+27.3	7.2-7.6 (5 H, m)
55	malto	$p\text{-}CH_2\text{-}C_6H_4\text{-}Cl$	68	83	+16.6	7.5 (4 H, m)
56	malto	$p\text{-}CH_2\text{-}C_6H_4\text{-}CH_3$	72	86-88	+29.5	2.25 (3 H, s), 7.15 (4 H, q)
57	malto	$p\text{-}CH_2\text{-}C_6H_4\text{-}OCH_3$	72	83-86	+29.2	3.75 (3 H, s), 7.15 (4 H, q)
58	malto	CH_2—(furyl, O)	74	54	+27.5	6.43 (2 H, m), 7.45 (1 H, m)
59	malto	CH_2—(thienyl, S)	70	60-65	+48.0	6.9-7.2 (2 H, m), 7.3-7.5 (1 H, m)
60	malto	$CH(CH_3)\text{-}C_6H_5$	65	110	+45.5	1.50-1.84 (3 H, m), 7.1-7.56 (5 H, m)
61	malto	$CH_2CH_2\text{-}C_6H_5$	69	72-75	+58.6	7.25 (5 H, br s)
62	lacto[d]	$CH_2CH_2CH_2CH_3$	73	90-95	+6.0	0.7-1.95 (7 H, m), 4.20 (2 H, t)
63	lacto	$CH_2CH(CH_3)_2$	75	87-92	-4.0	0.93 (6 H, d), 1.85-2.2 (1 H, m)
64	melibio[e]	$CH_2CH(CH_3)_2$	70	73	+49.1	0.93 (6 H, d), 1.8-2.3 (1 H, m)
65	malto (OAc)₇[f]	$CH_2CH(CH_3)_2$	56	131	+48.0	

[a] These nitrosoureas usually gave the characteristic IR absorption bands at 3300-3400 (OH), 1690-1700 (CO), and 1070-1080 (-O-) cm⁻¹. [b] Measured in methanol at 15-30 °C. [c] Malto represents a β-maltosyl moiety (see Table IV). [d] Lacto represents a β-lactosyl moiety (see Table IV). [e] Melibio represents a β-melibiosyl moiety (see Table IV). [f] Malto (OAc)₇ represents a hepta-O-acetyl-β-maltosyl moiety. [g] Signals due to $N(NO)CH_2CH_2Cl$.

confirmed the β-maltosyl structure of **37** and suggest that the disaccharide derivatives of nitrosoureas prepared in the present study would also be activated by the mechanism depicted in Scheme III.

Biological Results and Discussion

The disaccharide derivatives of nitrosoureas (**33-65**) were tested for their antitumor activities against leukemia L1210 and Ehrlich ascites carcinoma by the methods described previously.[8] The results are listed in Table III together with the comparative data for CCNU, ACNU, and GANU.

A large number of the maltosylnitrosoureas were remarkably active against both leukemia L1210 and Ehrlich ascites carcinoma. As regards the effect of the alkyl substituents (R), isobutyl and sec-butyl group appear to be of optimum size, since smaller or larger groups caused activity to fall off rapidly. Some alkoxylalkyl, alkenyl, and aralkyl groups also confer potent activity. Thus compounds **37, 38, 43, 45, 50, 58,** and **60** exhibit excellent

(11) Aoe, K.; Date, T.; Kotera, K., unpublished results.

Scheme V

37, X = H
65, X = COCH₃

66, X = H
67, X = COCH₃
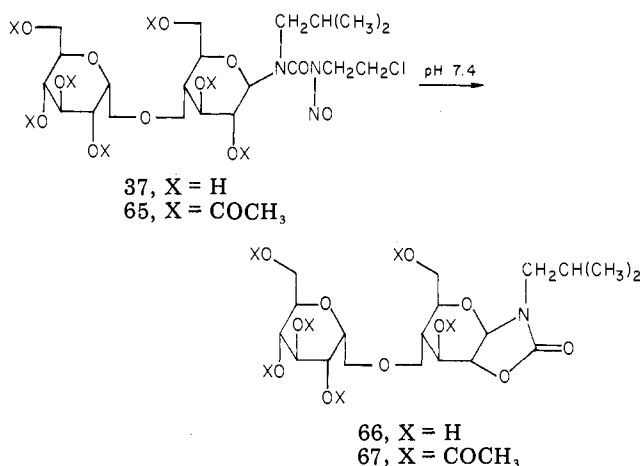
antitumor activity, their therapeutic ratios against leukemia L1210 and Ehrlich ascites carcinoma being more than 50 and more than 128, respectively. Sixty-day survivors

Table III. Antitumor Activities of Nitrosourea Derivatives of Disaccharides

compd	anti-L1210 activity [a]				anti-Ehrlich activity [b]		
	ILS_{30} [c]	OD [d]	ILS_{max}, %	therapeutic ratio [e]	MED [f]	MTD [g]	therapeutic ratio [h]
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	7.8	0.39	12.5	32
33	8.8	100	>710.8 [i]	11.4	6.25	200	32
34	4.9	100	>650.0	20.4	3.12	200	64
35	5.4	100	>700.0 [i]	18.5	3.12	100	32
36	4.5	100	>480.5	22.2	6.25	200	32
37	1.20	100	>733.3 [i]	83.3	0.78	200	256
38	1.50	100	>757.1 [i]	66.7	1.56	200	128
39	3.5	100	95.9	28.6	3.12	200	64
40	14	50	72.4	3.6	3.12	100	32
41		200	14.1	inact	6.25	200	32
42	2.0	100	>689.5 [i]	50.0	1.56	100	64
43	1.70	100	>757.1 [i]	58.8	1.56	200	128
44	3.1	200	>455.6	64.5	1.56	200	128
45	3.2	200	>757.1 [i]	62.5	1.56	200	128
46	17	200	123.8	11.8	3.12	200	64
47	2.7	100	>669.2 [i]	37.0	3.12	200	64
48	7.5	200	>261.8	26.7	3.12	200	64
49	3.8	200	>413.9	52.6	3.12	200	64
50	3.0	200	>700.0 [i]	66.7	1.56	200	128
51	3.5	100	>689.5 [i]	28.6	1.56	100	64
52	8.0	100	64.4	12.5	3.12	100	32
53	6.0	200	>689.5 [i]	33.3	3.12	200	64
54	7.3	200	>589.7	27.4	1.56	400	256
55	20	200	>219.0	10.0	1.56	200	128
56	10	200	>358.3	20.0	1.56	400	256
57	4.0	200	>600.0	50.0	1.56	400	256
58	4.0	200	>733.3 [i]	50.0	3.12	400	128
59	4.1	200	>277.8	48.9	1.56	400	256
60	2.7	200	>757.1 [i]	73.0	1.56	200	128
61	3.1	200	>349.3	64.5	3.12	200	64
62	100	200	40.5	2.0	50	200	4
63	160	200	32.9	1.3	100	200	2
64	350	400	34.0	1.1	400	400	1
65		400	18.0	inact			inact

[a] Leukemic cells (10^5) were inoculated ip into four male BDF_1 mice and ip administration was begun 24 h after the inoculation and performed once daily for 5 days. [b] The ascites cells (10^6) were inoculated ip into 5 female ICR mice and ip administration was begun 24 h after the inoculation and performed once daily for 5 days. [c] Daily dose, (mg/kg)/day × 5, providing 30% increase in life-span over the control. ILS (%) = (T/C-1) × 100. [d] Optimal dose, (mg/kg)/day × 5, = the daily dose providing the maximum increase in life-span. [e] Therapeutic ratio = OD/ILS_{30}. [f] Minimum effective dose, (mg/kg)/day × 5, = the minimum dose which shows 100% inhibition of the growth of the tumor. [g] Maximum tolerated dose, (mg/kg)/day × 5, = 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 60 days.

against leukemia L1210 were found at the optimal dose for many of the maltosyl nitrosourea derivatives. Acetylation of 37 resulted in a substantial loss of activity (65), endorsing the apparent significance of a free hydroxyl group in the sugar moiety as an essential structural feature for potent antitumor activity in this class of compounds.

In previous papers,[1,10] we have shown that the sugar moiety in the 3,3-disubstituted nitrosoureas act as a specific carrier to transport these compounds into tumor cells and that galactopyranosyl and arabinopyranosyl groups are the most favorable sugar moieties in aldohexoses and aldopentoses, respectively. In the disaccharide derivatives prepared in the present study, only maltose was found to be an effective sugar moiety, the lactosyl (62 and 63) and the melibiosyl (64) derivatives being almost inactive.

In Table IV are given comparative data for the antitumor activities of the three disaccharide derivatives bearing an isobutyl group together with those of the known[12] analogues (68 and 69) bearing a proton at the N-3 position. Interestingly, the favorable effect of a maltosyl moiety is seen only in the 3,3-disubstituted derivatives, because the activities of the unsubstituted analogues of maltosyl and

lactosyl nitrosoureas (68 and 69) are quite similar. In Table IV, ILS_{30} and minimum effective dose (MED) are the parameters possibly related to the cytotoxicity of the compounds against tumor cells, while an optimal dose (OD) and a maximum tolerated dose (MTD) are those related to the whole animal toxicity of the compounds. Both of the nitrosoureas (68 and 69) that bear a proton on the N-3 position exhibit small values of ILS_{30} and MED and relatively small values of OD and MTD. This appears to indicate that both 68 and 69 easily pass through the cell membrane and exhibit toxicity and antitumor activity. The lactosyl and melibiosyl nitrosoureas (63 and 64) bearing an isobutyl group exhibit very large values of ILS_{30}, OD, MED, and MTD. It seems likely that these compounds probably hardly pass through cell membrane as compared with the unsubstitued analogues (68 and 69) and exhibit only weak effects in both toxicity and activity. The maltosyl derivative (37) also shows very weak toxicity, but its antitumor activity is extremely potent as compared with 63 and 64. This indicates that the maltosyl derivative (37) possesses highly selective cytotoxicity to tumor cells. Although the reason for this selectivity is not clear, it may be related to the fact that among various disaccharides only maltose is known to be metabolized and utilized as an energy source in common mammalian cells.[13]

(12) Suami, T.; Machinami, T.; Hisamatsu, T. *J. Med. Chem.* 1979, 22, 247.

Table IV. Antitumor Activities [a] of Some Nitrosourea Derivatives of Disaccharides

$$\underset{\text{sugar}}{\overset{R}{\diagdown}} NCONCH_2CH_2Cl$$
$$\underset{NO}{|}$$

maltosyl =   lactosyl =   melibiosyl =

no.	sugar	R	anti-L1210 activity			anti-Ehrlich activity	
			ILS_{30} [b]	OD [b]	ILS_{max}, %	MED [b]	MTD [b]
37	maltosyl	$CH_2CH(CH_3)_2$	1.20	100	>733.3 [c]	0.78	200
63	lactosyl	$CH_2CH(CH_3)_2$	160	200	32.9	100	200
64	melibiosyl	$CH_2CH(CH_3)_2$	350	400	34.0	400	400
68	maltosyl	H	0.76	12.5	>290.0	0.78	25
69	lactosyl	H	1.00	12.5	>407.0	1.56	25

[a] Test conditions, etc., are all the same as those described in Table III. [b] In (mg/kg)/day × 5. [c] All treated mice survived more than 60 days.

Table V. Antitumor Activity of Some Nitrosourea Derivatives by Single or Multiple Treatment on L1210 [a]

$$\underset{\text{sugar}}{\overset{R}{\diagdown}} N-CO-N-CH_2CH_2Cl$$
$$\underset{NO}{|}$$

compd	sugar	R	day 1 only (ip)			Qd day 1-5 (ip)		
			ILS_{30} [b]	OD [b]	therapeutic ratio	ILS_{30} [c]	OD [c]	therapeutic ratio
37	malto [d]	$CH_2CH(CH_3)_2$	7.3	230	31.5	1.35	130	96.3
70	gluco [e]	$CH_2CH(CH_3)_2$	2.6	130	50.0	0.73	25	34.2
71	galacto [f]	$CH_2CH(CH_3)_2$	3.1	200	64.5	1.0	50	50.0
CCNU			9.3	50	5.4	4.9	25	5.1
ACNU			3.6	50	13.9	2.7	25	9.3
GANU			1.1	25	22.7	0.8	6.25	7.8

[a] Leukemic cells (10^5) were inoculated ip into six or eight male BDF_1 mice, and ip administration was begun 24 h after the inoculation. [b] In (mg/kg)/day × 1. [c] In (mg/kg)/day × 5. [d] Malto represents a β-maltosyl moiety. [e] Gluco represents a β-D-glucopyranosyl moiety. [f] Galacto represents a β-D-galactopyranosyl moiety.

Meanwhile, it is well known that nitrosourea derivatives commonly possess myelosuppressive activity which precludes their clinical use by multiple treatment. In fact, there has been no report on the nitrosourea derivatives whose therapeutic ratio obtained by multiple treatment is apparently larger than that obtained by single treatment even in experimental tumor systems. The antitumor activity of the maltosyl nitrosourea (**37**), the most interesting compound of this series, against leukemia L1210 was tested by single and multiple treatment, and the results are given in Table V together with the comparative data for the monosaccharide derivatives (**70** and **71**)[10] and the three positive controls. The therapeutic ratio of **37** obtained by multiple treatment was 3 times larger than that obtained by single treatment. This result further demonstrates the highly effective role of the maltosyl moiety in this series of compounds and suggests possible clinical utility of **37** by multiple treatment. Compound **37** is currently under clinical trials in Japan under the code name of TA-077.[14] Further synthesis of this new class of nitrosoureas bearing other sugar moieties is in progress.

Experimental Section

IR spectra were determined with a Hitachi IR-215 spectrometer

in Nujol mull. NMR spectra were recorded on a JEOL-PMX 60 spectrometer using tetramethylsilane as an internal standard in Me_2SO-d_6/D_2O 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.

General Procedure for the Preparation of 3-Substituted 1-(2-Chloroethyl)-3-β-glycosylureas (1–32). A mixture of sugar (0.01 mol), amine (0.012–0.015 mol), and 10 mL of methanol was stirred and heated at 65 °C for 20–40 min. The reaction mixture was concentrated to dryness under reduced pressure, and ethanol was added to the residue. The mixture was again concentrated to dryness, and the residue was dissolved in 30 mL of methanol. To the solution was added dropwise 2-chloroethyl isocyanate (0.012–0.015 mol) at 5 °C, and then the mixture was stirred for 1.5 h at room temperature and concentrated under reduced pressure. The residue was chromatographed on silica gel (solvent: AcOEt–benzene–MeOH). The ureas were generally obtained as unstable colorless amorphous powders and are listed in Table I with some characteristic data.

General Procedure for the Preparation of 3-Substituted 1-(2-Chloroethyl)-3-β-glycosyl-1-nitrosoureas (33–64). The glycosylurea (0.01 mol) was suspended in 50 mL of tetrahydrofuran at 0 °C and then anhydrous sodium acetate (0.08 mol) was added. Into the mixture was introduced dinitrogen tetroxide (0.085 mol) at −5 °C for 10 min under vigorous stirring. After 10 min, 15 mL of methanol was added to the mixture and then stirred at the same temperature for 10 min. To the mixture were added cold ethyl acetate (100 mL), anhydrous sodium acetate (0.08 mol), and water (20 mL) at −5 °C under 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–

(13) (a) Weser, E.; Sleisengar, M. H.; Dickstein, M.; Bartley, F. H. *J. Clin. Invest.* 1967, 46, 499. (b) Young, S. J. M.; Weser, E. *J. Clin. Invest.*, 1971, 50, 986.

(14) The detailed data for the antitumor effects and the toxicity of compound **37**, including myelosuppressive activity, will be reported elsewhere.

benzene–MeOH). The nitrosoureas (33–64) thus obtained were usually unstable light yellow amorphous powders and are listed in Table II together with the yields and some characteristic data.

Preparation of the Heptaacetate (65) of 1-(2-Chloroethyl)-3-isobutyl-3-β-maltosyl-1-nitrosourea (37). A mixture of 37 (2.65 g, 0.005 mol), acetic anhydride (15 mL), and pyridine (30 mL) was stirred at room temperature for 2 days. The reaction mixture was poured into water and extracted three times with 50 mL of ethyl acetate. The organic layer was washed with cold aqueous hydrochloric acid, water, and saturated NaCl solution, dried over MgSO$_4$, and concentrated. The residual oil was stirred with ether to give a colorless powder, which was collected by filtration and dried. The crude product was crystallized from methanol–ethanol to afford pure 65 as light yellow fine needles: 61.5% yield; mp 131 °C dec; $[\alpha]^{15}_D$ +48.0° (c 1.0, MeOH); IR (Nujol) 1750, 1700 (C=O) cm^{-1}; mass spectrum, m/e 825 (M$^+$), 795 (M$^+$ – NO). Anal. (C$_{33}$H$_{48}$O$_{19}$N$_3$Cl) C, H, N, Cl.

Decomposition of the Maltosylnitrosourea (37) in Phosphate-Buffered Solution (pH 7.4). The nitrosourea 37 (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 20 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-maltose-1,2-carbamate (66) in 75% yield as colorless crystals: mp 207–210 °C (ethanol); $[\alpha]^{15}_D$ +104.5° (c 0.98 MeOH); IR (Nujol) 1755 (C=O) cm^{-1}; NMR (Me$_2$SO-d_6/D$_2$O) δ 0.86 [d, J = 6.3 Hz, 6 H, CH(CH$_3$)$_2$], 1.7–2.1 [m, 1 H, CH(CH$_3$)$_2$], 4.65 (d, J = 8.5 Hz, 1 H, C$_1$ H), 5.10 (d, J = 3 Hz, 1 H, C$_{1'}$ H). Anal. (C$_{17}$H$_{29}$NO$_{11}$) C, H, N.

Heptaacetate (67) of 66: mp 180–181 °C; $[\alpha]^{15}_D$ +81.6° (c 1.0, MeOH); IR (Nujol) 1790, 1740 (C=O) cm^{-1}. Anal. (C$_{29}$H$_{41}$NO$_{17}$) C, H, N.

Preparation of Active Controls (CCNU, ACNU, and GANU) and Unsubstituted Analogues (68 and 69). These active controls were synthesized in our laboratory by the method reported in the previous paper.[10] The unsubstituted analogues 68 and 69 were prepared according to the method described in the literature.[12] Compound 68 had mp 95 °C dec and $[\alpha]^{29}_D$ +62° (c 0.5, H$_2$O) [lit. mp 96 °C dec; $[\alpha]^{23}_D$ +60° (c 0.5, H$_2$O)]. Compound 69 had mp 129–131 °C dec and $[\alpha]^{28}_D$ +4.7° (c 1.0, H$_2$O) [lit. mp 131 °C dec; $[\alpha]^{23}_D$ +4.0 (c 0.5, H$_2$O)].

Antiinflammatory Agents. 2.[1] Syntheses and Antiinflammatory Activity of Substituted 2-Aminophenylacetic Acid Derivatives

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Several substituted 2-aminophenylacetic acid derivatives were prepared and tested for in vitro prostaglandin synthetase inhibition activity and for in vivo antiinflammatory activity. The 2-amino substituent is beneficial to potency in the inhibition of prostaglandin synthetase for the 3-phenoxy, 4-phenyl, and 3-benzoyl series, but only the 3-benzoyl series shows increased antiinflammatory potency in the in vivo assay.

The synthesis and potent antiinflammatory and analgesic activities of 2-amino-3-benzoylphenylacetic acid (1, amfenac) have recently been reported.[1] Since there are few nonsteroidal antiinflammatory drugs (NSAIDs) containing an NH$_2$ group described in the literature, it became of interest to determine if the NH$_2$ moiety was responsible for the unexpected potency of 1.

Shen,[2,3] Scherrer,[4] and Appleton[5] have all proposed models in which NSAIDs bind with prostaglandin synthetase and, thus, inhibit prostaglandin production and concomitant inflammation. Compound 1 also inhibits prostaglandin synthetase in vitro,[6] and the NH$_2$ group could provide an additional point of attachment in binding to the receptor. Three series of compounds, the 2-amino-4-biphenylacetic acids and 2-amino-3-phenoxyphenylacetic acids, in addition to analogues of 1, were synthesized, and their antiinflammatory activities and prostaglandin synthetase inhibitory properties were determined to ascertain the influence of the NH$_2$ moiety on potency.

Chemistry. Scheme I illustrates a general method for the preparation of *o*-aminophenylacetic acids utilizing Gassman's procedure[7] for the synthesis of oxindoles. Using 3-aminobiphenyl (4), we obtained two isomers, 10 and 11, in a ratio of ~2:1 (by ^{1}H NMR), respectively. These

isomers could be separated by fractional crystallization, but the procedure was quite tedious. Since the desired isomer (11) was the minor component of the mixture, an unequivocal synthesis of 17 was sought and is presented in Scheme II.

Compounds 24 (Scheme III) and 26 (Scheme IV) were synthesized by standard procedures for pharmacological comparison.

Pharmacology. Acute antiinflammatory activity was determined in the Evans blue–carrageenan induced pleural effusion model as described by Sancilio and Fishman.[8] Each compound was dissolved or suspended in water

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(1) For part 1 in this series, see W. J. Welstead, Jr., H. W. Moran, H. F. Stauffer, L. B. Turnbull, and L. F. Sancilio, *J. Med. Chem.*, 22, 1074 (1979).

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