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Synthesis of α-glucosidase inhibitors: kojibiose-type pseudodisaccharides and a related pseudotrisaccharide[1]

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Abstract

Two kojibiose-type pseudo-disaccharides and a trisaccharide, containing a 5-amino-1,2,3,4-cyclopentanetetrol derivative or valienamine, linked by way of nitrogen bridges to the sugar residues, have been designed and synthesized as processing α-glucosidase I inhibitors. Synthesis of the pseudodisaccharides was carried out starting from the coupling products of the sugar isothiocyanates and an aminocyclitol, respectively, by cyclization with mercury(II) oxide to the cyclic isoureas and subsequent deprotection. Pseudokojibiose was prepared in a poor yield by reaction of a protected valienamine and a sugar epoxide, followed by deprotection. Although the pseudooligosaccharides are all strong inhibitors of α-glucosidase (baker's yeast), they did not have any inhibitory potency against either sucrase isomaltase (rat intestine) or processing α-glucosidase (rat liver microsomes).

Keywords: Pseudooligosaccharides; Carbaoligosaccharides; Carbohydrate mimics; α-Glucosidase inhibitors; Glycoprotein-processing glucosidase I inhibitors

1. Introduction

Trimming of glucose residues on the oligosaccharide moiety of human immunodeficiency virus (HIV) glycoprotein is crucial for HIV infection, and processing glucosidase inhibitors have been found to exhibit anti-HIV in vitro [2–5]. Based on these results, a number of N-substituted deoxynojirimycins, including the N-butyl derivative (*N*-butyl DNJ), have so far been tested as potential anti-HIV compounds [6]. Recently, studies on the elucidation of structure–activity relationships of derivatives of the trehalase inhibitor, trehazoline [7], have led to a development of highly potent α-glucosidase inhibitors composed of cyclic isourea derivatives of certain stereoisomers of 5-amino-1-(hydroxymethyl)-1,2,3,4-cyclopentanetetrols[2] having N-substituted cyclic isourea functions [8]. In particular, N-butyl cyclic isourea [9] of the 1L-(1,2,4,5/3) isomer has been shown to possess a

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[1] Pseudosugars, Part 38: For Part 37, see ref. [1].

[2] In this paper, nomenclature of pseudooligosaccharides follow IUPAC and IUB 1996 recommendations for carbohydrates (*Pure Appl. Chem.*, 68 (1996) 1919–2008; *Carbohydr. Res.*, 297 (1997) 1–92) and 1973 recommendations for cyclitols (*Pure Appl. Chem.*, 37 (1974) 285–297).

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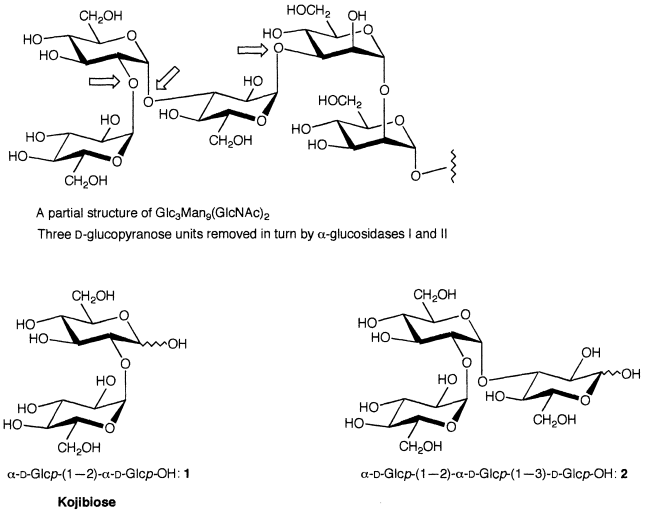


Scheme 1.

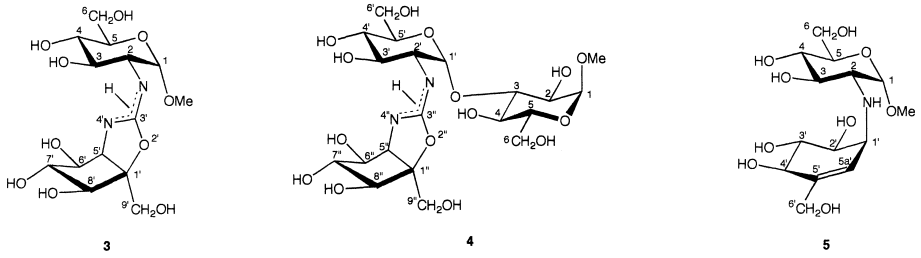
high inhibitory potential against both α-glucosidase (baker's yeast) and glucosidase I (yeast) (IC_{50} 4.2×10^{-6} M) [10], being almost comparable to *N*-butyl DNJ. In the examination of the potentials of pseudoamino sugars (valiolamine, valienamine, etc.) as processing glucosidase inhibitors [11], kojibiose has been shown to be a moderate inhibitor of glucosidase I (rat liver microsomes). This suggested that kojibiose-type pseudodisaccharides would act as transition-state analogues of these enzymes. Thus, pseudo di- and trisaccharides, composed of the *N*-(2-deoxy-α-D-glucos-2-yl) cyclic isoureas of 5-amino-1-(hydroxymethyl)-1,2,3,4-cyclopentanetetraol, were chosen to improve hopefully the specificity of the inhibitory potential against glucosidase I. In this study, three pseudooligosaccharides **3**, **4**, and **5** have been designed, as processing glucosidase inhibitors, to mimic the terminal sequence di- and trisaccharide portions of the *N*-glycan tetradecasaccharide chain Glc_3 $Man_9GlcNAc_2$: α-D-glucopyranosyl-(1→2)-α-D-glucopyranose (**1**) and α-D-glucopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranose (**2**) (Scheme 1).

Compounds **3** and **4** have cyclic isourea derivatives as their nonreducing moieties. In addition, pseudokojibiose **5**, containing unsaturated carba-amino sugar valienamine [12] in place of the nonreducing glucopyranose residue, i.e., the analog of the strong α-glucosidase inhibitor methyl acarviosin [13], was designed to compare its activity favorably to that of **3** (Scheme 2).

2. Results and discussion

Synthesis of methyl α-kojibioside mimic **3**, the nonreducing α-glucopyranose residue being replaced by the N-linked cyclic isourea moiety composed of 5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclopentanetetrol, was carried out by coupling of a newly prepared sugar isothiocyanate **10** and the aminocyclitol [7,8] **18**, and cyclization of the resulting thiourea in the presence of yellow mercury(II) oxide [7,14], followed by deprotection. Alternatively, the trisaccharide analogue **4** was prepared similarly from new disaccharide isothiocyanate **17** and **18**.

First, starting from known 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-β-D-glucopyranose [15,16] (**6**), two sugar isothiocyanates **10** and **17** were prepared. Thus, methanolysis of **6** with methanolic hydrochloric acid proceeded very slowly to give poor yields of methyl α- (**7**, 16%) and β-glucopyranoside derivatives (**8**, 10%), together with **6** recovered (62%). Conversion to the methyl glucosides would be improved by mainitaining the concentration of hydrochloric acid under prolonged reaction time. The α-anomer was benzylated with benzyl bromide and sodium hydride in DMF to give the known tribenzyl ether **9** [17,18] (93%), which was reduced with hydrogen sulfide in aq pyridine and subsequently treated with 1,1′-thiocarbonyldiimidazole [19] in dichloromethane at room temperture to give crystalline isothiocyanate **10** (93%). On the other

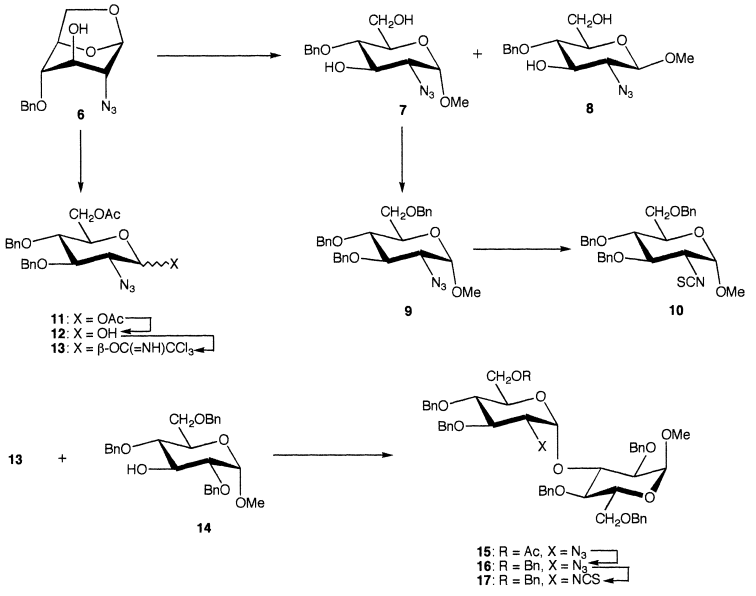


Scheme 2.

hand, acetolysis of the dibenzyl ether obtained [16] from **6** gave the diacetate **11** (~100%), which was treated with titanium tetrabromide [16] in chloroform containing acetic acid, giving a 57% yield of an anomeric mixture of the 6-acetate **12**. Compound **12** was then converted in the conventional manner [20] into the β-trichloroacetimidate **13** (68%). Condensation of **13** with methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside [21] **14** using trimethylsilyltrifluoromethanesulfonate in diethyl ether [20] gave the disaccharide derivative **15** (84%), the ^{1}H NMR spectrum of which revealed a doublet (δ 5.46, *J* 3.7 Hz) due to H-1′, supporting the α-glucopyranoside structure. Zemplén O-deacylation [22] of **15**, followed by benzylation, gave the hexabenzyl ether **16** in 65% yield. The azido group was reduced, and the resulting amine was similarly converted into the isothiocyanate **17** (94%) (Scheme 3).

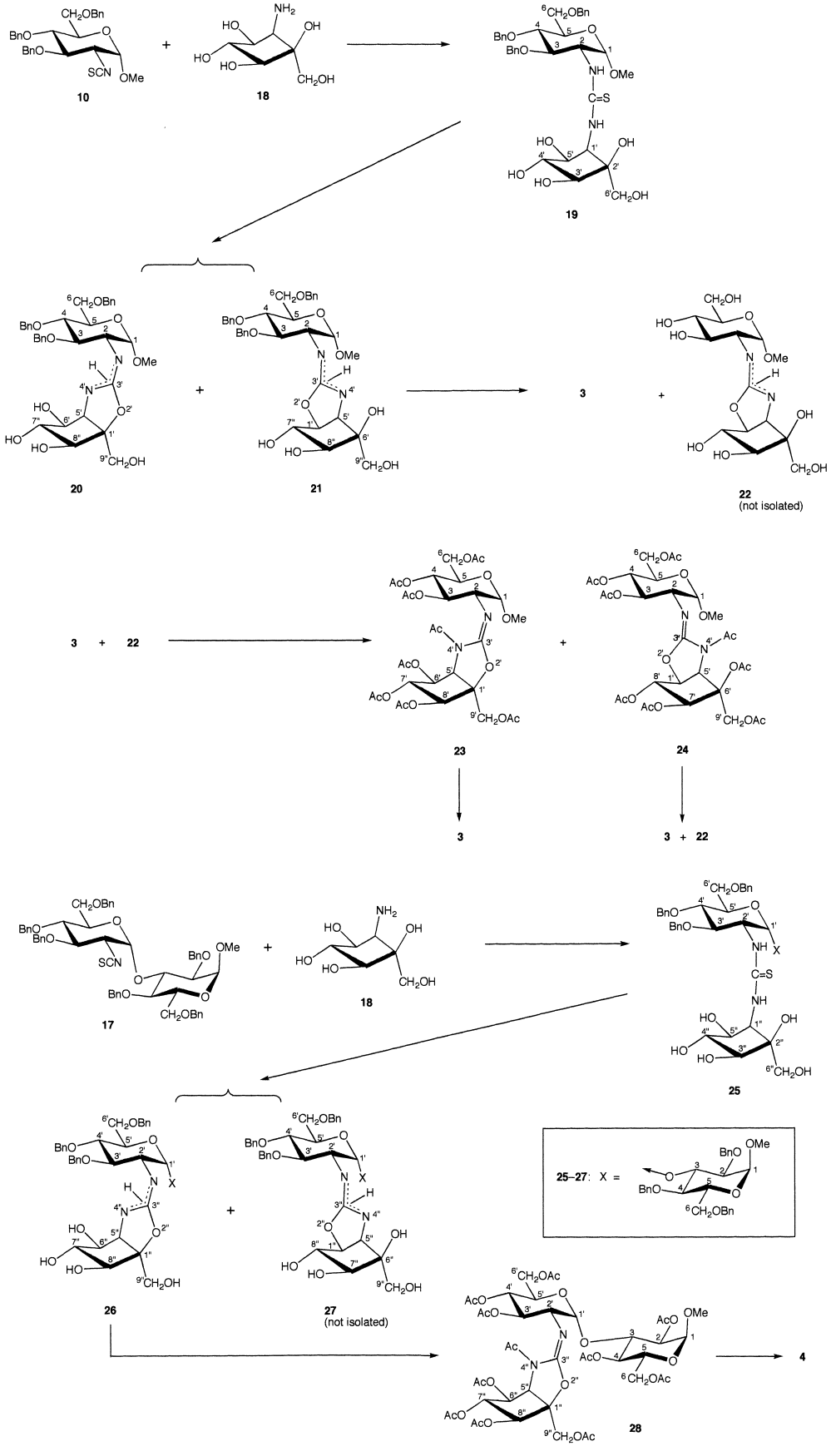
Coupling of the sugar isothiocyanates **10** and **17** with 1L-(1,2,4,5/3)-5-amino-1-hydroxymethyl-1,2,3,4-cyclopentanetetrol [7] (**18**) was conducted essentially similar to the preparation [7] of trehazolin derivatives. Thus, a mixture of **10** (0.28 mmol) and the aminocyclitol **18** (0.23 mmol) in aqueous 75% DMF was allowed to react for 21 h at room temp, giving the thiourea **19** (97%), which was then treated with excess of mercury(II) oxide in 1:6 acetone–diethyl ether to give rise to a 79% of the cyclic isoureas **20** and **21**. The mixture was reduced under Birch conditions, and the free bases **3** and **22** were subsequently acetylated to give, after separation by chromatography on silica gel column, the octa-*N*,*O*-acetyl derivatives **23** (53%) and **24** (9%) of the cyclic isoureas. Judging from the ^{1}H NMR data of **23** and **24**, they are considered to be single compounds, respectively, but there are no data to determine which isomeric form (syn- or anti-giometrical structure) they adopt. The product ratio seemed to largely depend on a stablity of the isomeric free bases **3** and **22** interconvertible under neutral to basic conditions. In general, the tautomers containing a tertiary hydroxyl in the ring are more stable [7]. Zemplén N,O-deacylation [22] of **23** at −15 °C, followed by purification on a column of Dowex-50W×2 (H^+) resin with 0.5 M aqueous ammonia, afforded **3** in 85% yield. Similar deacylation of **24** gave only **3** instead of **22**. Compound **3** is spectroscopically a single compound, no data being available to determine whether there exists the stable tautomer with regards to the position of unsaturation or a rapid interconversion between two possible tautomers (Scheme 4).

Similar coupling of molar equivalents of **17** and **18** gave the thiourea **25** (76%), which was cyclized by treatment with mercury(II) oxide to afford the isomeric mixture of cyclic isoureas **26** and **27**, Birch



Scheme 3.

[3] For the nomenclature of valienamine, an unsaturated 5a-carba-amino sugar, see ref. [24].


10
18
19
20
21
3
22
(not isolated)
3 + 22
23
24
3
3 + 22
17
18
25
25–27: X =
26
27
(not isolated)
28
4

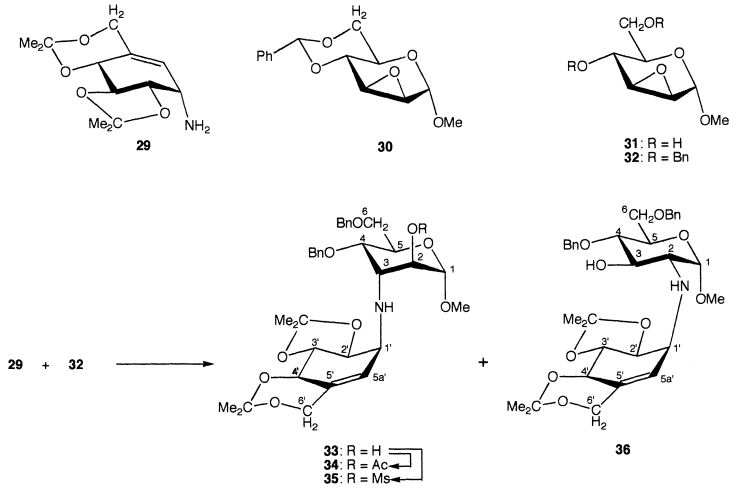
Scheme 4.

reduction of which gave, after subsequent acetylation and isolation, the undeca-*N*,*O*-acetyl derivative **28** (16%). The free base **4** (93%) was generated from **28**.

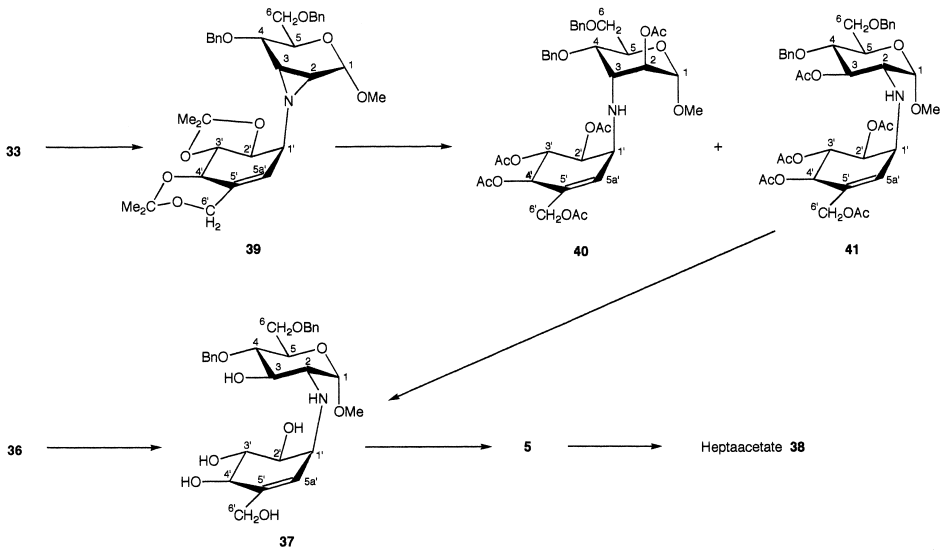
The disaccharide mimic **5**, containing valienamine moiety linked by way of an imino bridge, was initially prepared as a minor diequatorial opening product by condensation of the sugar epoxide [23] **32** and di-*O*-isopropylidenevalienamine [2,3:4,6-di-*O*-isopropylidene-5a-carba-*xylo*-hex-5(5a)-enopyranosylamine[3]] [25] **29**. In order to improve a yield of desired diequatorial opening products of the nucleophilic substitution reaction between **29** and methyl 2,3-anhydro-α-D-mannopyranoside [26] **31**, two protected compounds, the 4,6-*O*-benzylidene [23] **30** and 4,6-di-*O*-benzyl derivatives [27] **32**, were subjected to a coupling reaction with **29** in 2-propanol at 120 °C. Reaction of **29** and **30** or **31** afforded exclusively a single positional isomer the diaxially opened product with the D-*altro*-configuration in high yields. However, when the dibenzyl ether **32** was used, the desired minor product **36** was obtained in 6% yield, along with the major isomer **33** (66%). The structure of **33** was confirmed by the ^{1}H NMR spectrum of the *O*-acetyl derivative **34** conventionally derived from it. Conventional acetylation of valienamine derivatives related to validoxylamine [28] or methyl acarviosin [29] did not give rise to the corresponding *N*-acetyl derivatives, probably due to steric hindrance (Scheme 5).

Then, attempts were made to transform **33** into **36** via an aziridine derivative. Thus, conventional mesylation of **33** gave the mesylate **35**, which was treated with DBU in toluene at 60 °C to give the aziridine **39** (95%). Reaction of **39** with sodium acetate in 80% aq acetic acid for 3 days at 90 °C, followed by acetylation, produced about a 4:5 mixture of **40** and **41** (difficulty separable) in 74% yield. The mixture was O-deacetylated, and the products were separated by preparative TLC to give the dibenzyl ether **37** (32%) as the only pure compound, which was identical to the compound obtained from **36** by O-deisopropylidenation. Birch reduction of **37** and purification by the resin column afforded the targeted compound **5** (78%) as a hygroscopic powder. The structure was confirmed by the ^{1}H NMR spectrum of its heptaacetate **38** (Scheme 6).

Biological assay.—Compounds **3**, **4**, and **5** were first assayed for inhibitory activity against α-glucosidase (Baker's yeast). The former two containing a cyclic isourea moiety have been shown to be very strong inhibitors (Table 1). The inhibitory potential of **3** is almost comparable to that of the respective *N*-phenyl cyclic isourea [9,10]. The corresponding valienamine analogue **5** shows about 250-fold lower activity, conceibably demonstrating that the cyclic isourea derivative of **18** more resembles in these cases the half-chair transition state postulated during hydrolysis of α-glucosides. However, all compounds did not possess any inhibitory activity against both maltase (porcine) and processing glucosidase I (rat liver microsomes) [30]. They should surely be tested for other processing glycosidases from different species in future. The present results suggested that design of the transition-state mimicking would not always lead to a finding an inhibitor of processing glucosidase.



Scheme 5.



Scheme 6.

3. Experimental

General methods.—Melting points were determined with a Mel-Temp capillary melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-370. IR spectra were recorded with Jasco IR-810 or Hitachi Bio-Rad Degilab FTS-65. ^{1}H NMR spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Jeol JNM GSX-270 ft (270 MHz) instrument. TLC was performed on Silica Gel 60 GF (E. Merck, Darmstadt) with detection by charring with concd H_2SO_4. Column chromatography was conducted on Wakogel C-300 (silica gel, 300 Mesh, Wako Chemical, Osaka). Organic solutions were dried over anhyd Na_2SO_4, and evaporated at < 50 °C under diminished pressure.

*Methyl 2-azido-4-*O*-benzyl-2-deoxy-*α*-* (**7**) *and* β-D-*glucopyranosides* (**8**).—To methanolic hydrochloric acid, prepared by treatment of methanol (3 mL) with acetyl chloride (0.5 mL) at 0 °C, was added 1,6-anhydro-2-azido-4-*O*-benzyl-2-deoxy-*β*-D-glucopyranose [16] (**6**, 84 mg, 0.30 mmol), and the mixture was stirred for 18 h at 60 °C. The mixture was neutralized with triethylamine and then evaporated. The products were chromatographed on silica gel (9 g, 1:3 ethyl acetate–toluene) to give the *β*-glucoside **8** (9.4 mg, 10%) and the *α*-glucoside **7** (15 mg, 16%) as crystals, together with **6** (52 mg) that was recovered.

Table 1
Inhibitory activity against three enzymes [a]

Compound	*α*-Glucosidase (baker's yeast) [b] [IC_{50} (M)]	Sucrase and isomaltase (Rat intestine) [c]	Processing glucosidase (Rat liver microsomes) [d]
3	1.2×10^{-8}	NI	NI
4	1.8×10^{-7}	NI	NI
5	3.1×10^{-6}	NI	NI

[a] NI: No inhibitory activity was observed.
[b] Substrate: *p*-Nitrophenyl *α*-D-glucopyranoside.
[c] Substrate: Maltose.
[d] Substrate: $Glc_3Man_9GlcNAc_2$.

For compound **7**: mp 97–98 °C (from ethyl acetate); R_f 0.25 (1:3 ethyl acetate–toluene); $[\alpha]_D^{21}$ +147° (*c* 1.2, $CHCl_3$); IR (neat): *ν* 3500 (OH) and 2100 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): *δ* 7.42–7.25 (5 H, m, Ph), 4.80 and 4.75 (ABq, J_{gem} 11.7 Hz, PhCH_2), 4.77 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.09 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 8.8 Hz, H-3), 3.85 (dd, 1 H, $J_{5,6a}$ 2.9, J_{6gem} 12.1 Hz, H-6a), 3.77 (dd, 1 H, $J_{5,6b}$ 3.7 Hz, H-6b), 3.66 (ddd, 1 H, $J_{4,5}$ 9.9, H-5), 3.46 (dd, 1 H, H-4), 3.40 (s, 3 H, Me), 3.22 (dd, 1 H, H-2). Anal. Calcd for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.58. Found: C, 54.30; H, 6.20; N, 13.67.

For compound **8**: mp 140–141 °C (from ethanol); R_f 0.31 (1:3 ethyl acetate–toluene); $[\alpha]_D^{25}$ −14° (*c* 1.1, $CHCl_3$); IR (neat): *ν* 3350 (OH) and 2110 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$): *δ* 7.42–7.25 (m, 5 H, Ph), 4.82 and 4.73 (ABq, J_{gem} 11.4 Hz, PhCH_2), 4.24 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 3.91 (dd, 1 H, $J_{5,6a}$ 2.6, J_{6gem} 12.1 Hz, H-6a), 3.77 (dd, 1 H, $J_{5,6b}$ 4.0 Hz, H-6b), 3.57 (s, 3 H, Me), 3.55–3.47 (m, 2 H, H-3, 4), 3.27 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2). Anal. Found: C, 54.10; H, 6.24; N, 13.37.

*Methyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-*α*-D-glucopyranoside* (**9**).—To a solution of **7** (478 mg,

1.55 mmol) in DMF (10 mL) was added at 0 °C sodium hydride (250 mg, 6.2 mmol) followed by benzyl bromide (550 μL, 4.6 mmol), and the mixture was then stirred for 3 h at room temp. The mixture was processed in the conventional manner. The crude product was chromatographed on silica gel (40 g, 1:9 ethyl acetate–hexane) to give **9** (704 mg, 93%) as a syrup: ^{1}H NMR ($CDCl_3$): δ 7.20–7.15 (m, 15 H, 3×Ph), 4.88 and 4.84 (ABq, J_{gem} 11.0 Hz, PhCH_2), 4.82 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.80 and 4.51, and 4.63 and 4.48 (ABq, J_{gem} 11.0 Hz, 2×PhCH_2), 3.97 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 8.4 Hz, H-3), 3.81–3.64 (m, 4 H, H-4,5,6), 3.44 (dd, 1 H, H-2), 3.42 (s, 3 H, Me). The spectral data were shown to be partly identical to those reported for an authentic sample [17]. This compound was used in the next step without further purification.

Methyl 3,4,6-tri-O-*benzyl-2-deoxy-2-isothiocyanato*-α-D-*glucopyranoside* (**10**).—H_2S was introduced into a solution of crude **9** (331 mg, 0.677 mmol) in a mixture of pyridine (8 mL) and water (4 mL) for 7 h at room temperature, and then the mixture was purged with N_2 for 30 min. The mixture was evaporated to dryness, and the residue was chromatographed on silica gel (16 g, toluene → 1:6 ethanol–toluene) to give a crude amine (~300 mg). The amine was dissolved in dichloromethane (6 mL), and the solution was treated with 1,1′-thiocarbonyldiimidazole (347 mg, 1.95 mmol) for 1.5 h at room temp and then evaporated. The residual product was chromatographed on silica gel (18 g, 1:10 ethyl acetate–hexane) to give **10** (317 mg, 92.7%) as crystals: mp 75–77 °C (from hexane); R_f 0.63 (1:4 ethyl acetate–hexane); $[\alpha]_D^{22}$ + 164° (*c* 0.77, $CHCl_3$); IR (neat): ν 2075 cm^{-1} (N=C=S); ^{1}H NMR ($CDCl_3$): δ 7.41–7.12 (m, 15 H, 3×Ph), 4.90 and 4.83 (ABq, J_{gem} 11.0 Hz, PhCH_2), 4.61 and 4.48 (ABq, J_{gem} 12.1 Hz, PhCH_2), 3.98 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 8.8 Hz, H-3), 3.82–3.79 (m, 1 H, H-5), 3.75 (dd, 1 H, $J_{1,2}$ 3.3 Hz, H-2), 3.74 (dd, 1 H, $J_{5,6a}$ 3.7, J_{6gem} 10.7, H-6a), 3.64 (dd, 1 H, $J_{5,6b}$ 1.9 Hz, H-6b), 3.63 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.43 (s, 3 H, Me). Anal. Calcd for $C_{29}H_{31}NO_5S$: C, 68.90; H, 6.18; N, 2.77. Found: C, 68.85; H, 6.16; N, 3.06.

6-O-*Acetyl-2-azido-3,4-di*-O-*benzyl-2-deoxy*-D-*glucopyranose* (**12**).—To a solution of a mixture (**11**, 164 mg, 0.351 mmol) of 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α- and β-D-glucopyranose acetates, prepared from **6** according to the procedure of Paulsen and Stenzel [16] in 1:10 acetic acid–chloroform (2.2 mL), was added titanium tetrabromide (193 mg, 0.527 mmol), and the mixture was stirred for 12 h at room temp. The mixture was diluted with chloroform (50 mL), washed with saturated aq $NaHCO_3$, dried, and evaporated. The residue was dissolved in acetone (2 mL), and the solution was treated with silver carbonate (107 mg, 0.386 mmol) and water (7 μL, 0.386 mmol) for 1.5 h at room temp. The mixture was filtered through a Celite bed, and the filtrate was diluted with ethyl acetate (30 mL), washed with water, dried, and evaporated. The residue was chromatographed on silica gel (6.5 g, 1:5 ethyl acetate–hexane) to give a ca. 3:2 mixture **12** of the inseparable α- and β-anomers (85 mg, 57%) as a solid: mp 102–105 °C (from ethyl acetate); IR (neat): ν 3400 (OH), 2100 (N_3), and 1710 cm^{-1} (ester); ^{1}H NMR ($CDCl_3$): for α-anomer: δ 7.41–7.22 (m, 10 H, 2×Ph), 5.29 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.92 and 4.88, and 4.92 and 4.82 (2×ABq, J_{gem} 10.6 Hz, 2×PhCH_2), 4.34 (dd, 1 H, $J_{5,6a}$ 2.2, J_{gem} 12.1 Hz, H-6a), 4.21 (dd, 1 H, $J_{5,6b}$ 4.4 Hz, H-6b), 4.11 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 4.04 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 8.8 Hz, H-3), 3.55 (dd, 1 H, H-4), 3.43 (dd, 1 H, H-2), 2.04 (s, 3 H, Ac); for β-anomer: δ 7.41–7.22 (m, 10 H, 2×Ph), 4.88 and 4.60 (ABq, J_{gem} 9.5 Hz, PhCH_2), 4.86 and 4.58 (ABq, J_{gem} 10.6 Hz, PhCH_2), 4.59 (s, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6a}$ 1.5, J_{6gem} 12.1 Hz, H-6a), 4.18 (dd, 1 H, $J_{5,6b}$ 7.7 Hz, H-6b), 3.57–3.45 (m, 2 H, H-4,5), 3.47 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 9.2 Hz, H-3), 3.39 (dd, 1 H, $J_{1,2}$ 9.2 Hz, H-2), 2.03 (s, 3 H, Ac). Anal. Calcd for $C_{22}H_{25}N_3O_6$: C, 61.82; H, 5.90; N, 9.83. Found: C, 62.11; H, 6.02; N, 9.53.

6-O-*Acetyl-2-azido-3,4-di*-O-*benzyl*-β-D-*glucopyranose trichloroacetimidate* (**13**).—To a solution of **12** (63 mg, 0.15 mmol) in dichloromethane (2 mL) were added potassium carbonate (100 mg, 0.74 mmol, 5 molar equiv) and trichloroacetonitrile (60 μL, 0.6 mmol, 4 molar equiv), and the mixture was stirred for 7 h at room temp. The mixture was then filtered through a Celite bed, and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (8 g, 1:8 ethyl acetate–hexane) to give **13** (58 mg, 68%) as a syrup, $[\alpha]_D^{24}$ + 6.8° (*c* 1.5, $CHCl_3$); IR (neat): ν 2110 (N_3) and 1740 cm^{-1} (ester); ^{1}H NMR ($CDCl_3$): δ 7.40–7.25 (m, 10 H, 2×Ph), 5.62 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.94 and 4.85 (ABq, J_{gem} 10.6 Hz, PhCH_2), 4.85 and 4.59 (ABq, J_{gem} 11.0 Hz, PhCH_2), 4.32 (dd, 1 H, $J_{5,6a}$ 1.3, J_{6gem} 12.1 Hz, H-6a), 4.23 (dd, 1 H, $J_{5,6b}$ 3.7 Hz, H-6b), 3.69 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$

9.5 Hz, H-2), 3.64–3.55 (m, 2 H, H-4,5), 3.60 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 2.02 (s, 3 H, Ac).

Methyl 6-O-acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (**15**).—A mixture of **13** (149 mg, 0.26 mmol) and methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside [21] (**14**, 242 mg, 0.52 mmol) in diethyl ether (8 mL) was stirred in the presence of 4A molecular sieves (150 mg) under nitrogen for 1 h at room temp. The mixture was cooled to −15 °C, and 0.01 M trimethylsilyl trifluoromethanesulfonate (2.6 μL, 0.026 mmol, 0.1 molar equiv) was added, and the mixture was stirred for 45 min at the same temp. After neutralization with sodium hydrogencarbonate, the mixture was filtered and the filtrate was evaporated. The residue was chromatographed on silica gel (22 g, 1:17 ethyl acetate–toluene) to give **15** (191 mg, 84%) as a syrup: R_f 0.42 (1:5 ethyl acetate–toluene); $[\alpha]_D^{25}$ +48° (*c* 0.88, $CHCl_3$); IR (neat): ν 2100 (azide) and 1740 (ester) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.40–7.15 (m, 25 H, 5×Ph), 5.46 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.89 and 4.53 (ABq, J_{gem} 10.6 Hz, CH_2Ph), 4.89 (s, 2 H, CH_2Ph), 4.80 and 4.51 (ABq, J_{gem} 11.0 Hz, CH_2Ph), 4.59 and 4.52 (ABq, J_{gem} 11.7 Hz, CH_2Ph), 4.38 (m, 1 H, H-5′), 4.14 (dd, 1 H, $J_{2,3}$ 9.6, $J_{3,4}$ 8.1 Hz, H-3), 4.12 (dd, 1 H, $J_{5',6'a}$ 2.2, $J_{6'gem}$ 12.1 Hz, H-6′a), 3.97 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 8.8 Hz, H-3′), 3.89 (dd, 1 H, $J_{5',6'b}$ 3.7 Hz, H-6′b), 3.80–3.63 (m, 4 H, H-4,5,6), 3.52 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 9.6 Hz, H-2), 3.51 (dd, 1 H, $J_{4',5'}$ 10.3 Hz, H-4′), 3.34 (s, 3 H, OMe), 3.31 (dd, 1 H, $J_{1',2'}$ 3.7 Hz, H-2′), 2.00 (s, 3 H, Ac). Anal. Calcd for $C_{50}H_{55}N_3O_{11}$: C, 68.71; H, 6.34; N, 4.81. Found: C, 68.57; H, 6.38; N, 4.87.

Methyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (**16**).—A solution of **15** (168 mg, 0.193 mmol) in methanol (3 mL) was treated with methanolic M sodium methoxide for 2 h at room temp. After neutralization with Amberlite IR-120B (H^+) resin, the mixture was evaporated to dryness. The residue was dissolved in DMF (3 mL), and it was first treated with sodium hydride (23 mg, 0.58 mmol) for 0.5 h at 0 °C, and then with benzyl bromide (46 μL, 0.385 mmol) for 20 h at 0 °C. After addition of a small amount of methanol, the mixture was diluted with ethyl acetate (50 mL) and washed with water, dried, and evaporated. The residue was chromatographed on silica gel (15 g, 1:6 ethyl acetate–hexane) to give **16** (115 mg, 65%) as a syrup: R_f 0.81 (1:5 ethyl acetate–toluene); $[\alpha]_D^{21}$ +56° (*c* 0.89, $CHCl_3$); IR (neat): ν 2110 (azide) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.38–7.09 (m, 30 H, 6×Ph), 5.52 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.91 and 4.50 (ABq, J_{gem} 11.6 Hz, CH_2Ph), 4.90 and 4.86 (ABq, J_{gem} 11.7 Hz, CH_2Ph), 4.75 and 4.45 (ABq, J_{gem} 11.4 Hz, CH_2Ph), 4.71 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.65 and 4.29 (ABq, J_{gem} 12.1 Hz, CH_2Ph), 4.59 and 4.52 (ABq, J_{gem} 11.4 Hz, CH_2Ph), 4.56 and 4.51 (ABq, J_{gem} 12.1 Hz, CH_2Ph), 4.27–4.21 (m, 1 H, H-5′), 4.16 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 8.1 Hz, H-3), 3.98 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 8.8 Hz, H-3′), 3.81–3.64 (m, 5 H, H-4,5,6,4′), 3.51 (dd, 1 H, $J_{1,2}$ 3.7 Hz, H-2), 3.42–3.36 (m, 2 H, H-6′), 3.35 (s, 3 H, OMe), 3.34 (dd, 1 H, H-2′). Anal. Calcd for $C_{55}H_{59}N_3O_{10}$: C, 71.64; H, 6.45; N, 4.56. Found: C, 71.65; H, 6.47; N, 4.54.

Methyl 3,4,6-tri-O-benzyl-2-deoxy-2-isothiocyanato-α-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-α-D-glucopyranoside (**17**).—H_2S was slowly bubbled into a solution of the azide **16** (95 mg, 0.103 mmol) in 4:2:1 pyridine–water–triethylamine (5 mL) for 7 h at room temp. After purging the solution of H_2S with nitrogen for half an hour, the mixture was evaporated, and the residue was roughly chromatographed on a silica gel column (8 g, toluene → 1:20 ethanol—toluene). The crude amine obtained was dissolved in dichloromethane, and the resulting solution was treated with 1,1′-thiocarbonyldiimidazole (55.2 mg, 0.31 mmol) for 4 h at room temperature. The reaction mixture was evaporated, and the residue was chromatographed on a silica gel (9 g, 1:5 ethyl acetate–hexane) to give the isothiocyanate **17** (90 mg, 94%) as a colorless syrup: $[\alpha]_D^{25}$ +93° (*c* 1.2, $CHCl_3$); IR (neat): ν 2090 (N=C=S) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.40–7.05 (m, 30 H, 6×Ph), 5.53 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.89 and 4.86 (ABq, J_{gem} 12.7 Hz, PhCH_2), 4.82 and 4.42 (ABq, J_{gem} 11.0 Hz, PhCH_2), 4.75 and 4.52 (ABq, J_{gem} 11.4 Hz, PhCH_2), 4.74 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.65 and 4.27 (ABq, J_{gem} 12.1 Hz, PhCH_2), 4.60–4.49 (m, 4 H, 2×PhCH_2), 4.25–4.22 (m, 1 H, H-5′), 4.16 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 9.5 Hz, H-3), 3.95 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 9.2 Hz, H-3′), 3.83 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.75–3.67 (m, 3 H, H-5,6,4′), 3.62 (dd, 1 H, H-2′), 3.54 (dd, 1 H, H-2), 3.42–3.32 (m, 2 H, H-6′), 3.36 (s, 3 H, Me). Anal. Calcd for $C_{56}H_{59}NO_{10}S$: C, 71.70; H, 6.34; N, 1.49. Found: C, 71.77; H, 6.39; N, 1.51.

Methyl 3,4,6-tri-O-benzyl-2-deoxy-2-{N³-[(1R)-(1,2,3,5/4)-2,3,4,5-tetrahydroxy-2-(hydroxymethyl)-cyclopentyl]thioureido}-α-D-glucopyranoside (**19**).—A mixture of the isothiocyanate **10** (141 mg,

0.278 mmol) and 1L-(1,2,4,5/3)-5-amino-1-(hydroxymethyl)-1,2,3,4-cyclopentanetetrol [7] (**18**, 41.6 mg, 0.232 mmol) in 75% aq DMF was stirred for 21 h at room temp, and then evaporated. The residue was chromatographed on silica gel (7.5 g, 1:5 ethyl acetate–hexane → 1:4 ethanol–toluene) to give the thiourea **19** (154 mg, 97%) as a syrup, R_f 0.39 (1:4 ethanol–toluene) $[\alpha]_D^{22}$ +50° (*c* 0.81, $CHCl_3$); IR (neat): *ν* 3325 (OH and NH) and 1540 cm^{-1} (NH). Anal. Calcd for $C_{35}H_{44}N_2O_{10}S$: C, 61.39; H, 6.48; N, 4.09. Found: C, 61.17; H, 6.58; N, 3.99.

*(1*S*,5*R*,6*S*,7*R*,8*S*)-1-* (**20**) *and (1*S*,5*R*,6*S*,7*S*,8*S*)-6-Hydroxymethyl-3-(methyl 3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranos-2-yl)amino-2-oxa-4-azabicyclo[3.3.0]octane-6,7,8-triol* (**21**).—To a solution of the thiourea **19** (129 mg, 0.188 mmol) in 1:6 acetone–diethyl ether (3 mL) was added four portions of yellow mercury(II) oxide (120 mg, 0.56 mmol, totally 490 mg, 12 molar equiv) at intervals of 3, 8, 19 h, and the mixture was stirred for a total of 25 h. An insoluble material was removed by filtration through a Celite bed, and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (11 g, 1:4 ethanol–toluene) to give a mixture (96 mg, 77%) of **20** and **21** as a hygroscopic syrup: R_f 0.15 and 0.23 (1:2:10 acetic acid—ethanol–toluene); IR (neat) *ν* 3350 (OH and NH) and 1660 cm^{-1} (C=N). Anal. Calcd for $C_{35}H_{42}N_2O_{10}\cdot 0.5H_2O$: C, 63.72; H, 6.57; N, 4.25. Found: C, 63.34; H, 6.58: N, 4.23.

*(1*S*,5*R*,6*S*,7*R*,8*S*)-6,7,8-tri-*O*-Acetyl-1-* (**23**) *and (1*S*,5*R*,6*S*,7*S*,8*R*)-6,7,8-tri-O-acetyl-6-acetoxymethyl-3-(methyl 3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranos-2-yl)amino-2-oxa-4-azabicyclo[3.3.0]octane-6,7,8-triol* (**24**).—To liquid ammonia (5 mL) containing sodium (340 mg, 15 mmol) was added a solution of the mixture (96 mg, 0.15 mmol) of **20** and **21** in THF (2 mL) at −78 °C, and it was stirred for 15 h at the same temp. After addition of ammonium chloride (1.2 g, 22 mmol), the mixture was allowed to stand at room temperature, until all ammonia was removed by spontaneous evaporation. The residual product was dissolved in water (5 mL), the solution was washed with chloroform (3 mL×2), and the water layer was applied to a column of Dowex-50W×2 (H^+) resin and eluted with 0.25 M aq ammonia to give a mixture (38 mg, 63%) of the cyclic isoureas **3** and **22** as a white solid: R_f 0.12 (4:1 acetonitrile–water); IR (KBr): *ν* 3440 (OH and NH) and 1653 (C=N) cm^{-1}; ^1H NMR (D_2O): *δ* 4.32 (d, 0.3 H, $J_{1',5'}$ 9.2 Hz, H-5′), 4.16 (d, 0.7 H, $J_{5',6'}$ 7.3 Hz, H-5′).

The mixture was acetylated with acetic anhydride (1 mL) in pyridine (1 mL) for 4 h at room temp. The products were chromatographed on a silica gel column (5 g, 1:5 acetone–toluene) to give the octa-*N*,*O*-acetyl derivatives **23** (38 mg, 53%) and **24** (7 mg, 9%) as a syrup.

For compound **23**: R_f 0.38 (1:4 acetone–toluene); $[\alpha]_D^{19}$ +11.4° (*c* 1.3, $CHCl_3$); IR (neat): *ν* 1750 (ester) and 1690 (amide, C=N) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 5.51 (dd, 1 H, $J_{2,3}$ 10.3, *J*3,4 9.9 Hz, H-3), 5.46 (ddd, 1 H, $J_{5',6'}$ 7.3, $J_{6',7'}$ 7.3, $J_{6',8'}$ 0.7 Hz, H-6′), 5.36 (dd, 1 H, $J_{7',8'}$ 8.4 Hz, H-7′), 5.29 (dd, 1 H, H-8′), 5.09 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 4.95 (d, 1 H, H-5′), 4.75 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6a}$ 4.8, J_{6gem} 12.1 Hz, H-6a), 4.17 (s, 2 H, H-9′), 4.08 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 4.04 (ddd, 1 H, H-5), 3.92 (dd, 1 H, H-2), 3.37 (s, 3 H, Me), 2.49, 2.23, 2.12, 2.073, 2.070, 2.05, and 1.96 (7 s, 3, 3, 3, 3, 3, 3, 6, 3 H, 8×Ac). Anal. Calcd for $C_{30}H_{40}N_2O_{18}$: C, 50.28; H, 5.63; N, 3.91. Found: C, 50.12; H, 6.01; N, 3.77.

For compound **24**: R_f 0.29 (1:4 acetone–toluene), $[\alpha_D^{24}]$ +49° (*c* 0.4, $CHCl_3$); IR (neat): *ν* 1750 (ester) and 1700 (amide, C=N) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 5.52–5.45 (m, 2 H, H-7′,8′), 5.49 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.5 Hz, H-3), 5.20 (d, 1 H, $J_{1',5'}$ 8.4 Hz, H-5′), 5.03 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.93 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.80 and 4.64 (ABq, J_{gem} 11.5 Hz, H-9′), 4.69 (br d, H-1′), 4.31 (dd, 1 H, $J_{5,6a}$ 4.8, J_{6gem} 12.1 Hz, H-6a), 4.09 (dd, 1 H, $J_{5,6b}$ 2.4 Hz, H-6b), 4.01 (ddd, 1 H, H-5), 3.84 (dd, 1 H, H-2), 3.38 (s, 3 H, Me), 2.41, 2.13, 2.11, 2.10, 2.08, 2.07, 2.02, and 1.96 (8 s, each 3 H, 8 Ac). Anal. Found: C, 50.22; H, 5.87; N, 3.93.

*Methyl 2-deoxy-2-{(1*S*,5*R*,6*S*,7*R*,8*S*)-6,7,8-trihydroxy-1-(hydroxymethyl)-2-oxa-4-azabicyclo[3.3.0]octan-3-ylideneamino}-α-D-glucopyranoside* (**3**).—Compound **23** (24 mg, 0.032 mmol) was treated with methanolic sodium methoxide (0.2 mL) in methanol (1 mL) for 2.5 h at −15 °C. The product was chromatographed on Dowex-50W×2 (H^+) resin (2 mL, 0.5 M aq ammonia) to give **3** (11 mg, 85%) as a white powder, R_f 0.24 (1:2:10 acetic acid–water–acetonitrile), $[\alpha]_D^{20}$ +87.5° (*c* 0.56, water); IR (neat): *ν* 3450 (OH, NH) and 1690 (C=N) cm^{-1}; ^1H NMR (D_2O): *δ* 4.79 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.01 (d, 1 H, $J_{5',6'}$ 6.2 Hz, H-5′), 3.74 (dd, 1 H, $J_{5,6a}$ 2.8, J_{6gem} 12.3 Hz, H-6a), 3.69–3.50 (m, 6 H, H-5,6′,7′,8′,9′), 3.57 (dd, 1 H, $J_{5,6b}$ 5.1 Hz, H-6b), 3.54 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 8.4 Hz, H-3), 3.49 (dd, 1 H, H-2), 3.35 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 3.27 (s, 3 H, Me).

Methyl 3,4,6-tri-O-*benzyl-2-deoxy-2-{N³-[(1R)-(1,2,3,5/4)-2,3,4,5-tetrahydroxy-2-(hydroxymethyl)-cyclopentyl]thioureido}*-α-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*benzyl*-α-D-*glucopyranoside* (**25**).—A mixture of the isothiocyanate **17** (86.5 mg, 0.092 mmol) and the aminocyclitol **18** (17 mg, 0.095 mmol) in 3:3:2 DMF–THF–water (4 mL) was stirred for 5 days at room temperature, and then evaporated. The residue was chromatographed on silica gel (7 g, 1:4 ethyl acetate–hexane → 1:10 ethanol–toluene) to give the thiourea **25** (79 mg, 76%) as a syrup: R_f 0.61 (1:4 ethanol–toluene); $[\alpha]_D^{22}$ +16° (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{62}H_{72}N_2O_{15}S$: C, 66.65; H, 6.50; N, 2.51. Found: C, 66.88; H, 6.54; N, 2.49.

Methyl 3,4,6-tri-O-*benzyl-2-deoxy-2-[(1*S*,5*R*,6*S*,7*R*,8*S*)-6,7,8-trihydroxy-1-(hydroxymethyl)-* (**26**) *and Methyl 3,4,6-tri*-O-*benzyl-2-deoxy-2-[(1*S*,5*R*,6*S*,7*S*,8*S*)-6,7,8-trihydroxy-6-(hydroxymethyl)-2-oxa-4-azabicyclo[3.3.0]octan-3-ylideneamino]*-α-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*benzyl*-α-D-*glucopyranoside* (**27**).—The thiourea **25** (34 mg, 0.0304 mmol) was dissolved in 1:6 acetone–diethyl ether (1 mL), and seven portions of mercury(II) oxide (20 mg, 3.0 equiv) were added in the interval of 3 h for 30 h. An insoluble material was removed by filtration through a Celite bed, and the filtrate was evaporated to give a mixture (33 mg, ~100%) of **26** and **27**, R_f 0.47 and 0.36 (1:2:10 acetic acid–ethanol–toluene), IR (neat), *ν* 3350 (OH and NH) and 1690 (C=N) cm^{-1}. Anal. Calcd for $C_{62}H_{70}N_2O_{15}\cdot 1.5H_2O$: C, 67.07; H, 6.63; N, 2.52. Found: C, 66.80: H, 6.43; N, 2.60.

Methyl 3,4,6-tri-O-*acetyl-2-deoxy-2-[*N*-acetyl-(1*S*,5*R*,6*S*,7*R*,8*S*)-6,7,8-triacetoxy-1-(acetoxymethyl)-2-oxa-4-azabicyclo[3.3.0]octan-3-ylideneamino]*-α-D-*glucopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl*-α-D-*glucopyranoside* (**28**).—To liquid ammonia (5 mL) containing sodium (70 mg, 3.05 mg·atom, 100 equiv) was added a solution of a mixture (33 mg, 0.03 mmol) of the hexabenzyl ethers **26** and **27** in THF (2 mL) at −78 °C, and the mixture was stirred for 15 min at the same temperature. After addition of ammonium chloride (244 mg, 4.6 mmol), the mixture was allowed to reach room temp, the ammonia being removed by spontaneous evaporation. The residual product was diluted with water (5 mL), the solution was washed with chloroform (3 mL×2), and the water layer was evaporated to dryness. The residue was acetylated with acetic anhydride (0.5 mL) in pyridine (1 mL) overnight at room temp, and the product was purified by silica gel column chromatography (1.2 g, 1:5 acetone–toluene) to give the peracetate **28** (5.0 mg, 16%) as a syrup: R_f 0.63 (1:2 acetone-toluene), $[\alpha]_D^{19}$ +56° (*c* 0.25, $CHCl_3$), IR (neat) 1725 (OAc) and 1690 (C=N) cm^{-1}, ^1H NMR ($CDCl_3$) *δ* 5.45 (dd, 1 H, $J_{5'',6''}$ 7.7, $J_{6'',7''}$ 8.1 Hz, H-6″), 5.35 (dd, 1 H, $J_{7'',8''}$ 8.8 Hz, H-7″), 5.34 (dd, 1 H, $J_{2',3'}$ 9.9, $J_{3',4'}$ 8.8 Hz, H-3′), 5.29 (d, 1 H, H-8″), 5.07 (dd, 1 H, $J_{4',5'}$ 10.3 Hz, H-4′), 5.06 (dd, 1 H, $J_{3,4}$ 8.8, $J_{4,5}$ 10.3 Hz, H-4), 4.99 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.95 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.94 (d, 1 H, H-5″), 4.79 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 4.38–4.26 (m, 2 H, H-6′), 4.31 (dd, 1 H, H-3), 4.23–4.11 (m, 1 H, H-5′), 4.18 (dd, 1 H, $J_{5,6}$ 2.2, J_{6gem} 12.5 Hz, H-6), 3.92 (dd, 1 H, H-2′), 3.84 (ddd, 1 H, H-5), 3.37 (s, 3 H, Me), 2.59, 2.23, 2.17, 2.15, 2.13, 2.07, 2.06, 2.05, 2.04, 2.03, and 1.96 (11 s, each 3 H, 11×Ac). Anal. Calcd for $C_{42}H_{56}N_2O_{26}$: C, 50.20; H, 5.62; N, 2.79. Found: C, 50.29; H, 5.82; N, 2.90.

*Methyl 2-deoxy-2-[(1*S*,5*R*,6*S*,7*R*,8*S*)-6,7,8-trihydroxy-1-(hydroxymethyl)-2-oxa-4-azabicyclo[3.3.0]octan-3-ylideneamino]*-α-D-*glucopyranosyl-(1→3)*-α-D-*glucopyranoside* (**4**).—A solution of **28** (5.0 mg, 5.0 mmol) in methanol (1 mL) was treated with M methanolic sodium methoxide (0.2 mL) at −15 °C for 4 h. The solution was applied to a column of Dowex-50W×2 (H^+) resin that was eluted with 0.5 M aq ammonia to give **4** (2.5 mg, 93%) as a white solid: R_f 0.16 (1:2:10 acetic acid–water–acetonitrile), $[\alpha]_D^{22}$ +203° (*c* 0.19, water); IR (KBr disk) *ν* 3430 (NH and OH) and 1655 (C=N) cm^{-1}, ^1H NMR (D_2O): *δ* 5.24 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1′), 4.67–4.62 (m, 1 H, H-1), 4.05 (d, 1 H, $J_{5'',6''}$ 6.2 Hz, H-5″), 3.89–3.48 (m, 15 H, H-2,3,4,5,6,2′,3′,6′, 6″,7″,8″,9″), 3.41 (dd, 1 H, $J_{3',4'}$ 9.2, $J_{4',5'}$ 9.9 Hz, H-4′), 3.28 (s, 3 H, Me).

Methyl 4,6-di-O-*benzyl-3-deoxy-3-[2,3:4,6-di*-O-*isopropylidene-5a-carba*-α-D-*xylo-5(5a)-enopyranosylamino]*-α-D-*altropyranoside* (**33**) *and methyl 4,6-di*-O-*benzyl-2-deoxy-2-[2,3:4,6-di*-O-*isopropylidene-5a-carba*-α-D-*xylo-hex-5(5a)-enopyranosylamino]*-α-D-*glucopyranoside* (**36**).—A mixture of 2,3:4,6-di-*O*-isopropylidene-5a-carba-α-D-*xylo*-hex-5(5a)-enopyranosylamine[4] [23] (**29**, 74 mg, 0.29 mmol) and methyl 2,3-anhydro-4,6-di-*O*-benzyl-α-D-glucopyranoside (**32**, 85 mg, 0.24 mmol) in 2-propanol (0.50 mL) was heated in a sealed tube for 5 days at 120 °C, and then evaporated. The residual products were chromatographed on a silica gel column

[4] Compound **29** was provided by hydrogenolysis of the corresponding azido compound with Raney nickel catalyst and used directly without purification.

(14 g, 1:3 ethyl acetate–toluene) to give first the *gluco* isomer **36** (8.1 mg, 5.6%) as a slightly yellow syrup, and then the *altro* isomer **33** (96 mg, 66%) as a colorless syrup.

A 23 mg-portion of the major product **33** was acetylated with acetic anhydride in pyridine to give the 2-acetate **34** (21 mg, 87%) as a syrup.

For compound **34**: R_f 0.52 (1:3 ethyl acetate–toluene), $[\alpha]_D^{23}$ +122° (*c* 1.1, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 7.82–7.37 (m, 10 H, 2×Ph), 5.49 (br d, 1 H, $J_{1',5a'}$ 4.8 Hz, H-5a′), 5.20 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.7 Hz, H-2), 4.68 and 4.56 (ABq, J_{gem} 12.5 Hz, PhCH_2), 4.65 (br s, 1 H, H-1), 4.58 and 4.43 (ABq, J_{gem} 12.5 Hz, PhCH_2), 4.47 (br d, 1 H, $J_{3',4'}$ 8.1 Hz, H-4′), 4.41 and 4.12 (ABq, $J_{6'gem}$ 13.9 Hz, H-6′), 4.05–4.00 (m, 1 H, H-5), 4.02 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-3′), 3.82–3.75 (m, 3 H, H-4,6), 3.69 (dd, 1 H, $J_{1',2'}$ 4.4 Hz, H-1′), 3.53 (dd, 1 H, H-6), 3.31 (br t, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 2.08 (s, 3 H, Ac), 1.55, 1.49, 1.46, and 1.42 (4 s, each 3 H, 2×CMe_2). Anal. Calcd for $C_{36}H_{46}NO_{10}$: C, 66.24; H, 7.10; N, 2.14. Found: C, 66.10; H, 7.25; N, 2.45.

For compound **36**: R_f 0.61 (1:3 butanone–toluene), $[\alpha]_D^{21}$ +9.2° (*c* 1.2, $CHCl_3$), ^{1}H NMR ($CDCl_3$): δ 7.34–7.23 (m, 10 H, 2×Ph), 5.55 (d, 1 H, $J_{1',5a'}$ 4.8 Hz, H-5a′), 4.93 and 4.54 (ABq, J_{gem} 11.5 Hz, PhCH_2), 4.85 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.63 and 4.50 (ABq, J_{gem} 12.5 Hz, PhCH_2), 4.49 (d, 1 H, $J_{3',4'}$ 8.1 Hz, H-4′), 4.46 and 4.17 (ABq, 1 H, $J_{6'gem}$ 13.9 Hz, H-6′), 4.01 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-3′), 3.79 (dd, 1 H, $J_{1',2'}$ 5.1 Hz, H-1′), 3.76 (dd, 1 H, $J_{5,6a}$ 6.9, J_{6gem} 9.5 Hz, H-6a), 3.75 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 8.8 Hz, H-3), 3.73 (dd, 1 H, $J_{4,5}$ 8.1 Hz, H-4), 3.57 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.56 (m, 1 H, H-5), 3.37 (s, 3 H, Me), 2.73 (dd, 1 H, H-2), 1.55, 1.48, 1.47, and 1.42 (4 s, each 3 H, 2×CMe_2). Anal. Calcd for $C_{34}H_{45}NO_9$: C, 66.75; H, 7.41; N, 2.29. Found: C, 66.64; H, 7.47; N, 2.04.

Methyl 4,6-di-O-*benzyl-2-deoxy-2-[5a-carba*-α-D-*xylo-hex-5(5a)-enopyranosylamino]*-α-D-*glucopyranoside* (**37**).—A solution of **36** (24 mg, 0.040 mmol) in aq 80% acetic acid (2 mL) was stirred for 3 h at 60 °C, and then evaporated. Chromatography of the residual product on silica gel (2 g, 1:7 methanol–chloroform) gave **37** (20 mg, 96%) as a syrup: R_f 0.34 (1:3 ethanol–toluene), $[\alpha]_D^{21}$ +178° (*c* 0.6, methanol); IR (KBr): ν 3450 (OH and NH) and 1650 (NH) cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.36–7.19 (m, 10 H, 2×Ph), 5.93 (br d, 1 H, $J_{1',5a'}$ 2.9 Hz, H-5a′), 4.87 and 4.54 (ABq, J_{gem} 11.0 Hz, PhCH_2), 4.77 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.55 and 4.47 (ABq, J_{gem} 12.1 Hz, PhCH_2), 4.20 and 4.13 (ABq, J_{gem} 14.5 Hz, H-6′), 3.95 (d, 1 H, $J_{3',4'}$ 7.0 Hz, H-4′), 3.70–3.45 (m, 7 H, H-1,4,5,6, 1′,2′,3′), 3.38 (s, 3 H, Me), 2.60 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2). Anal. Calcd for $C_{28}H_{37}NO_9$: C, 63.26: H, 7.02; N, 2.63. Found: C, 62.99; H, 7.12; N, 2.64.

Methyl 4,6-di-O-*benzyl-2,3-dideoxy-2,3-[2,3:4,6-di*-O-*isopropylidene-5a-carba*-α-D-xylo-*hex-5(5a)-enopyranosylepimino]*-α-D-*allopyranoside* (**39**).—To a stirred solution of **33** (125 mg, 0.204 mmol) in pyridine (3 mL) at 0 °C was added methanesulfonyl chloride (20.5 μL, 0.26 mmol). Stirring was continued at 0 °C while three additional portions of the acid chloride (in all 51 μL, 0.65 mmol) were added at intervals of 4 h. After addition of methanol (0.2 mL), the mixture was diluted with ethyl acetate (60 mL), washed with water, dried, and evaporated. The residual crude mesylate **35** was dissolved in toluene (3 mL), and the solution was treated with DBU (46 μL, 0.31 mmol, 1.5 equiv) for 36 h at 60 °C. Then the mixture was evaporated and the residue was chromatographed on silica gel (8 g, 1:9 ethyl acetate–toluene) to give **39** (16 mg, 95%) as a syrup: R_f 0.46 (1:3 butanone–toluene), $[\alpha]_D^{27}$ +201° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.21 (m, 10 H, 2×Ph), 5.30 (d, 1 H, $J_{1',5a'}$ 4.4 Hz, H-5a′), 4.90 (d, 1 H, $J_{1,2}$ 4.8 Hz, H-1), 4.69 and 4.54 (ABq, J_{gem} 11.4 Hz, PhCH_2), 4.63 and 4.47 (ABq, J_{gem} 12.1 Hz, PhCH_2), 4.47 (dd, 1 H, $J_{3',4'}$ 8.1, $J_{2',4'}$ 3.3 Hz, H-4′), 4.45 (dd, 1 H, $J_{2',3'}$ 7.1 Hz, H-3′), 4.33 and 4.04 (ABq, J_{gem} 14.1 Hz, H-6′), 3.87–3.83 (m, 2 H, H-4,5), 3.69 (dd, 1 H, $J_{5,6a}$ 3.0, J_{6gem} 10.4 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.55 (ddd, 1 H, $J_{1',2'}$ 3.7 Hz, H-2′), 3.32 (s, 3 H, Me), 2.48 (dd, 1 H, $J_{1',2'}$ 3.7, $J_{1',5a'}$ 4.4 Hz, H-1′), 2.38 (dd, 1 H, $J_{2,3}$ 6.8 Hz, H-2), 2.07 (dd, 1 H, $J_{3,4}$ 1.8 Hz, H-3), 1.54, 1.52, 1.44, and 1.43 (4 s, each 3 H, 2×CMe_2). Anal. Calcd for $C_{34}H_{43}NO_8$: C, 68.78; H, 7.30; N, 2.36. Found: C, 68.65; H, 7.29; N, 2.58.

Reaction of the aziridine **39** *with sodium acetate in aq acetic acid.*—A mixture of **39** (62 mg, 0.105 mmol) and sodium acetate (215 mg, 2.62 mmol) in 80% aq acetic acid (5 mL) was stirred for 3 days at 90 °C, and then evaporated. The residue was treated with acetic anhydride (1 mL) and pyridine (3 mL) overnight at room temp, and the products were chromatographed on silica gel (4 g, 1:9 acetone–toluene) to give a ca. 4:5 mixture (58 mg, 74%) of methyl 2-*O*-acetyl-4,6-di-*O*-benzyl-3-deoxy-3-[2,3,4,6-tetra-*O*-acetyl-5a-carba-α-D-*xylo*-hex-5(5a)-enopyranosylamino]-α-D-altropyranoside (**40**) and methyl 3-*O*-acetyl-4,6-di-*O*-benzyl-2-deoxy-2-[2,3,4,6-tetra-*O*-acetyl-5a-carba-α-D-

xylo-hex-5(5a)-enopyranosylamino]-α-D-glucopyranoside (**41**) that was difficult to separate: R_f 0.46 (1:5 acetone–toluene). Anal. Calcd for $C_{38}H_{47}NO_{14}$: C, 61.53; H, 6.39; N, 1.89. Found: C, 61.27; H, 6.32; N, 1.90.

The mixture (57 mg) of **40** and **41** was treated with methanolic M sodium methoxide (0.2 mL) in methanol (2 mL) for 1 h at room temp. The solution was applied to a column of Dowex-50W×2 (H^+) resin (5 mL) and eluted with a mixture of 1:5 28% aq ammonia–methanol, and the products were further separated by a preparative TLC (silica gel, 1:5 ethanol–toluene, acidified by a drop of acetic acid; three irrigations) to give **37** (13 mg, 32%), identical in all respects to the compound previously obtained.

*Methyl 2-deoxy-2-[5a-carba-*α*-D-xylo-hex-5(5a)-enopyranosylamino]-*α*-D-glucopyranoside* (**5**).—A solution of **37** (20 mg, 0.037 mmol) in THF (1 mL) was added to liquid ammonia (5 mL) containing sodium (86 mg, 3.7 mmol) at −75 °C, and the mixture was stirred for 15 h at the same temperature. The reaction mixture was processed in the conventional manner, and the product was purified on a column of Dowex-50W×2 (H^+) resin (20 mL) with aq 0.5 M ammonia as eluant to give **5** (10 mg, 78%) as a hygroscopic powder: R_f 0.39 (1:3 water–acetonitrile), $[\alpha]_D^{21}$ +206° (*c* 0.5, water), IR (KBr disk): ν 3480 (OH and NH), 1610 (NH) cm^{-1}; ^1H NMR (D_2O): δ 5.83 (d, 1 H, $J_{1',5a'}$ 4.8 Hz, H-5a′), 4.72 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.10 and 3.98 (ABq, $J_{6'gem}$ 13.9 Hz, H-6′), 3.92 (d, 1 H, $J_{3',4'}$ 5.9 Hz, H-4′), 3.74 (dd, 1 H, $J_{5,6a}$ 2.0, J_{6gem} 12.3 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6b}$ 5.3 Hz, H-6b), 3.52 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-3′), 3.51–3.44 (m, 1 H, H-5), 3.48 (dd, 1 H, $J_{3,4}$ 10.3, $J_{4,5}$ 9.9 Hz, H-4), 3.42 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-3), 3.35 (br dd, 1 H, $J_{1',2'}$ 4.4 Hz, H-1′), 3.31–3.24 (m, 1 H, H-2′), 3.28 (s, 3 H, Me), 2.71 (dd, 1 H, H-2).

*Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-[2,3,4,6-tetra-*O*-acetyl-5a-carba-*α*-D-xylo-hex-5(5a)-enopyranosylamino]-*α*-D-glucopyranoside* (**38**).—Compound **5** (7.6 mg, 0.022 mmol) was acetylated in the conventional manner with acetic anhydride in pyridine to give, after chromatography (silica gel, 1.4 g, 1:8 acetone–toluene), the heptaacetate **38** (11 mg, 76%) as a syrup: R_f 0.48 (1:5 acetone–toluene); $[\alpha]_D^{24}$ +127° (*c* 0.53, $CHCl_3$), IR (neat): ν 3350 (NH), 1750 (ester) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.83 (d, 1 H, $J_{1',5a'}$ 5.1 Hz, H-5a′), 5.59 (d, 1 H, $J_{3',4'}$ 6.6 Hz, H-4′), 5.53 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-3′), 5.15 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 9.9 Hz, H-3), 4.99 (dd, 1 H, $J_{1',2'}$ 4.0 Hz, H-2′), 4.97 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.81 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.66 and 4.36 (ABq, $J_{6'gem}$ 12.8 Hz, H-6′), 4.27 (dd, 1 H, $J_{5,6a}$ 4.8, J_{6gem} 12.1 Hz, H-6a), 4.07 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 3.92 (ddd, 1 H, $J_{4,5}$ 9.9 Hz, H-5), 3.69 (br dd, H-1′), 3.42 (s, 3 H, Me), 2.83 (dd, 1 H, H-2), 2.17, 2.09, 2.07, 2.06, 2.04, and 2.02 (6 s, 6, 3, 3, 3, 3, and 3 H, 7×Ac). Anal. Calcd for $C_{28}H_{39}NO_{16}$: C, 52.09; H, 6.09; N, 2.17. Found: C, 51.96; H, 6.32; N, 2.40.

Biological assay.—Yeast α-glucosidase activity was assayed according to the reported method of Tsuji et al. [30], and rat intestine sucrase–isomaltase activity was determined under the same conditions. Processing glucosidase I activity was assayed as reported previously [30] with the following two modifications: (1) Triton X-100-solubilized, [^{14}C]-glucose-labeled vesicular stomatitis virus glycoprotein was used instead of oligosaccharides liberated by protease treatment as the substrate for the enzyme assay, and (2) 10% trichloroacetic acid-insoluble, nonliberated [^{14}C]-glucose radioactivity was determined.

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