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SYNTHESIS OF "DIHYDROACARBOSE", AN α-D-GLUCOSIDASE INHIBITOR HAVING A PSEUDO-TETRASACCHARIDE STRUCTURE

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ABSTRACT

"Dihydroacarbose" (**2**), an α-D-glucosidase inhibitor having a pseudo-tetrasaccharide structure, was synthesized by reductive coupling of 4^3-amino-1^1,6^1-anhydro-2^1,3^1,2^2,3^2,6^2,2^3,3^3-hepta-*O*-benzyl-4^3,6^3-dideoxy-β-maltotriose and 2D-(2,4/3,5)-2,3,4-tris(benzyloxy)-5-(trityloxymethyl)cyclohexanone with sodium-cyanoborohydride. The former intermediate was prepared from a partially benzylated 1^1,6^1-anhydro-β-maltotriose, and the latter was prepared from a chiral, pentasubstituted cyclohexene derived from D-glucose. The synthetic **2** was found to be a strong, non-competitive inhibitor (K_i = 1.13 × 10^{-6}M) against small-intestinal sucrase of rat.

INTRODUCTION

A pseudo-tetrasaccharide, acarbose (**1**), is one of the microbial secondary metabolites that exhibit strong inhibitory activity against such α-D-glucosidases as sucrase, maltase, and other similar oligosaccharidases found in the wall of the small intestine[1,2]. A pseudo-disaccharide residue of characteristic structure, in which the nitrogen atom of valienamine is bonded to carbon atom 4 of a 4,6-dideoxy-D-hexopyranose, constitutes the core part of **1**, as well as other homologous inhibitors. In 1982, we succeeded in preparing one of such homologs, namely, amylostatin (XG)[3], and, very recently, Ogawa and Shibata[4] communicated the synthesis of **1**.

On the other hand, the Bayer AG group catalytically hydrogenated **1** (to saturate the double bond of its valienamine moiety) and isolated its analog **2** having the cyclitol moiety of D-*gluco* configuration in only 2% yield[5]. Similarly to such other saturated α-D-glucosidase inhibitors of natural origin as oligostatins[6], compound **2** also exhibited potent enzyme-inhibitory activities[2,7]. In 1986, we pre-

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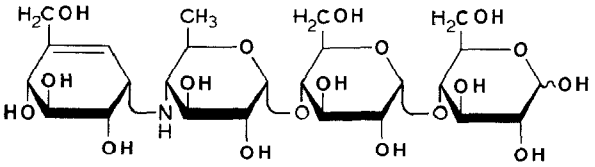

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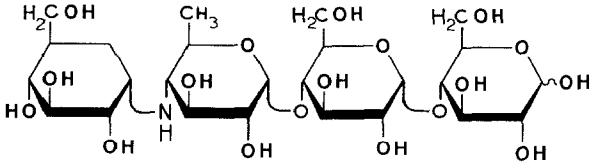

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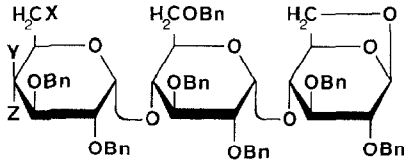
liminarily communicated the total synthesis of **2**, proposing "dihydroacarbose" as its trivial name[8]. We now deal with the details of that synthesis, and provide additional experimental results.

RESULTS AND DISCUSSION

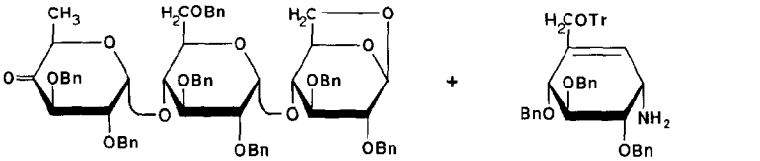
Our synthetic plan directed towards **2** comprised two large stages: first, cyclitol synthons and trisaccharide synthons were to be prepared from D-glucose and maltotriose, respectively, and second, the two synthons were to be coupled through an imino linkage. Most of the studies necessary for the first stage had



	X	Y	Z
3	OH	H	OH
4	I	H	OH
5	H	H	OH
6	H	I	H
7	H	H	N_3
8	H	H	NH_2

already been performed[9–11]; however, an efficient way had to be sought for the second stage. Generally, the most applicable avenue to such coupling should be reductive condensation of ketones and primary amines.

At first, the combination of a trisaccharide ketone and an aminocyclitol was tested for the coupling. The former synthon was prepared from a partially benzylated $1^1,6^1$-anhydro-β-maltotriose[11] (**3**) as follows. Regioselective iodination at C-6^3 of **3** with *N*-iodosuccinimide–triphenylphosphine under Hanessian and Lavallée's conditions[12], followed by reduction with lithium aluminum hydride in oxolane, gave



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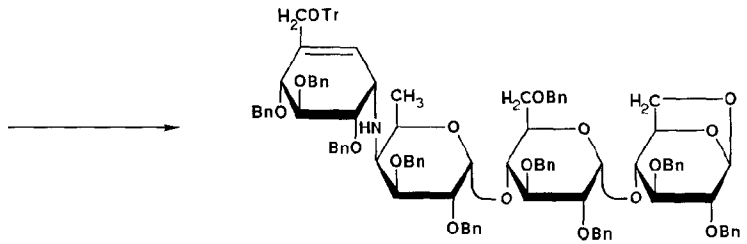

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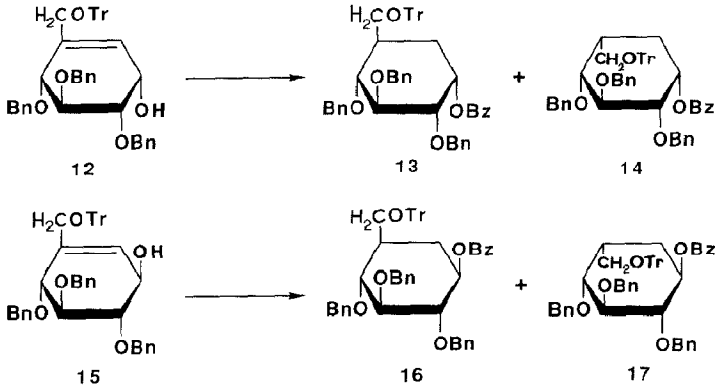
the 6^3-deoxy derivative (**5**) in 52% overall yield. The 4^3-hydroxyl group of **5** was oxidized by dimethyl sulfoxide–trifluoroacetic anhydride–triethylamine, giving the corresponding 4^3-ulopyranose (**9**) in 85% yield. As a model reaction for reductive coupling, a mixture of **9** and a readily accessible aminocyclitol, a valienamine derivative[10] (**10**), was treated with sodium cyanoborohydride, giving a pseudo-tetrasaccharide (**11**) in 1.1% yield, together with many unidentified products. It was determined that **11** was a fully protected 4^3-*epi*-acarbose having the D-*galacto* configuration by its ^{1}H-n.m.r. data, which contained the H-4^3 and -6^4 signals as a broad triplet at δ 3.09 with $J_{3^3,4^3} = J_{4^3,5^3} = \sim 3$ Hz and as a broad doublet at δ 5.76 with *J* 5.4 Hz, respectively. Consequently, the combination of a trisaccharide ketone and an aminocyclitol, such as **9** and **10**, proved to be unsuitable for the preparation of **2**.

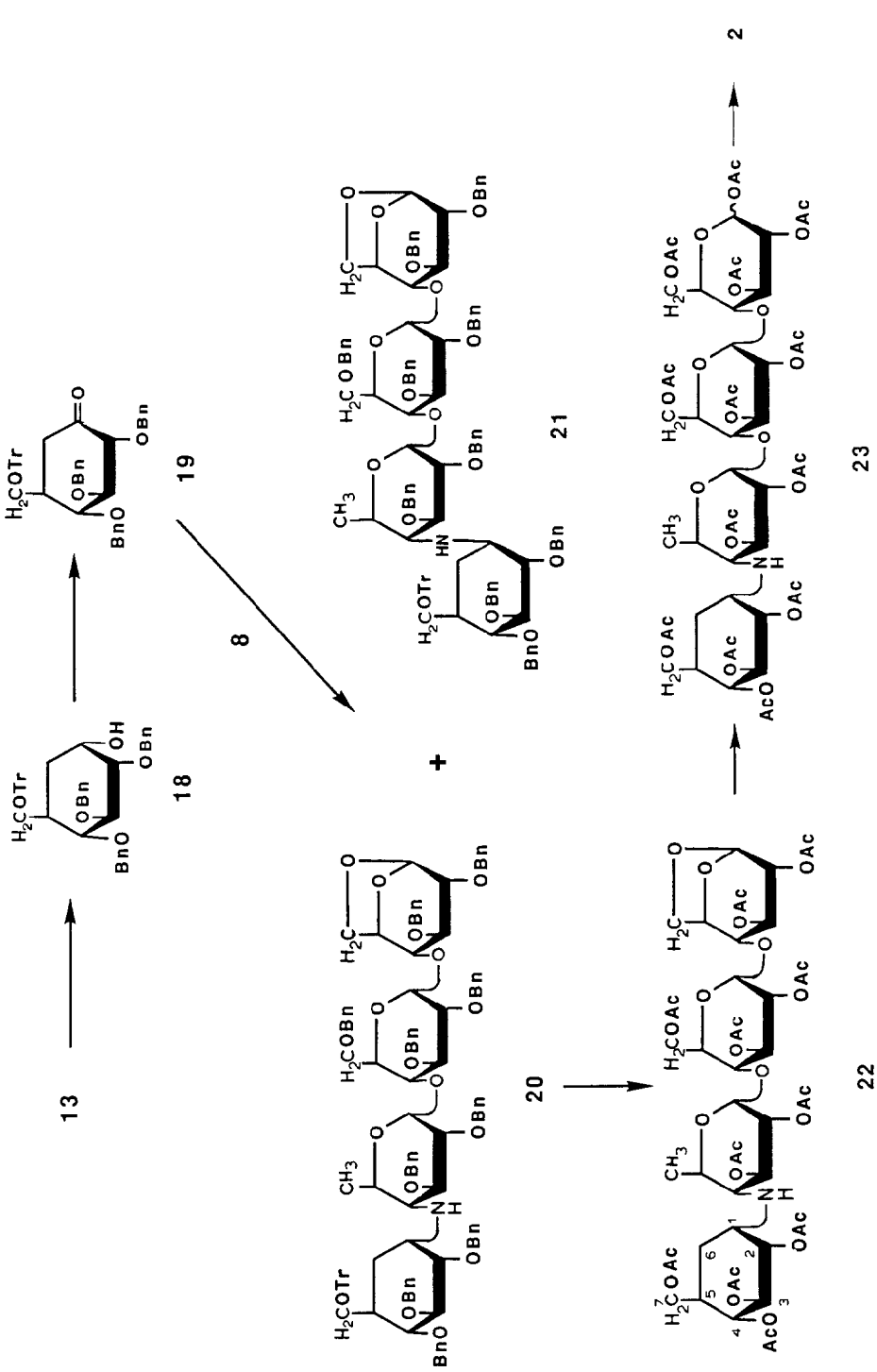
Then, our attention turned towards an alternative combination, an aminotrisaccharide and a cyclitol ketone, for coupling. The aminotrisaccharide synthon was prepared from the intermediate **5**. Thus, Garegg iodination[13] of **5** with 2,4,5-triiodoimidazole–imidazole–triphenylphosphine gave the $4^3,6^3$-dideoxy-4^3-iodo derivative **6**, which was converted into the 4^3-azido derivative (**7**) by a subsequent substitution reaction with sodium azide. The ^{1}H-n.m.r. spectrum of **7** exhibited signals due to H-6^3 and -4^3 as a doublet at δ 1.15 with *J* 6.1 Hz and as a triplet at δ

3.03 with J 9.8 Hz, respectively, showing the presence of a 4-azido-4,6-dideoxy-D-glucopyranose residue. The azido group of **7** was readily reduced by lithium aluminum hydride to give a 59% yield of the 4^3-amino derivative (**8**).



Preparation of the other synthon, a cyclitol ketone, required several steps. Catalytic hydrogenation of the unsaturated alcohol[9] (**12**) with Adams' catalyst afforded an inseparable mixture of the epimeric, saturated alcohols without hydrogenolysis of the benzyl or trityl groups. After the mixture had been benzoylated, the 1D-(1,2,4/3,5) and 1D-(1,2,4,5/3) isomers (**13** and **14**) were successfully isolated by chromatography on a column of silica gel in 43 and 41% yield, respectively. On the other hand, hydrogenation of the β-hydroxyl derivative (**15**) gave the 1L-(1,3,5/2,4) and 1L-(1,3/2,4,5) isomers (**16** and **17**) in 18 and 81% yield, respectively. The difference in the stereoselectivity of the hydrogenation reaction, as between **12** and **15**, could be ascribed to the different configurations of the 1-hydroxyl groups, because transfer of hydrogen occurs on a catalyst surface where the hydroxyl groups are coordinated. In contrast to the α-hydroxyl group, the α-oriented benzyloxy group at the other allylic position unfavorably functions against producing the desirable 5-β-configuration due to steric hindrance in the cases of both **12** and **15**. Compound **12** was preferable to **15** as a substrate and simple conversion of **15** into **12** had already been achieved[9]. Finally, the benzoate **13** was successively treated with methanolic sodium methoxide and then dimethyl sulfoxide–trifluoroacetic anhydride–triethylamine, to give the carbonyl synthon (**19**) in 73% overall yield.

The coupling reaction proceeded rather satisfactorily between **8** and **19**, in contrast to the former model reaction between **9** and **10**. In accordance with the informative paper of Kohn and Schmidt[14], the mixture of **8** and **19** (2 mol. equiv.) was treated with sodium cyanoborohydride in the presence of molecular sieves 3A at pH 6.2, giving two pseudo-tetrasaccharides in 30 and 4% yield. The spectral data and elemental analyses for the major product showed good agreement with those for the desired structure **20**. In particular, its ^{1}H-n.m.r. spectrum showed a broad triplet at δ 1.36 with $J_{6^4ax,1^4} < 2$ Hz, and $J_{5^4,6^4ax} = J_{6^4ax,6^4eq} = 13.0$ Hz and a broad doublet at δ 2.06 with $J_{6^4ax,6^4eq}$ 13.0 Hz, attributable to the methylene protons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attached to C-6[4]; these coupling constants revealed that **20** had an axially oriented substituent at C-1[4]. In the same way, the minor product was found to be the 1[4]-epimer (**21**) of **20** having an equatorially oriented substituent. Removal of the protecting groups of **20** was performed in the following steps. Benzyl and trityl groups were removed with sodium in liquid ammonia, and the product was isolated as the tridecaacetate (**22**), which underwent acetolysis of the 1,6-anhydro ring with 40:40:1 (v/v/v) acetic anhydride–acetic acid–conc. sulfuric acid at room temperature, giving an anomeric mixture of the a etate **23** (α/β = 9:1) in 84% yield. Finally, deacetylation of **23** by the Zemplén procedure gave **2**, which was purified by chromatography with CM-Sephadex C-25 (NH_4^+). The ^{1}H-n.m.r. spectrum of synthetic **2** (α/β = 2:3) was in good agreement with that of the hydrogenated product[5] of natural **1**, except for their anomeric ratio.

Inhibitory activity of the synthetic **2** against sucrase was examined according to a modified procedure of Dahlqvist *et al.*[15], using a crude enzyme preparation isolated from the small-intestinal mucosa of rats, with sucrose as substrate. Compound **2** proved to possess potent inhibitory activity against sucrase (K_i = 1.13 × 10^{-6}M). Moreover, a Lineweaver–Burk plot showed that **2** was a non-competitive inhibitor.

EXPERIMENTAL

General methods. — Melting points were determined with a Yamato micro melting-point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241MC polarimeter. I.r. spectra were recorded with a Shimadzu IR-27 spectrophotometer, for potassium bromide disks or on KRS (thallium bromide–iodide) for thin films. ^{1}H-N.m.r. spectra were recorded at 400 or 500 MHz with JEOL JNM-GX 400 or 500 spectrometers, using tetramethylsilane as the internal standard, for solutions in chloroform-*d*, unless otherwise noted. A secondary-ion (s.i.) mass spectrum was recorded with a Hitachi H-80 spectrometer at an ionizing voltage of 3 kV (primary ion of xenon) for **2**, or 1.5 kV for **11**, and 8–9 kV (secondary ion). Analytical and preparative t.l.c. were conducted on precoated plates of Silica Gel $60F_{254}$ (layer thickness, 0.25 mm and 0.5 mm; E. Merck, Darmstadt, Germany). Column chromatography was performed on Silica Gel 60 (70–230 mesh; E. Merck). Solvent extracts were dried with anhydrous sodium sulfate unless otherwise specified.

*1^1,6^1-Anhydro-2^1,3^1,2^2,3^2,6^2,2^3,3^3-hepta-*O*-benzyl-6^3-deoxy-6^3-iodo-β-maltotriose* (**4**). — To a solution of 1^1,6^1-anhydro-2^1,3^1,2^2,3^2,6^2,2^3,3^3-hepta-*O*-benzyl-β-maltotriose[11] (**3**) (7.26 g, 6.5 mmol) in *N*,*N*-dimethylformamide (150 mL) were added triphenylphosphine (3.41 g, 13 mmol) and *N*-iodosuccinimide (2.92 g, 13 mmol), and the mixture was stirred for 2 h at 65–75°. Methanol (10 mL) was added, and the mixture was stirred for 30 min at room temperature. The solvent was evaporated, and the resulting syrup was dissolved in ethyl acetate (200 mL). The extract was successively washed with aqueous sodium thiosulfate, aqueous sodium

hydrogencarbonate, and brine, dried (anhydrous magnesium sulfate), and evaporated. The residual syrup was chromatographed on a column of silica gel with 19:1 benzene–ethyl acetate, to give **4** as a colorless syrup (6.0 g, 75%); $[\alpha]_D^{18}$ +25° (*c* 1.08, chloroform); ν_{max}^{film} 3450 cm^{-1} (OH).

Anal. Calc. for $C_{67}H_{71}IO_{14}$: C, 65.58; H, 5.83; I, 10.34. Found: C, 65.63; H, 5.79; I, 10.09.

*1^1,6^1-Anhydro-2^1,3^1,2^2,3^2,6^2,2^3,3^3-hepta-*O*-benzyl-6^3-deoxy-6^3-β-maltotriose* (**5**). — To an ice-cold solution of **4** (5.88 g, 4.8 mmol) in anhydrous oxolane (150 mL) was added lithium aluminum hydride (400 mg), and the mixture was stirred overnight at room temperature. After cooling in an ice bath, ethyl acetate, methanol, and aqueous saturated potassium sodium tartrate were successively added dropwise to the resulting mixture. The mixture was diluted with diethyl ether, and the separated organic layer was successively washed with aqueous potassium sodium tartrate and brine, dried (anhydrous magnesium sulfate), and evaporated to dryness. The residue was subjected to column chromatography with 9:1 benzene–ethyl acetate, to give **5** as a colorless syrup (3.8 g, 72%); $[\alpha]_D^{24}$ +22.7° (*c* 1.07, chloroform); ν_{max}^{film} 3450 cm^{-1} (OH); δ_H 1.15 (d, 3 H, *J* 6.1 Hz, H-6^3), 2.13 (d, 1 H, *J* 2.7 Hz, OH), 3.11 (dt, 1 H, *J* 2.7, 9.0, and 9.0 Hz, H-4^3), 3.39 (br. s, 1 H, H-4^1), 3.40 (dd, 1 H, *J* 3.4 and 9.8 Hz, H-2^3), 3.57–3.74 (m, 8 H, H-2^1, 3^1, 6^{1}b, 2^2, 6^{2}a, 6^{2}b, 3^3, 5^3), 3.88 (d, 1 H, *J* 6.8 Hz, H-6^{1}a), 3.95 (dd, 1 H, *J* 9.0 and 9.8 Hz, H-4^2), 4.13 (m, 1 H, H-5^2), 4.18 (t, 1 H, *J* 9.0 Hz, H-3^2), 4.45–5.06 (m, 14 H, 7 C*H*$_2$Ph), 4.76 (d, 1 H, *J* 4.9 Hz, H-5^1), 5.00 (d, 1 H, *J* 3.9 Hz, H-1^2), 5.48 (s, 1 H, H-1^1), and 5.59 (d, 1 H, *J* 3.4 Hz, H-1^3).

Anal. Calc. for $C_{67}H_{72}O_{14}$: C, 73.07; H, 6.59. Found: C, 73.18; H, 6.58.

*O-(2,3-Di-O-benzyl-4,6-dideoxy-4-iodo-α-*D*-galactopyranosyl)-(1→4)-O-(2,3,6-tri-O-benzyl-α-*D*-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-*D*-glucopyranose* (**6**). — To a solution of **5** (1.1 g, 1 mmol), triphenylphosphine (1.05 g, 4 mmol), and imidazole (0.14 g, 2 mmol) in toluene (50 mL) was added triiodoimidazole[13] (0.89 g, 2 mmol), and the boiling mixture was stirred under reflux for 2 h and cooled. The precipitate was filtered off, and the filtrate was evaporated to dryness. A solution of the residue in diethyl ether was successively washed with saturated aqueous sodium thiosulfate, aqueous sodium hydrogencarbonate, and brine, dried, and evaporated. The residual syrup was chromatographed on a column of silica gel with 24:1 benzene–ethyl acetate, to give **6** as a colorless syrup (1.1 g, 82%); $[\alpha]_D^{20}$ +58.6° (*c* 1.18, chloroform); δ_H 1.05 (d, 3 H, *J* 6.1 Hz, H-6^3), 3.00 (dq, 1 H, *J* 6.1, 6.1, 6.1, and 1.3 Hz, H-5^3), 3.08 (dd, 1 H, *J* 9.5 and 3.7 Hz, H-3^3), 3.39 (d, 1 H, *J* 2.7 Hz, H-4^1), 3.51 (dd, 1 H, *J* 3.7 and 9.5 Hz, H-2^2), 3.60–3.71 (m, 5 H, H-2^1, 3^1, 6^{1}b, 6^{2}a, 6^{2}b), 3.76–3.81 (m, 2 H, H-4^2, 2^3), 3.89 (d, 1 H, *J* 7.1 Hz, H-6^{1}a), 4.07–4.13 (m, 2 H, H-3^2, 5^2), 4.36 (dd, 1 H, *J* 3.7 and 1.3 Hz, H-4^3), 4.43–4.91 (m, 14 H, 7 C*H*$_2$Ph), 4.78 (d, 1 H, *J* 4.9 Hz, H-5^1), 4.97 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.48 (s, 1 H, H-1^1), and 5.50 (d, 1 H, *J* 4.2 Hz, H-1^3).

Anal. Calc. for $C_{67}H_{71}IO_{13}$: C, 66.44; H, 5.91; I, 10.48. Found: C, 66.47; H, 5.93; I, 10.01.

1¹,6¹-Anhydro-4³-azido-2¹,3¹,2²,3²,6²,2³,3³-hepta-O-benzyl-4³,6³-dideoxy-β-maltotriose (**7**). — A solution of **6** (1.0 g, 0.83 mmol) and sodium azide (0.54 g) in hexamethylphosphoric triamide (20 mL) was stirred overnight at room temperature, and partitioned between diethyl ether and water. The organic layer was washed with brine, dried, and evaporated. The residual syrup was chromatographed on a column of silica gel with 24:1 benzene–ethyl acetate, giving colorless syrupy **7** (0.46 g, 49%); $[\alpha]_D^{21}$ +63° (*c* 0.50, chloroform); ν_{max}^{film} 2100 cm^{-1} (N_3); δ_H 1.15 (d, 3 H, *J* 6.1 Hz, H-6³), 3.03 (t, 1 H, *J* 9.8 Hz, H-4³), 3.40–3.42 (m, 2 H, H-2³, 4¹), 3.53–3.75 (m, 7 H, H-2¹, 3¹, 6¹b, 2², 6²a, 6²b, 5³), 3.78 (t, 1 H, *J* 9.8 Hz, H-3³), 4.13 (m, 1 H, H-5²), 4.17 (t, 1 H, *J* 9.3 Hz, H-3²), 4.47–5.06 (m, 14 H, 7 C*H*$_2$Ph), 4.74 (d, 1 H, *J* 4.9 Hz, H-5¹), 5.00 (d, 1 H, *J* 3.7 Hz, H-1²), 5.49 (s, 1 H, H-1¹), and 5.58 (d, 1 H, *J* 3.4 Hz, H-1³).

Anal. Calc. for $C_{67}H_{71}N_3O_{13}$: C, 71.45; H, 6.35; N, 3.73. Found: C, 71.82; H, 6.32; N, 3.49.

6³-Amino-1¹,6¹-anhydro-2¹,3¹,2²,3²,6²,2³,3³-hepta-O-benzyl-4³,6³-dideoxy-β-maltotriose (**8**). — Compound **7** (460 mg, 0.41 mmol) was treated with lithium aluminum hydride (200 mg) in the same way as in the preparation of **5**. The product was purified on a column of silica gel with 99:99:2 benzene–chloroform–methanol, giving **8** (270 mg, 59%); $[\alpha]_D^{23}$ +27° (*c* 0.60, chloroform); ν_{max}^{film} 3450 cm^{-1} (NH_2); δ_H 1.17 (d, 3 H, *J* 6.1 Hz, H-6³), 1.47 (br. s, 2 H, N*H*$_2$), 2.41 (t, 1 H, *J* 9.5 Hz, H-4³), 3.37–3.44 (m, 2 H, H-4¹, 2³), 3.51 (t, 1 H, *J* 9.5 Hz, H-3³), 3.57–3.75 (m, 7 H, H-2¹, 3¹, 6¹b, 2², 6²a, 6²b, 5³), 3.89 (d, 1 H, *J* 7.1 Hz, H-6¹a), 3.94 (t, 1 H, *J* 9.3 Hz, H-4²), 4.14 (m, 1 H, H-5²), 4.19 (t, 1 H, *J* 9.3 Hz, H-3²), 4.45–5.05 (m, 14 H, 7 C*H*$_2$Ph), 4.77 (d, 1 H, *J* 5.1 Hz, H-5¹), 5.01 (d, 1 H, *J* 3.7 Hz, H-1²), 5.49 (s, 1 H, H-1¹), and 5.59 (d, 1 H, *J* 3.4 Hz, H-1³).

Anal. Calc. for $C_{67}H_{73}NO_{13}\cdot 0.5H_2O$: C, 72.54; H, 6.72; N, 1.26. Found: C, 72.43; H, 6.60; N, 1.24.

O-*(2,3-Di-O-benzyl-6-deoxy-α-*D-xylo-*hexopyranosyl-4-ulose)-(1→4)-O-(2,3,6-tri-O-benzyl-α-*D-*glucopyranosyl)-(1→4)-1,6-anhydro-2,3-di-O-benzyl-β-*D-*glucopyranose* (**9**). — To a solution of dimethyl sulfoxide (1.5 mL, 21 mmol) in dichloromethane (10 mL) was added dropwise a dichloromethane solution (6 mL) of trifluoroacetic anhydride (2.5 mL, 17.7 mmol) at −78°, and the mixture was stirred for 15 min. A dichloromethane solution (10 mL) of **5** (3.3 g, 3 mmol) was added dropwise to the mixture, and it was stirred for 1 h at the same temperature. After dropwise addition of dichloromethane solution (10 mL) of triethylamine (4.5 mL), the mixture was warmed to room temperature, stirred for 30 min, poured into ice–water, and extracted with diethyl ether (2 × 50 mL). The extracts were combined, successively washed with 0.5M hydrochloric acid, aqueous sodium hydrogencarbonate, and brine, dried, and evaporated. The residual syrup was purified on a column of silica gel with 19:1 benzene–ethyl acetate, giving **9** as a colorless syrup (2.8 g, 85%); $[\alpha]_D^{23}$ +46° (*c* 2.5, chloroform); ν_{max}^{film} 1740 cm^{-1} (C=O); δ_H 1.12 (d, 3 H, *J* 6.6 Hz, H-6³), 3.40 (s, 1 H, H-4¹), 3.57 (dd, 1 H, *J* 3.7 and 9.7 Hz, H-2²), 3.60–3.75 (m, 6 H, H-2¹, 3¹, 6¹b, 6²a, 6²b, 2³), 3.92 (d, 1 H, *J* 7.1 Hz, H-6¹a),

3.97–4.07 (m, 2 H, H-4^2, 5^2), 4.15–4.23 (m, 2 H, H-3^2, 5^3), 4.32 (d, 1 H, *J* 9.8 Hz, H-3^3), 4.43–4.99 (m, 14 H, 7 C*H*$_2$Ph), 4.75 (d, 1 H, *J* 5.6 Hz, H-5^1), 4.98 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.49 (s, 1 H, H-1^1), and 5.69 (d, 1 H, *J* 3.4 Hz, H-1^3).

Anal. Calc. for $C_{67}H_{70}O_{14}$: C, 73.20; H, 6.42. Found: C, 73.14; H, 6.39.

Reductive coupling of **9** *and 1*L*-(1,3,4/2)-4-amino-1,2,3-tri*-O*-benzyl-6-(trityloxymethyl)-5-cyclohexene-1,2,3-triol* (**10**). — A mixture of **9** (165 mg, 0.15 mmol) and **10** (ref. 10; 69 mg, 0.1 mmol), dried by co-evaporation with toluene, was dissolved in 1:2 (v/v) dichloromethane–methanol (1.5 mL). Powdered molecular sieves 3A (50 mg) and sodium cyanoborohydride (10 mg) were added, and the pH was adjusted to 6.2 by addition of acetic acid. The mixture was stirred overnight at room temperature, filtered, and the filtrate partitioned between dichloromethane and water. The organic layer was successively washed with aqueous sodium hydrogencarbonate and brine, dried, and evaporated. Purification by chromatography on a column of silica gel with 19:1 benzene–ethyl acetate and by preparative t.l.c. with 23:2 benzene–ethyl acetate gave *O*-[2,3-di-*O*-benzyl-4,6-dideoxy-4-[1D-(1,2,4/3)-2,3,4-tris(benzyloxy)-5-(trityloxymethyl)-5-cyclohexenylamino]-α-D-galactopyranosyl]-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-di-*O*-benzyl-β-D-glucopyranose (**11**; 2 mg, 1.1%); R_F 0.49 in 17:3 benzene–ethyl acetate; δ_H 1.16 (d, 3 H, *J* 6.4 Hz, H-6^3), 1.50 (br. s, 1 H, N*H*), 3.09 (br. t, *J* ~2 Hz, 1 H, H-4^3), 3.38 (br. s, 1 H, H-4^1), 3.48 (dd, H-1, *J* 3.9 and 9.0 Hz, H-2^4), 3.51–3.86 (m, 14 H, H-2^1, 3^1, 6^{1}a, 6^{1}b, 2^2, 4^2, 6^{2}a, 6^{2}b, 2^3, 3^3, 5^3, 1^4, 7^{4}a, 7^{4}b), 4.11–4.17 (m, 4 H, H-3^2, 5^2, 3^4, 4^4), 4.43–4.17 (m, 21 H, H-5^1, 10 C*H*$_2$Ph), 4.98 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.44 (d, 1 H, *J* 3.4 Hz, H-1^3), 5.47 (s, 1 H, H-1^1), and 5.76 (br. d, 1 H, *J* 5.4 Hz, H-6^4); *m/z* (s.i.-m.s. in 3-nitrobenzyl alcohol matrix): 1770 [M$^+$, calc. for $C_{114}H_{115}NO_{17}$: 1769.8157].

*1*D*-(1,2,4/3,5)-* (**13**) *and 1*D*-(1,2,4,5/3)-1-*O*-benzoyl-2,3,4-tri*-O*-benzyl-5-(trityloxymethyl)cyclohexane-1,2,3,4-tetrol* (**14**). — A mixture of 1D-(1,2,4/3)-2,3,4-tri-*O*-benzyl-5-(trityloxymethyl)-5-cyclohexene-1,2,3,4-tetrol[9] (**12**; 480 mg, 0.7 mmol) and platinum(IV) oxide (5 mg) in ethyl acetate (10 mL) was shaken under an atmosphere of hydrogen at 1 atm. for 12 h at room temperature. The catalyst was filtered off and washed with ethyl acetate, and the filtrate and washings were combined and evaporated. To a solution of the residual syrup in dichloromethane (2 mL) and pyridine (2 mL) was added, dropwise, benzoyl chloride (0.4 mL). The mixture was stirred overnight at room temperature, poured into water, extracted with diethyl ether, and the extract successively washed with M hydrochloric acid, aqueous saturated sodium hydrogencarbonate, and brine, dried (anhydrous magnesium sulfate), and evaporated to dryness. The residue was subjected to column chromatography with 199:199:2 benzene–cyclohexane–ethyl acetate to give syrupy **13** (230 mg, 43%); $[\alpha]_D^{22}$ +45.7° (*c* 1.7, chloroform); ν_{max}^{film} 1720 cm^{-1} (C=O); δ_H 1.96 (dt, 1 H, *J* 2.0, 11.0, and 11.0 Hz, H-6*ax*), 2.10 (dt, 1 H, *J* 11.0, 3.7, and 3.7 Hz, H-6*eq*), 2.21 (m, 1 H, H-5), 3.34 (dd, 1 H, *J* 2.4 and 9.0 Hz, H-7b), 3.49 (dd, *J* 4.2 and 9.0 Hz, H-7a), 3.69 (dd, 1 H, *J* 3.2 and 9.8 Hz, H-2), 3.71 (dd, 1 H, *J* 9.8 and 9.5 Hz, H-3), 3.96 (t, 1 H, *J* 9.5 Hz, H-4), 4.32–4.95 (m, 6 H, 3 C*H*$_2$Ph), and 5.85 (m, 1 H, H-1).

Anal. Calc. for $C_{54}H_{50}O_6 \cdot 0.75H_2O$: C, 80.22; H, 6.42. Found: C, 80.25; H, 6.25.

Further elution with the same solvent gave **14** (220 mg, 41%); $[\alpha]_D^{22}$ +22° (*c* 0.80, chloroform); ν_{max}^{film} 1720 cm^{-1} (C=O); δ_H 1.63 (br. d, 1 H, *J* 11.2 Hz, H-6*eq*), 2.10 (br. q, 1 H, *J* 11.2 Hz, H-6*ax*), 2.37 (m, 1 H, H-5), 3.27 (m, 2 H, H-7a, 7b), 3.76 (m, 2 H, H-2,3), 3.96 (s, 1 H, H-4), 4.31–4.61 (m, 6 H, 3 C*H*$_2$Ph), and 5.42 (m, 1 H, H-1).

Anal. Calc. for $C_{54}H_{50}O_6 \cdot H_2O$: C, 79.78; H, 6.45. Found: C, 79.52; H, 6.38.

1L-(1,3,5/2,4)- (**16**) *and 1L-(1,3/2,4,5)-1-O-benzoyl-2,3,4-tri-O-benzyl-5-(trityloxymethyl)cyclohexane-1,2,3,4-tetrol* (**17**). — A mixture of 1L-(1,3/2,4)-tri-*O*-benzyl-5-(trityloxymethyl)-5-cyclohexene-1,2,3,4-tetrol[9] (**15**: 138 mg, 0.2 mmol) and platinum(IV) oxide (1 mg) was treated as already described, giving **17** (129 mg, 81%) and **16** (29 mg, 18%).

Compound **17** had $[\alpha]_D^{23}$ −27° (*c* 0.27, chloroform); δ_H 1.54 (m, 1 H, H-6*ax*), 2.5 (m, 1 H, H-5), 2.51 (td, 1 H, *J* 4.4, 4.4, and 13.2 Hz, H-6*eq*), 3.32–3.36 (m, 2 H, H-7), 3.67 (t, 1 H, *J* 8.3 Hz, H-2), 3.69 (t, 1 H, *J* 8.3 Hz, H-2), 3.77 (t, 1 H, *J* 8.3 Hz, H-3), and 5.49 (ddd, 1 H, *J* 4.6, 7.1, and 9.5 Hz, H-1).

Anal. Calc. for $C_{54}H_{50}O_6 \cdot H_2O$: C, 79.78; H, 6.45. Found: C, 79.49; H, 6.35.

Compound **16** had $[\alpha]_D^{23}$ −22° (*c* 0.40, chloroform); δ_H 1.79 (q, 1 H, *J* 11.9 Hz, H-6*ax*), 1.90 (m, 1 H, H-5), 2.23 (m, 1 H, H-6*eq*), 3.34 (dd, 1 H, *J* 4.4 and 8.8 Hz, H-7a), 3.41 (dd, 1 H, *J* 2.4 and 8.8 Hz, H-7b), 3.6 (m, 2 H, H-3,4), 3.76 (t, 1 H, *J* 9.6 Hz, H-2), and 5.21 (ddd, 1 H, *J* 5.1, 9.2, and 11.5 Hz, H-1).

Anal. Calc. for $C_{54}H_{50}O_6 \cdot H_2O$: C, 79.78; H, 6.45. Found: C, 79.82; H, 6.68.

1D-(1,2,4/3,5)-2,3,4-Tri-O-benzyl-5-(trityloxymethyl)cyclohexane-1,2,3,4-tetrol (**18**). — To a solution of **13** (630 mg, 0.8 mmol) in dichloromethane (10 mL) and methanol (20 mL) were added several drops of 28% methanolic sodium methoxide, and the mixture was stirred overnight at room temperature. The solvent was evaporated, and the residue was dissolved in dichloromethane; the solution was washed with brine and evaporated to dryness. The residual syrup was chromatographed on a column of silica gel with 49:1 benzene–ethyl acetate as the eluant to give **18** (450 mg, 81%) as a colorless syrup; $[\alpha]_D^{23}$ +16.7° (*c* 0.46, chloroform); ν_{max}^{film} 3500 cm^{-1} (OH); δ_H 1.72 (br.t, 1 H, *J* 13.2 Hz, H-6*ax*), 2.05 (dt, 1 H, *J* 13.2, 1.2, and 1.2 Hz, H-6*eq*), 2.22 (m, 1 H, H-5), 2.49 (br.s, 1 H, O*H*), 3.33 (dd, 1 H, *J* 8.8 and 2.5 Hz, H-7b), 3.40 (dd, 1 H, *J* 8.8 and 4.2 Hz, H-7a), 3.53 (dd, 1 H, *J* 9.5 and 3.2 Hz, H-2), 3.56 (dd, 1 H, *J* 9.5 and 9.3 Hz, H-3), 3.84 (t, 1 H, *J* 9.3 Hz, H-4), 4.19 (m, 1 H, H-1), and 4.31–4.89 (m, 6 H, 3 C*H*$_2$Ph).

Anal. Calc. for $C_{47}H_{46}O_5$: C, 81.71; H, 6.71. Found: C, 81.75; H, 6.71.

2D-(2,4/3,5)-2,3,4-tribenzyloxy-5-(trityloxymethyl)cyclohexanone (**19**). — Compound **18** (440 mg, 0.64 mmol) was treated with trifluoroacetic anhydride (1 mL, 7 mmol), dimethyl sulfoxide (0.6 mL, 8.5 mmol), and triethylamine (1.8 mL) as in the preparation of **9**; the raw material was subjected to column chromatography with 99:1 benzene–ethyl acetate, to give **19** (400 mg, 90%) as a colorless

syrup; $[\alpha]_D^{23}$ +33.2° (*c* 1.1, chloroform); ν_{max}^{film} 1730 cm^{-1} (C=O); δ_H 1.92 (m, 1 H, H-5), 2.48 (dd, 1 H, *J* 4.2 and 13.9 Hz, H-6*eq*), 2.73 (t, 1 H, *J* 13.9 Hz, H-6*ax*), 3.35 (dd, 1 H, *J* 2.5 and 9.0 Hz, H-7b), 3.47 (dd, 1 H, *J* 4.2 and 9.0 Hz, H-7a), 3.69 (t, 1 H, *J* 9.5 Hz, H-3), 3.96 (dd, 1 H, *J* 9.3 and 9.5 Hz, H-4), 4.22 (d, 1 H, *J* 9.5 Hz, H-2), and 4.36–4.97 (m, 6 H, 3 CH_2Ph).

Anal. Calc. for $C_{47}H_{44}O_5$: C, 81.95; H, 6.44. Found: C, 82.01; H, 6.54.

O-[*2,3-Di*-O-*benzyl*-*4,6-dideoxy*-*4*-[*1*D-*(1,2,4/3,5)*-*2,3,4-tris(benzyloxy)*-*5*-*(trityloxymethyl)cyclohexyl*]*amino*-α-D-*glucopyranosyl*]- (**20**) *and* O-[*2,3-di*-O-*benzyl*-*4,6-dideoxy*-*4*-[*1*L-*(1,3,5/2,4)*-*2,3,4-tris(benzyloxy)*-*5*-*(trityloxymethyl)cyclohexyl*]*amino*-α-D-*glucopyranosyl*]-*(1→4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)*-*(1→4)*-*1,6-anhydro*-*2,3-di*-O-*benzyl*-β-D-*glucopyranose* (**21**). — A mixture of **8** (300 mg, 0.3 mmol)and **19** (413 mg, 0.6 mmol), dried by azeotropic evaporation with toluene (3 × 20 mL) and by keeping overnight with phosphorus pentaoxide in a vacuum oven at 60°, was dissolved in methanol (1 mL) and dichloromethane (2 mL). Dried and powdered molecular sieves 3A (400 mg) and sodium cyanoborohydride (20 mg) were added to the solution, and the pH was adjusted to 6.2 by addition of acetic acid. The mixture was stirred for 7 h at room temperature, and filtered. The filtrate ws diluted with dichloromethane, successively washed with water, aqueous sodium hydrogencarbonate, and brine, dried, and evaporated to dryness. The residue was subjected to chromatography on a column of silica gel: elution with 97:3 benzene–ethyl acetate gave an inseparable mixture (205 mg, 50%, based on **19**) of **18** and its 1-epimer. Elution next with 19:1 benzene–ethyl acetate gave **20** as an amorphous mass (160 mg, 30%, based on **8**); $[\alpha]_D^{22}$ +39.2° (*c* 1.55, chloroform); ν_{max}^{KBr} 3350 cm^{-1} (NH); δ_H 1.20 (d, 3 H, *J* 6.1 Hz, H-6^3), 1.36 (br.t, 1 H, *J* 13.0, H-6^4*ax*), 2.06 (br.d, 1 H, *J* 13.0 Hz, H-6^4*eq*), 2.29 (m, 1 H, H-5^4), 2.35 (m, 1 H, N*H*), 3.18 (dd, 1 H, *J* 8.5 and 5.4 Hz, H-7^4b), 3.33–3.90 (m, 18 H, H-2^1, 3^1, 4^1, 6^1a, 6^1b, 2^2, 4^2, 6^2a, 6^2b, 2^3, 3^3, 4^3, 5^3, 1^4, 2^4, 3^4, 4^4, 7^4a), 4.07–4.14 (m, 2 H, H-3^2, 5^2), 4.22–5.14 (m, 21 H, H-5^1, 10 CH_2Ph), 5.01 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.44 (d, 1 H, *J* ~1 Hz, H-1^3), and 5.48 (s, 1 H, H-1^1).

Anal. Calc. for $C_{114}H_{117}NO_{17}$: C, 77.22; H, 6.65; N, 0.79. Found: C, 77.15; H, 6.66; N, 0.79.

Elution with 23:2 benzene–ethyl acetate gave **21** (20 mg, 4%) as an amorphous mass; $[\alpha]_D^{24}$ +25° (*c* 0.26, chloroform); δ_H 1.22 (d, 3 H, *J* 6.1 Hz, H-6^3), 2.97 (t, 1 H, *J* 9.5 Hz, H-4^3), 4.98 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.48 (s, 1 H, H-1^1), and 5.54 (d, 1 H, *J* 2.9 Hz, H-1^3).

Anal. Calc. for $C_{114}H_{117}NO_{17}\cdot H_2O$: C, 76.44; H, 6.70; N, 0.78. Found: C, 76.14; H, 6.76; N, 0.79.

Further elution with 49:1 chloroform–methanol gave unchanged **8** (190 mg, 57%).

O-[*2,3-Di*-O-*acetyl*-*4,6-dideoxy*-*4*-[*1*D-*(1,2,4/3,5)*-*2,3,4-tris(acetoxy)*-*5*-*(acetoxymethyl)cyclohexyl*]*amino*-α-D-*glucopyranosyl*]-*(1→4)*-O-*(2,3,6-tri*-O-*acetyl*-α-D-*glucopyranosyl)*-*(1→4)*-*1,6-anhydro*-*2,3-di*-O-*acetyl*-β-D-*glucopyranose, ethyl acetate solvate* (**22**). — A solution of **20** (160 mg, 0.09 mmol) in oxolane (5 mL) was added to liquid ammonia (~30 mL) at −78°. Sodium (200 mg) was added

portionwise to the mixture, and the dark blue solution was stirred for 1 h at the same temperature. Ammonium chloride (2 g) was added in portions, and the mixture was stirred overnight at room temperature, to give a white solid; this was dissolved in water (30 mL), and the solution was washed with diethyl ether, and placed on a column of Dowex 50W-X-4 (H^+) resin. The column was washed with water, eluted with 0.5M ammonium hydroxide, and the eluate evaporated to dryness. Pyridine (5 mL) and acetic anhydride (1 mL) were added to the residue, and the mixture was stirred for 4 d at room temperature, poured into ice–water, stirred for 2 h at room temperature, and extracted with diethyl ether. The extract was successively washed with M hydrochloric acid, aqueous sodium hydrogencarbonate, and brine, dried, and evaporated. The residue was chromatographed on a column of silica gel with 1:1 benzene–ethyl acetate, giving **22** as a white powder (52 mg, 53%); $[\alpha]_D^{23}$ +81.3° (*c* 0.60, chloroform); ν_{max}^{KBr} 1745 and 1240 cm^{-1} (AcO); δ_H 1.25 (t, *J* 7.22 Hz, EtOAc), 1.29 (d, 3 H, *J* 6.1 Hz, H-6^3), 1.38 (br.s, 1 H, N*H*), 1.44 (br.t, 1 H, *J* 14 Hz, H-6^4*ax*), 1.93 (br.d, 1 H, *J* 14 Hz, H-6^4*eq*), 1.96, 1.98, 1.99, 2.00, 2.03, 2.04, 2.05, 2.09, 2.11, 2.15, and 2.20 (11 s, 11 × 3 H, 11 C*H*$_3$CO), 2.38 (m, 1 H, H-4^3), 2.43 (m, 1 H, H-5^3), 3.49 (br.s, 2 H, H-4^1, 1^4), 3.64 (m, 1 H, H-5^3), 3.82 (dd, 1 H, *J* 7.6 and 5.9 Hz, H-6^{1}b), 3.93 (dd, 1 H, *J* 11.2 and 3.4 Hz, H-7^{4}b), 3.98–4.06 (m, 3 H, H-6^{1}a, 4^2, 7^{4}a), 4.17 (q, *J* 7.22 Hz, EtOAc), 4.26 (dd, 1 H, *J* 12.5 and 3.9 Hz, H-6^{2}b), 4.41 (m, 1 H, H-5^2), 4.57 (dd, 1 H, *J* 2.2 and 12.5 Hz, H-6^{2}a), 4.61 (s, 1 H, H-2^1), 4.72 (dd, *J* 10.0 and 3.7 Hz, H-2^2), 4.77 (dd, 1 H, *J* 10.2 and 4.2 Hz, H-2^3), 4.80 (d, 1 H, *J* 5.8 Hz, H-5^1), 4.85 (br.s, 1 H, H-3^1), 4.89 (dd, 1 H, *J* 4.4 and 10.3 Hz, H-2^4), 4.92 (dd, 1 H, *J* 10.3 and 9.5 Hz, H-4^4), 5.17 (m, 2 H, H-1^2, 3^3), 5.26 (d, 1 H, *J* 4.2 Hz, H-1^3), 5.34 (t, 1 H, *J* 10.3 Hz, H-3^4), 5.25 (s, 1 H, H-1^1), and 5.55 (dd, *J* 10.0 and 9.4 Hz, H-3^2).

Anal. Calc. for $C_{47}H_{65}NO_{28}\cdot C_4H_8O_2$: C, 51.90; H, 6.24; N, 1.19. Found: C, 52.13; H, 6.14; N, 1.27.

O-[*2,3-Di-O-acetyl-4,6-dideoxy-4-[1*D*-(1,2,4/3,5)-2,3,4-tris(acetoxy)-5-(acetoxymethyl)cyclohexyl]amino-α-*D*-glucopyranosyl]-(1→4)-O-(2,3,6-tri-O-acetyl-α-*D*-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-*D*-glucopyranose* (**23**). — Compound **22** (60 mg, 55 μmol) was dissolved in 40:40:1 (v/v) acetic acid–acetic anhydride–conc. sulfuric acid (2 mL). The mixture was stirred for 3 h at room temperature, poured into ice-cold aqueous sodium hydrogencarbonate, stirred overnight, and extracted with chloroform. The extract was successively washed with aqueous sodium hydrogencarbonate and brine, and evaporated. The residue was chromatographed on a column of silica gel with 49:49:2 benzene–chloroform–methanol as the eluent, to give **23** as a colorless syrup (55 mg, 84%); $[\alpha]_D^{23}$ +109.3° (*c* 1.14, chloroform); δ_H 1.29 (d, 3 H, *J* 6.4 Hz, H-6^3), 1.44 (br.t, 1 H, *J* 13 Hz, H-6^4*ax*), 1.83 (br.s, 1 H, N*H*), 1.95 (br.d, 1 H, *J* 13 Hz, H-6^4*eq*), 1.97, 1.98, 1.99, 2.00, 2.01, 2.02, 2.03, 2.048, 2.053, 2.09, 2.17, 2.21, and 2.24 (13 s, 13 × 3 H, 13 C*H*$_3$CO), 2.39 (t, 1 H, *J* 10.3 Hz, H-4^3), 2.42 (m, 1 H, H-5^4), 3.49 (br.d, 1 H, *J* 2 Hz, H-1^4), 3.59 (m, 1 H, H-5^3), 3.92–3.99 (m, 3 H, H-4^2, 5^2, 7^{4}b), 4.03 (dd, 1 H, *J* 5.4 and 11.2 Hz, H-7^{4}a), 4.07 (t, 1 H, *J* 8.8 Hz, H-4^1), 4.15 (m, 1 H, H-5^1), 4.20 (dd, *J* 2.7 and 12.2 Hz, H-6^{2}a), 4.32 (dd, 1 H, *J* 3.2 and 12.5 Hz, H-6^{1}a), 4.48 (dd,

1 H, *J* 2.4 and 12.5 Hz, H-6^{1}b), 4.53 (br.d, 1 H, *J* 12.2 Hz, H-6^{2}b), 4.74 (dd, 1 H, *J* 3.9 and 10.3 Hz, H-2^3), 4.75 (dd, 1 H, *J* 3.7 and 10.5 Hz, H-2^2), 4.89 (dd, 1 H, *J* 4.2 and 10.5 Hz, H-2^4), 4.92 (dd, 1 H, *J* 10.5 and 9.0 Hz, H-4^4), 4.96 (dd, 1 H, *J* 3.8 and 10.0 Hz, H-2^1), 5.20 (t, 1 H, *J* 10.3 Hz, H-3^3), 5.25 (d, 1 H, *J* 3.9 Hz, H-1^3), 5.32 (d, 1 H, *J* 3.7 Hz, H-1^2), 5.36 (t, 1 H, *J* 10.5 Hz, H-3^4), 5.41 (dd, 1 H, *J* 10.5 and 8.3 Hz, H-3^2), 5.52 (dd, 1 H, *J* 10.0 and 8.8 Hz, H-3^1), 5.75 (d, 0.1 H, *J* 8.1 Hz, H-1$^1\beta$), and 6.25 (d, 0.9 H, *J* 3.7 Hz, H-1$^1\alpha$).

Anal. Calc. for $C_{51}H_{71}NO_{31}$: C, 51.30; H, 5.99; N, 1.17. Found: C, 51.13; H, 5.99; N, 1.22.

O-[*4,6-Dideoxy-4-[1*D-*(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexyl]amino-α*-D-*glucopyranosyl]-(1→4)*-O-*α*-D-*glucopyranosyl-(1→4)*-D-*glucopyranose (dihydroacarbose)* (**2**). — To a solution of **23** (36 mg, 30 μmol) in methanol (10 mL) was added methanolic sodium methoxide (28%, 6 μL). The mixture was stirred for 2 d at room temperature and evaporated. The residue was dissolved in water (2 mL) and chromatographed on a column of CM-Sephadex C-25 (NH_4^+) with water as the eluant. The ninhydrin-positive fractions were combined and freeze-dried, to give white, powdery **2** (17 mg, 87%); $[\alpha]_D^{25}$ +120° (*c* 0.166, water, 24 h) [lit.[3] $[\alpha]_D^{25}$ +141.3° (*c* 0.3, water)]; δ_H* (D_2O) 1.15 (d, 3 H, *J* 6.4 Hz, H-6^3), 1.24 (t, 1 H, *J* 13.2 Hz, H-6^4*ax*), 1.65 (m, 1 H, H-5^4), 1.76 (d, 1 H, *J* 13.2 Hz, H-6^4*eq*), 2.27 (t, 1 H, *J* 10.5 Hz, H-4^3), 3.08–3.13 (m, 2 H, H-2^1, 7^{4}a), 3.17 (m, 1 H, H-1^4), 3.36 (dd, 1 H, *J* 3.9 and 10.0 Hz, H-2^4), 3.39–3.86 (m, 17 H, H-3^1, 4^1, 5^1, 6^{1}a, 6^{1}b, 2^2, 3^2, 4^2, 5^2, 6^{2}a, 6^{2}b, 2^3, 3^3, 5^3, 3^4, 4^4, 7^{4}b), 4.49 (d, 0.6 H, *J* 8.1 Hz, H-1$^1\beta$), 5.07 (d, 0.4 H, *J* 4.2 Hz, H-1$^1\alpha$), 5.15 (d, 1 H, *J* 3.7 Hz, H-1^2), and 5.24 (d, 1 H, *J* 3.9 Hz, H-1^3); *m/z* (s.i.–m.s. in glycerol matrix): 648 [(M + H)$^+$, calc. for $C_{25}H_{45}NO_{18}$: 647.2623].

Inhibition activity of **2** *against sucrase.* — Fasted rats of the Wistar strain weighing 200 g were sacrificed by decapitation. The entire small intestine was removed, and washed with cold 0.9% NaCl. The mucosa was scraped off with a slide glass, and homogenized in 10mM sodium phosphate buffer at pH 7.0, using a Teflon-pestled homogenizer. The homogenate was centrifuged at 10,000 *g* for 10 min, and the supernatant liquor was used as the enzyme solution. A mixture of the enzyme solution (0.05 mL), solutions of 12.5–200mM sucrose (0.1 mL), and 80mM sodium phosphate buffer (pH 7.0, 0.05 mL) in the presence or absence of **2** (4 μg mL^{-1}) was incubated for 20 min at 35°, boiled for 3 min to terminate the reaction, and centrifuged at 1500 *g* for 10 min. The amount of D-glucose in the supernatant liquor was assayed by the D-glucose oxidase method, using New Glucostat from Worthigton Biochemical Co. The apparent inhibition constant (K_i) calculated from the Lineweaver–Burk plot was 1.13×10^{-6}M.

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