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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME 1-*N*-SUBSTITUTED 2-ACETAMIDO-2-DEOXY-β-D-GLYCOPYRANOSYLAMINE DERIVATIVES AND RELATED ANALOGS

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ABSTRACT

Several 1-*N*-substituted derivatives [haloacetyl-, glycyl-, (dimethyl)amino-acetyl-, azidoacetyl-, trifluoroacetyl-, and trifluoromethylsulfonyl-] of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosylamine (**1**) were synthesized as potential metabolic inhibitors of cellular-membrane glycoconjugates. Several fully acetylated derivatives were found to inhibit growth of mouse mammary adenocarcinoma TA3, leukemia L-1210, or leukemia P-288 cells at 1–0.01 mM concentration *in vitro*. Some of these derivatives were less active after *O*-deacetylation. Analogs of **1** in which NH_2-1 was replaced by OH- or OAc-1 were also active on the same cell systems. The growth-inhibitory activity was correlated with inhibition of the incorporation of 2-amino-deoxy-D-glucose and L-leucine into a macromolecular fraction.

INTRODUCTION

Numerous studies have revealed significant differences between the plasma membranes of normal cells and those of neoplastic or transformed cells[1–3]. These differences may represent a basis for selectivity in cancer chemotherapy and immunotherapy. Many of these differences involve carbohydrate components of membrane glycoproteins and glycolipids that are asymmetrically located at the outer plasma-membrane surface. They are believed to be responsible for many biological and physiological phenomena, such as the expression of antigenicity, density-dependent inhibition of growth, differentiation, and social behavior (*e.g.*, invasiveness and metastasis). On the basis of these considerations, we have been engaged in the synthesis and biological evaluation of some membrane sugar-analogs as potential modifiers or inhibitors of plasma-membrane glycoconjugates[4–7]. These analogs may selectively interfere with glycoprotein or glycolipid biosynthesis (or both), or may be incorporated into the plasma membrane as fraudulent components[4–7].

The formation of the 2-acetamido-1-*N*-(4-L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine (Asn-GlcNAc) residue is the key step of the glycoprotein biosynthesis[8]. The involvement of dolichol phosphate in the attachment of the "core"

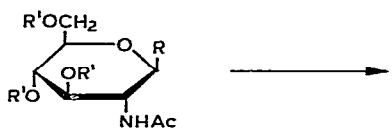
sugars to the asparagine residue of proteins has been reported by several workers[9]. Recently, tunicamycin, a mold metabolite that is known to contain 2-acetamido-2-deoxy-D-glucose residues, and 2-deoxy-D-glucose have been shown to inhibit these initial reactions[10,11]. In the present paper, the synthesis of 1-*N*-substituted 2-acetamido-2-deoxy-β-D-glucopyranosylamine derivatives that potentially could inhibit the formation of the link between sugars and the asparagine residue of the protein is described.

RESULTS AND DISCUSSION

Starting with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (**1**), several analogs were synthesized by acylation of the 1-amino group. These analogs had either highly polar groups, designed to simulate the polar nature of the asparagine moiety in Asn-GlcNAc (*e.g.*, the trifluoromethanesulfonyl group in **14**) or potential alkylating groups, such as the haloacetyl derivatives (**7**, **11**, and **15**). It is likely that these analogs could also inhibit other metabolic steps, such as phosphorylation catalyzed by hexokinase[12].

The key intermediate **1** was obtained by the catalytic reduction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[13] (**3**). Treatment of **1** with acetic anhydride, chloroacetic anhydride, and dichloroacetic anhydride in pyridine gave the acetyl[14] (**5**), chloroacetyl (**7**), and dichloroacetyl (**9**) derivatives, respectively. When **1** was treated with bromoacetic anhydride in pyridine, it gave the pyridinium salt of the bromoacetyl derivative **10** and 2-(carboxymethyl)pyridinium bromide. The formation of pyridinium salt could be avoided by carrying out the reaction in methanol–benzene solution. The iodoacetyl derivative **15** was obtained by treating **7** with dried, finely powdered sodium iodide in acetone. Similarly, treatment of **7** with dried, finely powdered lithium azide in acetone gave the azidoacetyl derivative **16**, and treatment with dimethylamine in *N*,*N*-dimethylformamide resulted in formation of the (dimethyl)aminoacetyl derivative **18**. Treatment of **1** with trifluoroacetic anhydride or trifluoromethanesulfonic anhydride in dichloromethane gave the expected trifluoroacetyl **13** and trifluoromethanesulfonyl **14** derivatives, respectively.

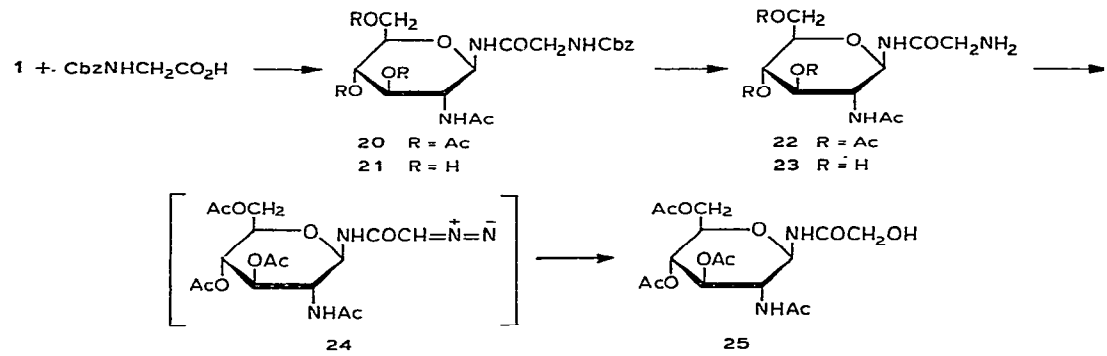
In an attempt to synthesize the diazoacetyl derivative of **1**, *N*-benzyloxycarbo-



1 R = NH_2, R' = Ac
2 R = NH_2, R' = H
3 R = N_3, R' = Ac
4 R = N_3, R' = H

5 R = R' = Ac
6 R = Ac, R' = H
7 R = $COCH_2Cl$, R' = Ac
8 R = $COCH_2Cl$, R' = H
9 R = $COCHCl_2$, R' = Ac
10 R = $COCH_2NC_5H_5^+Br^-$, R' = Ac
11 R = $COCH_2Br$, R' = Ac
12 R = $COCH_2Br$, R' = H

13 R = $COCF_3$, R' = Ac
14 R = SO_2CF_3, R' = Ac
15 R = $COCH_2I$, R' = Ac
16 R = $COCH_2N_3$, R' = Ac
17 R = $COCH_2N_3$, R' = H
18 R = $COCH_2NMe_2$, R' = Ac
19 R = $COCH_2NMe_2$, R' = H



nylglycine was condensed with **1** in the presence of dicyclohexylcarbodiimide in dichloromethane to give the *N*-benzyloxycarbonylglycinamido derivative[15] **20**. Hydrogenolysis of **20** gave the glycinamido derivative[15] **22**, which was diazotized with sodium nitrite and acetic acid in the presence of sodium acetate. The i.r. spectrum

TABLE I

BIOLOGICAL ACTIVITIES OF DERIVATIVES[a] OF 2-AMINO-2-DEOXY-D-GLUCOSE

Compound	*ID_{50} with TA3 or L-1210 cells (mM)*	*Incorporation (% control) in P-288 cells of*		*Growth (% of control)*
		2-Amino-2-deoxy-D-[^{14}C]-glucose	*L-[^{3}H]-Leucine*	
1	0.32	14	6	28
2[b]	> 1			
3	1 (~40%)	60	72	92
4[b]	> 1			
7	0.26	26	10	48
8[b]	> 1			
9	> 1	62	71	61
10	0.22[c]	82	94	77
11	0.019[c]	70	91	30
12[b]	0.62[c]			
13	0.2	48	65	95
14	0.007	99	105	46
15	0.026[c]	39	30	57
27	0.27	15	7	57
28[b]	> 1			
32	0.36	18	70	88
33	0.19	17	13	50
38	0.22	13	16	81
39[b]	> 1			

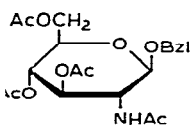
[a]The following compounds shown are ID_{50} at concentrations higher than mM, and had no appreciable effect on leukemia P-288 cells at an mM concentration: **5**, **6**[b], **16**, **17**[b], **18**, **19**[b], **20**, **21**[b], **22**, **29**, **30**, **31**[b], **34**, **35**[b], **36**, **37**[b], and **39**[b]. [b]*O*-Deacetylated derivatives; generally, the preceding compound in the table is the fully acetylated analog. [c]These results were obtained with L1210 leukemia cells.

of the crude product **24** showed a diazo peak at 2120 cm^{-1}, but **24** was insufficiently stable to be isolated. It was converted into the glycoloyl (hydroxyethanoyl) derivative **25** during attempted chromatography on a silica gel column.

Several of the compounds synthesized (**1**, **3**, **5**, **7**, **16**, **18**, **20**, and **22**) were *O*-deacetylated to **2**, **4**, **6**, **8**, **17**, **19**, **21**, and **23**, respectively, by treatment with triethylamine in aqueous methanol. Since the *O*-deacetylation of **11** could not be accomplished without complication, it was obtained by the treatment of 2-acetamido-2-deoxy-β-D-glucopyranosylamine[13] (**2**) with bromoacetic anhydride.

The compounds synthesized were tested for their growth inhibitory activity against mouse mammary adenocarcinoma TA3 or leukemia L-1210 cells *in vitro*. In addition, the effects of these analogs on growth, viability, and the incorporation of 2-amino-2-deoxy-D-glucose and L-leucine were studied in P-288 leukemia cells (Table I). The key intermediate **1**, the chloroacetyl **7**, bromoacetyl **11**, iodoacetyl **15**, trifluoroacetyl **13**, and trifluoromethanesulfonyl **14** derivatives inhibited growth at a concentration of 10–100 μM. The chloroacetyl **7**, bromoacetyl **11**, and iodoacetyl **15** derivatives are potential alkylating agents, as are the structurally related 2-amino-2-deoxy-D-glucose derivatives, streptozotocin[16], and the *N*-chloroethyl-*N*-nitrosourea derivative of D-glucose (GCNU; chlorozotocin)[17], which are finding applications in cancer chemotherapy. Recently, 2-deoxy-2-haloacetylhexoses were reported as being potential *in vivo* chemo-immunotherapeutic agents and as active agents against Ehrlich ascites carcinoma in BDF_1 mice[18]. The finding that **1** is inhibitory is of interest since it was established that its NH_2-1 group may be reactive, as shown in its dimerization reaction[13]. To establish the structure–activity relationship, the analogs of **1** with OH-1 (**27**), SH-1 (**29**), H-1 (**30**), α-OAc-1 (**32**), β-OAc-1 (**33**), α-OMe-1 (**34**), and β-OMe-1 (**36**) were investigated, and 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucose hydrochloride (**38**) was also prepared. Compounds **27**, **32**, **33**, and **38** showed growth-inhibitory activity at a concentration of 0.1mM, whereas derivatives **29**, **30**, **34**, and **36** were inactive at a mM concentration.

In order to study the biological effect of *O*-deacetylation, the following derivatives were tested (see Table I): 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[13] (**4**),



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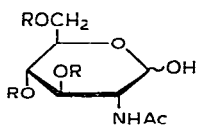
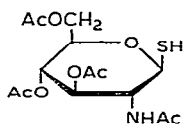


27 R = Ac
28 R = H



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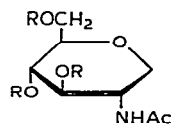
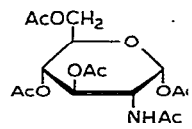


30 R = Ac
31 R = H



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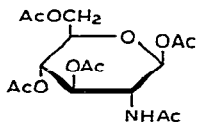

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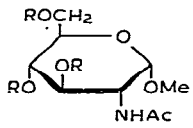
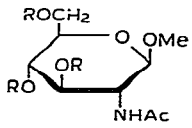
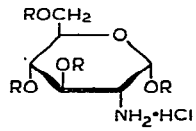


34 R = Ac
35 R = H



36 R = Ac
37 R = H



38 R = Ac
39 R = H

2-acetamido-2-deoxy-β-D-glucopyranosylamine[13] (**2**), 1,2-diacetamido-1,2-dideoxy-β-D-glucopyranose (**6**), 2-acetamido-1-*N*-(chloroacetyl)-2-deoxy-β-D-glucopyranosylamine (**8**), 2-acetamido-1-*N*-(bromoacetyl)-2-deoxy-β-D-glucopyranosylamine (**12**), 2-acetamido-1-*N*-(azidoacetyl)-2-deoxy-β-D-glucopyranosylamine (**17**), 2-acetamido-2-deoxy-1-*N*-[(dimethyl)aminoacetyl]-β-D-glucopyranosylamine (**19**), 2-acetamido-1-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-β-D-glucopyranosylamine (**21**), 2-acetamido-2-deoxy-1-*N*-glycyl-β-D-glucopyranosylamine (**23**), 2-acetamido-2-deoxy-D-glucose (**28**, *O*-deacetylated derivative of **27**, **32**, and **34**), 2-acetamido-1,5-anhydro-2-deoxy-D-glucitol (**31**), methyl 2-acetamido-2-deoxy-α- (**35**) and -β-D-glucopyranoside (**37**), and 2-amino-2-deoxy-D-glucose hydrochloride (**39**, *O*-deacetylated derivative of **38**). It is interesting to note that the *O*-deacetylated derivatives show less inhibitory activity than the corresponding acetylated analogs. It has been shown earlier by double-labeling experiments that the fully acetylated derivatives of sugars are *O*-deacetylated within the cells[19]. On the other hand, 2-acetamido derivatives of 2-amino-2-deoxy sugars are only poorly absorbed by the cells[20]. Thus, *O*-acetylation promotes uptake by passive diffusion. In addition, it has been shown that the acetylated 2-amino-2-deoxy-D-glucose derivatives are inhibitors of hexokinase[6], and this might provide another site of inhibitory activity before *O*-deacetylation has taken place.

It is probable that **1**, **27**, **32**, **33**, and **38** may be first converted to **27**, since all these compounds were found to inhibit TA3 cells at around 100 μM concentration. Thus, **27** may be generated from **1** by hydrolysis of the amino, and from **32** and **33** by hydrolysis of the acetoxyl groups. N to O acetyl migration in **38** at neutral or slightly alkaline condition (pH 7.4) could also produce **27** in the biophase. This might be followed by complete *O*-deacetylation and conversion to UDP-GlcNAc, as has been shown for 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose[19] (**32**). Alternatively, the acyclic aldehydo form of **27** may react with L-cysteine to form a thiazolidine, thus depleting the cell of this amino acid, which has been shown to be required for growth of certain tumor cells[21].

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined by the capillary method. I.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer, and n.m.r. spectra with Varian A-60A and Varian XL-100 instruments; ^{1}H-n.m.r. spectra at 100 MHz and ^{13}C-n.m.r. spectra (25.2 MHz) were determined by the Fourier transform (FT) mode; the positions of the peaks are expressed in δ from the tetramethylsilane or 1,4-dioxane signals. ^{19}F-N.m.r. spectra were determined at 94.1 MHz with $CFCl_3$ as internal standard. Optical rotation was measured with a Perkin–Elmer 141 polarimeter. Thin-layer chromatograms were performed on Merck HF-254 silica gel plates, and spots on chromatograms were detected with iodine vapor, by u.v. absorption, or by spraying with a ninhydrin solution.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide* (**3**) *and 2-*

*acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine* (**1**) were prepared by the procedure described earlier[13].

*2-Acetamido-1-*N*-acetyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosylamine*(**5**). — Acetic anhydride (2 mL) was added slowly with stirring to an ice-cold solution of **1** (1.0 g) in pyridine (10 mL, dried with potassium hydroxide). The reaction mixture was stirred for 1 h at 0°. The resulting gel was stirred at room temp. for 4 h, and then was evaporated *in vacuo*. The residue was extracted with chloroform (30 mL), and the solution was washed with cold water (5 mL), dried (Drierite), and evaporated. The residue was crystallized from alcohol (yield 0.97 g, 86.5%); m.p. 243–244°, $[\alpha]_D^{24}$ +4.3° (*c* 1.08, chloroform); lit.[14] m.p. 236–237°, $[\alpha]_D^{24}$ +18.5° (*c* 2.0, chloroform); lit.[22] m.p. 239–240°, $[\alpha]_D^{32}$ +17.9°, (*c* 1.0, chloroform); ν_{max}^{KBr} 3318 (NH), 3078, 2958, 2875 (CH), 1745 (acetoxyl CO), 1665, 1540 (amide CO), and 1240 cm^{-1} (acetate); ^{13}C-n.m.r. (Me_2SO-d_6): δ 170.0, 169.6, 169.5, 169.3 (CO), 78.0 (C-1), 73.5 (C-5), 72.3 (C-3), 68.5 (C-4), 61.9 (C-6), 52.2 (C-2), 22.6 (CH_3, NAc), 20.5, and 20.3 (CH_3, OAc).

*2-Acetamido-1-*N*-acetyl-2-deoxy-β-*D*-glucopyranosylamine* (**6**). — Compound **5** (0.2 g) was stirred with 10% triethylamine in 50% aqueous methanol (10 mL) for 6 h at room temperature, and the solution was evaporated *in vacuo*. Water (3 × 5 mL) was added to the residue, and was evaporated *in vacuo* to remove traces of triethylamine and methyl acetate. The residue was dissolved in water (10 mL), and the solution was washed with ethyl acetate. The aqueous layer was evaporated and the residue crystallized from ethanol (yield 0.12 g, 85%); m.p. 265–266°, $[\alpha]_D^{24}$ +26.4° (*c* 1.0, water); lit.[14] m.p. 232–233°, $[\alpha]_D^{19}$ −24° (*c* 2.0, water); ν_{max}^{KBr} 3480–3130 (br, NH, OH), 2950, 2855 (CH), 1660, and 1545 cm^{-1} (amide CO); ^{13}C-n.m.r. (D_2O): δ 176.1, 176.0 (CO), 79.6 (C-1), 78.8 (C-5), 75.5 (C-3), 70.8 (C-4), 61.8 (C-6), 55.6 (C-2), and 23.3 ($NHCOCH_3$).

Anal. Calc. for $C_{10}H_{18}N_2O_6$: N, 10.68. Found: N, 10.56.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(chloroacetyl)-2-deoxy-β-*D*-glucopyranosylamine* (**7**). — Chloroacetic anhydride (0.5 g) was added slowly to a stirred, ice-cold solution of **1** (1.0 g) in dry pyridine (15 mL, dried with potassium hydroxide). The stirring was continued for 2 h at 0°, and then for 4 h at room temp. The reaction mixture was evaporated *in vacuo*, and the residue was extracted with ethyl acetate (30 mL). The solution was washed with cold water (10 mL), and the water-wash was re-extracted with ethyl acetate (2 × 10 mL). The combined ethyl acetate extracts were dried (magnesium sulfate), and evaporated, and the residue was crystallized from ethyl acetate–ether (yield 0.8 g, 65%); m.p. 212°, $[\alpha]_D^{23}$ −12.8° (*c* 1.03, chloroform); ν_{max}^{KBr} 3340 (NH), 2955, 2890 (CH), 1745 (acetoxyl CO), 1660, 1539 (amide CO), 1235 (acetate), and 776 cm^{-1} (C-Cl); ^{1}H-n.m.r. ($CDCl_3$): δ 1.96 (s, 3 H, $NHCOCH_3$), 2.05, 2.08 (2 s, 9 H, $OCOCH_3$), 4.03 (s, 2 H, CH_2), 4.20 (2, H_2-6), 5.16 (t, 1 H, *J* 9 Hz, H-3), 5.15 (d, 1 H, *J* 9.5 Hz, H-1), 6.60 (d, 1 H, *J* 9 Hz, NHAc), 7.86 (d, 1 H, *J* 9 Hz, NH amide).

Anal. Calc. for $C_{16}H_{23}ClN_2O_9$: C, 45.44; H, 5.48; Cl, 8.38; N, 6.62. Found: C, 45.73; H, 5.70; Cl, 8.53; N, 6.50.

*2-Acetamido-1-*N-(*chloroacetyl*)-*2-deoxy-β-*D-*glucopyranosylamine* (**8**). — Compound **7** (0.2 g) was treated with triethylamine as described for **6** (yield 0.11 g, 78%); m.p. 220–221° (ethanol–ethyl acetate), $[\alpha]_D^{23}$ +28.2° (*c* 1.0, water); ν_{max}^{KBr} 3380, 3310, 3260 (br, CH, NH), 2959, 2855 (CH), 1689, 1659, 1545 (amide CO), and 755 cm^{-1} (C-Cl); ^{1}H-n.m.r. (D_2O): δ 1.96 (s, 3 H, NHCOCH_3), 4.08 (s, 2 H, COCH_2Cl), and 5.03 (d, 1 H, *J* 9 Hz, H-1).

Anal. Calc. for $C_{10}H_{17}ClN_2O_6$: C, 40.47; H, 5.79; Cl, 11.94; N, 9.44. Found: C, 40.30; H, 5.82; Cl, 11.75; N, 9.25.

*2-Acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-1-*N-(*dichloroacetyl*)-*β-*D-*glucopyranosylamine* (**9**). — A solution of dichloroacetic anhydride (1 mL) in dry benzene (5 mL, dried with sodium) was added drop by drop with stirring to an ice-cold solution of **1** (0.5 g) in dry pyridine (10 mL, dried with potassium hydroxide). The reaction mixture was stirred for 3 h at 0°, and then for 3 h at room temperature, and poured onto crushed ice (~20 g). The resulting solution was evaporated *in vacuo*, and the residue extracted with chloroform (30 mL). The extract was washed with cold water (5 mL), dried (Drierite), and evaporated *in vacuo*, and the residue gave crystals from ethyl acetate–ether (yield 0.52 g, 78.7%); m.p. 231–232°, $[\alpha]_D^{23}$ −5.7° (*c* 1.04, chloroform); ν_{max}^{KBr} 3288 (NH), 2995, 2960, 2882 (CH), 1754 (acetoxyl CO), 1690, 1660, 1550 (amide CO), and 1230 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, NHCOCH_3), 2.06, 2.10 (2 s, 9 H, OCOCH_3), 3.80 (m, 1 H, H-5), 4.22 (m, 2 H, H_2-6), 5.11 (m, 1 H, H-3), 5.12 (d, 1 H, *J* 9 Hz, H-1), 5.91 (s, 1 H, dichloroacetyl), 6.32 (d, 1 H, *J* 9 Hz, N*H*Ac), and 7.98 [d, 1 H, *J* 9 Hz, N*H*(dichloroacetyl)].

Anal. Calc. for $C_{16}H_{22}Cl_2N_2O_9$: C, 42.02; H, 4.85; Cl, 15.51; N, 6.13. Found: C, 42.19; H, 4.94; Cl, 15.66; N, 5.94.

*Attempted synthesis of 2-acetamido-3,4,6-tri-*O-*acetyl-1-*N-(*bromoacetyl*)-*2-deoxy-β-*D-*glucopyranosylamine* (**11**) *from* **1**. *Formation of 2-acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-1-*N-(*2-pyridiniumacetyl*)-*β-*D-*glycopyranosylamine bromide* (**10**) *and 2-*(*carboxymethyl*)*pyridinium bromide in an equimolar ratio.* — A solution of bromoacetic anhydride (0.4 g) in benzene (5 mL, dried with sodium) was added slowly with stirring to an ice-cold solution of **1** (0.5 g) in pyridine (10 mL, dried with potassium hydroxide). The reaction mixture was stirred for 4.5 h at 0° when it turned yellow, and a yellow precipitate separated out. The suspension was evaporated *in vacuo*, crushed ice (~20 g) added, and the resulting yellowish solution was extracted with chloroform (5 × 15 mL). The combined chloroform extract was dried (Drierite) and evaporated to dryness *in vacuo*. The residue was extracted with chloroform (3 × 25 mL) and the suspension filtered. The combined chloroform extract was evaporated to give an oily gum. The latter, left in the refrigerator for 48 h, formed a semi-crystalline mass, which was crystallized from methanol–ethyl acetate (yield 0.55 g, 65%); m.p. 189–190° (dec.), $[\alpha]_D^{25}$ +12.5° (*c* 1.08 methanol); ν_{max}^{KBr} 3440 (br, NH), 3300, 3220, 3135 (aromatic CH), 2975, 2870 (aliphatic CH), 1740 (acetoxyl CO), 1562 (amide CO), 1235 (acetate), 1595, 1493 (aromatic C=C), and 700 cm^{-1} (aromatic); ^{1}H-n.m.r. (D_2O): δ 1.95 (s, 3 H, NHCOCH_3), 2.03, 2.05, 2.07 (3 s, 9 H, COCH_3), 3.97 (m, 1 H, H-5), 4.17 (m, 3 H, H_2-6 and -2), 5.03 (t, 1 H, *J* 9 Hz,

H-4), 5.32 (t, 1 H, *J* 10 Hz, H-3), 5.37 (d, *J* 9 Hz, H-1), and 7.97–8.83 (m, aromatic H); ^{13}C-n.m.r. (D_2O): δ 175.4, 174.4, 173.8, 173.5, 170.3, 167.8 (CO, OAc, NHAc, pyridinium acetyl, and pyridinium acetic acid), 148.1, 147.6, 146.9, 146.7, 129.2 (pyridinium C), 79.2 (C-1), 74.6, 74.1 (C-3, C-5), 69.6 (C-4), 63.2, 63.0, 62.9 (C-6, CH_2 pyridinium *acetyl*, and pyridinium *acetic* acid), 53.5 (C-2), 23.6 (CH_3 of NHAc), 21.6, and 21.5 (CH_3 of OAc).

Anal. Calc. for $C_{28}H_{36}Br_2N_4O_{11} \cdot H_2O$: C, 42.98; H, 4.90; Br, 20.43; N, 7.16. Found: C, 43.35; H, 5.12; Br, 20.45; N, 7.20.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(bromoacetyl)-2-deoxy-β-*D*-glucopyranosylamine* (**11**). — A solution of bromoacetic anhydride (2 mL) in dry benzene (2 mL) was added slowly with stirring to an ice-cold solution of **1** (0.8 g) in dry benzene (6 mL) and anhydrous methanol (4 mL). The reaction mixture was brought slowly to room temperature, and the solution was stirred for 3 h and then evaporated *in vacuo*. The gummy residue was dissolved in chloroform (50 mL), and the solution washed with cold water (5 mL), dried (Drierite), and evaporated. The residue was triturated with petroleum ether (25 mL) resulting in a solid, crystalline material which separated out. It was filtered off, washed with petroleum, ether, and dried (yield 0.90 g, 83%); m.p. 216–217°, $[\alpha]_D^{25}$ −10.8° (*c* 1.02, chloroform); ν_{max}^{KBr} 3290 (NH), 3095, 2970, 2885 (CH), 1750 (acetoxyl CO), 1664, 1550 (amide CO), and 1235 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, NHCOC*H*$_3$), 2.05, 2.09 (2 s, 9 H, OCOC*H*$_3$), 3.80 (s, 2 H, Br*H*$_2$CCO), 3.84 (m, 1 H, H-5), 4.22 (m, 3 H, H$_2$-6, H-4), 5.07 (t, 1 H, *J* 8 Hz, H-3), 5.08 (d, 1 H, *J* 9 Hz, H-1), 6.34 (d, 1 H, *J* 9 Hz, N*H*Ac), and 7.73 (d, 1 H, *J* 9 Hz, N*H* bromoacetyl).

Anal. Calc. for $C_{16}H_{23}BrN_2O_9$: C, 41.12; H, 4.96; Br, 17.10; N, 5.99. Found: C, 41.08; H, 5.15; Br, 17.13; N, 5.75.

*2-Acetamido-1-*N*-(bromoacetyl)-2-deoxy-β-*D*-glucopyranosylamine* (**12**). — A solution of bromoacetic anhydride (1.5 mL) in dry benzene (2 mL) was added slowly with stirring to an ice-cold solution of **2** (0.6 g) in anhydrous methanol (3 mL) and dry benzene (5 mL). The reaction mixture was stirred for 1.5 h at 0°, and then for 1.5 h at room temperature, cooled in ice, and crushed ice (10 g) added to it. The resulting solution was evaporated *in vacuo*. The residue was dissolved in water (15 mL), and the solution washed with chloroform (2 × 10 mL) and evaporated *in vacuo*. The residue was crystallized from ethanol–ethyl acetate (yield 0.68 g, 73%); m.p. 203–204° (dec.), $[\alpha]_D^{23}$ +31.5° (*c* 1.0, chloroform); ν_{max}^{KBr} 3430–3170 (br, NH, OH), 2950, 2920, 2842 (CH), 1670, 1645, and 1535 cm^{-1} (amide CO); ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.80 (s, 3 H, NHCOC*H*$_3$), 3.86 (q, 2 H, C*H*$_2$Br), 4.59, and 4.99 (br, OH), 4.79 (t, 1 H, *J* 9 Hz, H-1), 7.84 (d, 1 H, *J* 9 Hz, N*H*Ac), and 8.53 (d, 1 H, *J* 9 Hz, N*H*COCH$_2$Br); ^{13}C-n.m.r. (D_2O): δ 175.8 (*C*OCH$_3$), 171.6 (*C*OCH$_2$Br), 80.0 (C-1), 78.9 (C-5), 75.2 (C-3), 70.7 (C-4), 61.7 (C-6), 55.6 (C-2), 28.8 (*C*H$_2$Br), and 23.4 (NHCO*C*H$_3$).

Anal. Calc. for $C_{10}H_{17}BrN_3O_6$: C, 35.19; H, 5.02; Br, 23.42; N, 8.22. Found: C, 35.02; H, 5.04; Br, 23.58; N, 8.02.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-(iodoacetyl)-β-*D*-glucopyranosylamine* (**15**). — Compound **7** (1 g) was added to a stirred solution of sodium iodide

(0.8 g) in anhydrous acetone (25 mL) at room temperature. Stirring was continued for 3 days, and the reaction mixture was evaporated *in vacuo*. The residue was extracted with chloroform (200 mL), and the solution washed with 5% sodium thiosulfate solution (20 mL) to remove traces of iodide ion, followed by water (10 mL), and dried (Drierite). The chloroform extract was evaporated *in vacuo*. The residue was triturated with anhydrous acetone, filtered off, washed with cold anhydrous acetone, and dried (yield 0.80 g, 65%); m.p. 251–252°, $[\alpha]_D^{24}$ −7.3° (*c* 1.1, chloroform); ν_{max}^{KBr} 3315, 3290 (NH), 3100, 2950, 2875 (CH), 1748 (acetoxyl CO), 1658, 1545 (amide CO), 1245 (acetate), and 600 cm^{-1} (C–I); ^{1}H-n.m.r. ($CDCl_3$): δ 2.02 (s, 3 H, $NHCOCH_3$), 2.06, 2.10 (2 s, 9 H, $OCOCH_3$), 3.65 (s, 2 H, ICH_2CO), 3.76 (m, 1 H, H-5), 4.16 (m, 2 H, H_2-6), 5.03 (t, 1 H, *J* 9 Hz, H-3), 5.05 (d, 1 H, *J* 9 Hz, H-1), 6.22 (d, 1 H, *J* 9 Hz, NHAc), and 7.45 (d, 1 H, *J* 9 Hz, $NHCOCH_2I$).

Anal. Calc. for $C_{16}H_{23}IN_2O_9$: C, 37.36; H, 4.50; I, 24.67; N, 5.44. Found: C, 37.54; H, 4.60; I, 24.58; N, 5.49.

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-(*azidoacetyl*)-*2-deoxy*-β-D-*glucopyranosylamine* (**16**). — A mixture of **7** (0.6 g) and lithium azide (0.2 g, dried and finely powdered) in dry acetone (25 mL, dried with Drierite) was heated with stirring for 16 h at 60–65°. The reaction mixture was cooled and evaporated *in vacuo*. The residue was dissolved in water (10 mL), and the solution was extracted with ethyl acetate (3 × 25 mL). The combined ethyl acetate extract was dried ($MgSO_4$) and evaporated *in vacuo*, and the residue was crystallized from ethyl acetate–ether (yield 0.45 g, 74%); m.p. 190–191°, $[\alpha]_D^{23}$ −2.1° (*c* 1.06, chloroform); ν_{max}^{KBr} 3340, 3290 (NH), 2950, 2880 (CH), 2110 (N_3), 1740 (acetoxyl CO), 1686, 1660, 1545 (amide CO), and 1240 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, $NHCOCH_3$), 2.05, 2.10 (2 s, 9 H, $OCOCH_3$), 3.92 (s, 2 H, N_3CH_2CO), 5.07 (2 t superimposed, 2 H, *J* 9 Hz, H-4, -3), 5.10 (d, 1 H, *J* 10 Hz, H-1 anomeric), 6.25 (d, 1 H, *J* 9 Hz, NHAc), and 7.67 (d, 1 H, *J* 9 Hz, $NHCOCH_2N_3$).

Anal. Calc. for $C_{16}H_{23}N_5O_9$: C, 44.75; H, 5.39; N, 16.31. Found: C, 44.97; H, 5.42; N, 16.18.

2-Acetamido-1-N-(*azidoacetyl*)-*2-deoxy*-β-D-*glucopyranosylamine* (**17**). — Compound **16** (0.2 g) was stirred with 10% triethylamine in aqueous methanol (20 mL) for 5 h and processed as described for **6** (yield 0.12 g, 85%); m.p. 231° (dec.), $[\alpha]_D^{25}$ +37.8° (*c* 1.0, water); ν_{max}^{KBr} 3240–3180 (OH, NH), 2955, 2850 (CH), 2110 (N_3), 1680, 1658, and 1540 cm^{-1} (amide CO).

Anal. Calc. for $C_{10}H_{17}N_5O_6$: C, 39.60; H, 5.66; N, 23.09. Found: C, 39.55; H, 5.82; N, 22.82.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-1*-N-[(*dimethylamino*)*acetyl*]-β-D-*glucopyranosylamine* (**18**). — A solution of **7** (0.8 g) in dry *N,N*-dimethylformamide (8 mL, dried with calcium hydride and distilled) containing dimethylamine (1 mL) was heated with stirring for 6 h at 70–72°. The reaction mixture was cooled and evaporated to dryness *in vacuo* at 40–45°. The residue was dissolved in water (5 mL) and extracted with ethyl acetate (4 × 25 mL). The combined ethyl acetate extract was dried ($MgSO_4$) and concentrated *in vacuo* at room temperature to a small

volume (~10 mL) when crystalline material separated out. This material was filtered off, washed with ether, and dried (yield 0.55 g, 67%); m.p. 195–196°, $[\alpha]_D^{23}$ +14.3° (*c* 1.09, chloroform); ν_{max}^{KBr} 3340, 3300 (NH), 2980, 2825 (CH), 2765 (NCH_3), 1745 (acetoxyl CO), 1659, 1545 (amide CO), and 1235 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, $NHCOCH_3$), 2.03, 2.07, 2.08 (3 s, 9 H, $OCOCH_3$), 2.27 [s, 6 H, $(CH_3)_2NH$], 2.95 (d, *J* 3 Hz, Me_2NCH_2CO), 3.83 (br, 1 H, H-5), 4.18 (2 H, H_2-6), 5.10 [d, 1 H, *J* 9 Hz, H-1 (anomeric)], 5.22 (2 t superimposed, 2 H, *J* 8 Hz, H-3, -4), 6.65 (d, 1 H, *J* 9 Hz, N*H*Ac), and 8.10 (d, *J* 9 Hz, Me_2NCH_2CONH).

Anal. Calc. for $C_{18}H_{29}N_3O_9$: C, 50.10; H, 6.77; N, 9.73. Found: C, 49.90; H, 6.91; N, 9.50.

*2-Acetamido-2-deoxy-1-*N-(*dimethylamino*)*acetyl-β-*D-*glucopyranosylamine* (**19**). — Compound **18** (0.25 g) was stirred with 10% triethylamine in 50% aqueous methanol (25 mL) for 6 h, and processed as described for **6** (yield 0.135 g, 74%, slightly hydroscopic); m.p. 210–211°, $[\alpha]_D^{25}$ +27.9° (*c* 0.95, water); ν_{max}^{KBr} 3480–3200 (OH, NH), 2955, 2845 (CH), 2795 (NCH_3), 1675, 1630, and 1569 cm^{-1} (amide CO); ^{1}H-n.m.r. (D_2O): δ 1.96 (s, 3 H, $NHCOCH_3$); 2.23 [s, 6 H, $NHCOCH_2N(CH_3)_2$], 3.08 (s, 2 H, $NHCOCH_2NMe_2$), and 5.03 (d, 1 H, *J* 10 Hz, H-1).

Anal. Calc. for $C_{12}H_{23}N_3O_6 \cdot 0.5\ H_2O$: C, 45.84; H, 7.71; N, 13.37. Found: C, 45.83; H, 7.50; N, 13.15.

*2-Acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-1-*N-(*trifluoroacetyl*)*-β-*D-*glucopyranosylamine* (**13**). — A solution of trifluoroacetic anhydride (1 mL) in dichloromethane (5 mL, dried with Drierite) was added drop by drop to a stirred ice-cold solution of **1** (0.5 g) in dry dichloromethane (5 mL). The reaction mixture was stirred for 3 h at 0°, and washed with ice-cold water (5 mL). The water-wash was back extracted with chloroform (2 × 10 mL). The combined dichloromethane and chloroform extract was dried (Drierite), and was concentrated at room temperature to a small volume. Ether was added to turbidity, and the mixture was kept at room temperature until crystalline material separated out. This material was filtered off, washed with ether, and dried (yield 0.3 g, 47%); m.p. 190°, $[\alpha]_D^{23}$ +1.1° (*c* 1.0, chloroform); ν_{max}^{KBr} 3330 (NH), 3080, 2980, 2960, 2880 (CH), 1750 (acetoxyl CO), 1719 (trifluoroacetamido CO), 1668, 1540 (amide 1540), 1230 (acetate), 1180, 1170, 1120, and 1110 cm^{-1} (CF); ^{19}F-n.m.r. ($CDCl_3$): δ 76.0 (s, $NHCOCF_3$).

Anal. Calc. for $C_{16}H_{21}F_3N_2O_9$: C, 43.44; H, 4.78; N, 6.33; F, 12.88. Found: C, 43.67; H, 4.98; N, 6.14; F, 13.06.

*2-Acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-1-*N-(*trifluoromethylsulfonyl*)*-β-*D-*glucopyranosylamine* (**14**). — A solution of trifluoromethanesulfonic anhydride (0.5 mL) in dry dichloromethane (5 mL) was added slowly, over a period of 10 min, to an ice-cold, stirred solution of **1** (0.5 g) in dry dichloromethane (5 mL), when a gummy mass separated out. The reaction mixture was stirred for 2 h at 0°, the supernatant liquid was decanted off, and the light-brown gummy residue was dried *in vacuo* at room temperature. It crystallized from ethyl acetate–petroleum ether, and was recrystallized from ethyl acetate–ether (yield 0.15 g, 21.7%); m.p. 175–176°, $[\alpha]_D^{24}$ +36.2° (*c* 1.0, methanol); ν_{max}^{KBr} 3260–3120 (NH), 2990 (CH), 1755 (acetoxyl

CO), 1600, 1575 (amide CO), 1240 (acetate), 1170 (SO_2CF_3), 1115 (CF_3), and 905 cm^{-1} (-S-N-); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, NHCOCH_3), 2.01, 2.33 (2 s, 9 H, OCOCH_3), and 5.23 (d, 1 H, J 10 Hz, H-1); ^{19}F-n.m.r. ($CDCl_3$): δ 78.5 (s, $NHSO_2CF_3$).

Anal. Calc. for $C_{15}H_{21}F_3N_2O_{10}S$: C, 37.65; H, 4.42; N, 5.85. Found: C, 37.70; H, 4.59; N, 5.82.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-β-*D*-glucopyranosylamine* (**20**). — Dicyclohexylcarbodiimide (0.3 g) was added to a stirred solution of **1** (0.5 g) and *N*-benzyloxycarbonylglycine (0.3 g) in dichloromethane (30 mL, dried with Drierite and distilled) cooled to −10°. The reaction mixture was stirred for 1 h at −10°, and then overnight (16 h) at room temperature. The precipitated *N,N*-dicyclohexylurea (0.3 g) was removed by filtration. To remove the residual diimide, acetic acid (4 drops) was added to the filtrate. The reaction mixture was kept for 1 h at room temperature, and then evaporated *in vacuo*. The residue was dissolved in chloroform (30 mL), and the solution washed with water (10 mL), 3% sodium hydrogencarbonate solution (10 mL), and water (5 mL), and dried (Drierite). The residue obtained after evaporation was crystallized from ethanol (yield 0.6 g, 77%); m.p. 202–203°, $[\alpha]_D^{24}$ −9.8° (*c* 1.01, chloroform); lit.[15] m.p. 196°, $[\alpha]_D$ +13.9° (pyridine); lit.[23] m.p. 205°, $[\alpha]_D^{25}$ −0.61° (*c* 0.3, chloroform); ν_{max}^{KBr} 3410, 3322 (NH), 2970, 2895 (CH), 1745, 1725 (acetoxyl CO), 1679, 1660, 1530 (amide CO), 1235 (acetate), 1050, 1038, and 702 cm^{-1} (aromatic); ^{1}H-n.m.r. (Me_2SO-d_6): δ 1.85 (s, 3 H, NHCOCH_3), 1.97, 2.00, 2.03 (3 s, 9 H, OCOCH_3), 4.92 (t, 1 H, J 9 Hz, H-4), 5.10 (s, 2 H, PhCH_2), 5.15 (d, 1 H, J 9 Hz, H-1), 5.22 (t, 1 H, J 9 Hz, H-3), 7.38 (m, 5 H, aromatic), 7.97 (d, 1 H, J 9 Hz, N*H*Ac), and 8.30 (d, 1 H, J 9 Hz, NH).

Anal. Calc. for $C_{24}H_{31}N_3O_{11}$: C, 53.62; H, 5.81; N, 7.81. Found: C, 53.84; H, 5.92; N, 7.87.

*2-Acetamido-1-*N*-(*N*-benzyloxycarbonylglycyl)-2-deoxy-β-*D*-glucopyranosylamine* (**21**). — A suspension of **20** (1.5 g) was stirred with 10% triethylamine in 50% aqueous methanol (100 mL) for 6 h, and then processed as described for **6** (yield 0.82 g, 71%); m.p. 238–239°, $[\alpha]_D^{25}$ +37.2° (*c* 1.0, water); lit.[15] m.p. 231–232°, $[\alpha]_D$ +43.4° (water); ν_{max}^{KBr} 3420, 3320, 3280 (br, NH, OH), 3065, 2970, 2882 (CH), 1689, 1662, 1641, 1545 (amide CO), 1460, 1453 (aromatic C=C), 898, 790, 750, 740, and 700 cm^{-1} (phenyl); ^{1}H-n.m.r. (D_2O): δ 1.95 (s, 3 H, NHCOCH_3), 3.48 (s, 2 H, glycyl CH_2), 3.77 (2 H, H_2-6), 5.05 (d, 1 H, J 9 Hz, H-1), 5.07 (s, 2 H, Ph-CH_2), and 7.38 (s, 5 H, aromatic).

Anal. Calc. for $C_{18}H_{25}N_3O_8$: C, 52.54; H, 6.14; N, 10.21. Found: C, 52.54; H, 6.17; N, 10.13.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-glycyl-β-*D*-glucopyranosylamine* (**22**). — A solution of **20** (0.25 g) in ethanol (75 mL) was hydrogenated for 16 h in the presence of palladium (40 mg) at room temperature and atmospheric pressure. After removal of the catalyst, the filtrate was evaporated to a small volume (~5 mL), when crystalline material separated out. This material was filtered off, washed with

ether, and dried. The product was recrystallized from ethanol (yield 0.16 g, 85%); m.p. 192–193°, $[\alpha]_D^{24}$ +1.4° (*c* 1.03, chloroform); lit.[23] m.p. 204–205°, $[\alpha]_D^{25}$ −0.94° (*c* 0.4, water); ν_{max}^{KBr} 3405, 3360, 3280 (NH), 1745 (acetoxyl CO), 1660, 1530, 1506 (amide CO), and 1230 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.62 (br s, 2 H, NH_2), 1.93 (s, 3 H, $NHCOCH_3$), 2.07, 2.08 (2 s, 9 H, $OCOCH_3$), 3.37 (s, 2 H, $COCH_2NH_2$), 6.92 (d, 1 H, *J* 9 Hz, N*H*Ac), and 8.17 (d, 1 H, *J* 9 Hz, N*H*$COCH_2NH_2$).

Anal. Calc. for $C_{16}H_{25}N_3O_9$: C, 47.63; H, 6.26; N, 10.42. Found: C, 47.90; H, 6.27; N, 10.27.

*2-Acetamido-2-deoxy-1-*N*-glycyl-β-*D*-glucopyranosylamine* (**23**). — Compound **22** (0.3 g) was stirred with 10% triethylamine in 50% aqueous methanol (25 mL) for 7 h and processed as described for **6** to give **23** as the acetate salt (yield 0.21 g, 83%); m.p. 226–227° (dec.), $[\alpha]_D^{20}$ +22.4° (*c* 1.02, water); ν_{max}^{KBr} 3320, 3260 (br, NH, OH), 2960, 2860 (CH), 1685, 1650, 1550 (amide CO), 1550, and 1400 cm^{-1} (CO of CO_2^-); ^{1}H-n.m.r. (D_2O): δ 1.87 (s, 3 H, CH_3CO) and 1.96 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{12}H_{23}N_3O_8$: C, 42.72; H, 6.87; N, 12.45. Found: C, 42.63; H, 7.04; N, 12.44.

*Attempted synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-(diazoacetyl)-β-*D*-glucopyranosylamine* (**24**). *Formation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-glycoloyl-β-*D*-glucopyranosylamine* (**25**). — Acetic acid (1.0 mL) and sodium nitrite (0.1 g) were added in successive small portions to a stirred suspension of **22** (0.2 g) in 2M sodium acetate solution (10 mL) while being cooled to 0°. The reaction mixture was stirred for 5 h at 0°, and then extracted with chloroform (4 × 20 mL). The extract was washed with ice-cold water (5 mL), dried (Drierite), and evaporated *in vacuo* at room temperature, when a gummy mass was obtained. Trituration with ether–petroleum ether gave a solid, which was filtered off, washed with petroleum ether, and dried, m.p. 128–130° (frothing); ν_{max}^{KBr} 2120 cm^{-1} (N_2); t.l.c. (1:9, v/v, methanol–chloroform) showed two spots (R_F 0.15 and 0.4). The product was chromatographed on a silica gel column (Bio-Sil A, 100–200 mesh; 1.3 × 40 cm) with 9:1 (v/v) methanol–chloroform as eluent. The fractions corresponding to the higher-R_F spot were pooled and evaporated. The residue (15 mg, ν_{max}^{KBr} 2120 cm^{-1}) was not sufficiently pure. The fractions corresponding to the lower-R_F spot were pooled, evaporated, and the residue crystallized from ethyl acetate–ether (yield 25 mg, 12.5%); m.p. 229°; ν_{max}^{KBr} 3535 (OH), 3318 (NH), 2980, 2895 (CH), 1750 (acetoxyl CO), 1660, 1547, 1512 (amide CO), 1230 cm^{-1} (acetate), and no peak corresponding to N_2.

Anal. Calc. for $C_{16}H_{24}N_2O_{10}$: C, 47.52; H, 5.99; N, 6.93. Found: C, 47.23; H, 5.99; N, 6.78.

*Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside* (**26**). — Acetic anhydride (15 mL) was added slowly with stirring to an ice-cold solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[24] (2.0 g) in dry pyridine (20 mL, dried with potassium hydroxide). Stirring was continued for 1 h at 0°, and then for 15 h at room temperature. The reaction mixture was evaporated *in vacuo*, and the

residue was dissolved in chloroform (40 mL). The solution was washed with water (10 mL), dried (Na_2SO_4), and evaporated. The residue crystallized from ether–petroleum ether (yield 2.4 g, 85%); m.p. 104–105°, $[\alpha]_D^{23}$ +120.0° (*c* 1.0, chloroform); ν_{max}^{KBr} 3320 (NH), 3080 (aromatic CH), 2965 (aliphatic CH), 1745 (acetoxyl CO), 1660, 1515 (amide CO, aromatic C=C), 1245 (acetate), 1050, and 705 cm^{-1} (aromatic); lit.[25] m.p. 111°, $[\alpha]_D^{26}$ +129° (*c* 1.11, pyridine), $[\alpha]_D^{20}$ +103° (*c* 0.59, chloroform); lit.[26] m.p. 108°, $[\alpha]_D^{20}$ +120° (*c* 1.0, chloroform).

Anal. Calc. for $C_{21}H_{27}NO_9$: N, 3.20. Found: N, 3.14.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyranose* (**27**). — (*a*) *Catalytic hydrogenolysis of* **26**. A solution of **26** (0.5 g) in glacial acetic acid (10 mL) was hydrogenolyzed for 4 days in the presence of palladium-on-charcoal catalyst (10%, 0.25 g) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. Water (3 × 10 mL) was added and evaporated to remove traces of acetic acid. The residue was crystallized from ether–petroleum ether (yield 0.35 g, 88%); m.p. 84–85° (preshrinkage at 65°), $[\alpha]_D^{23}$ +52.2° (*c* 1.01, chloroform); ν_{max}^{KBr} 3370 (br, NH, OH), 2970 (OH), 1750 (acetoxyl CO), 1660, 1542 (amide CO), and 1240 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.93 (s, 3 H, $NHCOCH_3$), 2.05, 2.08, 2.20 (3 s, 9 H, $OCOCH_3$), 3.93 (1 H, H-5), 4.17 (2 H, H_2-6), 5.17 (t, 1 H, *J* 9 Hz, H-4), 5.33 (t, 1 H, *J* 9 Hz, H-3); ^{13}C-n.m.r. ($CDCl_3$): δ 171.1, 170.9, 169.4 (CO), 91.3 (C-1), 71.1 (C-5), 68.5 (C-3), 67.3 (C-6), 52.4 (C-2), 22.9 ($NHCOCH_3$), and 20.7 ($OCOCH_3$); lit.[27] m.p. 65–75°, $[\alpha]_D^{20}$ +49.4° (*c* 2.1, chloroform); +50.4→+26.9° (4 h, *c* 0.5, water).

Anal. Calc. for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.11; N, 4.03. Found: C, 48.57; H, 6.35; N, 3.79.

(*b*) Compound **27** was prepared from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrochloride by treatment with sodium acetate according to the method of Leaback and Walker[27].

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranose* (**29**). — This compound was synthesized from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[28] by condensation with thiourea, followed by reductive cleavage[29–31].

*2-Acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-*D*-glucitol* (**30**). — This compound was prepared by the method of Horton and Wolfrom[29] by treating 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride with Raney nickel (W-2) (yield 78%); m.p. 162–163°, $[\alpha]_D^{22}$ +10.8° (*c* 1.1, chloroform); ν_{max}^{KBr} 3260 (NH), 2980, 2855 (CH), 1745 (acetoxyl CO), 1641, 1562 (amide CO), and 1228 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.93 (s, 3 H, $NHCOCH_3$), 2.03, 2.07 (2 s, 9 H, $OCOCH_3$), 3.20 (t, 1 H, *J* 12 Hz, H-2), 3.58 (m, 1 H, H-5), 4.25 (m, 3 H, H_2-6, H-2), 5.03 (d, *J* 10 Hz, H-1β), and 5.03 (q, *J* 9 Hz, H-3); ^{13}C-n.m.r. ($CDCl_3$): δ 171.7, 170.7, 170.4, 169.4 (CO), 76.6 (C-1), 74.3 (C-5), 68.7 (C-3), 68.3 (C-4), 62.6 (C-6), 50.3 (C-3), 23.1 ($NHCOCH_3$), 20.7, and 20.6 ($OCOCH_3$); lit.[29] m.p. 165–168°; lit.[31] m.p. 160–161°, $[\alpha]_D^{25}$ 0° (*c* 1.1, chloroform).

Anal. Calc. for $C_{14}H_{21}NO_8$: N, 4.22. Found: N, 4.01.

2-Acetamido-1,5-anhydro-2-deoxy-D-glucitol (**31**). — A suspension of **30** (0.5 g) in 10% triethylamine in 50% aqueous methanol (30 mL) was stirred for 7 h and then was processed as described for **6** (yield 0.25 g, 81%); m.p. 206–207° (EtOH) (lit.[31] m.p. 204–206°), $[\alpha]_D^{25}$ +8.4° (*c* 1.02, water); ν_{max}^{KBr} 3480–3170 (OH, NH), 2955, 2865 (CH), 1635, and 1561 cm^{-1} (amide CO); ^{1}H-n.m.r. (D_2O): δ 1.97 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_8H_{15}NO_5$: N, 6.82. Found: N, 6.54.

2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-α-D-glucopyranose (**32**). — This compound was prepared by treating **39** with acetic anhydride in pyridine by modified procedures of Westphal and Holzmann[33], and Inouye *et al.*[34]. Acetic anhydride (25 mL) was added slowly with stirring to an ice-cold solution of **39** (5 g) in dry pyridine (40 mL, dried with potassium hydroxide). The reaction mixture was stirred for 1 h at 0°, and for 2 days at room temperature, and was then evaporated *in vacuo*. The gummy residue was dissolved in chloroform (200 mL). The solution was washed with ice-cold water (2 × 15 mL), and evaporated. The residue was crystallized from benzene–ether (yield 8.1 g, 93%); m.p. 138–139°, $[\alpha]_D^{23}$ +87.4° (*c* 1.07, chloroform); ν_{max}^{KBr} 3435 (NH), 2998, 2922 (CH), 1740 (acetoxyl CO), 1670, 1510 (amide CO), and 1235 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, $NHCOCH_3$), 2.05, 2.08, 2.20 (3 s, 12 H, $OCOCH_3$), 6.22 (d, *J* 3 Hz, H-1), and 6.37 (1 H, *J* 9 Hz, NH); lit.[33] m.p. 139°, $[\alpha]_D^{20}$ +92°; lit.[34] m.p. 138–139°, $[\alpha]_D$ +92° (*c* 1.0, chloroform).

2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (**33**). — This compound was prepared by the method of Horton[32].

Methyl 2-acetamido-2-deoxy-α- (**35**) *and -β-D-glucopyranoside* (**37**). — Dowex-50 W X8 (H^+, 40 g) was added to a suspension of **28** (50 g) in methanol (250 mL), and the mixture was heated to reflux with stirring for 5 h. After being cooled, the mixture was filtered, and the residue was washed several times with methanol (5 × 40 mL). The combined filtrate and washings were evaporated *in vacuo*, and the residue was crystallized from methanol (yield 45.2 g, 85%); m.p. 166–167° (pre-shrinkage at 160°); ^{1}H-n.m.r. (D_2O) shows the product is a ~1:1 mixture of **35** and **37**.

Methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α- (**34**) *and -β-D-glucopyranoside* (**36**). — Acetic anhydride (25 mL) was added slowly to an ice-cold, stirred solution of **35** and **37** (5.0 g) in dry pyridine (35 mL, dried with potassium hydroxide). Stirring was continued for 1 h at 0°, and for 16 h at room temperature. The reaction mixture was evaporated *in vacuo*, and the residue was dissolved in chloroform (75 mL). The solution was washed with water (2 × 10 mL), and evaporated. T.l.c. (9:1, v/v, methanol–chloroform) of the residue showed two spots (R_F 0.58 and 0.83). The residue was chromatographed on a silica gel column (Bio-Sil A, 100–200 mesh, 1.9 × 56 cm) with 1:99 to 1:9 (v/v) methanol–chloroform as eluent. The fractions corresponding to the spot of high R_F (0.83) were pooled and evaporated, and the residue crystallized from ethyl acetate–petroleum ether to give **34** (yield 2.4 g, 32%), m.p. 114–115°, $[\alpha]_D^{22}$ +94.3° (*c* 1.06, chloroform); ν_{max}^{KBr} 3415 (NH), 2925, 2858 (CH), 1740 (acetoxyl CO), 1681, 1515 (amide CO), 1230 (acetate), and 844 cm^{-1}

(α-D-glycoside); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, NHCOCH_3), 2.04, 2.11 (2 s, 9 H, OCOCH_3), 3.42 (s, 3 H, OCH_3-1), and 5.77 (d, J 9 Hz, NH); lit.[35] m.p. 107–108°, $[\alpha]_D^{25}$ +100.2° (*c* 1.0, chloroform).

Anal. Calc. for $C_{15}H_{23}NO_8$: N, 4.05. Found: N, 3.89.

The fractions corresponding to the spot of lower R_F (0.58) were pooled and evaporated, and the residue was crystallized from ethyl acetate to give **36** (yield 2.1 g, 28.6%), m.p. 163–164°, $[\alpha]_D^{23}$ −10.2° (*c* 1.01, chloroform); ν_{max}^{KBr} 3300–3260 (NH), 2960, 2890 (CH), 1750 (acetoxyl CO), 1658, 1566 (amide CO), and 1235 cm^{-1} (acetate); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, NHCOCH_3), 2.05, 2.10 (2 s, 9 H, OCOCH_3), 3.05 (s, OCH_3-1), and 6.11 (d, J 9 Hz, NH); lit.[36] m.p. 163°, $[\alpha]_D^{16.5}$ −22° (*c* 1.0, methanol).

Anal. Calc. for $C_{15}H_{23}NO_8$: N, 4.05. Found: N, 3.91.

*1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-2-*D*-glucopyranose hydrochloride* (**38**). — This compound was prepared from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride by the method of Leaback and Walker[27]; ν_{max}^{KBr} 3060–2680, 2540, 2039 (NH_3), 1750 (acetoxyl CO), 1595, 1579, 1518 (NH_3), and 1230 cm^{-1} (acetate); ^{1}H-n.m.r. (D_2O): δ 2.05, 2.10, 2.22 (3 s, 12 H, OCOCH_3), 4.23 (2 H, H_2-6), 5.10 (t, 1 H, J 9 Hz, H-4), 5.55 (t, 1 H, J 9 Hz, H-3), and 6.35 (d, 1 H, J 4 Hz, H-1).

*2-Acetamido-2-deoxy-β-*D*-glucopyranosyl azide* (**4**) *and 2-acetamido-2-deoxy-β-*D*-glucopyranosylamine* (**2**). — These compounds were prepared by a published procedure[13].

Biological testing. — The compounds were tested on murine L-1210 leukemia, P-288 lymphoma, and TA3 mouse mammary adenocarcinoma cell lines maintained *in vitro* as follows:

L-1210 or TA3 cells. To an inoculum of 50000 cells in RPMI 1640 medium containing 10% heat-inactivated, fetal calf serum (1 mL) was added 1 mL of the same medium containing the compound to be tested and also containing a final concentration of 20mM Hepes buffer[37]. All solutions were sterilized by passing them through a 0.22-μm Millipore filter. No antibiotics were used. The tubes were incubated in an upright position for 3 days and growth was estimated by protein assay[38]. The growth in control cultures varied from 6- to 10-fold after 3 days. Each concentration was tested in triplicate. For compounds found inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50%-inhibitory concentration. Results are expressed in terms of ID_{50}, which corresponds to the molar concentration of the compound tested in the nutrient medium giving a 50% inhibition of cell growth as compared with the drug-free control. Results are reported in Table I.

P-288 cells. A. Cell growth and viability. For routine drug-testing, P-288 murine leukemic cells were suspended at a conc. ~10^5 cells/mL in fresh, glucose-free RPMI 1640 medium containing 10% fetal calf serum (pH 7.2). Aliquots (1 mL) were then transferred to disposable polyethylene tubes and were placed in a CO_2 incubator. One hour later, the sugar derivatives were added to a final concentration of 1 mM

(or as otherwise stated). Cell growth was monitored 24 h later by use of a Coulter cell-counter. The cell number increased 2–3-fold. The results (see Table I) are recorded as % of the control cell-number.

B. Macromolecular biosynthesis. 2-Amino-2-deoxy-D-[^{14}C]glucose (2μM, 1.1 · 10^6 d.p.m., Amersham Corp., Arlington Heights, IL 60005) and L-[^{3}H]leucine (0.37 μM, 2.6 · 10^6 d.p.m., New England Nuclear, Boston, MA 02118) were added to cell cultures to assess the effects of sugar derivatives on protein and glycoprotein biosynthesis. Five hours later, incubations were terminated by the addition of 10% trichloroacetic acid (2 mL). The acid-insoluble, radioactive precipitate was centrifuged off, and washed twice with 10% trichloracetic acid. The pellet was dissolved in sodium hydroxide and its radioactivity determined by scintillation counting methods.

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