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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 II*

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ABSTRACT

Allyl 2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside was prepared, and coupled with 2-methyl-(4-*O*-acetyl-3,6-di-*O*-benzyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline. The resulting, protected disaccharide allyl 2-acetamido-4-*O*-(2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside was *O*-deacetylated and the product coupled with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide in the presence of silver trifluoromethanesulfonate and 1,1,3,3-tetramethylurea, to give the trisaccharide, allyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside. *O*-Deacetylation, oxidation with acetic anhydride–dimethyl sulfoxide, and stereoselective reduction with sodium borohydride gave mainly allyl *O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside. Removal of the 2-butenyl groups was performed by treatment with potassium *tert*-butoxide in dimethyl sulfoxide, followed by isomerization of the allyl to a 1-propenyl group with tris(triphenylphosphine)rhodium chloride. Mild, acid treatment, and catalytic hydrogenation, gave the title trisaccharide.

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INTRODUCTION

The trisaccharide lipid derivative P^1-dolichyl P^2-[*O*-β-D-mannopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranosyl] pyrophosphate is a key intermediate in the biosynthesis of *N*-glycoproteins[2]. For the chemical synthesis thereof, it is first necessary to obtain 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**). This synthesis may be achieved either by the synthesis of (*a*) *O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, followed by its β-(1→4) coupling to 2-acetamido-2-deoxy-D-glucose, or (*b*) a derivative of *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (di-*N*-acetylchitobiose), followed by coupling of a β-D-mannopyranosyl group at O-4′. The second route provides intermediates particularly suitable for conversion into a trisaccharide phosphate for eventual synthesis of a dolichyl pyrophosphate "lipid intermediate", and is the one reported in this paper.

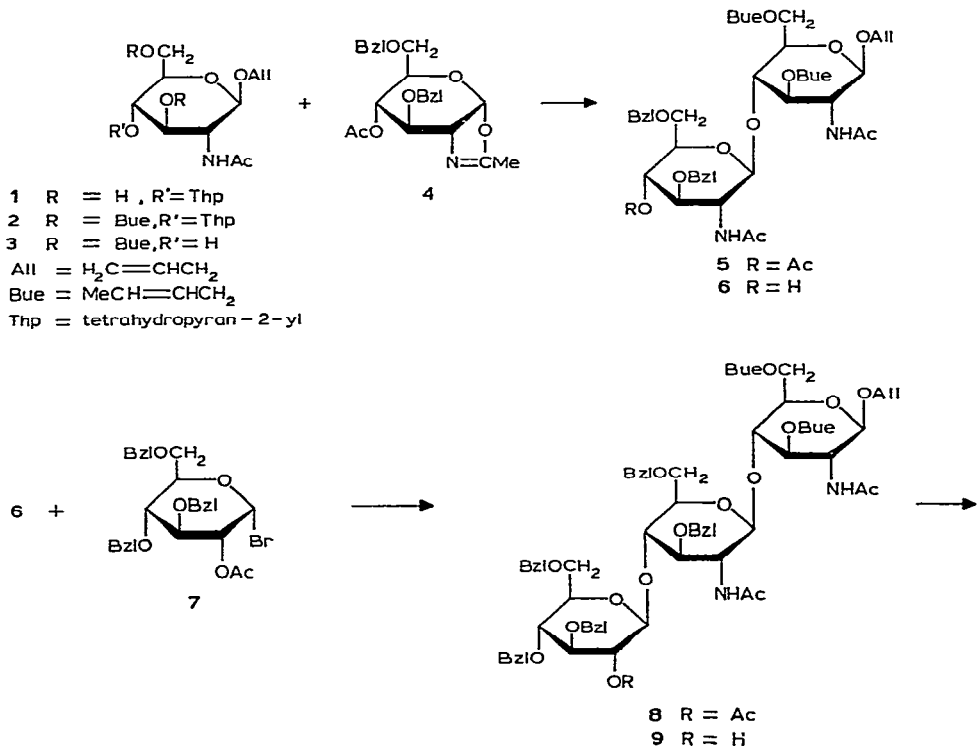
RESULTS AND DISCUSSION

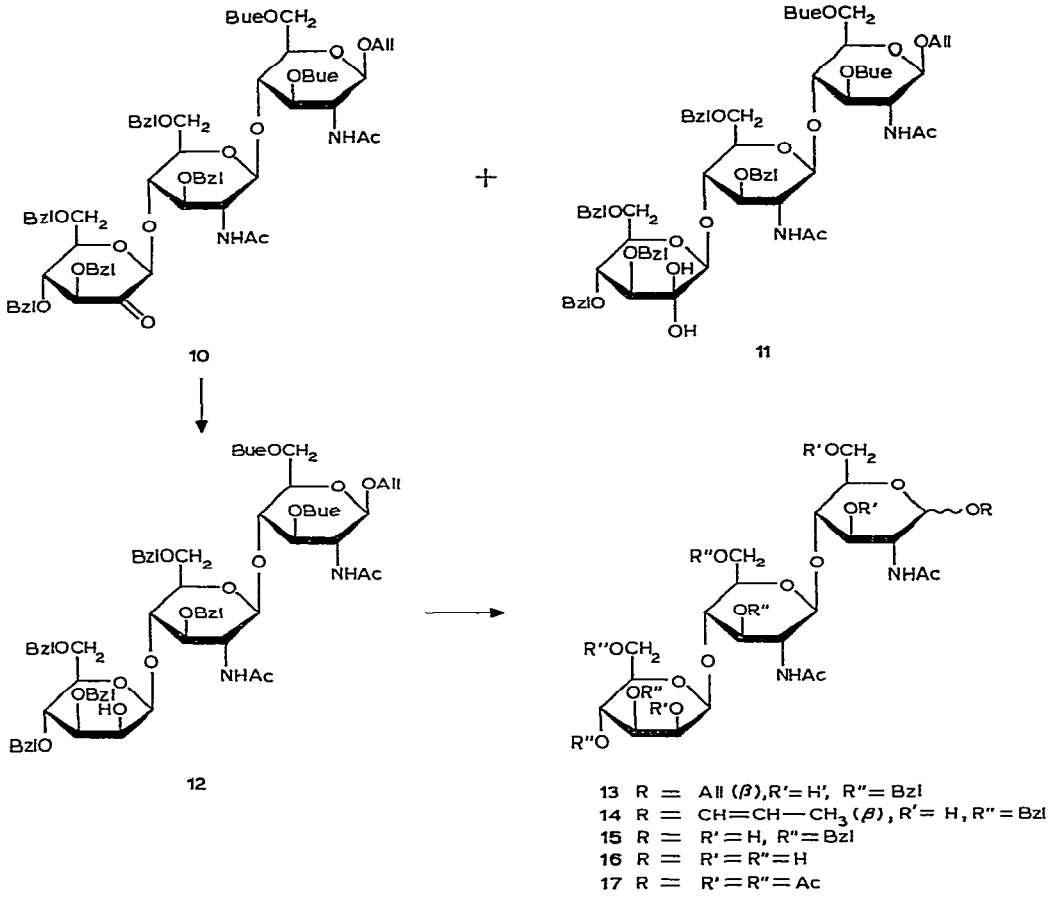
For the synthesis of a chitobiose derivative having only O-4′ available for glycosylation, it was necessary to employ synthetic intermediates that would not only yield trisaccharide **16**, but would also be suitable for eventual conversion into a per-*O*-acetylglycosyl phosphate, and thence into a "lipid intermediate". In previous work[3], we have shown that treatment of an oxazoline with dibenzyl phosphate is a good method for preparing per-*O*-acetylglycosyl phosphates as precursors of "lipid intermediates". The resulting per-*O*-acetylglycosyl dibenzyl phosphate may be readily converted into a per-*O*-acetyl-α-D-glycosyl phosphate, the α-D configuration being present in "lipid intermediates" that have a 2-acetamido-2-deoxy-D-glucopyranosyl phosphate residue at the reducing end of the oligosaccharide chain[4]. It was also shown[5] that, for this method of phosphorylation to give good results, the oxazoline residue should contain no substituents, other than acetyl groups on O-3 and O-6.

Therefore, the chitobiose derivative used for synthesis of **16** had to be readily convertible into an oxazoline and to have groups at O-3 and O-6 that could conveniently be replaced by *O*-acetyl groups *after* the steps of glucosylation and conversion of the added β-D-glucopyranosyl into a mannopyranosyl group, but *before* phosphorylation. This was necessary in order to avoid the premature deprotection of O-3 and O-6. A suitable group is 2-butenyl, which can be readily removed under strongly basic conditions[6].

The synthesis of a chitobiose derivative **6** fulfilling the requirements just mentioned was accomplished as follows. Allyl 2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside (**3**) was prepared by the crotylation of a suitably protected intermediate (**1**; see Experimental section), followed by deprotection of O-4.

The resulting compound was then coupled with the 3,6-di-*O*-benzyl oxazoline[7] **4** in 1,2-dichloroethane solution with *p*-toluenesulfonic acid as the catalyst, to give the protected chitobioside **5** in 30% yield. The stereospecificity of glycosylations with benzylated oxazolines had been established in related work[5,7], so a β-D-(1→4)-linkage could be assigned to **5**; this was supported by the negative optical rotation, sharp melting point, and homogeneity in t.l.c. (indicating a single anomer) of **5**, and by the ^{1}H-n.m.r. spectrum of the trisaccharide alditol derived from **16** (see later). *O*-Deacetylation of **5** gave **6**, which was treated with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[8] (**7**) in the presence of silver trifluoromethanesulfonate and 1,1,3,3-tetramethylurea[9]. The course of the reaction was monitored by t.l.c., which showed the partial conversion of **6** into a new compound having the properties of an allyl or 2-butenyl derivative. The unreacted, glycosyl acceptor **6** was recovered by column chromatography, which also gave the desired trisaccharide **8** as an amorphous solid having the expected i.r. and ^{1}H-n.m.r. spectra, optical rotation, and elemental analysis. However, it was not possible to determine at this stage whether or not any α-D anomer was present (see later). For conversion into a β-D-mannopyranosyl derivative, **8** was *O*-deacetylated, and the product (**9**) oxidized at C-2 by treatment with acetic anhydride–dimethyl sulfoxide[10]. For this oxidation to be successful, it was essential that the dimethyl sulfoxide be carefully predried[11]. In t.l.c., the oxidized product showed two closely migrating spots, probably corre-





13 R = All (β), R' = H, R" = Bzl
14 R = CH=CH—CH_3 (β), R' = H, R" = Bzl
15 R = R' = H, R" = Bzl
16 R = R' = R" = H
17 R = R' = R" = Ac

sponding[12] to the glyculose **10** and ketone hydrate **11**, but the (methylthio)methyl ether[8,13] was not a significant product. Reduction of **10** and **11** with sodium borohydride gave a good yield of the trisaccharide **12**, together with a small proportion of **9**. That this product was mainly the D-*manno* derivative was shown by removal of protecting groups (see later), acid hydrolysis, conversion into alditol acetates, and determination of D-mannose and D-glucose by gas–liquid chromatography. (When slightly damp dimethyl sulfoxide was employed in the oxidation step, a much larger proportion of the product was the D-*gluco* derivative.)

Removal of the 2-butenyl groups from **12** was achieved by treatment with potassium *tert*-butoxide in dimethyl sulfoxide[6], but, surprisingly, this did not always result in an efficient isomerization of the allyl into a 1-propenyl group[14]. For this reason, a small sample of the product resulting from the treatment of **12** with potassium *tert*-butoxide was routinely subjected to a trial hydrolysis with mercuric chloride[15]. On the occasions when this showed a large proportion of residual, allyl derivative **13**, the isomerization was performed by treatment of the diol **13** with tris(triphenylphosphine)rhodium chloride in the presence of diazabicyclo[2.2.2.]-

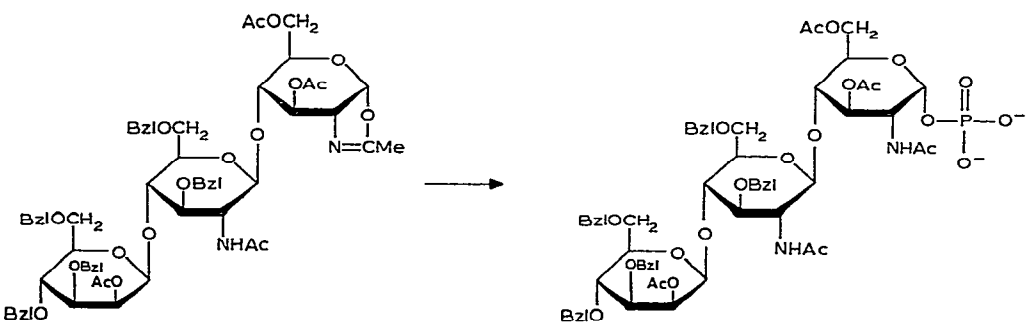
octane[16], after which the 1-propenyl group could be hydrolyzed from the product **14** by heating with aqueous acetic acid, to give the penta-*O*-benzyl trisaccharide **15**.

Hydrogenation of **15** gave the free trisaccharide **16** as an amorphous solid, found identical with the product of the alternative, synthetic route[1] by comparison of the optical rotation and t.l.c. migration, and the ^{1}H-n.m.r. spectrum of the alditol obtained from **16** by treatment with sodium borohydride. The 270-MHz n.m.r. data are in good agreement with the values reported for a natural *manno* oligosaccharide[17], and show the absence of any significant contamination by trisaccharides having α-D-manno- or α-D-gluco-pyranosyl-(1→4) residues.

The alditol derived from **16** was also examined by field-desorption mass-spectrometry; the spectrum showed peaks derived from the molecular ion, and fragmentation ions arising from D-mannopyranose, *O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucitol, and 2-acetamido-2-deoxy-D-glucitol residues.

Acetylation of **16** with acetic anhydride–pyridine gave a mixture of the anomers of the per-*O*-acetyltrisaccharide **17**, identical (t.l.c. migration) with the products obtained by the other route[1].

With regard to the relative efficiency of the synthetic routes described in this and the accompanying report[1], it is relevant to note that the glucosylation step, employing a modified Koenigs–Knorr reaction[9], gave satisfactory yields (44–62%) regardless of whether the aglycon was a monosaccharide[1] or, as in this report, a disaccharide. For the glycosylation steps involving an oxazoline, the yields were lower. Thus, the currently described glycosylation of allyl 2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-D-glucopyranoside (**3**) by 2-methyl-(2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**4**) gave disaccharide **5** in a yield of 30%. The comparable glycosylation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside by 2-methyl-[2-acetamido-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[1] gave a 25% yield of the desired trisaccharide. Similar yields were obtained for glycosylations with a trisaccharide oxazoline[18]. The fact that, in the synthesis described here, this relatively low-yield step occurs at an early stage, tends to favor this route over the alternative one[1].



18 19

Another advantage of the route described here is the potential conversion of the 1-propenyl trisaccharide **14** into an oxazoline by treatment of **14**, after acetylation, with mercuric chloride–mercuric oxide[15] in anhydrous acetonitrile, as recently described in a communication from one of our laboratories[7]. This should considerably shorten the synthetic route to **18**, which is the precursor of the desired trisaccharide phosphate **19**.

EXPERIMENTAL

General methods. — The techniques, instruments, and chromatographic procedures commonly employed in the Laboratory for Carbohydrate Research are described in the accompanying paper[1]. With minor variations, the same methods were used for the work conducted at the University of Wisconsin (preparation of compounds **1**–**6**). The mass spectrum was recorded with a Varian MAT 731 instrument equipped with a combined, electron-impact–field-ionization–field-desorption ion-source.

*Allyl 2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-*D*-glucopyranoside* (**3**). — To a solution of allyl 2-acetamido-2-deoxy-4-*O*-(tetrahydropyran-2-yl)-β-D-glucopyranoside[19] (**1**; 3.0 g, 8.7 mmol) in *N*,*N*-dimethylformamide (100 mL) were added powdered barium oxide (12 g), powdered, anhydrous barium hydroxide (4 g), and 1-bromo-2-butene ("crotyl bromide", 6 mL). The mixture was stirred at room temperature, and the progress of the reaction was monitored by t.l.c. with 9:1 chloroform–methanol as the developing solvent; the spray reagent (also for **5** and **6**) was 3:7 sulfuric acid–water; detection was achieved by charring. After 4.5 h (longer reaction-times were used in later preparations), the suspended barium salts were filtered off and washed with chloroform, and the filtrates were combined and evaporated to dryness (oil pump). The residue was extracted with a mixture of chloroform and water, and the chloroform phase was successively washed with water, M hydrochloric acid, and water, dried (sodium sulfate), and evaporated to a syrup.

To remove the tetrahydropyran-2-yl protecting group, a solution of the syrup in methanol (35 mL) and 60% aqueous acetic acid (80 mL) was heated for 1 h on a steam bath, and then evaporated. The residue was dissolved in chloroform, and the solution was successively washed with water, M aqueous sodium hydroxide, and water, dried (sodium sulfate), and evaporated. Crystallization of the residue from ethyl acetate–hexane gave pure **3** in 65% yield; additional product could be isolated from the mother liquors; m.p. 138–139°, $[\alpha]_D^{25}$ −25.8°, $[\alpha]_{436}^{25}$ −51.2° (*c* 0.5, chloroform); n.m.r.: δ 4.96 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 2.0 (s, 3 H, NHCOCH_3), and 1.8–1.6 (m, 6 H, CH_3–CH=CH–).

Anal. Calc. for $C_{19}H_{31}NO_6$: C, 61.76; H, 8.46; N, 3.79. Found: C, 61.47; H, 8.43; N, 3.66.

*Allyl 2-acetamido-4-*O*-(2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-3,6-di-*O*-(2-butenyl)-2-deoxy-β-*D*-glucopyranoside* (**5**). — Compound **4** (1.2 g, 2.8 mmol; freshly prepared by a modification of the method of Nashed

et al.[7]) was dissolved in dry 1,2-dichloroethane (3.3 mL). The allyl glycoside **3** (450 mg, 1.22 mmol) was dissolved in a 20mM solution of *p*-toluenesulfonic acid in dry 1,2-dichloroethane (1.6 mL), the oxazoline solution (1.6 mL) added, and the mixture boiled under reflux with the exclusion of atmospheric moisture. At 2 h, and again at 4 h, more oxazoline solution (1.05, and then 0.65 mL) was added. After 6 h, the acid was neutralized by the addition of a slurry of a weak-base, anion-exchange resin in acetone, the suspension was filtered, and the filtrate evaporated. Chromatography of the residue on silica gel (17:3 chloroform–acetone) gave a disaccharide fraction, and fractions containing unreacted **3**. Crystallization of the crude disaccharide from absolute ethanol gave **5** (yield 293 mg, 30.3%), m.p. 193–194°, $[\alpha]_D^{25}$ −28°, $[\alpha]_{436}^{25}$ −57.8° (*c* 0.5, chloroform); n.m.r.: δ 7.4–7.2 (m, 10 H, 2 Ph), 2.03, 1.93, 1.87 (3 s, 9 H, OCOCH_3 and 2 NHCOCH_3), and 1.8–1.6 (m, 6 H, CH_3–CH=CH–).

Anal. Calc. for $C_{43}H_{58}N_2O_{12}$: C, 64.97; H, 7.35; N, 3.52. Found: C, 64.80; H, 6.97; N, 3.54.

*Allyl 2-acetamido-4-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-3,6-di-*O*-(2-butenyl)-2-deoxy-β-*D*-glucopyranoside* (**6**). — A solution of **5** (300 mg) in M sodium methoxide in methanol (0.3 mL) was kept at room temperature. After 1 h, *O*-deacetylation was complete, as shown by t.l.c. (9:1 chloroform–methanol). Methanol was removed by evaporation, and the product was freed of sodium salts by a conventional partition between chloroform and water. The compound proved difficult to crystallize, but crystals were eventually obtained from a methanol–ether–hexane solution kept in a freezer; m.p. 160–164°, $[\alpha]_D^{25}$ −51.4°, $[\alpha]_{436}^{25}$ −107.8° (*c* 0.5, chloroform); ν_{max}^{KBr} 3500 (OH), 3280 (NH), 1660 (Amide I), 1565 (Amide II), 730, and 690 cm^{-1} (Ph); n.m.r.: δ 7.29 (m, 10 H, 2 Ph), 2.02 (s, 3 H, NHCOCH_3), 1.83 (s, 3 H, NHCOCH_3), and 1.67 (m, 6 H, 2 CH_3–CH=CH–).

Anal. Calc. for $C_{41}H_{56}N_2O_{11}$: C, 65.41; H, 7.50; N, 3.72. Found: C, 64.97; H, 7.21; N, 3.71.

Allyl O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-(2-butenyl)-2-deoxy-β-*D*-glucopyranoside* (**8**). — To a solution of **6** (700 mg, 0.93 mmol) in dry dichloromethane (10 mL) were added silver triflate (530 mg, 1.86 mmol; Aldrich Chemical Co., Milwaukee, WI 53233), 1,1,3,3-tetramethylurea (0.32 mL, 2.9 mmol; Eastman Kodak Co., Rochester, NY 14650), and a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[11] (**7**; 1 g, 1.86 mmol) in dichloromethane (10 mL). The suspension was stirred under nitrogen in the dark for 6 h at room temperature, diluted with dichloromethane, filtered through a layer of Celite, and the filtrate successively washed with water, a saturated solution of potassium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated, to give a syrup that was chromatographed on a column of silica gel with 2:1 toluene–acetone. From fractions 16 to 27, the trisaccharide **8** was obtained as an amorphous solid (510 mg, 44%), $[\alpha]_D^{20}$ −26.5° (*c* 1.22, chloroform); ν_{max}^{KBr} 3290 (NH), 1745 (OAc), 1650 (Amide I), 1555 (Amide II), 725, and 680 cm^{-1} (Ph); n.m.r.: δ 7.24 (m, 25 H, 5 Ph),

2.01 (s, 3 H, NHCOCH_3), 1.92 (s, 3 H, OCOCH_3), 1.81 (s, 3 H, NHCOCH_3), and 1.67 (m, 6 H, 2 CH_3–CH=CH–).

Anal. Calc. for $C_{70}H_{86}N_2O_{17} \cdot 2\ H_2O$: C, 66.54; H, 7.18; N, 2.22. Found: C, 66.60; H, 6.98; N, 2.00.

Fractions 35 to 48 gave 220 mg of unreacted starting-material **6**.

Allyl O-(*3,4,6-tri*-O-*benzyl*-β-D-*glucopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido-3,6-di*-O-(*2-butenyl*)-*2-deoxy*-β-D-*glucopyranoside* (**9**). — A solution of **8** (550 mg, 0.45 mmol) in 1:2 dichloromethane–methanol (4.5 mL) was treated with M sodium methoxide in methanol (0.3 mL) for 24 h at room temperature. The solution was passed through a column of cation-exchange resin (H^+), and the eluate evaporated. The residue (520 mg, 98%) gave a precipitate on adding ether–hexane; m.p. 161–164°, $[\alpha]_D^{20}$ −22° (*c* 0.8, chloroform); ν_{max}^{KBr} 3530 (OH), 3280 (NH), 1650 (Amide I), 1555 (Amide II), 1500, 725, and 680 cm^{-1} (Ph); n.m.r.: δ 7.27 (m, 25 H, 5 Ph), 2.05 (s, 3 H, NHCOCH_3), 1.75 (s, 3 H, NHCOCH_3), and 1.68 (m, 6 H, 2 CH_3–CH=CH–).

Anal. Calc. for $C_{68}H_{84}N_2O_{16}$: C, 68.90; H, 7.14; N, 2.36; O, 21.60. Found: C, 69.00; H, 7.25; N, 2.38; O, 21.61.

Allyl O-(*3,4,6-tri*-O-*benzyl*-β-D-*mannopyranosyl*)-(*1→4*)-O-(*2-acetamido-3,6-di*-O-*benzyl-2-deoxy*-β-D-*glucopyranosyl*)-(*1→4*)-*2-acetamido-3,6-di*-O-(*2-butenyl*)-*2-deoxy*-β-D-*glucopyranoside* (**12**). — A solution of **9** (250 mg, 0.21 mmol) in 1:2 acetic anhydride–dimethyl sulfoxide (6 mL) was kept overnight at room temperature. The solvents were evaporated, to give an amorphous residue; t.l.c. (20:1 chloroform–methanol) showed the complete disappearance of **9** and the presence of two closely migrating spots, probably corresponding to the glyculose **10** and its hydrate[15] **11**; ν_{max}^{KBr} 3300 (NH), 1745 (CO), 1655 (Amide I), 1560 (Amide II), 725, and 685 cm^{-1} (Ph).

A solution of **10** and **11** (250 mg, 0.21 mmol) in 1:1 dichloromethane–methanol (18 mL) was treated with sodium borohydride (90 mg) for 4 h at room temperature. The mixture was diluted with chloroform (50 mL), and successively washed with water (2 × 8 mL), 5% citric acid solution (4 × 8 mL), a saturated solution of potassium hydrogencarbonate (2 × 8 mL), and water (2 × 8 mL), dried (sodium sulfate), and evaporated. T.l.c. (2:1 toluene–acetone) revealed a very slight contamination of **12** by **9** (R_F 0.27 and 0.33, respectively; and the colors of the spots with the anisaldehyde spray were quite different); **12** was chromatographed on silica gel with 2:1 toluene–acetone, affording 180 mg (72%) of a product (pure according to t.l.c.) that gave an amorphous powder on addition of ether–hexane; m.p. 165.5–169°, $[\alpha]_D^{20}$ −31.6° (*c* 1.2, chloroform); ν_{max}^{KBr} 3500 (OH), 3270 (NH), 1655 (Amide I), 1560 (Amide II), 725, and 680 cm^{-1} (Ph); n.m.r.: δ 7.36, 7.34 (m, 25 H, 5 Ph), 6.55 (1 H, N*H*), 2.60 (s, 1 H, O*H*), 2.05 (s, 3 H, NHCOCH_3), 1.81 (s, 3 H, NHCOCH_3), and 1.67 (t, 6 H, 2 CH_3–CH=CH–).

Anal. Calc. for $C_{68}H_{84}N_2O_{16}$: C, 68.90; H, 7.14; N, 2.36; O, 21.60. Found: C, 68.91; H, 7.24; N, 2.27; O, 21.50.

O-β-D-*Mannopyranosyl*-(*1→4*)-O-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-

*(1→4)-2-acetamido-2-deoxy-β-*D*-glucopyranose* (**16**). — A solution of **12** (50 mg, 42 μmol) in dry dimethyl sulfoxide (0.3 mL) containing potassium *tert*-butoxide (50 mg, 0.45 mmol, Aldrich) was stirred under nitrogen for 2 h at 80°. The mixture was cooled, diluted with chloroform, washed with water until neutral, and the organic phase separated, dried (sodium sulfate), and evaporated. The following treatment was performed only when isomerization of the allyl to a 1-propenyl group was incomplete (see Results and Discussion); otherwise, treatment with acetic acid was the next step. The residue was dissolved in 7:3:1 ethanol–benzene–water (1.25 mL), tris(triphenylphosphine)rhodium chloride (4 mg) and 1,4-diazabicyclo[2.2.2]octane (6 mg) were added, and the mixture was boiled under reflux for 5 h. T.l.c. in 5:1 chloroform–methanol gave the same result as before the treatment (one major spot, R_F 0.50). The solvents were evaporated, and the residue was treated with 60% acetic acid for 4 h at 80°. T.l.c. in 5:1 chloroform–methanol then showed that the compound giving a spot having R_F 0.50 had been entirely converted into another compound, giving a spot having R_F 0.25, which corresponds to the penta-*O*-benzyl trisaccharide **15**. This was purified on a p.l.c. plate (20 × 20 cm, 2-mm thick) which was eluted with 5:1 chloroform–methanol. The product, detected by u.v. irradiation, was extracted from the silica gel with 2:1 chloroform–methanol, and the suspension filtered; the filtrate was evaporated, and the residue taken up in 5:1 chloroform–methanol. The suspension was filtered (sintered glass), and the filtrate evaporated, to give 22 mg of **15** (50% from **12**). Compound **15** (22 mg, 21 μmol) in acetic acid (2.5 mL) was hydrogenated in the presence of 10% palladium-on-charcoal (40 mg) at 2 atm. for 6 h. After removal of the catalyst, the filtrate was evaporated, affording, after drying by several additions and evaporations of toluene, 12 mg (95%) of **16**, which could be precipitated from a methanolic solution by addition of ether, as an amorphous solid (indefinite m.p.), $[\alpha]_D^{20}$ +0.5° (*c* 0.44, water); t.l.c. (3:3:2 2-propanol–ethyl acetate–water): R_F 0.20; it co-chromatographed with the product obtained by the other route[1].

To verify the composition of **16**, a sample (0.2 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 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 trimethylsilyl ethers were analyzed by g.l.c. on a column (300 × 0.3 cm) packed with Gas-Chrom Q (80–100 mesh) coated with 3% of OV-17. 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 0.41:1. A small peak was observed for methyl α- and β-D-glucopyranoside.

To evaluate the ratio of D-*manno* to D-*gluco* compounds, a further sample of **16** (0.2 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 then reduction of the released sugars with sodium borohydride (0.2 mg) in M ammonium hydroxide

(0.2 mL). The excess of sodium borohydride was decomposed by adding M acetic acid, and, after evaporation (N_2 gas), the boric acid was removed as methyl borate by first boiling with methanol (1 mL) containing 1 drop of acetic acid, and then evaporating (N_2 gas). This procedure was repeated three times. After being dried, the sample containing alditols was treated with acetic anhydride (1 mL) for 2 h at 100°, followed by evaporation. The alditol acetates were extracted from inorganic material with chloroform, and analyzed by g.l.c. on a column (150 × 0.3 cm) packed with Gas-Chrom Q coated with 3% of OV-225. The ratio of the area of the peak for mannitol acetate to that for glucitol acetate was 13.3:1.

Compound **16** (9 mg) was reduced with sodium borohydride (5 mg) in water (1 mL) overnight at room temperature. Then, M acetic acid (0.1 mL) was added, the solution was de-ionized by passing through a small column of cation-exchange resin (H^+), the eluate was evaporated, and the residue was twice taken up in methanol and evaporated; n.m.r. (270-MHz; D_2O, 30°): δ 4.77 (s, 1 H, $J_{1'',2''} < 1$ Hz, H-1″), 4.63 (d, 1 H, $J_{1',2'}$ 7.5 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, $J_{2'',3''}$ 3 Hz, H-2″), 2.06 (s, 3 H, $NHCOCH_3$), and 2.05 (s, 3 H, $NHCOCH_3$). The spectrum also showed very small signals for H-1″ at δ 5.43 and 4.92 that may have resulted from traces of contaminating compounds having α-D-glucopyranosyl or α-D-mannopyranosyl groups, respectively; the compound prepared by the alternative route[1] showed an identical spectrum; m.s. (field desorption): *m/e* 611.5 (M + Na), 593.5 (M + Na — H_2O), 568.5 (M + Na — $COCH_3$), 550.5 (M + Na — $COCH_3$ — H_2O), 409 [*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-D-glucitol], 366 [*O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose], 222.5 (2-acetamido-2-deoxy-D-glucitol), and 202 (D-mannose + Na).

Anal. Calc. for $C_{22}H_{40}N_2O_{16} \cdot 1.5\ H_2O$: C, 42.92; H, 7.04; N, 4.55. Found: C, 42.97; H, 7.01; N, 4.40.

A portion (2 mg) of trisaccharide **16** was acetylated with 1:1 acetic anhydride–pyridine (0.3 mL) for 2 days at room temperature. A mixture of the α- and β-D anomers of **17** (R_F 0.35 and 0.30, respectively) was obtained in the ratio of ~2:1, corresponding, in t.l.c. (2 elutions with 10:1 chloroform–methanol), to the anomeric mixture obtained by acetylation of the trisaccharide synthesized by the other route[1].

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