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THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-6-*O*-β-D-MANNOPYRANOSYL-D-GLUCOSE*†

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ABSTRACT

4,6-Di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide was condensed with benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside in the presence of silver carbonate to give crystalline benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-α-D-glucopyranoside in 32% yield. Removal of the protective *O*-acetyl and cyclic carbonate groups gave the crystalline benzyl α-glycoside of the disaccharide, which was catalytically hydrogenolyzed to yield the crystalline, title compound. Proof of the anomeric configuration of the interglycosidic linkage was obtained by comparison of the physical, spectral, and chromatographic properties of the disaccharide and its derivatives with those of the previously prepared α-D-linked analog.

INTRODUCTION

The "core" region of some glycoproteins having one or more carbohydrate chains linked to the protein backbone through a 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-D-glucopyranosylamine linkage have been shown to have a β-D-mannopyranosyl residue linked to the di-*N*-acetylchitobiosyl residue bound to the L-asparagine residue[2]. Synthetic disaccharides having the general structure 2-acetamido-2-deoxy-*O*-β-D-mannopyranosyl-D-glucose are of great interest for testing the specificity of the β-D-mannosidases used in establishing the chemical structure of the "core region" and to search for lectins specific for the β-D-mannopyranosyl residue,

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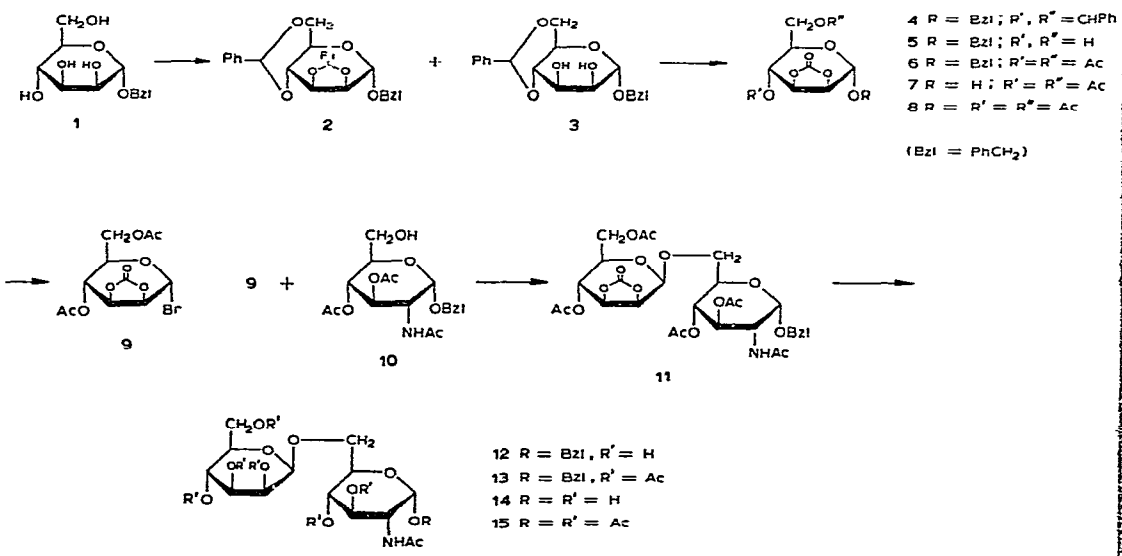
and as starting materials for the synthesis of oligosaccharides, which are subsequently linked to a peptide backbone[3], or to a dolichyl phosphate residue[4]. Such disaccharides have not been synthesized up to the present and this paper describes the synthesis of the β-D-(1→6) derivative.

RESULTS AND DISCUSSION

One of the approaches in the preparation of 1,2-*cis*-glycosides is the elimination of the steric control of the C-2 substituent[5]. Thus, Gorin and Perlin[6] and Bebault and Dutton[7] were able to synthesize oligosaccharides containing an *O*-α-D-mannopyranosyl residue by condensation of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (**9**), which has a nonparticipating substituent on O-2, with an appropriate monosaccharide derivative. Examination[6] of the molecular model of **9**, as well as n.m.r. studies of related, cyclic carbonates[8], showed a considerable flattening of the pyranose ring in the $^4C_1$(D) conformation, resulting in inability to form a planar, carbonium ion.

Compound **9** was synthesized by two known procedures[6,7] (one published[7] after this study had been completed) and by a new procedure. Benzyl α-D-mannopyranoside (**1**), prepared from D-mannose in 57% yield by the boron trifluoride etherate method, was treated with benzaldehyde and anhydrous formic acid, according to the method described by Buchanan and Schwartz[9] for the methyl glycoside, to give a mixture of the 4,6-*O*-benzylidene (**3**) and 2,3:4,6-di-*O*-benzylidene (**2**) derivatives in the ratio of 3:1. Conversion of the monobenzylidene derivative **3** into the cyclic carbonate **4** was achieved either by treating its solution in pyridine with phosgene[10] or with ethyl chloroformate and triethylamine in 1,4-dioxane[7,11]. The former gave a better yield than the latter method, owing to formation of the cyclic



carbonate **4** in agreement with the recent study by Komura *et al.*[8] of the synthesis of cyclic carbonates. Removal of the 4,6-*O*-benzylidene group of **4** by treatment with 60% acetic acid gave **5**, which was acetylated to give the diacetate **6**. Hydrogenolysis of **6** gave 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α,β-D-mannopyranose, from which the α anomer **7** was obtained in crystalline form. Assignment of the α-D configuration was based on the mutarotation, as well as on the n.m.r.-spectral data which showed, in addition to other characteristic signals, H-1 as a one-proton doublet having $J_{1,2}$ 1.2 Hz. Acetylation of **7** gave the crystalline triacetate **8** which, on treatment with hydrogen bromide in acetic acid, yielded the bromide **9**.

Condensation of **9** with benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**10**) in dichloromethane in the presence of silver carbonate, followed by chromatography, gave crystalline benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**11**) in 32% yield. The i.r. spectrum of **11** showed absorption bands corresponding to the expected structural band of the five-membered, cyclic carbonate ring on the D-mannose residue, in addition to the bands corresponding to the NH, Amide I, Amide II, and phenyl groups of the benzyl 2-acetamido-2-deoxy-D-glucoside residue. The n.m.r. spectrum of **11** contained the characteristic signals for one phenyl group, one imino proton, and five acetyl groups. Treatment of **11** with a catalytic amount of sodium methoxide removed both the cyclic carbonate and the *O*-acetyl groups, to give the crystalline benzyl α-D-glycoside **12** of the title disaccharide. G.l.c. of a mixture of the per-*O*-(trimethylsilyl) derivatives of **12** and of the α-D-linked isomer[12] showed a difference in retention time, the α-D being eluted before the β-D anomer. Comparison of the g.l.c. properties of the benzyl glycosides of both derivatives was preferred to a comparison of the free disaccharides, because the formation of anomeric mixtures of the trimethylsilyl glycosides would have added to the complexity of the chromatograms.

TABLE I

MOLECULAR ROTATIONS OF PREPARED COMPOUNDS COMPARED WITH THE SUM OF THOSE OF THEIR CONSTITUENTS, AND OF DISACCHARIDE **14** AND ITS DERIVATIVES **13** AND **15** COMPARED WITH THOSE OF THE α ANALOG AND ITS DERIVATIVES

Compound	$[M]_D$ (*degrees*) $\times 10^{-2}$
Methyl 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-D-mannopyranoside[a] (ref. 7) + compound **10**[a] (ref. 14)	+245
Compound **11**[a]	+307
Methyl α-D-mannopyranoside[b] (ref. 13) + benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[b] (**16**) (ref. 14)	+723
Methyl β-D-mannopyranoside isopropyl alcoholate[b] (**17**) (ref. 15) + compound **16**	+434
Benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside[c] (ref. 12)	+791

Table continued on p. 108.

TABLE I (*continued*)

Compound	$[M]_D$ (*degrees*) $\times 10^{-2}$
Compound **12**[c]	+412
Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside[a] (ref. 16)+compound **10**	+622
Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside[a] (**18**) (ref. 16)+compound **10**	+277
Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside[a] (ref. 12)	+615
Compound **13**[a]	+298
Compound **17**+2-acetamido-2-deoxy-α-D-glucopyranose[c] (ref. 17)	+5
Compound **17**+2-acetamido-2-deoxy-β-D-glucopyranose[c] (ref. 18)	−184
Compound **14**[c], immediately after dissolution	+8
Compound **14**, at equilibrium	+23
Compound **18**+2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[a] (ref. 19)	+170
Compound **18**+2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[a] (ref. 20)	−183
Compound **15**[a]	+102

[a]Optical rotation determined in chloroform; [b]in water; [c]in methanol.

Acetylation of **12** gave the crystalline hexaacetate **13**, and hydrogenolysis of the benzyl group gave the crystalline, free disaccharide **14**. Comparison of the molecular rotation of the free disaccharide **14** with the sum of the molecular rotations of the constituents (see Table I) suggests an α-D configuration for the hexosamine moiety. The disaccharide was further characterized as a heptaacetate obtained in the crystalline, α-D form **15**.

Comparison of the physical constants of **12**, **13**, **14**, and **15** with those of the corresponding α-D-linked disaccharide[12], and comparison of the molecular rotations with the sum of the molecular rotations of the constituents (see Table I), fully confirmed the β configuration assigned to the D-mannopyranosyl residue.

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting points". Optical rotations were determined, for solutions in 1-dm semimicrotubes, with a Perkin–Elmer Model 141 polarimeter; the chloroform used was analytical-reagent grade and contained ~0.75% of ethanol. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. N.m.r. spectra were recorded with a Varian T-60 spectrometer for solutions in chloroform-*d* or pyridine-d_5, with tetramethylsilane as the internal standard. Column chromatography was performed on

Silica Gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The ratio of weight of substance to weight of silica gel was 1:80 to 1:120. The ratio of diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 3–30 ml per g of substance to be chromatographed. The homogeneity of the products was verified by t.l.c. on precoated Silica Gel G plates (Silplate 22; E. Merck, Darmstadt, Germany; layer thickness 0.25 mm); the solvent travel-distance was ~5 cm. The spots were detected by spraying the plates with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol and heating them on a hot plate for a few minutes. Evaporations were conducted *in vacuo*, with a bath temperature below 45°. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

Benzyl α-D-mannopyranoside (**1**). — Dry D-mannose (50 g) was suspended in benzyl alcohol (500 ml, dried with molecular sieve 4 Å for 48 h) and treated with boron trifluoride etherate (7 ml). The mixture was heated with stirring for 2 h at 95°, 2% hydrogen bromide in benzyl alcohol (50 ml) was added, and heating was continued for an additional hour. The mixture was allowed to cool to room temperature, diluted with 1:1 (v/v) ether–hexane, and kept overnight at 4°. The product, which separated, was filtered off, washed with ether, and crystallized from ethyl acetate–ether to give 43 g (57%) of **1**, m.p. 132–133°, $[\alpha]_D^{25}$ +73.5° (*c* 1.5, water); lit.[6] m.p. 131–132°, $[\alpha]_D^{26}$ +74.0° (*c* 1.3, water); i.r. data: ν_{max}^{KBr} 3350 (broad, OH), and 760 and 750 cm^{-1} (Ph); t.l.c. in 4:1 (v/v) ethyl acetate–methanol: R_F 0.48.

Benzyl 4,6-O-benzylidene-α-D-mannopyranoside (**3**). — Dry **1** (12 g) was rapidly dissolved in 99% anhydrous formic acid (50 ml), cooled to 0°, and treated, under a stream of nitrogen, with freshly distilled benzaldehyde (50 ml). The mixture was stirred for 5 min at 0° and for 2 min at room temperature, and then slowly added, with vigorous stirring, to a cooled mixture of hexane (800 ml) and 30% potassium carbonate solution (300 ml). The precipitated product was filtered off, and successively washed with water (200 ml) and hexane (300 ml); the filtrate and washings were kept (in order that we might isolate therefrom the di-*O*-benzylidene derivative **2**). A solution of the solid product in chloroform (300 ml) was washed with water (2 × 100 ml), dried (sodium sulfate), and evaporated; the residue was triturated with hexane (2 × 100 ml), the suspension filtered, and the solid crystallized from dichloromethane–benzene, to give 5.4 g (34%) of **3** as prismatic needles, m.p. 147–148°, $[\alpha]_D^{25}$ +79° (*c* 1.2, chloroform); i.r. data: ν_{max}^{KBr} 3390 (broad, OH), 1500, 1450, 760, 750, 695, and 660 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.37–7.19 (10-proton multiplet, 2 Ph); 5.53 (1-proton singlet, PhCH), 4.86 (1-proton doublet, $J_{1,2}$ 1.5 Hz, H-1), 3.86 (4-proton multiplet, CH_2 of benzyl group and H_2-6), and 2.99 (2-proton singlet, deuteratable, 2 OH); t.l.c.: R_F 0.34 [19:1 (v/v) chloroform–ethanol] and 0.62 [1:1 (v/v) ether–ethyl acetate].

Anal. Calc. for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19; O, 26.79. Found: C, 66.93; H, 6.22; O, 26.67.

Benzyl 2,3:4,6-di-O-benzylidene-α-D-mannopyranoside (**2**). — The hexane layer of the filtrate and washings from the previous step was separated, washed with water

(3 × 100 ml), dried (sodium sulfate), and evaporated. The excess of benzaldehyde was distilled off under high vacuum, affording a residue. Chromatography on a column of silica gel with benzene as the eluent gave 2.3 g of **2**, which crystallized from dichloromethane–pentane as needles, m.p. 174–176°, $[\alpha]_D^{25}$ +34° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 1500, 1455, 740, and 685 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.67–7.10 (15-proton multiplet, 3 Ph), 6.22 (1-proton singlet, 2,3-*O*-Ph-CH), 5.56 (1-proton singlet, 4,6-*O*-Ph-CH), 5.15 (1-proton doublet, $J_{1,2}$ 1.5 Hz, H-1), and 4.20–3.60 (4-proton multiplet, CH_2 of benzyl group and H_2-6); t.l.c.: R_F 0.44 (chloroform) and 0.18 (benzene).

Anal. Calc. for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87; O, 21.50. Found: C, 72.64; H, 5.92; O, 21.59.

Benzyl 4,6-O-benzylidene-2,3-O-carbonyl-α-D-mannopyranoside (**4**). — *Method A.* A stirred solution of **3** (6 g) in dry pyridine (30 ml), cooled to 0°, was treated with a 12.5% solution of phosgene in benzene (20 ml) during 30 min. The mixture was allowed to warm to room temperature, and was then heated for 30 min at 40°. After dilution with chloroform (300 ml), the mixture was slowly added to a stirred, cooled, saturated solution of sodium hydrogencarbonate (200 ml). The chloroform layer was separated, washed with water (4 × 100 ml), and dried (sodium sulfate). Evaporation of the chloroform and of the residual pyridine by repeated addition and distillation of toluene gave a residue which crystallized from dichloromethane–hexane as needles (5.9 g, 92%), m.p. 143–144°, $[\alpha]_D^{25}$ +19° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 1835 (five-membered, cyclic carbonate), 1505, 1455, 750, and 690 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.60–7.00 (10-proton multiplet, 2 Ph), 5.53 (1-proton singlet, PhCH), 5.17 (1-proton singlet, H-1), 4.53–3.66 (4-proton multiplet, CH_2 of benzyl group and H_2-6); t.l.c.: R_F 0.52 (chloroform) and 0.74 [19:1 (v/v) chloroform–ethanol].

Anal. Calc. for $C_{21}H_{18}O_7$: C, 65.97; H, 4.74; O, 29.29. Found: C, 65.51; H, 5.32; O, 28.98.

Method B. To a solution of **3** (6 g) in 1,4-dioxane (30 ml) was added a 3% solution of triethylamine in benzene (100 ml). The mixture was cooled to 0°, ethyl chloroformate (50 ml) was added with stirring during 45 min, and the mixture was stirred for an additional 15 min. After dilution with benzene (300 ml), the mixture was washed successively with water (4 × 100 ml), M hydrochloric acid (2 × 100 ml), water (4 × 100 ml), saturated sodium hydrogencarbonate solution (2 × 100 ml), and water (4 × 100 ml), dried (sodium sulfate), and evaporated to a syrup which was dissolved in ether (50 ml), and nucleated, to give 4.2 g (66%) of **4**, m.p. and mixed m.p. 142–144°, and the same physical constants as those observed for **4** obtained by Method *A*.

Benzyl 2,3-O-carbonyl-α-D-mannopyranoside (**5**). — A suspension of **4** (2 g) in 60% acetic acid (20 ml) was heated for 1 h at 100°, diluted with water (300 ml), and lyophilized to give a solid which was chromatographed on a column of silica gel. Elution with 9:1 (v/v)chloroform–ethanol gave 1.25 g (81%) of pure **5**, which crystallized from ether–pentane, m.p. 123–124°, $[\alpha]_D^{25}$ +79° (*c* 1.9, methanol); lit.[6]: m.p. 122–123°, $[\alpha]_D^{26}$ +77° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 3620, 3490 (OH), 1800 (five-membered,

cyclic carbonate), 790 and 760 cm^{-1} (Ph); n.m.r. data (pyridine-d_5): δ 8.03 (1-proton singlet, deuteratable, OH), 7.60–7.06 (5-proton multiplet, Ph), 6.50 (1-proton singlet, deuteratable, OH), and 4.76–3.93 (4-proton multiplet, CH_2 of benzyl group and H_2-6); t.l.c.: R_F 0.45 [9:1 (v/v) chloroform–ethanol].

Benzyl 4,6-di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranoside (**6**). — Acetic anhydride (5 ml) was added to a solution of **5** (800 mg) in dry pyridine (3 ml). The mixture was kept overnight at room temperature, and poured onto crushed ice. The precipitate was filtered off, washed with water, and crystallized from methanol–ether–pentane to give 1 g (97%) of **6** as needles, m.p. 113–114°, $[\alpha]_D^{26}$ +37° (*c* 1.7, chloroform); lit.[6]: m.p. 114–115°, $[\alpha]_D^{26}$ +37° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 1800 (five-membered, cyclic carbonate), 760, and 690 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.36–7.10 (5-proton multiplet, Ph), 5.16 (1-proton singlet, H-1), and 2.05 and 2.02 (6 protons, 2 OAc); t.l.c.: R_F 0.63 [19:1 (v/v) chloroform–ethanol].

4,6-Di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranose (**7**). — A solution of **6** (1.5 g) in 96% ethanol (100 ml) containing acetic acid (10 ml) was treated with 10% palladium-on-charcoal (1 g) and hydrogenolyzed for 72 h at room temperature/2.0 atm. The catalyst was filtered off (Celite bed) and the filtrate evaporated. A similar hydrogenolysis, but performed without acetic acid, gave an identical residue as shown by t.l.c. in 4:1 (v/v) chloroform–acetone, which indicated a minor proportion of **6**, two closely moving spots (R_F 0.30 and 0.26) corresponding to the α and β anomers of **7**, and two minor, slower-moving spots, which were unidentified. Chromatography of the combined residues on a column of silica gel with 2:1 (v/v) chloroform–ethyl acetate gave fractions containing the anomeric forms of **7**. After evaporation, crystallization from chloroform–ether or dichloromethane–ether–hexane gave 800 mg (50%) of **7** as needles, m.p. 104–105°, $[\alpha]_D^{25}$ −22 → +8° (*c* 1.9, chloroform); lit.[6]: syrup, $[\alpha]_D^{26}$ −7.5° (*c* 1.0, chloroform); i.r. data: ν_{max}^{KBr} 3545 (OH), 1800 (five-membered, cyclic carbonate), and 1740 cm^{-1} (OAc); n.m.r. data (chloroform-*d*): δ 5.50 (1-proton doublet, $J_{1,2}$ 1.2 Hz, H-1), 3.93 (1-proton singlet, deuteratable, OH), and 2.05 (6-proton multiplet, 2 OAc); t.l.c.: R_F 0.40 [10:1 (v/v) chloroform–methanol] and 0.30 [4:1 (v/v) chloroform–acetone].

Anal. Calc. for $C_{11}H_{14}O_9$: C, 45.52; H, 4.86; O, 49.61. Found: C, 45.50; H, 4.85; O, 49.48.

1,4,6-Tri-O-acetyl-2,3-O-carbonyl-α-D-mannopyranose (**8**). — A mixture of **7** (200 mg) with 3:2 (v/v) acetic anhydride–pyridine (5 ml) was kept overnight at room temperature. Evaporation, followed by drying of the residue by repeated addition and distillation of toluene, afforded a residue that crystallized from dichloromethane–ether–pentane to give 110 mg (48%) of **8**, m.p. 115–117°, $[\alpha]_D^{25}$ +15.0° (*c* 1.1, chloroform); lit.[6]: syrup, $[\alpha]_D^{26}$ +7.5° (*c* 1.0, chloroform); lit.[7]: m.p. 117.5–118.5°, $[\alpha]_D^{23}$ +15.6° (*c* 2.5, chloroform); i.r. data: ν_{max}^{KBr} 1815 (five-membered, cyclic carbonate), 1750, and 1725 cm^{-1} (OAc); t.l.c.: R_F 0.52 [1:1 (v/v) ether–ethyl acetate], 0.66 [19:1 (v/v) chloroform–ethanol], and 0.38 (chloroform).

Anal. Calc. for $C_{13}H_{16}O_{10}$: C, 46.99; H, 4.85; O, 48.15. Found: C, 46.91; H, 4.86; O, 47.99.

4,6-Di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide (**9**). — To a solution of **8** (2.5 g) in dichloromethane (300 ml), cooled to 0°, was added a 32% solution of hydrogen bromide in acetic acid (60 ml), and the reaction was monitored by t.l.c. in 1:1 (v/v) ether–ethyl acetate (R_F of **8**, 0.52; R_F of **9**, 0.73). After 4 h, the solution was washed with ice–water (3 × 100 ml), cold, saturated sodium hydrogencarbonate solution (3 × 100 ml), and ice–water (3 × 100 ml), dried (sodium sulfate), treated with carbon (Darco G-60), and evaporated to a syrup of **9** (2 g, 79%), $[\alpha]_D^{25}$ +88° (*c* 1.0, chloroform); lit.[6]: $[\alpha]_D^{26}$ +55° (*c* 1.0, chloroform); lit.[7]: $[\alpha]_D^{23}$ +89.8° (*c* 3.3, chloroform).

Benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**11**). — A solution of benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside[12] (**10**, 1 g) in dichloromethane (30 ml) was stirred for 10 min in the dark with silver carbonate (3 g) and Drierite (4 g). To this mixture was added a solution of bromide **9** (2 g) in dichloromethane (25 ml) under anhydrous conditions during 2 h, and stirring was continued for an additional 16 h. The suspension was filtered (Celite bed), and the inorganic residue washed with dichloromethane. The combined filtrate and washings were evaporated to a semicrystalline residue. Crystallization from dichloromethane–ethyl acetate gave 1.5 g of needles, m.p. 193–210°, which showed three defined spots in t.l.c. with 19:1 (v/v) chloroform–ethanol. Attempted purification by recrystallization failed. Chromatography on a column of silica gel with 4:1 (v/v) chloroform–acetone gave **8** (60 mg), **7** (230 mg), and a major fraction that crystallized from dichloromethane–ether–pentane to give **11** (590 mg, 32%) as needles, m.p. 243–244°, $[\alpha]_D^{25}$ +46° (*c* 1.4, chloroform); i.r. data: ν_{max}^{KBr} 3325 (NH), 1775 (five-membered, cyclic carbonate), 1740 (OAc), 1650 (Amide I), 1540 (Amide II), 740, and 690 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.50–7.20 (5-proton multiplet, Ph), 5.70 (one-proton doublet, *J* 9.0 Hz, NH), and 2.10, 2.01, 2.00, 1.84, and 1.79 (15 protons, NAc and 4 OAc); t.l.c.: R_F 0.36 [19:1 (v/v) chloroform–ethanol] and 0.27 [4:1 (v/v) chloroform–acetone].

Anal. Calc. for $C_{30}H_{37}NO_{16}$: C, 53.97; H, 5.58; N, 2.09; O, 38.34. Found: C, 53.90; H, 5.58; N, 1.99; O, 38.34.

Finally, 20 mg of **10** was recovered.

Benzyl 2-acetamido-2-deoxy-6-O-β-D-mannopyranosyl-α-D-glucopyranoside (**12**). — To a solution of **11** (240 mg) in methanol (20 ml), cooled to 4°, was added 0.1M sodium methoxide in methanol (3 ml). After being kept overnight at 4°, the cold solution was de-ionized by passage through a column (2.5 × 1 cm) of Dowex 50 (H^+) cation-exchange resin. Evaporation gave 155 mg (91%) of **12**, which crystallized from methanol as needles, m.p. 218–219°, $[\alpha]_D^{25}$ +87° (*c* 1.2, methanol); i.r. data: ν_{max}^{KBr} 3350 (broad, NH and OH), 1650 (Amide I), 1550 (Amide II), 740, and 690 cm^{-1} (Ph).

Anal. Calc. for $C_{21}H_{31}NO_{11}$: C, 53.26; H, 6.59; N, 2.95; O, 37.21. Found: C, 53.05; H, 6.57; N, 2.92; O, 36.96.

The α analog, benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-

glucopyranoside[12] crystallized with 0.5 molecule of water, m.p. 117–118°, $[\alpha]_D^{20}$ +167° (*c* 0.7, methanol). G.l.c. of the per-*O*-(trimethylsilyl) derivatives of **12** and the α analog was performed with a Perkin–Elmer Model 900 gas chromatograph equipped with a flame-ionization detector, on a stainless-steel column (120 × 0.3 cm) packed with Chromosorb GHP (100–120 mesh) coated with 1% of OV-11 (Supelco Inc., Bellefonte, PA 16823), operated isothermally at 250°, with nitrogen as the carrier gas; the $t'_{Per\text{-}O\text{-}(trimethylsilyl)raffinose}$ was 2.63 for **12**, and 1.92 for the α analog.

*Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-D-glucopyranoside* (**13**). — Acetic anhydride (5 ml) was added to a solution of **12** (95 mg) in dry pyridine (3 ml); the solution was kept overnight at room temperature, and evaporated, and the residue was dried by several additions and evaporations of toluene. Crystallization from dichloromethane–ether–pentane gave 130 mg (89%) of needles, m.p. 147–148°, $[\alpha]_D^{25}$ +41° (*c* 1.1, chloroform); i.r. data: ν_{max}^{KBr} 3300 (NH), 1740 (OAc), 1645 (Amide I), 1540 (Amide II), 750, and 690 cm^{-1} (Ph); t.l.c.: R_F 0.45 [19:1 (v/v) chloroform–ethanol].

Anal. Calc. for $C_{33}H_{43}NO_{17}$: C, 54.61; H, 5.97; N, 1.92; O, 37.41. Found: C, 54.50; H, 5.95; N, 1.82; O, 37.38.

The α analog, benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside[12], had m.p. 76–78° and $[\alpha]_D^{20}$ +143° (*c* 1.2, chloroform).

*2-Acetamido-2-deoxy-6-*O*-β-D-mannopyranosyl-D-glucopyranose* (**14**). — A solution of **12** (190 mg) in a mixture of absolute ethanol (47 ml), water (2 ml), and acetic acid (1 ml) was hydrogenolyzed in the presence of 10% palladium-on-charcoal (100 mg) for 72 h at room temperature/2.0 atm. The catalyst was filtered off on a layer of Celite, and the filtrate was evaporated, and dried by several additions and evaporations of toluene. Crystallization from methanol gave 127 mg (83%) of **14**, m.p. 184–185° (dec.), $[\alpha]_D^{25}$ +4° (no mutarotation; *c* 0.4, water); i.r. data: ν_{max}^{KBr} 3300 (broad, NH and OH), 1645 (Amide I), and 1510 cm^{-1} (Amide II).

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.85; H, 6.57; N, 3.65; O, 45.90. Found: C, 43.80; H, 6.57; N, 3.58; O, 45.86.

The α analog, 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranose[12], crystallized with 0.5 molecule of water, m.p. 142–144°, $[\alpha]_D^{20}$ +38 → +35° (*c* 1.2, 50% methanol).

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-α-D-glucopyranose* (**15**). — Dry **14** (77 mg) was stirred with 1:1 (v/v) pyridine–acetic anhydride (7 ml) for 24 h at room temperature. The mixture was evaporated, and the residue was dried by several additions and evaporations of toluene, and chromatographed on a column of silica gel with 19:1 (v/v) chloroform–ethanol. The fractions (108 mg, 79%) having R_F 0.28 in t.l.c. in the same solvent system were crystallized from dichloromethane–ether–pentane; m.p. 102–103°, $[\alpha]_D^{25}$ +15° (*c* 1.4, chloroform); i.r. data: ν_{max}^{KBr} 3300 (NH), 1750 (OAc), 1650 (Amide I), and 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.48; H, 5.84; N, 1.95; O, 42.32.

The α analog, 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucose had m.p. 75–77°, $[\alpha]_D^{20}$ +68° (*c* 0.6, chloroform).

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