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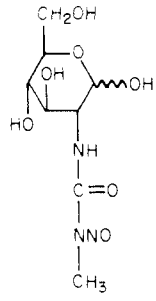
Alkyl Streptozotocin Analogues with Improved Biological Activities

Morio Iwasaki, Masaki Ueno, Kenji Ninomiya, Junzo Sekine, Yasuhiro Nagamatsu, and Goro Kimura*

Research Laboratories, Tokyo Tanabe Co., Ltd., Kita-ku, Tokyo 115, Japan. *Received July 14, 1975*

Alkyl 16α- and -β-glycosides of a series of N^3-alkyl homologues of streptozotocin were synthesized from glucosamine hydrochloride. These compounds, when tested against ascites Sarcoma 180, Ehrlich ascites carcinoma, or leukemia L1210, exhibited potent antitumor activities, and antibacterial and diabetogenic activities were eliminated. Furthermore, the acute toxicities of these compounds were lower than that of streptozotocin. The methyl, ethyl, n-propyl, and n-butyl glycosides of streptozotocin, whether α- or β-anomers, all showed higher antitumor activities than streptozotocin itself. The most active compound was found to be the methyl β-streptozotocin.

Streptozotocin is a broad-spectrum antibiotic[1] and has been shown by degradation[2,3] and synthesis[3-5] to have structure 1. It exhibits marked antileukemic activity[6] but suffers from its observed damaging effects on the β cells of the islets of Langerhans and its diabetogenic activity.[7,8]



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Recently, there have been reports of studies on isomers and analogues of 1 with results showing varying degrees of antileukemic, diabetogenic, and antibacterial activities.[1,9-11] Notable among these, analogues of 1 obtained by reacting either methyl α- and β-glycosides[9-11] or various other sugars with the methylnitrosoureido group[10,11] were studied. These analogues exhibited activity against Ehrlich ascites carcinoma and leukemia L1210 in mice.[10,11] Furthermore, in vitro studies with the methyl glycosides of 1 revealed that the cytotoxic activities of the β-anomers were the same as that of 1, whereas the activity of the α-anomers was twice that of 1 against cultures of leukemia L1210; yet neither derivative showed any diabetogenic activity.[10] These studies were followed by reports of the synthesis of six isomers of 1 containing the methyl α-glycosidic linkage[11,12] which were also found to exhibit activity against Ehrlich ascites carcinoma in mice,[12] in addition to being 20–40% more active against leukemia L1210 than 1.[11]

Independently, we were interested in the biological effects of alkyl streptozotocin analogues, their in vivo antitumor activities, and also their toxicities. Accordingly, we synthesized 16 compounds and studied their antitumor activities, toxicities, and diabetogenic and antibacterial activities. The melting points and the specific rotations were also recorded and compared with the known values for the methyl α- and β-streptozotocins[9,10] in order to explore their relationships.

Chemistry. All of the streptozotocin analogues were synthesized by the procedure depicted in Scheme I. The starting material, glucosamine hydrochloride (2), was allowed to react with carbobenzyloxy chloride in an aqueous solution of sodium carbonate according to the method of Chargaff et al.[14] to yield N-carbobenzyloxy-D-glucosamine (3). Employing Fischer's[15] procedure, 3 was methylated with anhydrous methanol with a catalytic amount of hydrogen chloride to obtain methyl N-carbobenzyloxy-D-glucosaminide. Similarly, methanol was replaced by ethanol, 1-propanol, or 1-butanol to give the corresponding alkyl N-carbobenzyloxy-D-glucosaminide. In the above reaction, it has been reported[16] that if the reaction were carried out at an elevated temperature, the α-anomer predominated, whereas if carried out below room temperature, the β-anomer predominated. This phenomenon was verified with the primary alcohols used in our reaction. The products from the above reactions were purified by column chromatography to separate the anomers; and the alkyl N-carbobenzyloxy-α- and -β-D-glucosaminides (**4a–7a** = α-anomers; **4b–7b** = β-anomers), identified by their optical rotation values, were obtained. After catalytic decarbobenzoxylation, the products were allowed to react, according to the method of Suami et al.,[9] with various alkyl isocyanates to obtain the alkyl 2-deoxy-2-(3-alkylureido)-α- and -β-D-glucopyranosides (**8a**, **10a–16a** = α-anomers; **8b–15b** = β-anomers). These were then allowed to react with a slight excess of sodium nitrite in dilute acetic acid solution at 0–10 °C. After treatment with a cation exchanger (H^+ type) to remove sodium ions, the alkyl 2-deoxy-2-(3-alkyl-3-nitrosoureido)-α- and -β-D-glucopyranosides (**17a**, **19a–25a** = α-anomers; **17b–24b** = β-anomers) were obtained by concentration of the resultant solutions. The products could be stored in a desiccator for 20–30 months without any apparent signs of decomposition.

As shown in Tables I–III, respectively, the β-anomers of the same compound group exhibited higher melting points or decomposition temperatures than the α-anomers. That is to say, **4b–7b** had melting points 20–32 °C higher than **4a–7a** among the alkyl N-carbobenzyloxy-α- and -β-D-glucosaminides (Table I); **8b–15b** had melting points 9–50 °C higher than **8a** and **10a–16a** among the alkyl 2-deoxy-2-(3-alkylureido)-α- and -β-D-glucopyranosides (Table II); and compounds **17b–24b** were likewise 8–55 °C higher in their decomposition temperatures than **17a** and **19a–25a** among the alkyl 2-deoxy-2-(3-alkyl-3-nitrosoureido)-α- and -β-D-glucopyranosides (Table III).

Furthermore, as can be seen in Tables I–III, compared with each alkyl derivative in Table III, the corresponding derivative in Table II invariably exhibited 38–87 °C higher melting points or decomposition temperatures. The

Table I. Yields of Alkyl *N*-Carbobenzyloxy-D-glucosaminides and Their Physical Constants[a]

Compd	R_1[a]	Anomer	Mp, °C	$[\alpha]^{25}D$, deg (*c* 1.0, MeOH)	Yield, %	Formula	Analyses
4a	CH_3	α	155-156[b]	+89[c]	29	$C_{15}H_{21}NO_7$	C, H, N
4b	CH_3	β	175-176[d]	−23[e]	45	$C_{15}H_{21}NO_7$	C, H, N
5a	C_2H_5	α	134-135	+100	60	$C_{16}H_{23}NO_7$	C, H, N
5b	C_2H_5	β	163-164	−24	14	$C_{16}H_{23}NO_7$	C, H, N
6a	*n*-C_3H_7	α	148-149	+111	54	$C_{17}H_{25}NO_7$	C, H, N
6b	*n*-C_3H_7	β	168-170	−22	18	$C_{17}H_{25}NO_7$	C, H, N
7a	*n*-C_4H_9	α	144-145	+110	43	$C_{18}H_{27}NO_7$	C, H, N
7b	*n*-C_4H_9	β	176-177	−22	18	$C_{18}H_{27}NO_7$	C, H, N

[a] Structures of compounds depicted in Scheme I. [b] Lit. mp 156-158 °C,[9] 154-155 °C.[15] [c] Lit. $[\alpha]^{25}D$ +92.6° (*c* 2.4, pyridine),[9] +80° (pyridine).[15] [d] Lit. mp 166-167.5 °C,[9] 166-168 °C.[15] [e] Lit. $[\alpha]^{25}D$ −22.8° (*c* 1.0, pyridine),[9] −38° (pyridine).[15]

Table II. Yields of Alkyl 2-Deoxy-2-(3-alkylureido)-D-glucopyranosides and Their Physical Constants[a]

Compd	R_1[a]	R_2[a]	Anomer	Mp, °C	$[\alpha]^{25}D$, deg (H_2O)	Ir, cm^{-1} $\nu_{C=O}$	Ir, cm^{-1} δ_{NH}	Yield, %	Formula	Analyses
8a	CH_3	CH_3	α	194-195[b]	+98 (*c* 1.0)[c]	1620	1585	76	$C_9H_{18}N_2O_6$	C, H, N
8b	CH_3	CH_3	β	243-245[d]	−35 (*c* 1.0)[e]	1620	1580	87	$C_9H_{18}N_2O_6$	C, H, N
9b	CH_3	C_2H_5	β	222-224	−33 (*c* 1.0)	1620	1570	93	$C_{10}H_{20}N_2O_6$	C, H, N
10a	CH_3	*n*-C_3H_7	α	200-202	+57 (*c* 0.5)	1615	1570	35	$C_{11}H_{22}N_2O_6$	C, H, N
10b	CH_3	*n*-C_3H_7	β	209-211	−45 (*c* 0.5)	1620	1580	58	$C_{11}H_{22}N_2O_6$	C, H, N
11a	CH_3	*n*-C_4H_9	α	196-198	+60 (*c* 0.5)			39	$C_{12}H_{24}N_2O_6$	C, H, N
11b	CH_3	*n*-C_4H_9	β	206-208	−53 (*c* 0.5)			63	$C_{12}H_{24}N_2O_6$	C, H, N
12a	C_2H_5	CH_3	α	185-187	+125 (*c* 0.5)	1615	1585	74	$C_{10}H_{20}N_2O_6$	C, H, N
12b	C_2H_5	CH_3	β	219-222	−42 (*c* 0.5)	1620	1570	75	$C_{10}H_{20}N_2O_6$	C, H, N
13a	C_2H_5	C_2H_5	α	189-192	+84 (*c* 0.5)	1620	1560	62	$C_{11}H_{22}N_2O_6$	C, H, N
13b	C_2H_5	C_2H_5	β	200-203	−37 (*c* 0.5)			70	$C_{11}H_{22}N_2O_6$	C, H, N
14a	*n*-C_3H_7	CH_3	α	175-177	+116 (*c* 0.5)	1620	1580	40	$C_{11}H_{22}N_2O_6$	C, H, N
14b	*n*-C_3H_7	CH_3	β	212-214	−35 (*c* 0.5)	1620	1575	44	$C_{11}H_{22}N_2O_6$	C, H, N
15a	*n*-C_4H_9	CH_3	α	165-167	+134 (*c* 1.0)			47	$C_{12}H_{24}N_2O_6$	C, H, N
15b	*n*-C_4H_9	CH_3	β	214-216	−45 (*c* 0.5)	1630	1570	44	$C_{12}H_{24}N_2O_6$	C, H, N
16a	*n*-C_4H_9	*n*-C_4H_9	α	173-175	+95 (*c* 0.5)	1615	1570	39	$C_{15}H_{30}N_2O_6$	C, H, N

[a] Structures of compounds depicted in Scheme I. [b] Lit. mp 194.5-196 °C,[9] 194-196 °C.[10] [c] Lit. $[\alpha]^{23}D$ +97.5° (*c* 1.0, H_2O),[9] $[\alpha]D$ +129° (*c* 0.88, DMF).[10] [d] Lit. mp 239.5-241 °C,[9] 244-245 °C.[10] [e] Lit. $[\alpha]D$ −52° (*c* 0.64, DMF).[10]

Table III. Yields of Alkyl 2-Deoxy-2-(3-alkyl-3-nitrosoureido)-D-glucopyranosides and Their Physical Constants[a]

Compd	R_1[a]	R_2[a]	Anomer	Mp, °C	$[\alpha]^{25}D$, deg (*c* 0.5, H_2O)	Ir, cm^{-1} $\nu_{C=O}$	Ir, cm^{-1} δ_{NH}	Ir, cm^{-1} ν_{NNO}	Yield, %	Formula	Analyses
17a	CH_3	CH_3	α	130-133[b]	+107[c]	1690	1530	1475	48	$C_9H_{17}N_3O_7$	C, H, N
17b	CH_3	CH_3	β	185-187[d]	−21[e]	1710	1540	1480	68	$C_9H_{17}N_3O_7$	C, H, N
18b	CH_3	C_2H_5	β	135-137	−24	1705	1545	1480	68	$C_{10}H_{19}N_3O_7$	C, H, N
19a	CH_3	*n*-C_3H_7	α	118-120	+88	1700	1535	1480	48	$C_{11}H_{21}N_3O_7$	C, H, N
19b	CH_3	*n*-C_3H_7	β	128-130	−20	1700	1540	1490	58	$C_{11}H_{21}N_3O_7$	C, H, N
20a	CH_3	*n*-C_4H_9	α	124-125	+72	1695	1530	1475	40	$C_{12}H_{23}N_3O_7$	C, H, N
20b	CH_3	*n*-C_4H_9	β	132-134	−34	1700	1540	1490	52	$C_{12}H_{23}N_3O_7$	C, H, N
21a	C_2H_5	CH_3	α	120-123	+136	1690	1525	1470	50	$C_{10}H_{19}N_3O_7$	C, H, N
21b	C_2H_5	CH_3	β	160-162	−34	1700	1540	1480	30	$C_{10}H_{19}N_3O_7$	C, H, N
22a	C_2H_5	C_2H_5	α	114-116	+135	1695	1530	1475	50	$C_{11}H_{21}N_3O_7$	C, H, N
22b	C_2H_5	C_2H_5	β	122-124	−26	1700	1540	1485	19	$C_{11}H_{21}N_3O_7$	C, H, N
23a	*n*-C_3H_7	CH_3	α	112-114	+142	1690	1525	1480	55	$C_{11}H_{21}N_3O_7$	C, H, N
23b	*n*-C_3H_7	CH_3	β	127-129	−20	1705	1535	1485	51	$C_{11}H_{21}N_3O_7$	C, H, N
24a	*n*-C_4H_9	CH_3	α	126-129	+175	1690	1540	1480	39	$C_{12}H_{23}N_3O_7$	C, H, N
24b	*n*-C_4H_9	CH_3	β	138-141	−32	1710	1540	1490	64	$C_{12}H_{23}N_3O_7$	C, H, N
25a	*n*-C_4H_9	*n*-C_4H_9	α	114-117	+127	1700	1525	1485	56	$C_{15}H_{29}N_3O_7$	C, H, N

[a] Structures of compounds depicted in Scheme I. [b] Lit. mp 129-133 °C dec,[9] 175-185 °C dec.[10] [c] Lit. $[\alpha]^{25}D$ +107° (*c* 0.5, H_2O),[9] $[\alpha]D$ +117° (*c* 0.63, H_2O).[10] [d] Lit. mp 149 °C dec,[9] 185-193 °C dec.[10] [e] Lit. $[\alpha]^{25}D$ −23.7° (*c* 0.5, H_2O),[9] lit. $[\alpha]D$ −4° (*c* 0.57, DMF).[10]

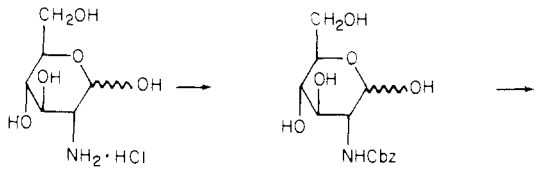
specific rotations of the α-anomers of the alkyl compounds in Table III also invariably exhibited rotations 9–41° greater on the positive scale than the corresponding alkyl compounds in Table II, whereas the β-anomers of those compounds in Table III had values 8–25° lower on the negative scale than the compounds in Table II.

In Table III the infrared absorptions ($\nu_{C=O}$, $\nu_{N=O}$, and δ_{NH}) of the β-anomers showed a slight shift to higher wavenumbers than the α-anomers.

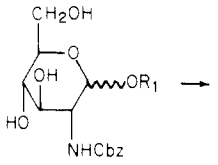
Results

Antitumor Activity. The effect of the 16 compounds on ascites Sarcoma 180 transplanted into mice is presented in Table IV. At doses of 25 or 50 mg/kg/day, **17a,b**, **21a,b**, **23a**, and **24a,b** showed activities equal to or higher than 1. These compounds all contained the 3-methyl-3-

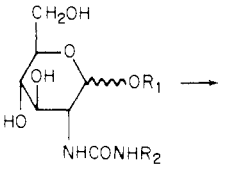
Scheme I



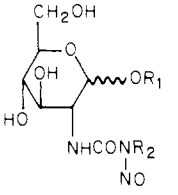
2 3 (Cbz = $COOCH_2C_6H_5$)



	α-anomer	β-anomer	R_1
4	a	b	CH_3
5	a	b	C_2H_5
6	a	b	n-C_3H_7
7	a	b	n-C_4H_9



	α-anomer	β-anomer	R_1	R_2
8	a	b	CH_3	CH_3
9		b	C_2H_5	C_2H_5
10	a	b	CH_3	n-C_3H_7
11	a	b	CH_3	n-C_4H_9
12	a	b	C_2H_5	CH_3
13	a	b	C_2H_5	C_2H_5
14	a	b	n-C_3H_7	CH_3
15	a	b	n-C_4H_9	CH_3
16	a		n-C_4H_9	n-C_4H_9



	α-anomer	β-anomer	R_1	R_2
17	a	b	CH_3	CH_3
18		b	CH_3	C_2H_5
19	a	b	CH_3	n-C_3H_7
20	a	b	CH_3	n-C_4H_9
21	a	b	C_2H_5	CH_3
22	a	b	C_2H_5	C_2H_5
23	a	b	n-C_3H_7	CH_3
24	a	b	n-C_4H_9	CH_3
25	a		n-C_4H_9	n-C_4H_9

nitrosoureido moiety attached to the 2 position of glucose, and the hydroxy group at the anomeric carbon had been blocked with an alkyl function. However, the change in the number of carbon atoms of the alkyl function apparently did not affect the activities of the compounds against ascites Sarcoma 180. In contrast, if the N^3-methyl group were substituted with another alkyl function, the activity of these compounds, without exception, decreased regardless of the *O*-alkyl group at the anomeric carbon. There were no observable differences in activities, however, between the α- and β-anomers.

Table V records the activities of ten different analogue compounds against Ehrlich ascites carcinoma transplanted in mice. Compounds **17b** and **24b** exhibited marked activities (+++) at a dosage of 150 mg/kg. These two compounds possessed the 3-methyl-3-nitrosoureido moiety in the molecule and were β-anomers. Compounds **17a**, **23a**, and **24a** were α-anomers and exhibited lesser activities (++). As was the case against ascites Sarcoma 180, compounds containing other N^3-substituents, i.e., ethyl (**22a**), *n*-propyl (**19a**, **20b**), or *n*-butyl (**25a**), all exhibited lower activities against Ehrlich ascites carcinoma than did **1**.

Results of increase in life-span tests (ILS) on 12 of the compounds against leukemia L1210 are shown in Table VI. Those analogues having activities comparable to that of **1** were **17a,b**, **21a**, **23a,b**, and **24a,b**. They all exhibited activities of +++ and they were all 3-methyl-3-nitrosoureido compounds. Other N^3-alkyl-substituted analogues, such as ethyl (**18b**, **22b**), *n*-propyl (**19b**), and *n*-butyl (**20b**), were tested but these were not as active as either **1** or the N^3-methyl derivatives.

Thus, it can be seen that against ascites Sarcoma 180, Ehrlich ascites carcinoma, or leukemia L1210, the antitumor activities of the alkyl streptozotocin analogues were independent of the *n*-alkyl group attached to the anomeric carbon but were influenced by the alkyl substituent on the 3-methyl-3-nitrosoureido moeity. Thus, replacement of the N^3-methyl by ethyl, *n*-propyl, or *n*-butyl caused a drop in the antitumor activities of these compounds.

Table IV. Antitumor Activity of Streptozotocin and Its Alkyl Analogues against Ascites Sarcoma 180 (S 180A)[a]

Compd	Dosage,[b] mg/kg/day	Anti-[c] tumor act.	Growth ratio, T/C, %	Mortality
17a	25	++	37	0/6
17b	25	++	22	0/6
18b	100	+	65	0/6
19a	50	+	55	0/6
19b	50	-	69	0/6
20a	125	-	75	0/6
20b	50	+	54	0/6
21a	50	-+	38	0/6
21b	25	++	37	0/6
22a	50	+	63	0/6
22b	50	+	59	0/6
23a	25	++	32	0/6
23b	50	++	32	0/6
24a	50	++	20	0/6
24b	25	-+	40	0/6
25a	50	-	80	0/6
1	25	++	40	0/6
1	50	++	26	0/6

[a] S 180A (1×10^7 cells) was implanted into ICR mice, six animals per group. [b] Five injections, saline, ip (qd, days 1-5). [c] 100-66 = -, 65-41 = +, 40-11 = ++, 10-0 = +++ based on growth ratio (T/C, %).

Table V. Antitumor Activity of Alkyl Streptozotocin Derivatives against Ehrlich Ascites Carcinoma (EAC)[a]

Compd	Dosage,[b] mg/kg/day	Anti-[c] tumor act.	Growth ratio, T/C, %	Mortality
17a	150	+-	16	0/6
17b	150	-++	1	0/6
19a	125	-	61	0/6
19b	125	-	72	0/6
20b	125	-	52	0/6
22a	125	+	60	0/6
23a	125	++	37	0/6
24a	150	+-	16	0/6
24b	150	-++	4	0/6
25a	125	-	53	0/6

[a] Ehrlich ascites carcinoma (1×10^7 cells) was implanted into ICR mice, six animals per group. [b] Five injections, saline, ip (qd, days 1-5). [c] 100-66 = -, 65-41 = -, 40-11 = ++, 10-0 = +++ based on growth ratio (T/C, %).

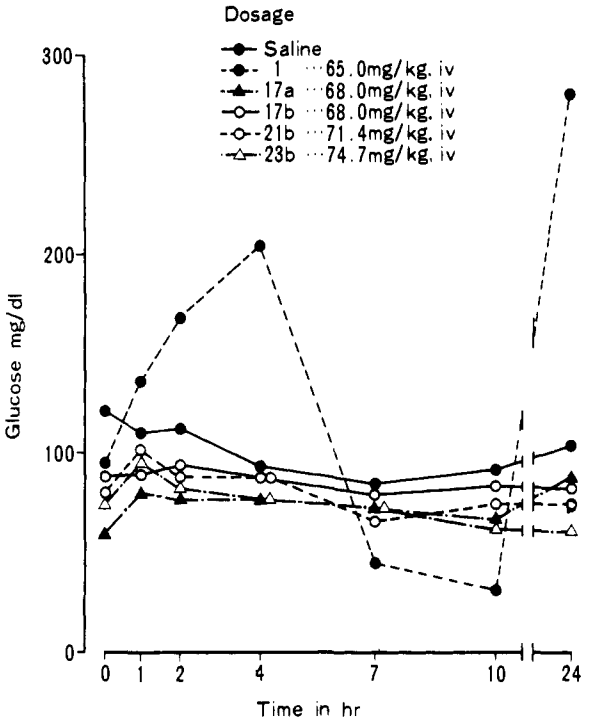


Figure 1. Effect of streptozotocin (**1**) and its alkyl derivatives on blood sugar concentration in starved rats. Blood sugar concentration was determined by the glucose oxidase method.[19] All test compounds were administered in the same molar quantities.

Table VI. Antitumor Activity of Streptozotocin and Its Alkyl Analogues against Leukemia L1210 (L1210)[a]

Compd	Dosage,[b] mg/kg/day	Anti-[c] tumor act.	ILS (%) over control	Mortality (in 5 days)
17a	50	+++	35	0/6
17b	50	+++	40	0/6
18b	50	+	14	0/6
19b	100	−	7	0/6
20b	50	+	12	0/6
21a	125	+++	64	0/6
22a	100	+	14	0/6
22b	100	+	10	0/6
23a	50	++	29	0/6
23b	50	+++	30	0/6
24a	50	+++	30	0/6
24b	50	+++	33	0/6
1	50	+++	44	0/6
1	100	+++	76	0/6
1	200	−	0	0/6

[a] L1210 (1×10^5 cells) was implanted into BDF_1 mice, six animals per group. [b] Five injections, saline, ip (qd, days 1-5). [c] 0-9% = −, 10-19% = +, 20-29% = ++, >30% ≡ +++ based on ILS.

Effects on Blood Sugar Concentration. Figure 1 shows the blood sugar concentrations over 24 h of normal rats and of rats treated with alkyl streptozotocin or with **1**, subsequent to a single dose of the compounds. Compound **1** could be seen to greatly increase the blood sugar concentrations in the treated animals whereas the four alkyl streptozotocins tested (**17a,b**, **21b**, and **23b**) produced practically the same sugar concentrations as that in the controls. Other alkyl streptozotocin analogues were also found to exhibit similar behavior.

In order to study the chronic effects of the test compounds on blood sugar concentrations, **17b** and **1** were administered to test animals for a 1-month period. The results of this study are presented in Figure 2. Compound **1**, when administered at a dosage of 20 mg/kg, qd, for 1 month, caused marked increases in the blood sugar concentrations, while at dosages of 30–120 mg/kg under similar conditions, **17b** was found to have essentially no effect on the blood sugar concentrations in the test animals.

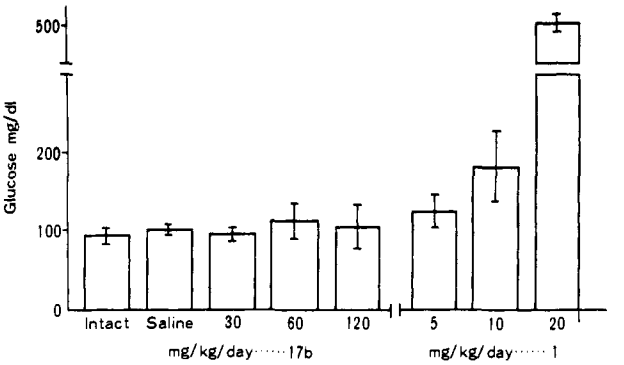


Figure 2. Effect of streptozotocin (**1**) and methyl 2-deoxy-2-(3-methyl-3-nitrosoureido)-β-D-glucopyranoside (**17b**) on blood sugar concentration in rats when administered intraperitoneally for a 1-month period. Blood sugar concentration determined by the glucose oxidase method.[19]

Table VII. Minimum Inhibitory Concentration (MIC)[a] of Streptozotocin (**1**) and Its Alkyl Derivatives for Various Bacterial Strains[b]

Bacterial strains	1
Bacillus subtilis ATCC 6633	12.5
Escherichia coli NIHJ JC-2	12.5
Klebsiella pneumoniae ATCC 10031	>200
Proteus vulgaris AHU 1469	0.4
Pseudomonas fluorescens NIHJ B-254	6.3
Salmonella typhi IFM 3020	>200
Sarcina lutea ATCC 9341	>200
Shigella sonnei	>200
Staphylococcus aureus FDA 209P JC-1	1.6
Streptococcus faecalis ATCC 10541	

[a] MIC given in μg/ml. [b] Broth dilution method was employed for the determination. Medium used was heart infusion broth. Incubation was carried out at 32 °C for 24 h. Compounds **17a,b**, **18b**, **21a,b**, and **23b** had no activity (MIC >1000) against any of these bacterial strains.

Antibacterial Activity. Compounds **1**, **17a,b**, **18b**, **21a,b**, and **23b** were tested against various bacterial organisms to determine their antibacterial activities. The results are presented in Table VII. Compound **1** was found to be active against *Proteus vulgaris* AHU 1469, *Pseudomonas fluorescens* NIHJ B-254, and *Staphylococcus aureus* FDA 209P JC-1 even at low concentrations. However, the alkyl streptozotocin analogues examined were all void of antibacterial activities even at concentrations of 1000 μg/ml.

Acute Toxicity. The acute toxicities of **1**, and of the alkyl streptozotocin analogues **17a,b**, **18b**, **21a,b**, **23a,b**, **24a,b**, and **25a**, are presented in Table VIII. The LD_{10} and LD_{50} values for the alkyl streptozotocin analogues were 2–10 times higher than those for **1**, indicating that they had comparatively lower toxicities.

From the above physiological experimental data, one can conclude that O-alkylation of the anomeric carbon of **1** results in compounds with enhanced antitumor activities with elimination of antibacterial and diabetogenic activities and reduced toxicities. This is in contrast with unmodified **1**, which has been known to cause damage to the β cells of islets of Langerhans in the pancreas and also to elevate the blood sugar concentration.[7,8]

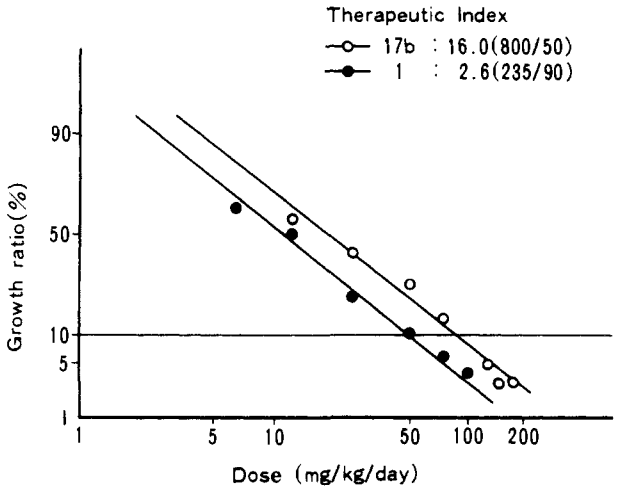


Figure 3. Dose-response curve of ascites Sarcoma 180 to streptozotocin (1) and methyl 2-deoxy-2-(3-methyl-3-nitrosoureido)-β-D-glucopyranoside (17b). LD_{10}/ED_{90} = 10% growth ratio.

Table VIII. Acute Toxicities of Streptozotocin (1) and Its Alkyl Derivatives

Compd	LD_{10} (female mouse), mg/kg ip	LD_{50} (female mouse), mg/kg ip	LD_{50}, mg/kg iv
17a	1380	1930	
17b	800	1700	1620 (female mouse), 400 (male dog)
18b	1380	1700	
21a	2000	2050	
21b	1550	2050	
23a	1020	1467	
23b	1240	2150	
24a	1200	2000	
24b	1100	1580	
25a	410	660	
1	235	360	275 (female mouse), 50 (male dog)

In our screening of the alkyl streptozotocin analogues, we found that methyl β-streptozotocin (**17b**) was more effective as an antitumor agent than **1**, possessed very low toxicity, and exhibited no adverse effect on blood sugar concentration. These results are depicted in Figures 3 and 4 where **17b** is compared against **1** relative to antitumor activity against ascites Sarcoma 180 and leukemia L1210 tumor systems. Compound **17b** was more active against ascites Sarcoma 180 than **1** at all dosages tested and had a therapeutic index (LD_{10}/ED_{90}) of about six times that of the parent compound. Against leukemia L1210, both compounds exhibited similar activities at doses of 20–100 mg/kg, but the therapeutic ratio of the modified compound was three times that of the parent compound. Therefore **17b** could be a worthwhile compound for further studies.

Experimental Section

Melting points were taken on a micromelting point apparatus (Mitamura Riken Co., Ltd.) and are uncorrected. Specific rotations were determined on an automatic polarimeter Model Dip-180 (Nippon Bunko Kogyo Co., Ltd.). Infrared spectra were taken on a Model EPI-Ga infrared spectrometer (Hitachi) and were determined as KBr disks. Thin-layer chromatography (TLC) was performed using 5 × 20 cm glass plates coated with silica gel B-5 (Wako Pure Chemicals), and the R_f values were determined after visualization by spraying and charring with a solution of 4% NH_4VO_3 in 50% aqueous H_2SO_4.

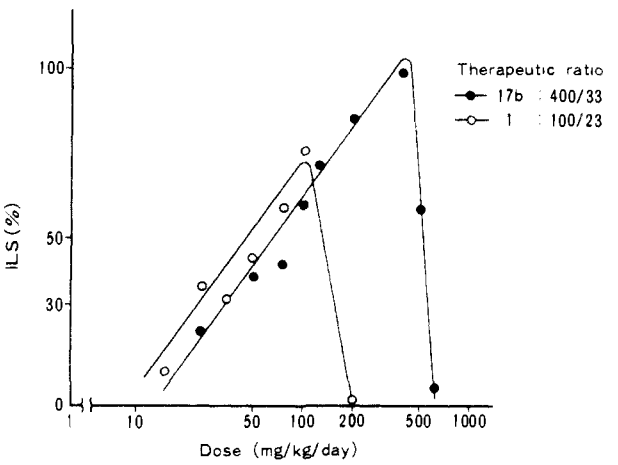


Figure 4. Dose-response curve of leukemia L1210 to streptozotocin (1) and methyl 2-deoxy-2-(3-methyl-3-nitrosoureido)-β-D-glucopyranoside (17b). Optimal dose/ILS_{30}.

Ethyl *N*-Carbobenzyloxy-α- and -β-D-glucosaminide (5a,b). A suspended solution of *N*-carbobenzyloxy-D-glucosamine[14] (50 g, 0.16 mol) in anhydrous EtOH (1.6 l.) and concentrated HCl (50 ml, d^{15}_4 1.18) was maintained at 40° for 6 days with stirring. TLC showed **5a** and **5b** with R_f's of 0.53 and 0.39, respectively. The reaction mixture was neutralized with basic $PbCO_3$, inorganic salts were filtered off, and the filtrate was evaporated in vacuo. The resulting residue was isolated with column chromatography on silica gel (Kiesel gel-60, Merck; CH_2Cl_2–MeOH, 20:1). The two anomers were crystallized (EtOH) to give **5a** (32.7 g, 60%) and **5b** (7.7 g, 14%), respectively.

General Preparative Method for Alkyl *N*-Carbobenzyloxy-α- and -β-D-glucosaminide (4a–7a = α-Anomers; 4b–7b = β-Anomers). These compounds were prepared by the method described for **5a** and **5b**. Their physical data were compiled in Table I.

Ethyl 2-Deoxy-2-(3-methylureido)-α-D-glucopyranoside (12a). A solution of **5a** in MeOH (300 ml) was hydrogenated at a pressure of 3.5 kg/cm² for 10 h over Pd black (1 g). After removal of the catalyst by filtration, MeNCO (8 ml, 125 mmol) was added dropwise into the filtrate and the mixture stirred for 2 h at 20–25°. Then, the reaction mixture was evaporated in vacuo at a temperature not exceeding 35° and the crystalline residue was recrystallized (EtOH) to give **12a** (17 g, 73%).

General Preparative Method for Alkyl 2-Deoxy-2-(3-alkylureido)-α- and -β-D-glucopyranoside (8a, 10a–16a = α-Anomers; 8b–15b = β-Anomers). These compounds were prepared by the method described for **12a**. Their physical data were compiled in Table II.

Ethyl 2-Deoxy-2-(3-methyl-3-nitrosoureido)-α-D-glucopyranoside (21a). Into an ice-cooled solution of **12a** (11.0 g, 42 mmol) in 50% aqueous AcOH, $NaNO_2$ (3.9 g, 57 mmol) was slowly added. After the addition of $NaNO_2$, the reaction mixture was further stirred for 3 h while maintaining the same temperature. Then, the mixture was treated with Amberlite IR-120 (H^+ type) ion-exchange resin (30 g, Rohm and Haas) to remove the Na^+ ion, and the reaction mixture again stirred for another 1 h while being chilled as before. After removal of the resin by filtration, the resultant filtrate was evaporated in vacuo below 35° and the residue crystallized (EtOH) to obtain **21a** (6.1 g, 50%).

General Preparative Method for Alkyl 2-Deoxy-2-(3-alkyl-3-nitrosoureido)-α- and -β-D-glucopyranoside (17a, 19a–25a = α-Anomers; 17b–24b = β-Anomers). These compounds were prepared by the method described for **21a**. Their physical data were compiled in Table III.

Biological Methods. Antitumor activity analyses were performed employing the methods of the National Cancer Center, Tokyo,[17,18] with ascites Sarcoma 180, Ehrlich ascites carcinoma, and leukemia L1210 as tumor systems. Ascites Sarcoma 180 and Ehrlich ascites carcinoma were inoculated into mice which were sacrificed on the 7th day. The treated animals were compared with controls to calculate the total packed cell volume (TPCV) from the total volume of ascites (TV) and percent packed cell

volume (PCV) according to the following formula.

$$TPCV = TV \times PCV$$

The effects of the nitrosoureas were determined by calculating the ratio of the tumor weight of treated animals to that of the controls (T/C, %).

$$T/C, \% = [TPCV\ (treated)/TPCV\ (control)] \times 100$$

The effect against leukemia L1210 was determined by the percent increase in life span (ILS) which was determined by the following formula.

$$ILS = [[life\ span\ (treated)/life\ span\ (control)] - 1] \times 100$$

For a single administration schedule, the blood sugar concentration was determined with groups of five male Wistar strain rats weighing about 200 g. These were not fed for 16 h prior to administration of the test compounds, which were given in a physiological saline solution. Administration was by means of intravenous injection into the femoral vein. Blood samples were taken from the caudal vein at specific time intervals and the blood sugar concentrations were determined by the glucose oxidase method.[19]

The effect of the test compounds on the blood sugar concentration after a 1-month administration period was determined with groups of five male Wistar strain rats weighing about 150 g. The test compounds were administered intraperitoneally in a physiological saline solution for 30 days, qd, days 1–30. Blood samples were taken 24 h after the last administration of the test compound and the blood sugar concentration was determined as before.

Acute toxicities were determined using groups of ten female JCL-ICR mice. The test compounds were administered intraperitoneally in a physiological saline solution. The LD_{50} was calculated according to the method of Litchfield and Wilcoxon[20] from the death rate over a 7-day period.

In vitro antibacterial activities were determined by the broth dilution method. Twofold serial dilutions of each test compound were prepared in heart infusion broth (Eiken Chemical Co., Tokyo) in test tubes. A 0.1-ml volume of a cell suspension containing the test bacteria which had been cultivated for 24 h in heart infusion broth and 100-fold diluted with physiological saline solution was inoculated into the tubes aseptically and the tubes were incubated at 30 °C for 18 h. The minimum inhibitory concentration (MIC) was expressed in μg/ml for the dosage at which the growth of test cultures was completely inhibited.

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Potential Histamine H_2-Receptor Antagonists.[1] 3. Methylhistamines

Graham J. Durant,* John C. Emmett, C. Robin Ganellin, Anthony M. Roe, and R. Antony Slater

The Research Institute, Smith Kline & French Laboratories Ltd., Welwyn Garden City, Hertfordshire, England.
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Syntheses are described for all the mono- and some di- and trimethylhistamines. New methods are given for the known N^{π}, N^{τ}-, N^{α}-, 2-, and 4-methylhistamines and for the novel compounds, β-methyl-, 4,N^{α}-dimethyl-, and 4,N^{α},N^{α}-trimethylhistamines. Agonist activities are reported for stimulation of histamine H_1 (guinea-pig ileum) and H_2 (rat gastric acid secretion) receptors. H_2-Receptor agonist activities indicate that a methyl group is more readily accommodated at the 4 and N^{α} positions than elsewhere in the histamine molecule and that receptor binding is substantially retained with a methyl substituent in these positions. Thus, for the design of potential antagonists, two sites are identified as being worthwhile exploring for the introduction of lipophilic substituents.

Certain pharmacological actions of histamine are mediated by histamine receptors, now classified into two types, H_1 and H_2.[2,3] The effect of histamine at its H_1 receptor may be blocked specifically by conventional antihistaminic drugs, such as pyrilamine,[2] but H_2-receptor effects are not blocked by these drugs. An investigation in these laboratories into the classification and blockade of histamine H_2 receptors has culminated in the synthesis and characterization of H_2-receptor antagonist drugs typified by burimamide,[3] metiamide,[4] and cimetidine.[5]

In this series of publications we describe various approaches used in attempts to design H_2-receptor antagonists. Our starting point throughout this work has been the natural agonist molecule, histamine, and we have attempted to modify it chemically in ways which, to our intuition, seemed potentially capable of providing an antagonist. One approach taken was to incorporate large nonpolar lipophilic substituents into the histamine mol-