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S-, *N*-, AND *O*-GLYCOSYL DERIVATIVES OF 2-ACETAMIDO-2-DEOXY-D-GLUCOSE WITH HYDROPHOBIC AGLYCONS AS POTENTIAL CHEMOTHERAPEUTIC AGENTS AND *N*-ACETYL-β-D-GLUCOSAMINIDASE INHIBITORS*

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ABSTRACT

S-, *N*-, and *O*-Glycosyl derivatives of 2-acetamido-2-deoxy-D-glucose with hydrophobic aglycons have been obtained as potential, plasma-membrane active agents. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (**6**) was converted into benzyl, diphenylmethyl, triphenylmethyl, and other thioglycosides. Acylation of **6** gave adamantoyl and haloacetyl derivatives. A similar series of *N*- and *O*-glycosyl derivatives was obtained from the corresponding NH_2-1 and OH-1 analogs of **6**, such as *O*- and *N*-dinitrophenyl, *O*- and *N*-adamantoyl, and *N*-4-methylbenzylidene derivatives. Several *N*- and *S*-glycosyl derivatives were found to inhibit mouse mammary adenocarcinoma (TA3) cells *in vitro* as well as *N*-acetyl-β-D-glucosaminidase from beef liver.

INTRODUCTION

Numerous studies have indicated differences between the plasma membranes of normal cells and neoplastic cells[1–3] and their importance in tumorigenesis[2,4]. This is the basis for our program to develop potential plasma-membrane modifiers and inhibitors as an approach to cancer chemotherapy and immunotherapy[4–7]. The carbohydrate components of the membrane glycoproteins and glycolipids are exposed on the cell-surface, and probably play a key role in the expression of antigenicity, density-dependent inhibition of growth, and their social behavior (*e.g.*, invasiveness and metastasis).

Previously, the synthesis of carbohydrate analogs that may be incorporated into the glycoconjugates of the plasma membrane and, thus, alter the cell-surface characteristic has been reported[4,5]. This paper describes attachment of hydrophobic groups to C-1 of the 2-acetamido-2-deoxy-D-glucose molecule (**1**). Due to

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the hydrophobic nature of these derivatives, they may compete with the glycolipids for binding to the outer membrane. Since the properties of these compounds also resemble those of the sugar–lipid intermediates involved in the biosynthesis of an important class of glycoproteins[8], the possibility of tunicamycin-like inhibitory activity was also considered, particularly since this antibiotic has likewise hydrophobic groups that are attached to 2-acetamido-2-deoxy-D-glucose residues[9].

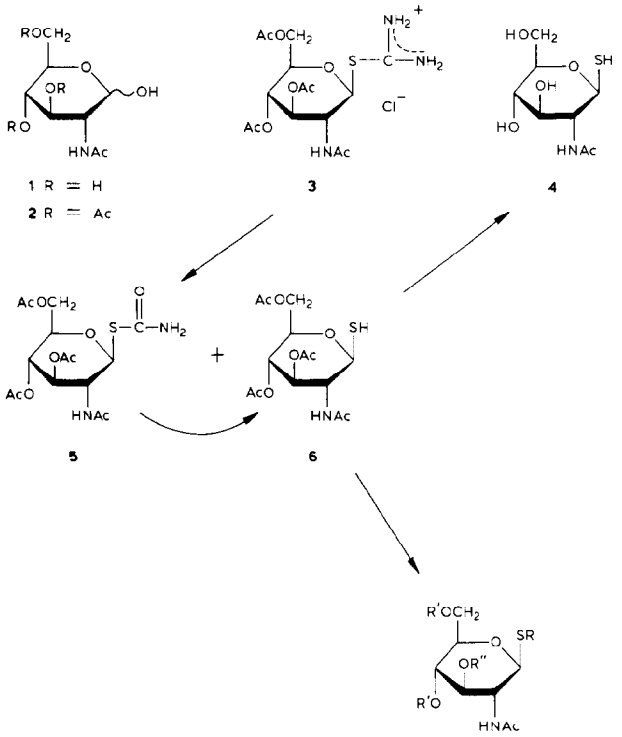
As an example of the introduction of a hydrophobic group into **1** the *S*-trityl group was of particular interest, since *S*-tritylcysteine has been found to have antitumor activity[10]. Similarly, the *S*-2,4-dinitrophenyl group was introduced in the belief that, in addition to its being hydrophobic, it might act as an alkylating agent as well, since *S*-2,4-dinitrophenyl-4-thiodeoxyuridylate has been reported to act as such[11].

In addition to determining the antitumor activity of the synthesized compounds *in vitro* and *in vivo*, we were also interested in their potential as *N*-acetylglucosaminidase inhibitors, since the level of the enzyme has been found to be elevated in certain metastatic tumors, and the enzyme may be involved in the modification of cell-surface carbohydrates that may be critical for cell-to-cell interactions (*e.g.*, detachment, seeding)[12]. Also, the possibility of exploring some of these compounds as antiviral agents should be mentioned, since certain phenyl glycosides have shown significant activity against the *influenza* and *herpes simplex* viruses, and their activity may be related to their ability to affect the viral envelope[13].

RESULTS AND DISCUSSION

Syntheses. — The syntheses of 1-*S*-substituted derivatives started with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose (**6**) which has been prepared from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[14] and thiourea to give the thiouronium salt[15] **3**. Hydrolytic cleavage of the latter in the presence of potassium pyrosulfite[16] gave, in addition to **6**, the *S*-carbamoyl derivative **5**. Further hydrolysis of **5** yielded the sulfhydryl compound **6**. Thus, the *S*-carbamoyl compound represents an intermediate stage in the hydrolysis. Derivatization of the sulfhydryl group in **6** with chlorotriphenylmethane, *p*-anisylchlorodiphenylmethane, 1-adamantanecarbonyl chloride, and 2-chloroacetic anhydride could be readily achieved in pyridine to give the corresponding *S*-trityl (**7**), *S*-*p*-anisyldiphenylmethyl (**9**), *S*-adamantoyl (**11**), and *S*-chloroacetyl (**12**) derivatives.

Since the derivatization with bromodiphenylmethane in pyridine gave the corresponding pyridinium salt and not the desired compound, the reaction was carried out in aqueous acetone in the presence of potassium carbonate to give the *S*-diphenylmethyl derivative **13**. Similarly, the *S*-benzyl (**15**), *S*-(1-naphthylmethyl) (**17**), *S*-(2-naphthylmethyl) (**19**), and *S*-(9-anthrylmethyl) (**21**) derivative were obtained. Conversely, tritylation of **6** with chlorotriphenylmethane in aqueous acetone was complicated by the formation of the disulfide[17,18] of **6**. Treatment of



1 R = H
2 R = Ac

3

4

5

6

7 R = Tr, R′ = Ac
8 R = Tr, R′ = H
9 R = *p*-MeOC$_6$H$_4$Ph$_2$C, R′ = Ac
10 R = *p*-MeOC$_6$H$_4$Ph$_2$C, R′ = H
11 R = C$_{10}$H$_{15}$CO, R′ = Ac
12 R = ClCH$_2$CO, R′ = Ac
13 R = Ph$_2$CH, R′ = Ac
14 R = Ph$_2$CH, R′ = H
15 R = Bn, R′ = Ac
16 R = Bn, R′ = H
17 R = C$_{10}$H$_7$CH$_2$ (1-naphthylmethyl), R′ = Ac
18 R = C$_{10}$H$_7$CH$_2$ (1-naphthylmethyl), R′ = H
19 R = C$_{10}$H$_7$CH$_2$ (2-naphthylmethyl), R = Ac
20 R = C$_{10}$H$_7$CH$_2$ (2-naphthylmethyl), R = H
21 R = C$_{14}$H$_9$CH$_2$ (9-anthrylmethyl), R′ = Ac
22 R = C$_{14}$H$_9$CH$_2$ (9-anthrylmethyl), R′ = H
23 R = 2,4-(NO$_2$)$_2$C$_6$H$_3$, R′ = Ac
24 R = 2,4-(NO$_2$)$_2$C$_6$H$_3$, R′ = H

6 with fluoro-2,4-dinitrobenzene in acetone in the presence of triethylamine gave the expected *S*-2,4-dinitrophenyl derivatives **23**. Most of the aforementioned compounds were *O*-deacetylated with triethylamine in aqueous methanol.

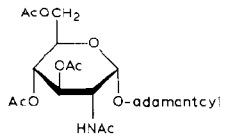
The outstanding feature of the ^{1}H-n.m.r. spectra of these thioglycosides, as compared to those of the glycosides, is the upfield shift of the signal of H-1, to the δ 4.6 region, whereas the signals from H-3 and -4 appear downfield from it at δ ~5.1. Although the ^{13}C-n.m.r. spectra (Table I) are characterized by a considerable shift upfield from the signal of C-1 due to the introduction of the sulfur atom, C-1 is still the most deshielded ring carbon in both acetylated and *O*-deacetylated derivatives. In contrast, substitution of a sulfur atom in the ring, as in 5-thio-D-glucose, has a much greater shielding effect, sometimes making C-1 the most shielded of all ring carbon atoms[19]. Various substitutions of the sulfur atom, as in

TABLE I

^{13}C-CHEMICAL SHIFTS[a] FOR SOME 1-THIOGLYCOSIDES

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCOCH$_3$*	*NHCOCH$_3$*
O-Acetyl derivatives								
6	78.5	55.4	73.2	68.3	74.6	61.8	22.4 20.4	22.6
7	83.3	51.6	73.1	68.3	74.1	61.7	20.2	22.5
17	82.5	51.9	73.5	68.6	74.6	62.0	20.2 20.3 20.4	22.5
19	82.1	51.9	73.5	68.6	74.6	62.0	20.2 20.4	22.5
21	82.8	51.8	73.5	68.7	74.9	62.1	20.4 20.6	22.6
O-Deacetylated derivatives								
8	84.7	54.1	75.2	69.8	80.0	60.5		23.0
10	84.6	54.0	75.3	69.8	80.0	60.6		22.9
16	82.2	54.0	75.3	70.6	81.0	61.2		22.8
18	82.5	54.1	75.3	70.6	81.1	61.3		22.9
20	81.9	54.1	75.3	70.6	81.2	61.3		22.9

[a]From the signal of internal Me_4Si, all spectra were determined for solutions in $(CD_3)_2SO$.



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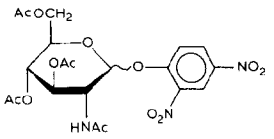

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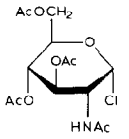

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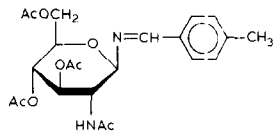

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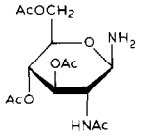

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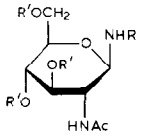
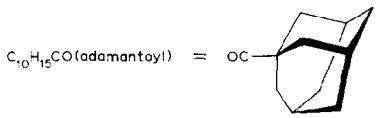
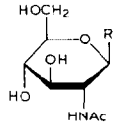


30 R = 2,4-$(NO_2)_2$-C_6H_3, R' = Ac
31 R = $C_{10}H_{15}CO$, R' = Ac
32 R = $C_{10}H_{15}CO$, R' = H





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17, 18, and **21**, generally had very little effect on ^{13}C resonances, other than on C-1. However, introduction of the bulky trityl group, as in **7**, caused an upfield shift of most carbon resonances. An opposite effect on C-4, -5, and -6 was evident in the *O*-deacetylated trityl and methoxytrityl derivatives (**8** and **10**, respectively).

Attempts to prepare the 1-*O*-trityl derivative by treating 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (**2**) with chlorotriphenylmethane in pyridine were not successful, presumably because the OH group is less nucleophilic than the SH group. Alternatively, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose was converted into 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[20], which was treated with triphenylmethanol in the presence of *p*-toluenesulfonic acid in nitromethane; this approach likewise proved to be unsuccessful.

On treatment with the sodium salt of 2,4-dinitrophenol in *N*,*N*-dimethylformamide, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**27**) gave **26** in 9% yield as a mixture of α and β anomers. In another attempt to obtain **26**, **2** was treated with fluoro-2,4-dinitrobenzene in acetone in the presence of triethylamine; only traces of **26** were obtained as judged by the intensity of the t.l.c. spot. However, on treatment with adamantoyl chloride in pyridine, **2** gave the *O*-adamantoyl derivative **25** in good yield. Both methyl and benzyl glycosides were obtained from 2-acetamido-2-deoxy-D-glucose (**1**) in good yield as has been described earlier[21].

The introduction of a hydrophobic moiety into 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (**29**) was complicated by the instability of the product and its ready conversion into a dimer[22]. Thus, on treatment with chlorotriphenylmethane in pyridine, **29** gave a gummy product, the ^1H-n.m.r. spec-

TABLE II

INHIBITORS OF *N*-ACETYL-β-D-GLUCOSAMINIDASE[a]

Compound (R of **33***)*	K_i*(mM)*
H[b]	0.67 ± 0.05
OMe[b]	10.6 ± 2.2
OH(**1**; α anomer)	3.3 ± .22
SH (**4**)	6.1 ± .97
NH_2[c]	0.18 ± 0.01
NHAc[b]	4.1 ± .49
N_3[c]	3.1 ± .33
$NHCOCH_2N_3$[b]	5.5 ± 1.1
$NHCOCH_2Cl$[b]	5.9 ± 1.1
$NHCOCH_2Br$[b]	6.7 ± 9.5
$NHCOCH_2NHCOCH_2C_6H_5$[b]	8.2 ± 1.2
$NHCOCH_2\overset{+}{N}H_3CH_2CO_2^-$[b]	2.01 ± .11
$2,4\text{-}(NO_2)_2C_6H_3S$ (**24**)	1.2 ± 0.06

[a]Conditions of assay are described in the Experimental Part. [b]Ref. 21. [c]Ref. 22.

trum of which indicated it to be the desired *N*-trityl derivative, but it slowly decomposed at room temperature and could not be characterized. However, the *N*-2,4-dinitrophenyl (**30**) and *N*-adamantoyl (**31**) derivatives could be obtained under similar reaction conditions. Compound **31** was *O*-deacetylated by treatment with 10% triethylamine in aqueous methanol at room temperature to give **32**. Condensation of **29** with 4-methylbenzaldehyde gave the Schiff base **28**. Its reduction with sodium borohydride gave a product that was too unstable to be isolated.

Biological activity. — Compounds prepared in this study have been tested as inhibitors of *N*-acetyl-β-D-hexosaminidase (hexosaminidase; EC 3.2.1.52) from beef liver (Table II), and as inhibitors of growth of mouse-mammary-adenocarcinoma (TA-3) cells grown in culture and *in vivo* for antitumor activity. Inadequate solubility of many of these glycosides in aqueous medium prevented them from being tested in all systems.

All compounds that were found to be adequately soluble have been tested as hexosaminidase inhibitors and their K_i values are shown in Table II. Computer analysis of the data indicated that all of them inhibited with competitive kinetics. The most potent inhibitor was found to be the glycosylamine derivative **29**, followed by 2-acetamido-1,5-anhydro-2-deoxy-D-glucitol. Substitution of NH_2-1 with various residues decreased the extent of inhibition, and at the same time revealed considerable bulk-tolerance with regard to the length of the side-chain. However, substitution of SH-1 by the 2,4-dinitrophenyl residue increased somewhat the inhibitory activity.

Inhibition of growth of TA-3 cells were expressed as ID_{50} value; if this value indicated a concentration greater than 1mM, the compound was considered to be inactive. The pseudothiouronium (**3**) and the 1-chloroacetylthio (**12**) derivatives inhibited the TA-3 cells at concentrations 360 and 250μM, respectively. The fully acetylated diphenylmethyl derivative **13** had an ID_{50} value of 800μM, but the inhibitory activity of the *O*-deacetylated compound **14** was greatly reduced. The *O*-deacetylated *S*-trityl derivative **8** inhibited the cells at a concentration of 53μM, and the corresponding *p*-anisyldiphenyl derivative **10** at that of 16μM. A compound containing a trityl group at O-6, as for example 2-acetamido-2-deoxy-6-*O*-trityl-D-glucopyranose, was inactive as a growth inhibitor of these cells. Thus, the position and the mode of linkage of the trityl group appear to be important for determining inhibitory activity. The following compounds were tested *in vitro* and *in vivo*, and found to be "inactive": **11, 15, 16, 17, 18, 19, 20, 21, 22, 23, 28, 30, 31,** and **32**.

Administration of the *O*-deacetylated *S*-trityl derivative **8** intraperitoneally at 50 mg/kg daily for 4 days to mice bearing leukemia L1210 prolonged their life-span by 15%.

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined by the capillary method Optical rotations were measured with a Perkin–Elmer 141

polarimeter. I.r. spectra were recorded with a Perkin–Elmer 457 spectrophotometer, and n.m.r. spectra with Varian A-60A and XL-100 instruments. ^{1}H-N.m.r. spectra at 100 MHz and ^{13}C-n.m.r. spectra at 25.1 MHz were obtained in the Fourier transform (F.t.) mode, with the positions of the peaks expressed in δ values from the signals of tetramethylsilane or 1,4-dioxane. Thin-layer chromatography was performed on Merck Silica gel HF-254 plates, and spots on chromatograms were detected by any of the following methods: iodine vapor, u.v. absorption, spraying with a ninhydrin solution, and charring after spraying with 1% sulfuric acid in water–ethanol and heating.

Hydrolysis of S-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)thiouronium chloride* (**3**) *in the presence of potassium pyrosulfite. Formation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranose* (**6**) *and 2-acetamido-3,4,6-tri-*O*-acetyl-1-carbamoyl-2-deoxy-1-thio-β-*D*-glucopyranose* (**5**). — *S*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)thiouronium chloride[15] (**3**; 16.5 g) was added slowly to a vigorously stirred solution of potassium pyrosulfite (8.3 g) in water (33 mL) to which chloroform (50 mL) was added. The mixture was heated to 85° for 20 min. After being cooled, the chloroform layer was transferred to a conical flask and was kept at room temperature for 0.5 h, at the end of which time **5** precipitated. It was filtered off, washed with ether, and dried (yield, 1.6 g, 10.5%); m.p. 187–188° (after crystallization from ethanol), $[\alpha]_D^{24}$ +7.1° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3400, 3280, 3200 (NH), 3065, 2960, 2890 (CH), 1750 (C=O, acetyl), 1690 (–S–CO–), 1665, 1540 (C=O, amide), 1235 cm^{-1} (AcO); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.77 (s, 3 H, NHCOCH_3), 1.92, 1.97, 2.00 (3 s, 9 H, 3 OCOCH_3), 3.35 (m, 1 H, H-5), 3.88 (t, 1 H, *J* 9 Hz, H-2), 5.18 (d, 1 H, *J* 10 Hz, H-1), 4.07 (2 H, H_2-6), 4.83 (t, 1 H, *J* 9 Hz, H-4), 5.15 (t, 1 H, *J* 9 Hz, H-3), 7.62 (s, 2 H, $CONH_2$-1), and 8.03 (d, 1 H, *J* 9 Hz, N*H*Ac); ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 169.7, 169.2, 168.9 (C=O), 163.7 ($SCONH_2$), 83.5 (C-1), 74.7 (C-5), 73.5 (C-3), 68.3 (C-4), 61.7 (C-6), 50.8 (C-2), 22.5 (NHCO*C*H_3), and 20.3 (OCO*C*H_3).

Anal. Calc. for $C_{15}H_{22}N_2O_9S$: C, 44.32; H, 5.47; N, 6.89. Found: C, 44.23; H, 5.71; N, 6.94.

The aqueous layer from the preceding experiment was extracted with chloroform (3 × 20 mL). The combined chloroform filtrates and extracts were washed with water (10 mL), dried, and evaporated. The residue (**6**), crystallized from ethyl acetate, was filtered off, and washed with ether (yield 8.50 g, 62%); m.p. 173°, $[\alpha]_D^{23}$ −16.0° (*c* 1.08, chloroform) (lit.[16] m.p. 160–162°); ν_{max}^{KBr} 3320 (NH), 2958 (CH), 2568 (SH), 1740 (C=O, acetoxyl), 1660, 1530 (C=O, amide) and 1236 cm^{-1} (AcO); ^{1}H-n.m.r. ($CDCl_3$): δ 2.00 (s, 3 H, NHCOCH_3), 2.06, 2.12 (2 s, 9 H, 3 OCOCH_3), 2.58 (d, 1 H, *J* 9 Hz, SH-1), 3.72 (m, 1 H, H-5), 4.16 (m, 3 H, H_2-6, H-2), 4.62 (t, 1 H, *J* 9 Hz, H-4), 5.12 (q, 1 H, *J* 9 Hz, H-3), 5.11 (d, 1 H *J* 9 Hz, H-1), 5.89 (d, 1 H, *J* 9 Hz, N*H*COCH$_3$); ^{13}C-n.m.r.: δ 170.5, 169.1 (C=O), 79.8 (C-1), 76.0 (C-5), 73.4 (C-3), 68.4 (C-4), 62.3 (C-6), 56.8 (C-2), 23.2 (NHCO*C*H_3); 20.7, 20.6 (OCO*C*H_3).

Hydrolysis of **5** *into* **6**. — The carbamoyl derivative **5** (0.5 g) was added slowly to a vigorously stirred and heated (85°) solution of potassium pyrosulfite (0.25 g) in water (1 mL) to which chloroform (5 mL) was added. After 45 min, the mixture was cooled and extracted with chloroform (3 × 15 mL). The combined chloroform extract was washed with water (5 mL), dried (Drierite), and evaporated *in vacuo*. The residue crystallized from ethyl acetate–ether (yield 0.20 g, 45%); m.p. 171–172°. It was identified as **6** by mixed m.p., and i.r., and ^{1}H-n.m.r. spectra.

*2-Acetamido-2-deoxy-1-thio-β-*D*-glucopyranose* (**4**). — This compound was synthesized from **6** according to the procedure described by Akagi *et al.*[17].

*Triphenylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**7**). — A mixture of **6** (3 g) and chlorotriphenylmethane (2.5 g) in dry pyridine (100 mL, dried over potassium hydroxide) was stirred for 4 days at room temperature. After evaporation of the mixture to dryness, the residue was dissolved in chloroform (50 mL), and the solution washed with water, and dried (Drierite). The chloroform extract showed on t.l.c. (9:1, v/v, chloroform–methanol) one major and two minor spots. After evaporation, the residue was chromatographed on a silica gel column (Bio-Sil A; 100–200 mesh; 55 × 1.9 cm), with 9:1 (v/v) chloroform–methanol as eluent. Fractions corresponding to the major t.l.c. spot were pooled and evaporated, and the residue crystallized from ether–petroleum ether (yield 2.1 g, 42%); m.p. 184–185°, $[\alpha]_D^{23}$ −13.5° (*c* 1.02; chloroform); ν_{max}^{KBr} 3262, 3218 (NH), 3080, 3060 (arom. CH), 2940, 2875 (CH), 1762, 1748 (C=O, acetoxyl), 1659, 1560 (C=O, amide), 1494, 1448 (arom. C=C), 1240 (AcO), 1050, 750, and 705 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (s, 3 H, NHCOC*H*$_3$), 1.96, 1.97, 1.98 (3 s, 9 H, 3 OCOC*H*$_3$), 2.97 (b, 1 H, H-5), 3.48 (d, 1 H, *J* 10 Hz, H-1), 3.93 (m, 2 H, H_2-6), 4.32 (q, 1 H, *J* 10 Hz, H-2), 4.70, 5.02 (2 t, 2 H, *J* 10 Hz, H-3, -4), 4.91 (d, 1 H, *J* 9 Hz, N*H*COCH$_3$), and 7.25–7.56 [m, 15 H, $(C_6H_5)_3C$].

Anal. Calc. for $C_{33}H_{35}NO_8S$: C, 65.43; H, 5.84; N, 2.31. Found: C, 65.21; H, 6.02; N, 2.43.

*Triphenylmethyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**8**). — A suspension of **7** (0.6 g) in 10% triethylamine in 50% aqueous methanol (25 mL) was stirred for 1 h at room temperature. The clear solution was stirred for another 5 h, and evaporated *in vacuo* when the product had precipitated. Water (10 mL) was added and evaporated to remove traces of triethylamine and methyl acetate, and the process was repeated 3–4 times. The precipitate was filtered off, washed with water, dried, and it crystallized from ethanol (yield 0.4 g, 84%); m.p. 212–213°, $[\alpha]_D^{25}$ −25.0° (*c* 1.01, *N,N*-dimethylformamide); ν_{max}^{KBr} 3410, 3290 (br., NH, OH), 3090, 3060, 3030 (arom. CH), 2940, 2880 (aliph. CH), 1658, 1650, 1570 (C=O, amide), 1494, 1444 (C=C, arom.), 1080, 1064, 1039, 1028, 740, and 700 cm^{-1} (arom.).

Anal. Calc. for $C_{27}H_{29}NO_5S$: C, 67.61; H, 6.09; N, 2.92. Found: C, 67.50; H, 6.20; N, 2.99.

p-*Anisyldiphenylmethyl 2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-1-thio-β*-D-*glucopyranoside* (**9**). — A solution of **6** (5 g) and *p*-anisylchlorodiphenylmethane (4.7 g) in dry pyridine (50 mL; dried over potassium hydroxide) was stirred for 4 days at room temperature and processed as described for **7**. The product crystallized from ethanol–water (yield 4.1 g, 46%); m.p. 110°, $[\alpha]_D^{27}$ −103.9° (*c* 1.02, chloroform); ν_{max}^{KBr} 3300 (br., NH), 3070, 3040, 2960, 2850 (CH), 1750 (C=O, acetoxyl), 1667, 1550 (C=O, amide), 1608, 1510, 1445 (subst. phenyl), 920, 830, 795, and 703 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (s, 3 H, $NHCOCH_3$), 1.97, 1.98, 1.99 (3 s, 9 H, $OCOCH_3$), 2.98 (m, 1 H, H-5), 3.52 (d, 1 H, *J* 10 Hz, H-1), 3.82 (s, 3 H, OCH_3), 3.98 (m, 2 H, H_2-6), 4.31 (q, 1 H, *J* 10 Hz, H-2), 4.72, 5.03 (2 t, 2 H, *J* 10 Hz, H-3, -4), 4.95 (d, 1 H, $NHCOCH_3$), and 6.78–7.44 [m, 14 H, $CH_3OC_6H_4(C_6H_5)_2CH$].

Anal. Calc. for $C_{34}H_{37}NO_9S$: C, 64.23; H, 5.86; N, 2.20. Found: C, 64.48; H, 5.70; N, 2.04.

p-*Anisyldiphenylmethyl 2-acetamido-2-deoxy-1-thio-β*-D-*glucopyranoside* (**10**). — A suspension of **9** (2.6 g) in 10% triethylamine in 50% aqueous methanol (75 mL) was stirred for 6 h at room temperature, and processed as described for **8**. The product was suspended in ether, mixed well, and filtered. The residue was washed with petroleum ether and dried (yield 1.65 g, 79%); m.p. 203°, $[\alpha]_D^{23}$ −24.3° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3410, 3280 (br., NH, OH), 3100, 3065, 3040, 2940, 2880, 2842 (CH), 1655, 1652, 1569 (C=O, amide), 1605, 1509, 1491, 1462, 1445 (subst. Ph), 795, 760, 748, and 700 cm^{-1} (Ph).

Anal. Calc. for $C_{34}H_{37}NO_9S$: C, 64.23; H, 5.88; N, 2.20. Found: C, 64.48; H, 5.70; N, 2.04.

2-Acetamido-3,4,6-tri-O-*acetyl-1*-S-*adamantanecarbonyl-2-deoxy-1-thio-β*-D-*glucopyranose* (**11**). — A solution of **6** (1 g) and 1-adamantanecarbonyl chloride (0.95 g) in pyridine (10 mL; dried over potassium hydroxide) was stirred for 5 h at room temperature. Crushed ice and ice-cold water (20 mL) were added to the mixture, which was evaporated *in vacuo* at room temperature. The oily, gummy residue was dissolved in chloroform (30 mL), and the solution washed with water (10 mL), and dried (Drierite). T.l.c. (9:1, v/v, chloroform–methanol) of the chloroform extract showed one major and one minor spot. The residue obtained after evaporation of the chloroform extract was chromatographed on a silica gel column (1.9 × 55 cm) with chloroform, followed by 9:1 chloroform–methanol as eluent. Fractions corresponding to the major spot were pooled and evaporated, and the residue crystallized from ethanol–water (yield 0.95 g, 63%); m.p. 109–110°; $[\alpha]_D^{24}$ −7.4° (*c* 1.0, chloroform); ν_{max}^{KBr} 3400–3280 (NH), 2920, 2860 (adamantane CH), 1775 (C=O, acetoxyl), 1695 (SC=O), 1670 (sh), 1550 (C=O, amide), and 1235 cm^{-1} (AcO); ^{1}H-n.m.r. ($CDCl_3$): δ 1.75 (adamantane H), 1.90 (s, 3 H, $NHCOCH_3$), 2.03, 2.07 (2 s, 9 H, $OCOCH_3$), 3.83 (b, 1 H, H-5), 4.18 (m, 2 H, H_2-6), 5.17 (d, 1 H, *J* 10 Hz, H-1), and 5.90 (d, 1 H, *J* 9 Hz, NHAc).

Anal. Calc. for $C_{25}H_{35}NO_9S \cdot H_2O$: C, 55.23; H, 6.87; N, 2.58. Found: C, 54.84; H, 6.64; N, 2.49.

*2-Acetamido-3,4,6-tri-*O*-acetyl-1-*S*-(chloroacetyl)-2-deoxy-1-thio-β-*D*-glucopyranose* (**12**). — Chloroacetic anhydride (0.35 g) was added slowly in small portions to an ice-cold, stirred solution of **6** (0.5 g) in dry pyridine (5 mL, dried over potassium hydroxide). The reaction mixture was stirred for 1 h at 0°, and then for 2 h at room temperature, followed by evaporation *in vacuo*, and repeated additions and evaporations of water, to remove traces of pyridine. The residue was dissolved in chloroform (30 mL), and the solution washed with cold water (5 mL), dried (Drierite), and evaporated. T.l.c. (ethyl acetate) showed one major and a few minor spots. After chromatography on a silica gel column (1.25 × 37 cm) with ethyl acetate as eluent, the fractions corresponding to the major spot were pooled and evaporated, and the residue was crystallized from ethyl acetate–ether (yield 0.12 g, 19%); m.p. 166–167°; $[\alpha]_D^{23}$ −14.4° (*c* 1, chloroform); ν_{max}^{KBr} 3355 (NH), 2960, 2885 (CH), 1742 (C=O, acetoxyl), 1705 (–S–CO–), 1664, 1529 (C=O, amide), 1238 (AcO), and 777 cm^{-1} (C–Cl); ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, $NHCOCH_3$), 2.07, 2.10 (2 s, 9 H, 3 $OCOCH_3$), 3.88 (b, 1 H, H-5), 4.20 (m, 2 H, H_2-6), 4.27 (s, 2 H, $ClCH_2CO$), 4.48 (m, 1 H, H-2), 5.30 (d, 1 H, *J* 10 Hz, H-1), 5.20 (t, 1 H, *J* 6 Hz, H-3), and 6.35 (d, 1 H, *J* 10 Hz, $NHCOCH_3$).

Anal. Calc. for $C_{16}H_{22}ClNO_3S$: C, 43.68; H, 5.05; N, 3.18. Found: C, 43.95; H, 5.18; N, 3.31.

Reaction of **6** *and bromodiphenylmethane in pyridine. Formation of diphenylmethylpyridinium bromide.* — A solution of **6** (1 g) and bromodiphenylmethane (1 g) in dry pyridine (20 mL, dried over potassium hydroxide)) was stirred for 3 days at room temperature. The mixture was evaporated *in vacuo*, and water (2 × 20 mL) was added and evaporated *in vacuo* to remove traces of pyridine. The crystalline residue was dissolved in chloroform (50 mL), and the solution washed with water (10 mL), dried (Drierite), and evaporated *in vacuo*. The residue crystallized from methanol–ethyl acetate (yield 1.15 g, 87%, based on bromodiphenylmethane); m.p. 211–212° (lit.[23] m.p. 215–216°); i.r. and ^{1}H-n.m.r. spectra indicated that the compound is diphenylmethylpyridinium bromide; an aqueous solution gave a halide test with silver nitrate; ν_{max}^{KBr} 3130, 3045, 3020 (aromatic CH), 1628 (arom. C=C). 1125, 1083, 1040 (pyridine CH) 770, 750, 710 (phenyl); ^{1}H-n.m.r. ($CDCl_3$): δ 7.37 (m, 10 H, arom. H), 8.10–9.20 (m, 5 H, pyridine H's), 8.42 (s, 1 H, C*H*); ^{13}C-n.m.r. ($CDCl_3$): δ 146.5, 144.2, 135.1, 129.5, 129.0, 128.7 (arom. C), and 75.8 (Ph_2CH).

*Diphenylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**13**). — A solution of potassium carbonate (0.63 g) in water (5 mL) was added slowly to a stirred solution of **6** (1.8 g) and bromodiphenylmethane (1.3 g) in acetone (10 mL) at room temperature, and stirring was continued for 6 h. The mixture was diluted with ice-cold water (10 mL) and extracted with chloroform (3 × 30 mL). The combined chloroform extract was washed with water (10 mL), dried (sodium sulfate), and evaporated *in vacuo*. On t.l.c. (9:1 chloroform–methanol), the residue gave three spots (R_F 0.28, 0.44, and 0.78). The spots R_F 0.28 and 0.78 were identified as starting materials **6** and bromodiphenylmethane. The reaction

product was chromatographed on a silica gel column (1.9 × 55 cm) with 9:1 chloroform–methanol as eluent. Fractions corresponding to the reaction product (middle spot on t.l.c., R_F 0.44) were pooled and evaporated, and the residue crystallized from ethyl acetate–ether (yield 0.74 g, 28%); m.p. 190–191°, $[\alpha]_D^{24}$ −95.5° (*c* 1, chloroform); ν_{max}^{KBr} 3260, 3220 (NH), 3110–3065 (arom. CH), 2960 (CH), 1745 (C=O, acetoxyl), 1660, 1564 (C=O, amide), 1492, 1445 (C=C, arom.), 1235 (AcO), 1040, 915, and 750 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.97 (s, 3 H, NHCOCH_3), 2.02, 2.17 (2 s, 9 H, OCOCH_3), 3.43 (b, 1 H, H-5), 4.22 (m, 2 H, H_2-6), 5.12 (d, 1 H, *J* 9 Hz, H-1), 5.58 (s, 1 H, Ph_2CH), 5.63 (d, 1 H, *J* 9 Hz, N*H*COCH$_3$), and 7.27–7.63 [m, 10 H, $(C_6H_5)_2$CH].

Anal. Calc. for $C_{27}H_{31}NO_8S$: C, 61.22; H, 5.91; N, 2.64. Found: C, 61.05; H, 6.04; N, 2.51.

*Diphenylmethyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**14**). — A suspension of **13** (0.25 g) in 10% triethylamine in 50% aqueous methanol (25 mL) was stirred for 6 h at room temperature and processed as described for **8**. The product crystallized from methanol–ether (yield 0.15, 77%); m.p. 196–197°, $[\alpha]_D^{24}$ −103.7° (*c* 1.03, *N,N*-dimethylformamide); ν_{max}^{KBr} 3490–3200 (NH, OH), 3105–3030 (arom. CH), 2962, 2938, 2879 (CH), 1645, 1573 (C=O, amide), 1495, 1450 (C=C, arom.), 1060, 1030, 880, 700, and 682 cm^{-1} (Ph).

Anal. Calc. for $C_{21}H_{25}NO_5S \cdot 0.5H_2O$: C, 61.14; H, 6.35; N, 3.39. Found: C, 61.55; H, 6.30; N, 3.27.

*Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**15**). — This compound was prepared from **6** by treatment with benzyl bromide according to the method of Matta *et al.*[16].

*Benzyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**16**). — A suspension of **15** (1 g) in 10% triethylamine in 50% aqueous methanol (35 mL) was stirred for 6 h at room temperature, and processed as described for **8**. The product crystallized from methanol (yield 0.7 g, 97%); m.p. 233–234° (lit.[16] m.p. 225–227°), $[\alpha]_D^{24}$ −132° (*c* 1.03, *N,N*-dimethylformamide); ν_{max}^{KBr} 3460–3190 (NH, OH), 3090, 3035 (arom. CH), 2960–2860 (CH), 1650, 1550 (C=O, amide), 1493, 1454 (C=C, arom.), 1050, 950, 890, 712, 700 cm^{-1} (Ph); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.90 (s, 3 H, NHCOCH_3), 3.16 (b, 2 H, H_2-6), 3.30 (b, 1 H, H-5), 3.88 (d, 2 H, *J* 5 Hz, $CH_2C_6H_5$), 4.21 (d, 1 H, *J* 10 Hz, H-1), 4.72 (t, 1 H, *J* 6 Hz, OH-6), 5.04 (b, 2 H, OH-3 and -4), 7.32 (s, 5 H, C_6H_5), and 7.73 (d, 1 H, N*H*COCH$_3$, *J* 10 Hz).

Anal. Calc. for $C_{15}H_{21}NO_5S$: C, 55.03; H, 6.47; N, 4.28; S, 9.78. Found: C, 54.76; H, 6.47; N, 4.23; S, 9.67.

*1-Naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**17**). — A solution of potassium carbonate (1.9 g) in water (5 mL) was added slowly to a stirred solution of **6** (5 g) and 1-(chloromethyl)naphthalene (2.5 g) in acetone (50 mL) at room temperature, whereupon the solution became turbid, and the product precipitated after 15 min. It was filtered off, washed with acetone (10 mL), water (20 mL), and acetone (10 mL), and dried (yield 6.1 g, 88%); m.p. 232–233° $[\alpha]_D^{26}$ −128.1° (*c* 1.01, *N,N*-dimethylformamide); ν_{max}^{KBr} 3299

(NH), 1740 (C=O, acetoxyl), 1660, 1549 (C=O, amide), 1440 (C=C, arom.), 1250 (AcO), 1095, 1070, 1048, 912, 803, and 780 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.78 (s, 3 H, NHCOCH_3), 2.00, 2.02, 2.16 (3 s, 9 H, 3 OCOCH_3), 3.59 (m, 1 H, H-5), 4.33 (m, 6 H, H-2, -4, H_2-6, C$H_2C_{10}H_7$), 5.09 (d, 1 H, *J* 10 Hz, H-1), 5.05 (2 H, H-3, N*H*COCH$_3$), and 7.82 (m, 7 H, $C_{10}H_7$).

Anal. Calc. for $C_{25}H_{29}NO_8S$: C, 59.62; H, 5.82; N, 2.78. Found: C, 59.35; H, 6.09; N, 2.66.

*1-Naphthylmethyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**18**). — A suspension of **17** (2 g) in 10% triethylamine in 10% aqueous methanol (300 mL) was stirred at room temperature for 24 h, and processed as described for **8**. The product crystallized from methanol–ethyl acetate (yield 1.2 g, 78%); m.p. 252°, $[\alpha]_D^{23}$ −184.1° (*c* 1.02, *N,N*-dimethylformamide); ν_{max}^{KBr} 3370, 3300 (br. OH, NH), 2948, 2875 (CH), 1655, 1548 (C=O, amide), 1455 (C=C, arom.), 890, 806, 795, 781, and 695 cm^{-1} (arom.); ^{1}H-n.m.r. [$(CD_3)_2$ SO]: δ 1.72 (s, 3 H, NHCOCH_3), 4.22 (d, 1 H, *J* 10 Hz), and 7.82 (m, 7 H, $C_{10}H_7$).

Anal. Calc for $C_{19}H_{23}NO_5S \cdot 0.2\ H_2O$: C, 59.89; H, 6.14; N, 3.67. Found: C, 59.66; H, 6.09; N, 3.60.

*2-Naphthylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**19**). — A stirred solution of **6** (5 g) and 2-(bromomethyl)naphthalene (3 g) in acetone (30 mL) was treated with a solution of potassium carbonate (1.9 g) in water (5 mL). The product was processed as described for **17** (yield 6.4 g, 92%); m.p. 220–221°, $[\alpha]_D^{25}$ −82.1° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3345 (NH), 3065 (CH arom.), 2970, 2955, 2945, 2885 (CH, aliph.), 1745 (C=O, acetoxyl), 1660, 1525 (C=O, amide), 1440 (C=C, arom.), 1240 (AcO), 1050, 918, 822, 760, and 688 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.90 (s, 3 H, NHCOCH_3), 1.99, 2.00, 2.12 (3 s, 9 H, 3 OCOCH_3), 3.57 (m, 1 H, H-5), 4.14 (m, 6 H, H-2, -4, H_2-6, C$H_2C_{10}H_7$), 5.05 (m, 1 H, H-3), 5.09 (d, 1 H, *J* 7 Hz, H-1), 5.48 (d, 1 H, *J* 9 Hz, N*H*COCH$_3$), and 7.67 (m, 7 H, $C_{10}H_7$).

Anal. Calc for $C_{25}H_{29}NO_8S$: C, 59.62; H, 5.80; N, 2.78. Found: C, 59.39; H, 5.84; N, 2.71.

*2-Naphthylmethyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**20**). — This compound was prepared from **19** as described for **18** (yield 1.1 g, 71%); m.p. 245°, $[\alpha]_D^{23}$ −95.3° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3460, 3380–3220 (br. NH, OH), 3060, 2955, 2875 (CH), 1678, 1650, 1540 (C=O, amide), 1462 (C=C, arom.), 1110, 1081, 1062, 1033, 1000, 741, and 736 cm^{-1} (arom.); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.76 (s, 3 H, NHCOCH_3), 4.13 (d, 1 H, *J* 10 Hz, H-1), and 7.73 (m, 7 H, $C_{10}H_7$).

Anal. Calc. for $C_{19}H_{23}NO_5S$: C, 60.45; H, 6.14; N, 3.71. Found: C, 60.16; H, 6.22; N, 3.64.

*9-Anthrylmethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-*D*-glucopyranoside* (**21**). — A solution of potassium carbonate (0.22 g) in water (1 mL) was added slowly at room temperature to a stirred solution of **6** (0.58 g) and 9-(chloromethyl)anthracene (0.34 g) in acetone (10 mL). After the reaction mixture had

been stirred for 15 min, a yellow precipitate formed, which was filtered, and was washed with water (10 mL), acetone (15 mL), and ether (2 × 10 mL) (yield 0.7 g, 76%); m.p. 301–302°, $[\alpha]_D^{26}$ −92.5° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3300 (NH), 2960, 2885 (CH), 1745 (C=O, acetoxyl), 1662, 1539 (C=O, amide), 1449 (C=C, arom.), 1240 (AcO), 918, 898, 740, and 730 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 1.75 (s, 3 H, NHCOCH_3), 1.98, 2.01, 2.18 (3 s, 9 H, 3 OCOCH_3), 3.50 (m, 1 H, H-5), 4.24 (m, 5 H, H-2, H_2-6, C$H_2C_{14}H_9$), 4.79 (m, 1 H, H-4), 5.11 (d, 1 H, *J* 10 Hz, H-1), 5.14 (m, 2 H, H-3, N*H*COCH_3), and 7.96 (m, 9 H, $C_{14}H_9$).

Anal. Calc. for $C_{29}H_{31}NO_8S \cdot H_2O$: C, 60.92; H, 5.83; N, 2.45. Found: C, 60.73; H, 5.46; N, 2.29.

*9-Anthrylmethyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**22**). — A suspension of **21** (0.2 g) in 10% triethylamine in 10% aqueous methanol (40 mL) was stirred for 24 h at room temperature and processed as described for **8** to give yellow crystals (yield 0.11 g, 71%); m.p. 295–296° (dec.), $[\alpha]_D^{24}$ −121.0° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3380, 3290 (br. NH, OH), 3090, 3060, 2940, 2890 (CH), 1651, 1548 (C=O, amide), 1450 (C=C, arom.), 892, 800, and 740 cm^{-1} (arom.); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.70 (s, 3 H, NHCOCH_3), 4.32 (d, 1 H, *J* 10 Hz, H-1), and 8.10 (m, 9 H, $C_{14}H_9$).

Anal. Calc. for $C_{23}H_{25}NO_5S$: C, 64.61; H, 5.89; N, 3.27. Found: C, 64.44; H, 5.77; N, 3.00.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*S*-(2,4-dinitrophenyl)-1-thio-β-*D*-glucopyranose* (**23**). — Triethylamine (2 drops) was added to a stirred solution of **6** (0.35 g) and fluoro-2,4-dinitrobenzene (0.3 g) in dry acetone (20 mL; dried over Drierite) at room temperature. The mixture was stirred for 1 h, when a light yellow precipitate started to separate out. Stirring was continued for another 4 h, and the yellow precipitate was filtered off, washed with ice-cold acetone, and dried (yield 0.43 g, 86%); m.p. 234°, $[\alpha]_D^{23}$ −58.8° (*c* 1.02, pyridine); ν_{max}^{KBr} 3310 (NH), 3120, 3090, 3040 (CH, arom.), 2940, 2880 (CH, aliph.), 1754 (C=O, acetoxyl), 1660, 1595 (C=O, amide), 1530, 1348, 870 (NO_2), 1230 (AcO), 1450, 750, and 740 cm^{-1} (subst. Ph); ^{1}H-n.m.r. (CD_3OD–$CDCl_3$): δ 1.93 (s, 3 H, NHCOCH_3), 2.06, 2.12 (2 s, 9 H, 3 OCOCH_3), 4.13 (m, 4 H, H-5, -2, H_2-6), 5.11 (t, 1 H, *J* 9 Hz, H-4), 5.12 (t, 1 H, *J* 9 Hz, H-3), 5.41 (d, 1 H, *J* 10 Hz, H-1), and 8.96 (m, 3 H, C_6H_3).

Anal. Calc. for $C_{20}H_{23}N_3O_{12}S$: C, 45.35; H, 4.39; N, 7.94. Found: C, 45.32; H, 4.42; N, 7.89.

*2,4-Dinitrophenyl 2-acetamido-2-deoxy-1-thio-β-*D*-glucopyranoside* (**24**). — A suspension of **23** (0.4 g) in 10% triethylamine in 50% aqueous methanol (50 mL) was stirred for 7 h at room temperature, and processed as described for **8**. The product crystallized from ethanol (yield 0.25 g, 82%); m.p. 185° (dec.), $[\alpha]_D^{23}$ −69.4° (*c* 0.97, Me_2SO); ν_{max}^{KBr} 3440–3170 (OH, NH), 2950–2870 (CH), 1660, 1595 (C=O, amide), 1525, 1349, 877 (NO_2), 1455, 750, and 748 cm^{-1} (subst. Ph); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.82 (s, 3 H, NHCOCH_3), 3.56 (m, 6 H, H-2, -3, -4, -5, H_2-6), 5.07 (d, 1 H, *J* 10 Hz, H-1), 7.81 (d, 1 H, *J* 9 Hz, N*H*COCH_2), and 8.39 (m, 3 H, C_6H_3).

Anal. Calc. for $C_{14}H_{17}N_3O_9S$: C, 41.68; H, 4.24; N, 10.41. Found: C, 41.39; H, 4.33; N, 10.13.

2,4-Dinitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranoside (**26**). — Sodium methoxide (0.6 g) was added to a solution of 2,4-dinitrophenol (2 g) in methanol (50 mL), and the mixture was evaporated *in vacuo* at room temperature. Solid sodium 2,4-dinitrophenoxide separated out. After the addition of a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (2.54 g) in *N,N*-dimethylformamide (10 mL; dried over calcium hydride), the resulting solution was stirred for 64 h at room temperature, and evaporated *in vacuo* at ~40°. Water (40 mL) was added to the residue, and a yellowish, crystalline material separated out; it was filtered off, washed with water, acetone (5 mL), ether, and dried (yield 0.5 g, 9%); m.p. 164°, $[\alpha]_D^{27}$ −4.6° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{KBr} 3320 (NH), 3120, 3090, 3070, 3020, 2970, 2960, 2900 (CH), 1755 (C=O, acetoxyl), 1668, 1545 (C=O, amide), 1608, 1488, 1462 (C=C, arom.), 1530, 1350, 839 (NO_2), 1235 (AcO), 839, 749, 720, 705 cm^{-1} (Ph); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.78 (s, 3 H, $NHCOCH_3$), 1.96, 2.02 (2 s, 9 H, 3 $OCOCH_3$), 4.06 (m, 4 H, H-2, -5, H_2-6), 4.97 (t, 1 H, *J* 9 Hz, H-4), 5.22 (t, 1 H, *J* 9 Hz, H-3), 5.68 (d, 1 H, *J* 9 Hz, H-1), 6.60 (d, 1 H, *J* 9 Hz, N*H*), 8.01 (d, 1 H, *J* 9 Hz, arom. H), 8.50 (q, 1 H, *J* 9 Hz, arom. H), 8.79 (d, 1 H, *J* 3 Hz, arom. H); ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 169.7, 169.4, 169.3, 169.0 (C=O), 152.7, 141.2, 139.3, 128.6, 122.5, 120.6, 117.5 (arom. C), 98.4, 97.9 (C-1), 71.8, 71.3, 69.8, 68.1, 67.9, 66.6, 64.2, 62.8, 61.3 (C-3, -4, -5, and -6), 52.6, 50.2 (C-2), 22.3 ($NHCOCH_3$), 20.3, 20.2 ($OCOCH_3$). From the ^{13}C-n.m.r. spectrum the ratio of α to β anomer was estimated to be 3:17. The product appears to be unstable in di($^2H_3$)methyl sulfoxide over a period of 4–5 days.

Anal. Calc. for $C_{20}H_{23}N_3O_{13}$: C, 46.78; H, 4.51; N, 8.18. Found: C, 46.73; H, 4.48; N, 8.11.

2-Acetamido-3,4,6-tri-O-acetyl-1-O-adamantanecarbonyl-2-deoxy-α-D-glucopyranose (**25**). — 1-Adamantanecarbonyl chloride (2 g) was added to an ice-cold, stirred solution of **2** (3 g) in dry pyridine (25 mL, dried over potassium hydroxide). The mixture was stirred for 1 h at 0°, and then for 6 h at room temperature, and then cooled in an ice bath. Crushed ice and ice-cold water (~30 g) were added when the product had precipitated. It was filtered off, washed with ice-cold water, dried, and crystallized from ethanol–water. T.l.c. of the product in 99:1 (v/v) dichloromethane–methanol showed a major spot (R_F 0.4) and a few minor spots. The product was chromatographed on silica gel with dichloromethane followed by 99:1 (v/v) dichloromethane–methanol. The fractions corresponding to the major spot were pooled. The product was isolated as the α anomer and crystallized from ethanol (yield 3.1 g, 70%); m.p. 156-157°, $[\alpha]_D^{25}$ +66.7° (*c* 1.04, chloroform): ν_{max}^{KBr} 3280 (NH), 3060, 2910, 2858 (adamantane CH), 1755, 1735 (C=O, acetoxyl and adamantanecarbonyl), 1650, 1540 (C=O, amide), 1225 cm^{-1} (AcO); ^{1}H-n.m.r. ($CDCl_3$): δ 1.78, 1.82, 1.94, 1.98, 2.08, 2.09 [6 s, 27 H, $NHCOCH_3$, 3 $OCOCH_3$, $C_{10}H_{15}$ (adamantane)], 4.0 (m, 1 H, H-5), 4.19 (m, 2 H, H_2-6), 4.52 (m, 1 H, H-2),

5.25 (m, 2 H, H-3, -4), 5.46 (d, 1 H, *J* 8 Hz, N*H*COCH$_3$), 6.22 (d, 1 H, *J* 4 Hz, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ 174.8, 171.6, 170.4, 169.7, 169.0 (C=O), 89.9 (C-1), 70.8 (C-5), 69.7 (C-3), 67.6 (C-4), 61.6 (C-6), 51.4 (C-2), 31.8, 36.4, 36.3, 27.8, 22.9, 20.7, 20.6 (NHCO*C*H$_3$, CO*C*H$_3$, and *C*H$_2$ and *C*H of adamantane).

Anal. Calc. for $C_{25}H_{35}NO_{10}$: C, 58.92; H, 6.92; N, 2.75. Found: C, 59.09; H, 6.95; N, 2.74.

2-Acetamido-3,4,5-tri-O-*acetyl-2-deoxy-1*-N-*(2,4-dinitrophenyl)-β*-D-*glucopyranosylamine* (**30**). — Triethylamine (2 drops) was added to a stirred solution of **29** (0.5 g) and fluoro-2,4-dinitrobenzene (0.5 g) in acetone (20 mL; dried over Drierite). The reaction mixture was stirred at room temperature for two weeks. Then, it turned yellowish orange, and some precipitate separated out. The solution was concentrated to a small volume (~5 mL) when the product crystallized. It was filtered off, washed with ether, dried, and recrystallized from acetone–ether (yield 0.35 g, 47%); m.p. 188°, $[\alpha]_D^{25}$ −78.5° (*c* 1.02, *N,N*-dimethylformamide); ν_{max}^{KBr} 3300 (NH), 3085 (arom. CH), 2960, 2890 (CH), 1775 (C=O, acetoxyl), 1660, 1555 (C=O, amide), 1595, 1525, 1345, 840 (NO_2), 1230 (AcO), 1525, and 750 cm^{-1} (Ph).

Anal. Calc. for $C_{20}H_{24}N_4O_{12}$: C, 46.87; H, 4.72; N, 10.93. Found: C, 46.89; H, 4.84; N, 10.88.

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-*adamantanecarbonyl-2-deoxy-β*-D-*glucopyranosylamine* (**31**). — A solution of 1-adamantanecarbonyl chloride (0.32 g) in dry benzene (5 mL; dried over calcium hydride) was added slowly with stirring to an ice-cold solution of **29** (0.5 g) in dry pyridine (5 mL; dried over potassium hydroxide). The mixture was stirred for 1 h at 0°, and for 12 h at room temperature, and then evaporated *in vacuo*. Water (3 × 10 mL) was added to the residue and was evaporated *in vacuo*, and the gummy residue crystallized from ethanol–water (yield 0.55 g, 74%); m.p. 188–189°, $[\alpha]_D^{24}$ −8.8° (*c* 1.03, chloroform); ν_{max}^{KBr} 3600, 3340 (br., NH), 2918, 2860 (adamantane CH), 1755 (C=O, acetoxyl), 1658 (C=O, amide), and 1232 cm^{-1} (AcO).

Anal. Calc. for $C_{25}H_{36}N_2O_9$: C, 59.03; H, 7.13; N, 5.50. Found: C, 58.85; H, 6.98; N, 5.27.

2-Acetamido-1-N-*adamantanecarbonyl-2-deoxy-β*-D-*glucopyranosylamine* (**32**). — A solution of **31** (0.3 g) in 10% triethylamine in 50% aqueous methanol (20 mL) was stirred at room temperaure for 6 h, and processed as described for **8**. On t.l.c. (1:1, v/v, methanol–chloroform, iodine vapor-positive), the residue showed two spots (R_F 0.17 and 0.83). It was chromatographed on a silica gel column (1.9 × 40 cm; Bio-Sil A; 100–200 mesh) with 3:1 chloroform–methanol as eluent. The fractions corresponding to the spot of higher R_F (0.83) were pooled and evaporated, and the residue crystallized by trituration with petroleum ether to give hygroscopic **32** (yield 0.12 g, 52%); m.p. 195–196° (foaming at 125°).

Anal. Calc. for $C_{19}H_{30}N_2O_6 \cdot H_2O$: C, 56.98; H, 8.05; N, 6.99. Found: C, 55.90; H, 7.40; N, 6.66.

2-Acetamido-3,4,6-tri-O-*acetyl-2-deoxy-1*-N-*(4-methylbenzylidene)-β*-D-*glu-*

copyranosylamine (**28**). — 4-Methylbenzaldehyde (0.5 mL) was added dropwise to an ice-cold, stirred solution of **29** (0.8 g) in absolute ethanol (25 mL). The stirring was continued for 4 h at 0°, and the product precipitated (yield 0.69 g, 76%); m.p. 249°, $[\alpha]_D^{26}$ −88.3° (*c* 1.03, *N,N*-dimethylformamide): ν_{max}^{KBr} 3330 (NH), 3035 (arom. CH), 2975, 2895 (CH), 1745 (C=O, acetoxyl), 1665, 1530 (C=O, amide) 1610, 1530 (C=C, arom.), 1245 (AcO), and 820 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 1.89 (s, 3 H, NHCOCH_3), 2.11, 2.10 (2 s, 9 H, 3 OCOCH_3), 2.38 (s, 3 H, CH_3C$_6$H$_4$), 3.91 (m, 2 H, H-2, -5), 4.27 (m, 2 H, H$_2$-6), 4.98 (d, 1 H, *J* 8 Hz, H-1), 5.16 (t, 1 H, *J* 9 Hz, H-4), 5.38 (q, 1 H, *J* 10 Hz, H-3), 5.91 (d, 1 H, N*H*COCH$_3$), 7.40 (q, 4 H, *J* 8 Hz, arom. H), and 8.36 (s, 1 H, C*H*C$_6$H$_4$CH$_3$).

Anal. Calc. for $C_{22}H_{28}N_2O_8$: C, 58.91; H, 6.29; N, 6.24. Found: C, 58.62; H, 6.19; N, 6.00.

Enzyme assay. — *N*-Acetyl-β-D-glycosaminidase (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) from beef kidney was a commercial product (Boehringer-Mannheim) containing 4 units/mg of protein. The substrate used was *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucoside, and the assay procedure that of von Figura[24]; K_m = 1.4 ±0.2mM. The enzyme kinetic data were analyzed with a HP-85 microcomputer using a nonlinear-curve-fitting package developed by Greco *et al.*[25].

Biological assay. — Mouse mammary adenocarcinoma (TA-3) cells were grown in cell culture in Eagle's medium, and the extent of inhibition was expressed as an ID_{50} value as has been described earlier[26].

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