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THE SYNTHESIS OF *O*-β-D-MANNOPYRANOSYL-(1→4)-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→4)-2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE. PART I*

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ABSTRACT

Benzyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**5**) was synthesized by the treatment of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**3**) with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide in the presence of silver trifluoromethanesulfonate and *s*-collidine. *O*-Deacetylation, followed by oxidation with acetic anhydride–dimethyl sulfoxide, and stereoselective reduction with sodium borohydride, converted **5** into benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**8**). Catalytic hydrogenation of **8** gave 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucopyranose, which was converted into 2-methyl-[3,6-di-*O*-acetyl-1,2-dideoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-D-glucopyrano]-[2,1-*d*]-2-oxazoline (**13**), by treatment with hydrogen chloride–acetyl chloride, followed by chloride-ion catalysis. Condensation of **13** with **3** gave the trisaccharide, benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**14**). Removal of the *O*-acetyl and *O*-benzyl groups from **14** gave the title compound.

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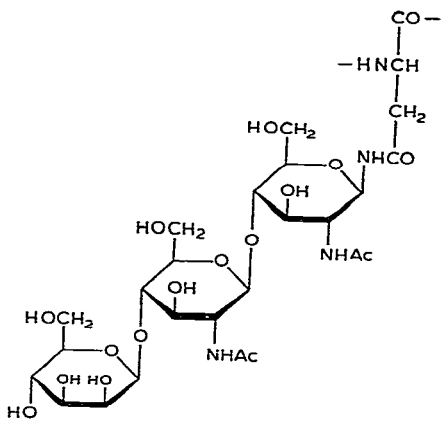

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INTRODUCTION

The trisaccharide *O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**16**) occurs in the "core" saccharide region of most of the *N*-glycoproteins[1], *i.e.*, those glycoproteins in which the carbohydrate–peptide linkage consists of an *N*-glycosyl bond between 2-acetamido-2-deoxy-D-glucose and L-asparagine. The sugar residue involved in the linkage is the one at the "reducing end" of the trisaccharide to give structure **1**.

The chemical synthesis of **16** is important, because it provides a pure compound for the structural investigation of glycoproteins and lipid intermediates[2]. The synthetic compound is also a useful, exogenous substrate for β-D-mannosidase[3], as a probe for lectins[4], and for linking to a solid support for affinity chromatography of the mannosyltransferases involved in the biosynthesis of the oligosaccharide "lipid intermediates" active in protein glycosylation[5]. Most importantly, **16** is the starting material for chemical synthesis of a trisaccharide "lipid intermediate" (see following paper[6]).

RESULTS AND DISCUSSION

The synthesis of *O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**16**) requires performing two glycosylations of D-glucopyranose residues at O-4, which is well known to be the least reactive position[7], and forming a β-D-mannopyranosyl derivative. With very few exceptions[8], mannosylations with D-mannopyranosyl halides have yielded α-D-mannosides, or α,β mixtures[9].

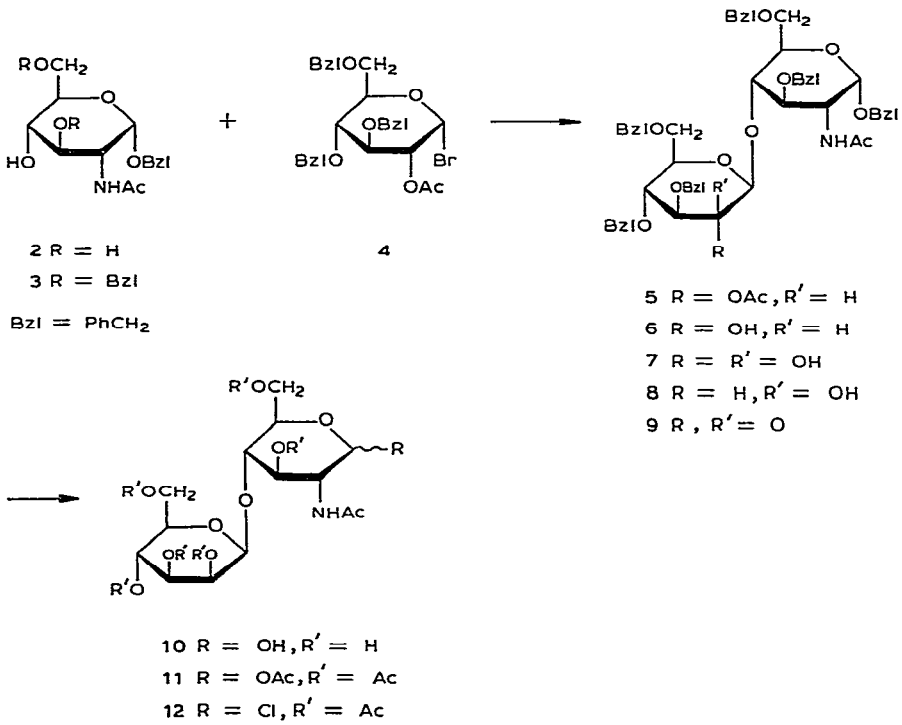
The problem of synthesizing (1→4)-linked D-glucopyranosyl derivatives was approached by use of the Koenigs–Knorr and oxazoline procedures, in both cases employing recent improvements of these methods[10,11]. For synthesis of the β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose residue, we used the method that involves the synthesis of a β-D-glucopyranosyl compound, followed by

its conversion into a D-mannopyranosyl derivative by a sequence of oxidation and stereoselective reduction, resulting in epimerization[12] at C-2.

It is apparent that the construction of the trisaccharide **16** could be achieved by two alternative approaches, involving either (*a*) the synthesis of *O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose, conversion into an oxazoline, and glycosylation of a suitably protected derivative of 2-acetamido-2-deoxy-D-glucose, or (*b*) the synthesis of a suitably protected chitobiose derivative, and glycosylation of O-4′. Both routes were employed (see following paper[6]), and it is the former procedure that is reported here.

For the synthesis of *O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**10**), the published procedure[12] was modified, where possible, to improve the efficiency and convenience of the synthetic steps. Thus, the preparation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**3**) from benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**2**) employed a selective, partial benzylation[11,13], and avoided the long process of tritylation at O-6, benzoylation or formation of a tetrahydropyran-2-yl ether at O-4, detritylation, benzylation at O-6, and, finally, deprotection at O-4, that had been employed previously[12,14]. Glycosylation of **3** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[15] (**4**) was performed by a modified Koenigs–Knorr reaction, employing silver trifluoromethanesulfonate (silver triflate) as the catalyst[10]. The reaction was rapid, the yield of the benzylated disaccharide **5** was good, and **5** was readily purified by column chromatography. Furthermore, when **5** was *O*-deacetylated, and the resulting disaccharide **6** oxidized with acetic anhydride–dimethyl sulfoxide[12], the desired glyculose **9** and a compound having the properties of the ketone hydrate[16] **7** were the only identifiable products, and formation of the (methylthio)methyl ether[12,15] was not observed. Borohydride reduction of **7** and **9** gave benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**8**), together with a small proportion of the D-glucose derivative **6**, which was readily removed by column chromatography or recrystallization; examination of the reduction products by g.l.c. of the trimethylsilyl ethers (after acid hydrolysis) showed that the proportion of the D-mannose derivative **8** was consistently 80–90% in various experiments. Finally, removal of the *O*-benzyl groups from **8** by catalytic hydrogenolysis gave the free disaccharide **10**, identical with the previously synthesized compound[12] according to migration in t.l.c. Because the melting point was slightly lower than previously reported[12,17], the identity of **10** was confirmed by (*a*) acid-catalyzed hydrolysis followed by gas–liquid chromatography of the released sugars as their trimethylsilyl ethers, and (*b*) reduction to the alditol with sodium borohydride, and examination of the high-field ^{1}H-n.m.r. spectrum.

For conversion of **10** into the oxazoline **13**, it was necessary to prepare the per-*O*-acetylglycosyl chloride derivative **12**. When this preparation was attempted by treatment of **10** with acetyl chloride[11], formation of the per-*O*-acetyl compound **11** was a major side-reaction. When acetyl chloride was saturated with gaseous hydrogen chloride[18], this side-reaction was somewhat suppressed, but by far the best method



2 R = H
3 R = Bzl
Bzl = $PhCH_2$

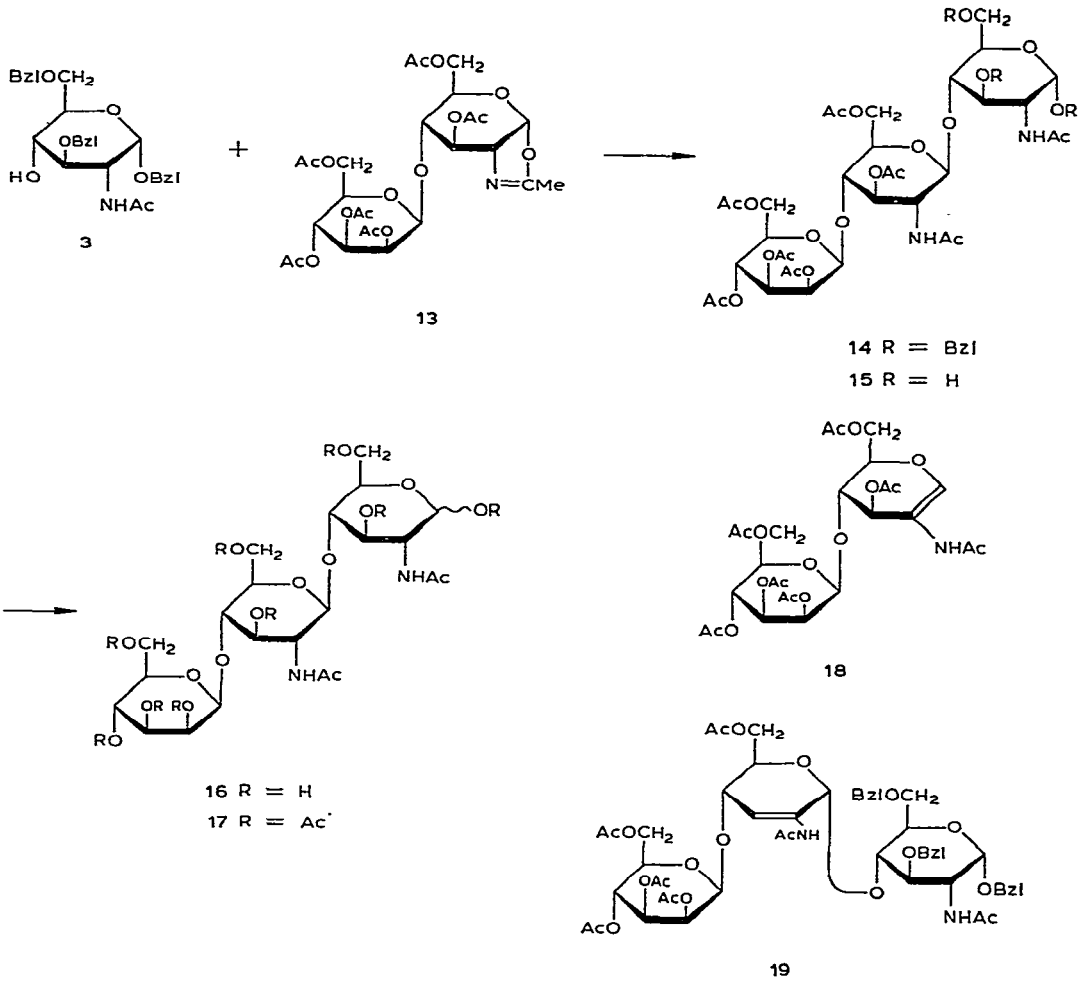
4

5 R = OAc, R' = H
6 R = OH, R' = H
7 R = R' = OH
8 R = H, R' = OH
9 R, R' = O

10 R = OH, R' = H
11 R = OAc, R' = Ac
12 R = Cl, R' = Ac

for converting **10** into the glycosyl chloride **12** was by suspension of **10** in acetyl chloride, treatment with a small volume of concentrated hydrochloric acid, and overnight reaction in a sealed tube. Conversion of **12** into the oxazoline **13** was readily achieved by chloride-ion catalysis at room temperature[11,19], and **13** was suitable for glycosylation of **3**, without prior chromatographic purification. The glycosylation was performed in 1,2-dichloroethane, with anhydrous *p*-toluenesulfonic acid as the catalyst[11], to give the partially benzylated trisaccharide **14** in a yield of 20–25%, after chromatographic purification. In this reaction, the temperature was found to be critical. By heating to 75–80°, the formation of the glucal **18**, identified by its characteristic reaction with spray reagents in t.l.c. (see Experimental section) was greatly increased; in this case, after prolonged heating, a by-product, presumably arising from condensation of the alcohol **3** with glucal **18**, was isolated, in addition to the trisaccharide **14**. Structure **19** was assigned to this product on the basis of elemental and gas–liquid chromatographic analysis, 270-MHz ^{1}H-n.m.r. spectrum, and previous reports[20] of the acid-catalyzed reaction of alcohols with glucals.

The trisaccharide **14** was *O*-deacetylated, and the benzyl groups were removed by catalytic hydrogenolysis, to give *O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (**16**). When a sample of **14** was hydrogenated prior to *O*-deacetylation, the product contained a considerable proportion of the 2-acetamido-2-deoxy-D-mannopyranose derivative (as shown by the 270-MHz ^{1}H-n.m.r. spectrum and by g.l.c. analysis), even though



the *O*-deacetylation was performed under mild conditions (0.02M sodium methoxide in methanol at room temperature).

The trisaccharide **16**, obtained as an amorphous solid, was found identical with the product of the alternative, synthetic route[6] by comparison of optical rotation, t.l.c. migration, g.l.c. of per-*O*-trimethylsilyl derivatives, and ^{1}H-n.m.r. spectrum of the alditol obtained from **16** by treatment with sodium borohydride. The n.m.r. data also confirmed the anomeric purity of the (1→4)-β-D-linkages in **16**, and were in good agreement with the values reported for a natural D-manno-oligosaccharide[21].

Alternatively, the benzyl groups were removed from **14** by catalytic hydrogenolysis, to give **15**, and acetylation of **15** with acetic anhydride–pyridine gave the per-*O*-acetyl trisaccharide **17** as a mixture of anomers, from which the α-D anomer was isolated by preparative-layer chromatography. Compound **17** was shown by t.l.c. to be identical with the product prepared by the other route[6].

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP2 hot-stage equipped with a microscope, and correspond to "corrected melting points". Optical rotations were determined in 1-dm, semimicro tubes with a Perkin–Elmer No. 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer spectrophotometer, Model 237. N.m.r. spectra were recorded at 60 MHz with a Varian T-60 spectrometer, with chloroform-*d* (containing 1% of tetramethylsilane as the internal standard) as the solvent. The high-field, n.m.r. experiments were performed at the N.M.R. Facility for Biomolecular Research located at the F. Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139. For spectra in deuterium oxide, chemical shifts are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulfonate (indirectly, to acetone in deuterium oxide; δ 2.22). The cation-exchange resin used was AG 50W-X8 (200–400 mesh, Bio-Rad Laboratories, Richmond, CA 98804). Evaporations were conducted *in vacuo* with the bath temperature kept below 30°. Dichloromethane, acetonitrile, and 1,2-dichloroethane were dried by distillation in the presence of phosphorus pentaoxide and addition of 3A molecular sieve (No. M-9882, Sigma Chemical Co., St. Louis, MO 63178). Dimethyl sulfoxide was dried by distillation *in vacuo* and addition of 4A molecular sieve (No. M-0133, Sigma). Other solvents were dried (where stated) by treatment with molecular sieve followed by addition of calcium hydride (in lump form, Fisher Scientific Co., Pittsburgh, PA 15219). The microanalyses were performed by Dr. W. Manser, CH-8704 Zurich, Switzerland, and by Galbraith Laboratories, Inc., Knoxville, TN 37921.

Chromatographic methods. — T.l.c. and preparative t.l.c. were performed on precoated plates of Silica Gel G, 0.25-mm thick (E. Merck AG, Darmstadt, Germany); for t.l.c., the plates supplied were cut to a length of 6 cm before use, but otherwise were used without pretreatment. All proportions of solvents are v/v. Preparative-layer chromatography (p.l.c.) was performed on precoated Silica Gel F254 PLC plates, 2-mm thick (Merck), or on precoated plates of Silica Gel F254, 0.5-mm thick (Merck). The spray reagent, unless otherwise stated, was 1:1:18 anisaldehyde–sulfuric acid–ethanol[22], and the plates were heated to 125°. Unsaturation was detected by spraying with a solution of 1% potassium permanganate in 2% aqueous sodium hydrogencarbonate. When plates were eluted more than once, they were dried in air between each elution. Column chromatography was performed on silica gel (0.05–0.2 mm, 70–325 mesh, Merck). Gas–liquid chromatography was performed with a Perkin–Elmer Model 900 instrument, equipped with a flame-ionization detector.

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**2**). — The following procedure gave a product that contained a high proportion of the desired α-D anomer and was more direct than the method of Gross and Jeanloz[23]. A mixture of 2-acetamido-2-deoxy-D-glucose (25.0 g), benzyl alcohol (500 mL, dried with molecular sieve), and boron trifluoride etherate (4.0 mL) was heated for 2 h at 100°, with stirring[11]. T.l.c. (60:25:4 chloroform–methanol–water) of a small aliquot showed

a majority of the desired compound (co-chromatographed beside an authentic sample), but also some trailing spots and unreacted starting-material. A 2% solution of hydrogen chloride gas in benzyl alcohol (6.3 mL) was added, and the mixture was kept for an additional 2 h at 100°. T.l.c. then showed a major spot for the desired compound, some trailing spots of contaminants, and only a trace of the starting material. The solution was cooled, treated with diethyl ether to incipient turbidity, and kept overnight in a freezer. The precipitate was filtered off, washed, and recrystallized from absolute ethanol, to give a white, crystalline product (15.3 g), m.p. 181°, $[\alpha]_D^{20}$ +198° (*c* 4.52, methanol). The filtrate from the original solids was treated with 2-isopropoxypropane, to give a flocculent precipitate that was filtered off and crystallized from absolute ethanol to give a second batch of crystals (3.2 g). A third batch (6.0 g) was similarly recovered from the reaction mixture; m.p. of second and third crops 181–183°, total yield 24.5 g (69.8%). The product was homogenous in t.l.c. (60:25:4 chloroform–methanol–water, R_F 0.44); lit.[23] m.p. 187–189°, $[\alpha]_D^{23}$ +170° (*c* 0.9, water), $[\alpha]_D^{23}$ +183° (*c* 1.1, pyridine); lit.[24] m.p. 183–184°, $[\alpha]_D^{23}$ +168.5° (*c* 0.9, water).

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (3). — Compound 3 was prepared from 2 by the usual synthetic route employing selective, partial benzylation[11,13]. However, the isolation of 3 by fractional recrystallization[13] could not be reproduced, and an alternative, chromatographic procedure was developed. The crude product (2.6 g) was chromatographed on a column (3.5 × 55 cm) of silica gel. Elution was started with 8:1 toluene–acetone and, after 300 mL had been added to the column, the solvent was changed to 4:1 toluene–acetone, the column fractions being monitored by t.l.c. (9:1 chloroform–methanol). The fractions containing pure 3 were pooled and evaporated, to give 0.84 g of 3 that was crystallized from toluene; m.p. 142°, $[\alpha]_D^{20}$ +112° (*c* 1.26, chloroform); lit.[14] m.p. 145–145.5°, $[\alpha]_D^{20}$ +114° (*c* 1, chloroform); t.l.c. (9:1 chloroform–methanol): R_F 0.65; ν_{max}^{KBr} 3483 (OH), 3310 (NH), 3043, 2953, 2916, 2885, 1653 (Amide I), 1548 (Amide II), 1501, 1460, 1378, 1360, 1323, 1218, 1173, 1130, 1107, 1055, 1028, 978, 960, 770, 728, and 685 cm^{-1} (Ph); n.m.r.: δ 5.45 (d, 1 H, *J* 9 Hz, N*H*), 4.86 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.77 (d, 1 H, $J_{a',b'}$ 11.7 Hz, PhC$H_{a'}H_{b'}$O), 4.68 (d, 1 H, $J_{a,b}$ 11.7 Hz, PhCH_aH_bO), 4.62 (d, 1 H, $J_{a',b'}$ 11.7 Hz, PhC$H_{a'}H_{b'}$O), 4.56 (s, 2 H, PhCH_2), 4.40 (d, 1 H, $J_{a,b}$ 11.7 Hz, PhCH_aH_bO), 4.30 (m, 1 H, $J_{1,2}$ 3.5 Hz, H-2), 4.0–3.5 (m, 5 H, 3 pyranose-ring H and CH_2), 3.0 (s, 1 H, deuteratable, O*H*-4), and 1.80 (s, 3 H, NHCOCH_3).

*Benzyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (5). — To a solution of 3 (980 mg, 2 mmol) in dry dichloromethane (10 mL) were added silver triflate (1.14 g, 4 mmol; Aldrich Chemical Co., Milwaukee, WI 53233), 2,4,6-trimethylpyridine (0.52 mL, 4 mmol), and a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[15] (4; 2.15 g, 4 mmol) in dichloromethane (10 mL). The suspension was stirred in the dark under nitrogen for 2 h at 0°, and then overnight at room temperature, diluted with dichloromethane, and filtered through a layer of Celite; the filtrate was

successively washed with water, M sulfuric acid (2 × 50 mL), a saturated solution of potassium hydrogencarbonate (2 × 50 mL), and water, dried (sodium sulfate), and evaporated to give a syrup which was chromatographed on a column of silica gel with 4:1 toluene–acetone, giving 1.2 g (62%) of **5**, which crystallized from aqueous methanol; m.p. 128–129°, $[\alpha]_D^{20}$ +84° (*c* 3, chloroform); ν_{max}^{KBr} 3315 (NH), 1740 (OAc), 1650 (Amide I), 1535 (Amide II), 725, and 685 cm^{-1} (Ph); n.m.r.: δ 7.32 (m, 3 OH, 6 Ph), 1.94 (s, 3 H, $OCOCH_3$), and 1.78 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{58}H_{63}NO_{12}$: C, 72.10; H, 6.85; N, 1.32; O, 19.53. Found: C, 71.96; H, 6.58; N, 1.48; O, 19.80.

The last fractions eluted from the column were combined, to give 200 mg of unreacted starting-material **3**.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**6**). — A solution of **5** (1.2 g, 1.24 mmol) in 1:2 dichloromethane–methanol (21 mL) was treated with M sodium methoxide in methanol (7 mL) for 42 h at room temperature. The solution was passed through a column of cation-exchange resin, and the eluate evaporated. The residue (1 g) crystallized from methanol, to give 850 mg of **6** (74%), m.p. 139–140°, $[\alpha]_D^{20}$ +88° (*c* 1.02, chloroform); ν_{max}^{KBr} 3470 (OH), 3325 (NH), 1655 (Amide I), 1550 (Amide II), 725, and 690 cm^{-1} (Ph); n.m.r.: δ 7.30 (m, 30 H, 6 Ph), 2.98 (s, 1 H, *OH*), and 1.72 (s, 3 H, $NHCOCH_3$). In t.l.c. (4:1 toluene–acetone), **6** had the same R_F as **5**, namely, 0.31.

Anal. Calc. for $C_{56}H_{61}NO_{11}$: C, 72.79; H, 6.65; N, 1.51; O, 19.04. Found: C, 72.65; H, 6.76; N, 1.46; O, 19.04.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-arabino-hexopyranosyl-2-ulose)-α-D-glucopyranoside (**9**). — A solution of **6** (850 mg, 0.92 mmol) in 1:2 acetic anhydride–dimethyl sulfoxide (24 mL) was kept overnight at room temperature. The solvents were evaporated, and, after being dried by repeated addition and evaporation of toluene, the residue was crystallized from methanol, to give **9** (700 mg, 82%), m.p. 164–166.5°, $[\alpha]_D^{20}$ +68° (*c* 0.93, chloroform); ν_{max}^{KBr} 3300 (NH), 1750 (C=O), 1650 (Amide I), 1550 (Amide II), 725, and 685 cm^{-1} (Ph); n.m.r.: δ 7.20 (m, 30 H, 6 Ph) and 1.70 (s, 3 H, $NHCOCH_3$); t.l.c. (20:1 chloroform–methanol) showed two closely migrating spots, probably corresponding to the glyculose **9** and its hydrate[16] **7**.

Anal. Calc. for $C_{56}H_{59}NO_{11}$: C, 72.94; H, 6.45; N, 1.52; O, 19.09. Found: C, 72.89; H, 6.46; N, 1.55; O, 19.16.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(3,4,6-tri-O-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**8**). — A solution of **9** and its hydrate **7** (750 mg, 0.81 mmol) in 1:1 dichloromethane–methanol (52 mL) was treated with sodium borohydride (260 mg) for 4 h at room temperature. The mixture was diluted with chloroform (200 mL), and successively washed with water (2 × 25 mL), 5% citric acid solution (4 × 25 mL), a saturated solution of potassium hydrogencarbonate (2 × 25 mL), and water (2 × 25 mL), dried (sodium sulfate), and evaporated. T.l.c. (4:1 toluene–acetone) revealed a very slight contamination of **8** by **6** (R_F 0.33 and 0.36, respectively; and the colors of the spots with the anisaldehyde spray were quite

different); **8** crystallized from methanol, affording 480 mg (64%), m.p. 156–159°, $[\alpha]_D^{20}$ +80° (*c* 0.98, chloroform); ν_{max}^{KBr} 3440 (OH), 3300 (NH), 1650 (Amide I), 1550 (Amide II), 720, and 680 cm^{-1} (Ph); n.m.r.: δ 7.20 (m, 30 H, 6 Ph) and 1.74 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{56}H_{61}NO_{11}$: C, 72.79; H, 6.65; N, 1.51; O, 19.04. Found: C, 72.73; H, 6.76; N, 1.50; O, 18.82.

Chromatography of the mother liquors on a column of silica gel with 4:1 toluene–acetone gave an additional 150 mg of **8** (total yield, 84%).

*2-Acetamido-2-deoxy-4-*O*-β-*D*-mannopyranosyl-*D*-glucopyranose* (**10**). — A solution of **8** (400 mg, 0.43 mmol) in acetic acid (30 mL) was hydrogenated in the presence of 10% palladium-on-charcoal (200 mg; Fluka AG, Buchs SG, Switzerland) for 48 h at room temperature and 2.0 atm. The catalyst was filtered off through a layer of Celite, and the filtrate evaporated, to afford, after drying by several additions and evaporations of toluene, 150 mg (90%) of **10**, which crystallized from ether–80% ethanol; m.p. 162–165.5°, $[\alpha]_D^{20}$ +1.6° (no mutarotation; *c* 0.86, water); lit.[12] m.p. 169–170°, $[\alpha]_D^{22}$ +11° (*c* 4.9, water); lit.[17] m.p. 167–169°, $[\alpha]_D^{25}$ +0.4° (*c* 5.4, water); t.l.c. (3:3:2 2-propanol–ethyl acetate–water): R_F 0.45.

To verify the composition of **10**, a sample (1 mg) was treated with M hydrochloric acid (0.3 mL) for 4 h at 100°, followed by evaporation *in vacuo* in the presence of potassium hydroxide, and acetylation with 1:1 acetic anhydride–pyridine (0.2 mL). The resulting, acetylated sugars were then subjected to methanolysis with M hydrogen chloride in methanol (1 mL) for 20 h at 80°, followed by evaporation, *N*-reacetylation with acetic anhydride–pyridine for 2 min at room temperature, evaporation, and per-*O*-(trimethylsilyl)ation. The per(trimethylsilyl) derivatives were analyzed by g.l.c. in a column (300 × 0.3 cm) packed with Gas-Chrom Q (80–100 mesh) coated with 3% of OV-17. The column temperature was programmed to rise from 120 to 290° at 8°/min. The ratio of the area of the peaks of methyl α- and β-D-mannopyranoside to that of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside was 1.21:1. No peak was observed for methyl α- and β-D-glucopyranoside.

A sample of **10** (5 mg) was reduced with sodium borohydride (10 mg) in water (1 mL) overnight at room temperature. M Acetic acid (0.1 mL) was added, and the solution was de-ionized by passage through a small column of cation-exchange resin (H^+). The eluate was evaporated, and the residue taken up twice in methanol and evaporated; n.m.r. (D_2O, 30°): δ 4.79 (1 H, $J_{1',2'}$ <1 Hz, H-1'), 4.07 (d, 1 H, $J_{2',3'}$ 3 Hz, H-2'), and 2.05 (s, 3 H, $NHCOCH_3$).

*2-Methyl-[3,6-di-*O*-acetyl-1,2-dideoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-mannopyranosyl)-α-*D*-glucopyrano]-[2,1-*d*]-2-oxazoline* (**13**). — To a mixture of **10** (100 mg, 0.26 mmol) and acetyl chloride (15 mL) was added concentrated hydrochloric acid (0.3 mL), and the mixture was stirred for 48 h at room temperature in a sealed tube. The solution was evaporated, and the residual reagents were removed by several additions and evaporations of toluene. Evaporation *in vacuo* in the presence of potassium hydroxide gave 160 mg of the glycosyl chloride **12** (94%); n.m.r.: δ 6.12 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1) and 2.17–1.97 (21 H, $NHCOCH_3$ and $OCOCH_3$).

A solution of **12** (160 mg, 0.24 mmol) in dry acetonitrile (12 mL) was stirred with tetraethylammonium chloride (50 mg; Aldrich) and sodium hydrogencarbonate (50 mg) for 1 h at room temperature. The solution was diluted with dichloromethane (100 mL) and washed twice with water (15 mL), dried (sodium hydrogencarbonate), and evaporated, to give a residue of **13** (140 mg, 87%). T.l.c. (20:1 chloroform–methanol) showed a major product (R_F 0.55) corresponding to the oxazoline **13**, slightly contaminated by the per-*O*-acetyl compound **11**. An analytical sample was obtained by column chromatography on silica gel with 10:10:1 chloroform–ether–methanol; it crystallized from dichloromethane–ether; m.p. 77–79°, $[\alpha]_D^{20}$ −15° (*c* 1.03, chloroform); ν_{max}^{KBr} 1750 (OAc) and 1670 cm^{-1} (C=N); n.m.r.: δ 5.86 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 2.18, 2.06, 2.01, and 1.96 (21 H, OCOCH_3 and CH_3 of oxazoline).

Anal. Calc. for $C_{26}H_{35}NO_{16} \cdot 0.5\ CH_2Cl_2$: C, 48.22; H, 5.50; N, 2.12; O, 38.79. Found: C, 48.27; H, 5.70; N, 1.92; O, 38.53.

Benzyl O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-(1→4)-O-(*2-acetamido-3,6-di*-O-*acetyl-2-deoxy*-β-D-*glucopyranosyl*)-(1→4)-*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-α-D-*glucopyranoside* (**14**) *and benzyl* O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-(1→4)-O-(*2-acetamido-6*-O-*acetyl-2,3-dideoxy*-α-D-erythro-*hex-2-enopyranosyl*)-(1→4)-*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-α-D-*glucopyranoside* (**19**). — To a solution of **13** (200 mg, 0.32 mmol) in dry 1,2-dichloroethane (4 mL) was added **3** (180 mg, 0.36 mmol). The mixture was treated with a solution (prepared by fusing *p*-toluenesulfonic acid hydrate at 110° *in vacuo* in the presence of phosphorus pentaoxide, and dissolving the residue in dry toluene) of anhydrous *p*-toluenesulfonic acid (7 mg) in toluene (0.1 mL) to give a pH of 4, and stirred at 75–80° under an atmosphere of nitrogen. Very quickly, t.l.c. in 7:7:1 toluene–ether–methanol revealed the formation of the glucal **18** (R_F 0.18; positive reaction with the potassium permanganate spray, and characteristic, green color with the anisaldehyde spray), derived from the oxazoline **13** (R_F 0.29). The temperature was decreased to 60–65° and, after 1 h, a condensation product (R_F 0.21) began to appear in t.l.c. The solution was stirred for 8 h, after which time t.l.c. showed the almost complete disappearance of **13**, the formation of **14** and another product (R_F 0.32; positive reaction with the potassium permanganate spray), and, at the same time, a decrease in the glucal **18**. The resulting, brown solution was cooled to room temperature, made neutral with a few drops of pyridine, and evaporated. The residue was chromatographed on a column of silica gel, the elution being started with 7:7:1 toluene–ether–methanol. The first fractions contained the unreacted starting-material (**3**; 120 mg, 66%). The succeeding fractions gave an amorphous by-product (R_F 0.32) which was identified as a condensation product (**19**) derived from the glucal **18** (38 mg; 11% based on **13**); $[\alpha]_D^{20}$ +28° (*c* 0.4, chloroform); ν_{max}^{KBr} 3315 (NH), 1745 (OAc), 1700 (C=C), 1675 (Amide I), 1545 (Amide II), 730, and 685 cm^{-1} (Ph); n.m.r.: δ 7.40, 7.33 (m, 15 H, 3 Ph), and 2.18–1.99 (21 H, 2 NHCOCH_3 and 5 OCOCH_3).

Anal. Calc. for $C_{53}H_{64}N_2O_{20} \cdot 2\ H_2O$: C, 58.67; H, 6.32; N, 2.58; O, 32.44. Found: C, 58.70; H, 6.54; N, 2.38; O, 32.23.

Hydrolysis and g.l.c. analysis were performed as described for **10**; the ratio of per(trimethylsilyl) derivatives of methyl α,β-D-mannopyranoside to methyl 2-acetamido-2-deoxy-α,β-D-glucopyranoside was 1.08:1, showing that one residue of 2-acetamido-2-deoxy-D-glucose had been lost. In support of this conclusion, g.l.c. showed the presence of a new, unidentified, hexosamine derivative that was eluted at 159°. For comparison, the per(trimethylsilyl) ethers of methyl α- and β-D-mannopyranoside were eluted at 119 and 123°, respectively, and the per(trimethylsilyl) ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside at 182°.

The eluent was changed to 7:7:2 toluene–ether–methanol, and the trisaccharide **14** was eluted from the next fractions (90 mg; 25% based on **13**). It was not completely pure, and so it was purified by chromatography on a 2-mm, p.l.c. plate with 20:1 chloroform–methanol. Extraction from the silica gel with 5:1 chloroform–methanol, filtration (Celite), and evaporation gave **14** (70 mg, 20%), which was precipitated with ether as an amorphous compound, $[\alpha]_D^{20}$ +23° (*c* 0.9, chloroform); ν_{max}^{KBr} 3400 (NH), 1750 (OAc), 1670 (Amide I), 1540 (Amide II), 730, and 685 cm^{-1} (Ph); n.m.r.: δ 7.35, 7.20 (m, 15 H, 3 Ph), 2.15, 2.02, 1.98, 1.93, 1.90, 1.68, and 1.63 (24 H, 2 NHCOCH_3 and 6 OCOCH_3).

Anal. Calc. for $C_{55}H_{68}N_2O_{22}$: C, 59.56; H, 6.18; N, 2.52; O, 31.73. Found: C, 59.50; H, 6.19; N, 2.46; O, 31.57.

O-β-D-*Mannopyranosyl*-(*1→4*)-O-(*2-acetamido-2-deoxy-β-D-glucopyranosyl*)-(*1→4*)-*2-acetamido-2-deoxy-D-glucopyranose* (**16**). — A solution of **14** (54 mg, 48 μmol) in methanol (2 mL) was treated with M sodium methoxide in methanol (0.4 mL) overnight at room temperature. The solution was passed through a small column of Dowex-50 (H^+) cation-exchange resin, and the eluate evaporated, to give 41 mg of the deacetylated trisaccharide, pure by t.l.c. (R_F 0.82 in 3:3:2 2-propanol–ethyl acetate–water). This product was dissolved in acetic acid (3 mL), and hydrogenated in the presence of 10% palladium-on-charcoal (30 mg) overnight at room temperature and 2.5 atm. The catalyst was filtered off, the filtrate evaporated, and the residue taken up several times in toluene and evaporated. The crude product was purified by column chromatography on silica gel with 3:3:2 2-propanol–ethyl acetate–water, affording 15 mg of pure **16** (54%) as an amorphous solid, $[\alpha]_D^{20}$ +0.2° (*c* 1.5, water); t.l.c. (3:3:2 2-propanol–ethyl acetate–water): R_F 0.20. The product co-chromatographed with the compound obtained by the other route[6]. To verify the composition of **16**, a sample (0.5 mg) was treated with M hydrochloric acid (0.25 mL) for 4 h at 100°, and the released sugars were analyzed by g.l.c. by the same method as for compound **10**. The ratio of the area of the peaks of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside to that of methyl α- and β-D-mannopyranoside was 2.12:1. A small peak was observed for methyl α- and β-D-glucopyranoside; ratio of D-*manno* to D-*gluco* compound: 26:1.

Compound **16** (1.5 mg) was reduced with sodium borohydride (5 mg) in water (1 mL) overnight at room temperature. M Acetic acid (0.1 mL) was added, and the solution was de-ionized by passing it through a small column of cation-exchange resin (H^+); the eluate was evaporated, and the residue taken up twice in

methanol and evaporated. The 270-MHz ^{1}H-n.m.r. spectrum of the product showed (D_2O, 30°): δ 4.78 (s, 1 H, $J_{1'',2''}$ <1 Hz, H-1''), 4.63 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1'), 4.25 (q, 1 H, $J_{1a,2} = J_{1b,2} = J_{2,3}$ = 5.25 Hz, H-2), 4.06 (d, 1 H, $J_{2'',3''}$ 2.7 Hz, H-2''), 2.06 (s, 3 H, NHCOCH_3), and 2.05 (s, 3 H, NHCOCH_3).

*2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-[2-acetamido-3,6-di-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-mannopyranosyl)-β-*D*-glucopyranosyl]-α-*D*-glucopyranose* (**17**). — A solution of **14** (55 mg, 49 μmol) in glacial acetic acid (3 mL) was hydrogenated in the presence of 10% palladium-on-charcoal (50 mg; Fluka) overnight at room temperature and 2.0 atm. The catalyst was filtered off through a Celite layer, and the filtrate evaporated, affording, after being dried by several additions and evaporations of toluene, 42 mg (99%) of **15**, pure by t.l.c. (R_F 0.23 in 5:1 chloroform–methanol).

A solution of **15** (20 mg, 23 μmol) in 1:1 pyridine–acetic anhydride (0.4 mL) was kept for 2 days at room temperature, and then evaporated. The residue was dried by several additions and distillations of toluene, and then the α anomer **17** was isolated on a 0.5-mm, preparative-layer plate with 10:1 chloroform–methanol (2 elutions). Extraction of the upper band from the silica gel with 5:1 chloroform–methanol, filtration (Celite), and evaporation gave **17** (14 mg, 64%), obtained as an amorphous powder by precipitation from ether–hexane, $[\alpha]_D^{20}$ −7° (*c* 1.26, chloroform); n.m.r. (270 MHz; $CDCl_3$): δ 6.10 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 6.06 (d, 1 H, N*H*), 5.66 (d, 1 H, N*H*), 5.40 (d, 1 H, $J_{2'',3''}$ 3.1 Hz, H-2''), 4.67 (s, 1 H, $J_{1'',2''}$ <1 Hz, H-1''), 2.19, 2.15, 2.13, 2.10, 2.09, 2.04, 2.03, 1.98, 1.96, and 1.93 (33 H, 2 NHCOCH_3 and 9 OCOCH_3); t.l.c. (10:1 chloroform–methanol, 2 elutions): R_F 0.35 (*cf.*, R_F 0.30 for the β anomer).

Anal. Calc. for $C_{40}H_{56}N_2O_{25} \cdot 0.5\ C_6H_{14}$: C, 51.23; H, 6.30; N, 2.78. Found: C, 51.19; H, 6.46; N, 2.55.

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