Carbohydrate Research, 52 (1976) 103–114
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SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-3-*O*-β-D-MANNOPYRANOSYL-D-GLUCOSE*

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(Received August 9th, 1976; accepted for publication, August 20th, 1976)

ABSTRACT

Condensation of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**2**) gave an α-D-linked disaccharide, further transformed by removal of the carbonyl and benzylidene groups and acetylation into the previously reported benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside. Condensation of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose or 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide with **2** gave benzyl 2-acetamido-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside. Removal of the acetyl group at O-2, followed by oxidation with acetic anhydride–dimethyl sulfoxide, gave the β-D-*arabino*-hexosid-2-ulose **14**. Reduction with sodium borohydride, and removal of the protective groups, gave 2-acetamido-2-deoxy-3-*O*-β-D-mannopyranosyl-D-glucose, which was characterized as the heptaacetate. The anomeric configuration of the glycosidic linkage was ascertained by comparison with the α-D-linked analog.

INTRODUCTION

The core region of the carbohydrate chains linked to an asparagine residue of the peptide backbone of various glycoproteins has been shown to be composed of a β-D-mannopyranosyl group linked (1→4) to the nonreducing end of a di-*N*-acetylchitobiosyl residue[2]. Subsequently, a disaccharide having the structure of a 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose was obtained by enzymic

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*Amino Sugars CIV. Synthesis of *O*-β-D-Mannopyranosyl Oligosaccharides, Part II. For Part I, see ref. 1. This is publication No. 711 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts. This work was supported by research grants from the National Institute of Arthritis, Metabolism, and Digestive Diseases (AM-03864 and AM-05067), National Institutes of Health, U. S. Public Health Service.

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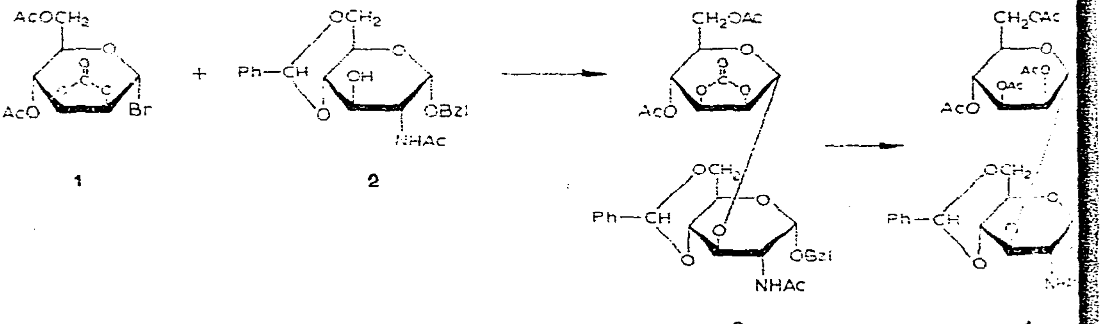
degradation of 2-acetamido-2-deoxy-poly(D-mannopyranosyl)-D-glucose oligosaccharides isolated from the urine of patients suffering from mannosidosis, a lysosomal storage disease[3]. In addition to the compounds obtained by enzymic degradation of glycopeptides or oligosaccharides[2,3], 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose was synthesized by degradation[4] of 4-*O*-β-D-mannopyranosyl-D-mannose, isolated from a natural source, to a D-arabinose derivative, followed by elongation of the chain. The synthesis of disaccharides having a 2-acetamido-2-deoxy-*O*-β-D-mannopyranosyl-D-glucose structure was undertaken[1] in order to establish the specificity of the β-D-mannosidases used in the elucidation of the structure of naturally occurring glycoproteins and oligosaccharides, and to search for lectins specific for the β-D-mannopyranosyl residue. These disaccharides may also serve as starting materials for the synthesis of larger oligosaccharides[5] and glycopeptides[6]. We describe here the synthesis of the 3-*O*-β-D-mannopyranosyl derivative **18**.

RESULTS AND DISCUSSION

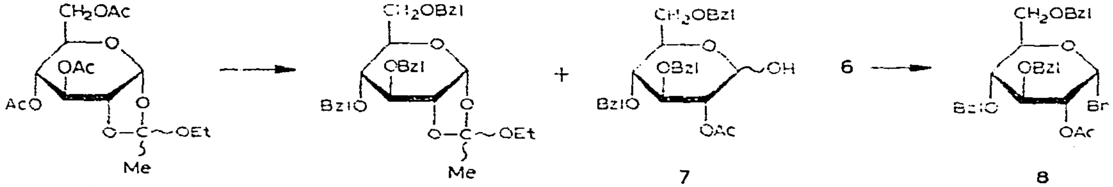
Previous reports[1,7,8] described the condensation of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (**1**), which has a nonparticipating, *O*-carbonyl protective group at O-2, with various derivatives in the synthesis of β-D-mannopyranosyl-containing oligosaccharides. Condensation of **1** with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[9] (**2**) in the presence of mercuric cyanide gave, however, the α anomer, namely, crystalline benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**3**). The optical rotation indicated an α-D- rather than a β-D-glycosidic linkage, and the nature of the linkage was ascertained by saponification of **3** followed by acetylation, to give the known[10] benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**4**). Attempts at condensing **1** with **2** in the presence of silver carbonate failed, **2** being recovered unchanged. On the basis of previous work[7,8], these results suggested that, under the same reaction conditions, the stereochemical course of a Koenigs–Knorr condensation depends not only on the structure and reactivity of the glycosyl halide but also on the structure of the glycose reacting with this halide.



1 **2** **3** **4**

In order to obtain the β-D-linked disaccharide **18**, another approach was selected. Previous investigations[11,12] had shown that β-D-mannopyranosyl-containing oligosaccharides may be prepared by oxidation at C-2 of a β-D-glucopyranosyl group to give the corresponding β-D-*arabino*-hexopyranosyl-2-ulose group, followed by reduction. Recently, in a study of the reduction of various hexopyranosid-2-uloses with metal borohydrides, Miljković *et al.*[13] found that the reaction proceeds stereospecifically to give one of the two isomeric hexopyranosides. This stereospecificity, explained by a unidirectional approach to the carbonyl group by the hydride ion, may depend on electrostatic interactions, torsional strain, and nonbonded interactions of the hexosidulose molecule and, accordingly, β-D-*arabino*-hexopyranosid-2-uloses gave β-D-mannopyranosides stereospecifically.

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose[12] (**6**) was prepared in one step from 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose[14] (**5**) by treatment with α-bromotoluene (benzyl bromide) and potassium hydroxide in tetrahydrofuran, according to the method described for the synthesis of the β-D-mannopyranose analog[15]: 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-glucose (**7**) was obtained as a minor by-product (3%). Compound **6** had the same properties as those of the compound prepared[12] by the more-laborious, two-step procedure.

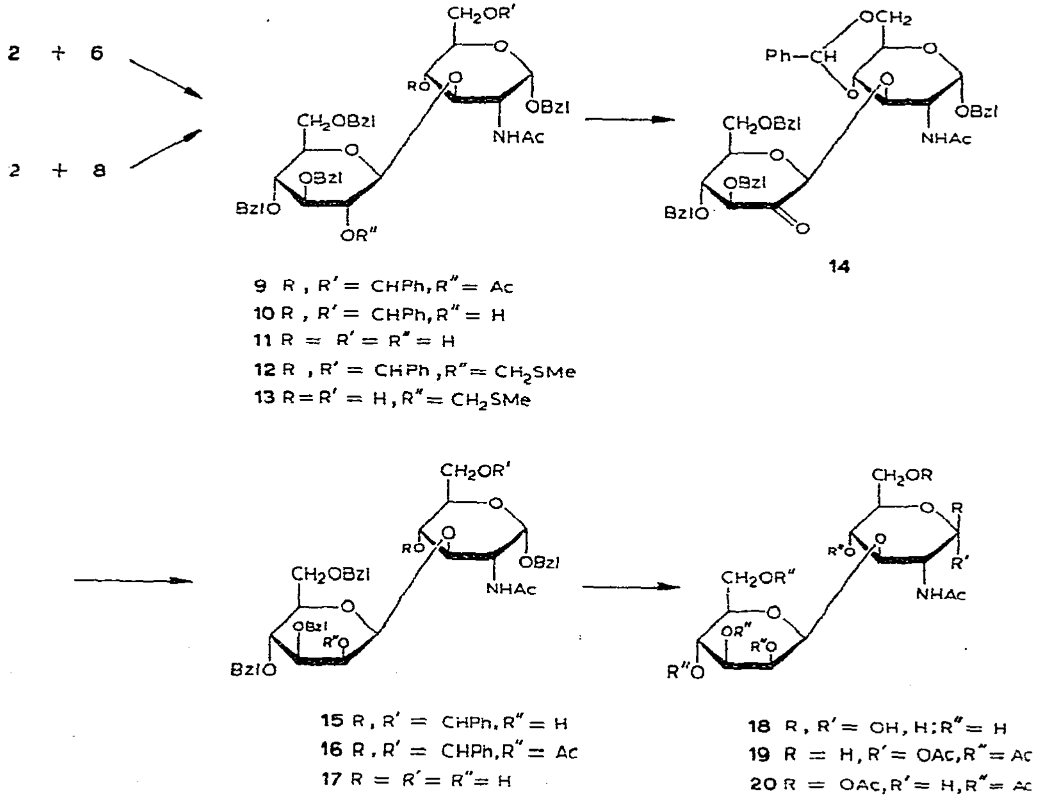


5 **6** **7** **8**

Condensation of **6** with **2** in nitromethane, followed by treatment with mercuric bromide, according to a modified method[14] of glycosylation, gave benzyl 2-acetamido-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-α-D-glucopyranoside (**9**) in a yield of 12–14%. This compound possesses a readily removable acetyl group at O-2 of the β-D-glucopyranosyl group, whereas the remaining hydroxyl groups thereof are protected by benzyl groups. The low yield from the condensation is in agreement with a similar yield, previously obtained[12] with **6** as the glycosylating agent, that was attributed[12] to the formation, through intramolecular glycosylation, of the 1,6-anhydro-D-glucose derivative. A better yield (82%) of **9** was, however, obtained by Koenigs–Knorr condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**8**) with **2** in the presence of mercuric cyanide; **8** had been obtained by treatment of **6** with hydrogen bromide in acetic acid. *O*-Deacetylation of **9** gave **10**, and subsequent removal of the 4,6-*O*-benzylidene group of **10** gave benzyl 2-acetamido-2-deoxy-3-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**11**), which was compared to the β-D-mannopyranosyl analog **17**. Oxidation of **10** with dimethyl sulfoxide–acetic anhydride[16] gave the expected benzyl

2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-benzyl-β-D-*arabino*-hexopyranosyl-2-ulose)-α-D-glucopyranoside (**14**), which showed a strong, carbonyl-group absorption at 1750 cm^{-1}, plus the 2-(methylthio)methyl derivative **12**; this kind of by-product has frequently been isolated as a result of this method of oxidation[17,18]. Compound **12** could not be purified, but was characterized as **13** after removal of the benzylidene group. The structure of **13** was confirmed by n.m.r. spectroscopy and elementary analysis.

Stereospecific reduction of the hexosidulose **14** with sodium borohydride gave the protected, crystalline β-D-mannopyranosyl disaccharide **15**, which was acetylated to give **16**, or partially hydrolyzed to give **17**. Comparison of the β-D-glucopyranosyl derivatives **9**, **10**, and **11** with the β-D-mannopyranosyl analogs **16**, **15**, and **17**, respectively, showed large differences in the melting points and optical rotations. Hydrogenolysis of **17** in the presence of palladium-on-charcoal gave amorphous 2-acetamido-2-deoxy-3-*O*-β-D-mannopyranosyl-D-glucose (**18**). Methanolysis of **18**, followed by trimethylsilylation, gave compounds that showed, in g.l.c., peaks corresponding to those of methyl α- and β-mannopyranoside, and methyl-2-acetamido-2-deoxy-α- and β-glucopyranoside; the ratio of the peak area of the mannosides to those of the 2-acetamido-2-deoxyglucosides was ~1:1. No peaks corresponding to



9 R, R' = CHPh, R" = Ac
10 R, R' = CHPh, R" = H
11 R = R' = R" = H
12 R, R' = CHPh, R" = CH$_2$SMe
13 R = R' = H, R" = CH$_2$SMe

14

15 R, R' = CHPh, R" = H
16 R, R' = CHPh, R" = Ac
17 R = R' = R" = H

18 R, R' = OH, H; R" = H
19 R = H, R' = OAc, R" = Ac
20 R = OAc, R' = H, R" = Ac

D-glucose derivatives could be detected. The transformation of a β-D-glucopyranosyl into a β-D-mannopyranosyl group should not affect the β-D configuration of the glycosidic linkage, and this was verified by comparison of the properties of **18** with those of the previously prepared α-D analog[10]. G.l.c. of the per-*O*-(trimethylsilyl) derivatives showed, on two different columns, that the α-D-linked disaccharide is eluted before the β-D isomer.

Acetylation of **18**, followed by chromatographic separation, gave the two isomeric, crystalline heptaacetates **19** and **20**. A comparison (see Table I) of the molecular rotations of the disaccharide **18** and of its acetates **19** and **20** with the sum of the molecular rotation of the constituents and with the molecular rotations of the α-D-linked analogs[10], confirmed the β-D configuration of the glycosidic linkage of **18**.

TABLE I

MOLECULAR ROTATIONS OF **18**, **19**, AND **20** COMPARED WITH THE SUM OF THE MOLECULAR ROTATIONS OF THE CONSTITUENTS AND WITH THE MOLECULAR ROTATIONS OF THE α ANALOGS

Compound	$[M]_D$ (*degrees*) $\times 10^{-2}$
Methyl α-D-mannopyranoside[a] (ref. 19) +2-acetamido-2-deoxy-D-glucose[a] (**21**), at equilibrium (ref. 20)	+243
Methyl β-D-mannopyranoside[a] (ref. 20) +compound **21**	−45
2-Acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose[b], at equilibrium (ref. 10)	+222
Compound **18**[a], at equilibrium	−53
Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside[c] (**22**) (ref. 22) +2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[c] (ref. 23)	+170
Compound **22**+2-acetamido-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[c] (ref. 24)	−183
Compound **19**[c]	+129
Compound **20**[c]	−8

[a]Optical rotation determined in water; [b]in 60% methanol; [c]in chloroform.

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, semimicro tubes, with a Perkin–Elmer Model 141 polarimeter. I.r. spectra were recorded, for potassium bromide discs or thin films, with a Perkin–Elmer Model 237 spectrophotometer. N.m.r. spectra were recorded with a Varian T-60 spectrometer for solutions in chloroform-*d*, with tetramethylsilane as the internal standard. G.l.c. of the per-*O*-(trimethylsilyl) derivatives was performed with a Perkin–Elmer Model 900 gas chromatograph, equipped with a flame-ionization detector, on stainless-steel columns of different lengths and packings, and with nitrogen as the carrier gas. 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 the diameter of the column to its length was 1:8 to 1:18. The volume of the fractions eluted was 3–4 ml per g of substance to be chromatographed. All proportions for solvent mixtures are in v/v. The homogeneity of the products was verified by t.l.c. on plates precoated with Silica Gel G (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 the bath temperature below 45°. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(4,6-di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**3**). — A solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[9] (**2**, 500 mg) and mercuric cyanide (1.2 g) in 1:1 benzene–1,2-dichloroethane (150 ml) was heated at atmospheric pressure until 50 ml of the solvents had distilled. To the mixture was added during 2 h at room temperature, with stirring, a solution of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide[1] (**1**, 1.5 g) in dry 1,2-dichloroethane. The mixture was stirred for a further 48 h, and treated with additional amounts of mercuric cyanide (1 g) and **1** (1 g), and stirring was continued for 24 h. The mixture was diluted with chloroform (300 ml), and filtered, and the filtrate was successively washed with water (3 × 50 ml), a saturated solution of sodium hydrogencarbonate (2 × 50 ml), a saturated solution of potassium iodide (3 × 50 ml), and water (3 × 50 ml), dried (sodium sulfate), and evaporated, to give a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, affording 353 mg (42%) of **3**; it crystallized from chloroform–methanol as needles, m.p. 287–289° (dec.), $[\alpha]_D^{25}$ +65° (*c* 1.05, chloroform); ν_{max}^{KBr} 3300 (NH), 1840 (5-membered, cyclic O–CO–O), 1740 (OAc), 1655 (Amide I), 1550 (Amide II), 725, and 690 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.44.

Anal. Calc. for $C_{33}H_{37}NO_{14} \cdot CH_3OH$: C, 58.03; H, 5.87; N, 1.99. Found: C, 57.61; H, 5.48; N, 1.99.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**4**). — A suspension of **3** (135 mg) in methanol (20 ml) was treated with a 0.1M solution (2 ml) of sodium methoxide in methanol for 4 h at room temperature. The insoluble product was filtered off, washed with water and methanol, and dried in high vacuum over phosphorus pentaoxide. A solution of this product in dry pyridine (5 ml) was treated with acetic anhydride (3 ml) for 16 h at room temperature, and then evaporated. The residue crystallized from methanol to give 113 mg (77%) of needles, m.p. 171–172°, showing no depression of the m.p. on admixture with an authentic sample[10]; $[\alpha]_D^{25}$ +67° (*c* 1.1, chloroform); lit.[10] m.p. 171°, $[\alpha]_D^{25}$ +68° (*c* 1.4, chloroform).

Anal. Calc. for $C_{36}H_{43}NO_{15}$: C, 59.24; H, 5.95; N, 1.91; O, 32.89. Found: C, 59.20; H, 5.95; N, 1.84; O, 32.86.

Attempted condensation of **1** *with* **2** *in the presence of silver carbonate.* — A solution of compound **2** (500 mg) in dichloromethane (50 ml) was stirred with silver carbonate (1 g) and Drierite (3 g) for 1 h at room temperature, and then treated with a solution of **1** (1.5 g) in dichloromethane (30 ml) for 3 h. Stirring was continued for a further 48 h. The mixture was filtered, and the inorganic residue was washed with chloroform (100 ml). The filtrate and washing were combined, and successively washed with water (2 × 50 ml), a saturated sodium hydrogencarbonate solution (3 × 50 ml), and water (2 × 50 ml), dried (sodium sulfate), and evaporated, to give a semicrystalline residue that afforded unchanged **2** (480 mg) upon trituration with methanol.

3,4,6-Tri-O-benzyl-1,2-O-(1-ethoxyethylidene)-α-D-glucopyranose (**6**) *and 2-O-acetyl-3,4,6-tri-O-benzyl-D-glucopyranose* (**7**). — A solution of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucose[14] (**5**, 12.5 g) and benzyl bromide (13 ml) in dry tetrahydrofuran (50 ml) was treated with powdered potassium hydroxide (25 g), and boiled for 4 h under reflux with stirring. After being cooled, the mixture was diluted with dichloromethane (300 ml), and successively washed with water (5 × 100 ml), saturated sodium hydrogencarbonate solution (2 × 50 ml), and water (2 × 50 ml), dried (potassium carbonate), and evaporated, to give a yellow oil that was chromatographed on a column of silica gel with 4:1 toluene–ether containing 0.1% of triethylamine, affording 27 g (79%) of **6** as a pale-yellow oil, $[\alpha]_D^{25}$ +35° (*c* 1.5, chloroform); lit.[12] $[\alpha]_D^{20}$ +33° (*c* 6.6, chloroform); ν_{max}^{film} 1740 (orthoester), 735, and 685 cm^{-1} (Ph).

Compound **7** (205 mg; 3%) was obtained as the slow-moving fraction, and crystallized from ether as needles, m.p. 128–129°; $[\alpha]_D^{22}$ +55° (*c* 1.1, chloroform); ν_{max}^{KBr} 3360 (OH), 1725 (OAc), 725, and 630 cm^{-1} (Ph); t.l.c. in 4:1 toluene–ether: R_F 0.52.

Anal. Calc. for $C_{29}H_{32}O_7$: C, 70.71; H, 6.55; O, 22.73. Found: C, 70.86; H, 6.55; O, 22.77.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl bromide (**8**). — A solution of **6** (2.0 g) in dichloromethane (50 ml) was cooled to 0°, treated with a 34% solution of hydrogen bromide in glacial acetic acid (5 ml), and stirred for 30 min. The mixture was diluted with dichloromethane (250 ml), successively washed with ice–water (2 × 100 ml), a saturated sodium hydrogencarbonate solution (2 × 100 ml), and water (2 × 100 ml), dried (magnesium sulfate), filtered, and evaporated. Bromide **8** was obtained as a syrup (1.9 g, 87%); as it is unstable, it was used without further purification.

Benzyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**9**). — *Method A.* A solution of **2** (500 mg) and **6** (900 mg) in dry nitromethane (20 ml) was distilled at atmospheric pressure while the volume was kept constant by continuous addition of dry nitromethane. After distillation of 300 ml of nitromethane, the mixture was treated with mercuric bromide (90 mg), and boiled under reflux for 16 h. Evaporation of the solvent gave a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 153 mg (14%) of **9**, which crystallized from benzene–methanol, m.p. 168–169°, $[\alpha]_D^{25}$ $-29°$ (*c* 2.6, chloroform); ν_{max}^{KBr} 3300 (NH), 1740 (OAc), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.25 (m, 25 H, 5 Ph), 1.93 (s, 3 H, OAc), and 1.88 (s, 3 H, NAc). Repetition of the same experiment with 798 mg of **2** and 1.500 g of **6** gave 210 mg (12%) of **9**.

Anal. Calc. for $C_{51}H_{55}NO_{12}$: C, 70.09; H, 6.34; N, 1.60; O, 21.97. Found: C, 69.97; H, 6.40; N, 1.55; O, 22.11.

Method B. A mixture of 1:1 (v/v) benzene–nitromethane (180 ml), **2** (1.0 g), and mercuric cyanide (1 g) was distilled at atmospheric pressure until the volume was ~130 ml, cooled to room temperature, treated with **8** (2.5 g), stirred for 72 h, and treated with additional amounts of mercuric cyanide (500 mg) and **8** (1 g); stirring was continued for 24 h. Chloroform (350 ml) was added, followed by successive washing with water (2 × 50 ml), a saturated hydrogencarbonate solution (2 × 50 ml), a saturated potassium iodide solution (2 × 50 ml), and water (4 × 50 ml), and drying (sodium sulfate). Evaporation of the solvents gave a syrupy residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 1.8 g (82%) of **9**, m.p. and mixed m.p. 168°; indistinguishable from **9** obtained by Method A. Repetition of this reaction with 2.5 g of **2** and a total amount of 6 g of **8** afforded 4.5 g (82%) of **9**.

*Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**10**). — To a solution of **9** (1.5 g) in 1:1 dichloromethane–methanol (30 ml) was added M sodium methoxide in methanol (2 ml). The mixture was kept at room temperature for 16 h, and then diluted with dichloromethane (300 ml), washed with water (3 × 100 ml), and dried (sodium sulfate). Evaporation of the solvent gave 1.27 g (89)% of **10** which crystallized from dichloromethane–methanol as needles, m.p. 174–176°, $[\alpha]_D^{25}$ $+64°$ (*c* 2.4, chloroform); ν_{max}^{KBr} 3500 (OH), 3300 (NH), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.42.

Anal. Calc. for $C_{49}H_{53}NO_{11}$: C, 70.74; H, 6.42; N, 1.68; O, 21.15. Found: C, 70.70; H, 6.33; N, 1.59; O, 21.59.

*Benzyl 2-acetamido-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**11**). — A suspension of **10** (104 mg) in 60% acetic acid (20 ml) was heated with stirring for 1 h at 80°. The solution was evaporated, and the residue was dried by repeated addition and distillation of toluene. Chromatography on a column of silica gel with 19:1 chloroform–ethanol gave 86 mg (92%) of **11**, which could not be crystallized, but was obtained as amorphous material from dichloromethane–ether–pentane; $[\alpha]_D^{25}$ $+48°$ (*c* 2.4, chloroform); ν_{max}^{KBr} 3425 (OH), 3295 (NH), 1645 (Amide I), 1555 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c.: R_F 0.42 (19:1 chloroform–ethanol) and 0.72 (9:1 chloroform–ethanol).

Anal. Calc. for $C_{42}H_{49}NO_{11}$: C, 67.82; H, 6.64; N, 1.88; O, 23.66. Found: C, 67.71; H, 6.55; N, 1.83; O, 23.50.

*Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(methylthio)methyl-β-*D*-glucopyranosyl]-α-*D*-glucopyranoside* (**12**) *and benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-benzyl-β-*D-arabino-*hexopyranosyl-2-ulose)-α-*D*-glucopyranoside* (**14**). — A solution of **10** (1.2 g) in 1:2 acetic anhydride–dimethyl sulfoxide (48 ml) was kept for 72 h at room temperature, evaporated to dryness, and the residue triturated with water (30 ml), and then dissolved in chloroform (200 ml). The solution was washed with water (4 × 50 ml), dried (sodium sulfate), and evaporated. Chromatography of the residue on a column of silica gel with 19:1 chloroform–ethanol gave 240 mg (19%) of **12** as the faster-moving fraction, and 640 mg (52%) of **14** as the slower-moving one. Attempted purification of **12** by two further chromatographies, or by crystallization, was unsuccessful, and compound **12** was, therefore, used as such for the preparation of the debenzylidenated derivative **13**.

Crystallization of **14** from dichloromethane–methanol–pentane gave needles, m.p. 195–196°, $[\alpha]_D^{25}$ +5.0° (*c* 0.5, chloroform); ν_{max}^{KBr} 3300 (NH), 1750 (C=O), 1650 (Amide I), 1550 (Amide II), 775, and 685 cm^{-1} (Ph); t.l.c.: R_F 0.56 (29:1 chloroform–ethanol) and 0.66 (19:1 chloroform–ethanol). Repetition of this experiment with 1.5 g of **10** for 20 h gave a better yield of **14** (1.02 g, 67%) and a slightly lower yield of the by-product **12** (225 mg, 14%).

Anal. Calc. for $C_{49}H_{51}NO_{11}\cdot H_2O$: C, 69.45; H, 6.30; N, 1.65; O, 22.64. Found: C, 69.52; H, 5.98; N, 1.82; O, 22.58.

*Benzyl 2-acetamido-2-deoxy-3-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(methylthio)methyl-β-*D*-glucopyranosyl]-α-*D*-glucopyranoside* (**13**). — A suspension of **12** (200 mg) in 60% acetic acid (50 ml) was heated for 1 h at 80°, and then evaporated. The residue was dried by several additions and distillations of toluene, and chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 152 mg (84%) of **13** which crystallized from dichloromethane–ether, m.p. 179–181° (dec.), $[\alpha]_D^{25}$ +47° (*c* 2.2, chloroform); ν_{max}^{KBr} 3510, 3410 (OH), 3300 (NH), 1650 (Amide I), 1545 (Amide II), 725, and 680 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.33 (m, 20 H, 4 Ph) and 1.96 (s, 6 H, NAc and Me).

Anal. Calc. for $C_{44}H_{53}NO_{11}S$: C, 65.74; H, 6.64; N, 1.74; S, 3.99. Found: C, 65.76; H, 6.55; N, 1.81; S, 3.90.

*Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-mannopyranosyl)-α-*D*-glucopyranoside* (**15**). — A solution of **14** (848 mg) in 1:1 dichloromethane–methanol (30 ml) was cooled to 0°, treated with a solution of sodium borohydride (500 mg) in methanol (20 ml) for 30 min, and kept for 2 h at room temperature. It was diluted with dichloromethane (300 ml), and the solution was successively washed with water (3 × 100 ml), 1% citric acid solution (2 × 50 ml), a saturated sodium hydrogencarbonate solution (2 × 50 ml), and water (2 × 100 ml), dried (sodium sulfate), and evaporated, to give a residue that showed only one spot in t.l.c. with 19:1 chloroform–ethanol (R_F 0.46). It crystallized from dichloromethane–methanol as needles (682 mg, 82%), m.p. 198–199°, $[\alpha]_D^{25}$ +46° (*c* 2.2, chloroform); ν_{max}^{KBr} 3450 (OH), 3300 (NH), 1650 (Amide I), 1545 (Amide II), 775, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{49}H_{53}NO_{11}$: C, 70.74; H, 6.42; N, 1.68; O, 21.15. Found: C, 70.69; H, 6.44; N, 1.66; O, 21.20.

*Benzyl 2-acetamido-3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-*D*-mannopyranosyl)-4,6-O-benzylidene-2-deoxy-α-*D*-glucopyranoside* (**16**). — Acetic anhydride (3 ml) was added to a solution of **15** (104 mg) in dry pyridine (5 ml), and the mixture was kept for 16 h at room temperature. Evaporation, drying of the residue by several additions and distillations of toluene, and chromatography on a column of silica gel with 29:1 chloroform–methanol, gave 91 mg (83%) of **16**. It crystallized from ether–pentane, m.p. 130–132°, $[\alpha]_D^{25}$ +17° (*c* 2.1, chloroform); ν_{max}^{KBr} 3300 (NH), 1750 (OAc), 1650 (Amide I), 1550 (Amide II), 780, and 680 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 7.30 (m, 25 H, 5 Ph), 2.03 (s, 3 H, OAc), and 1.98 (s, 3 H, NAc); t.l.c. in 29:1 chloroform–ethanol: R_F 0.36.

Anal. Calc. for $C_{51}H_{55}NO_{12}$: C, 70.09; H, 6.34; N, 1.60; O, 21.97. Found: C, 69.90; H, 6.25; N, 1.52; O, 21.83.

*Benzyl 2-acetamido-2-deoxy-3-O-(3,4,6-tri-O-benzyl-β-*D*-mannopyranosyl)-α-*D*-glucopyranoside* (**17**). — A mixture of **15** (416 mg) with 60% acetic acid (40 ml) was heated for 1 h at 60°, and then evaporated. The residue was dried by repeated addition and distillation of toluene, and then chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 324 mg (87%) of **17**. It crystallized from dichloromethane–ether–pentane, m.p. 165–166°, $[\alpha]_D^{25}$ +63° (*c* 2.8, chloroform); ν_{max}^{KBr} 3440 (OH), 3300 (NH), 1650 (Amide I), 1545 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c.: R_F 0.20 (19:1 chloroform–ethanol) and 0.44 (9:1 chloroform–ethanol).

Anal. Calc. for $C_{42}H_{49}NO_{11}$: C, 67.82; H, 6.64; N, 1.88; O, 23.66. Found: C, 67.79; H, 6.72; N, 1.84; O, 23.56.

*2-Acetamido-2-deoxy-3-O-β-*D*-mannopyranosyl-*D*-glucose* (**18**). — A solution of **17** (372 mg) in methanol (30 ml) and acetic acid (5 drops) was hydrogenolyzed with hydrogen in the presence of 10% palladium-on-charcoal (150 mg) for 72 h at room temperature and 2.0 atm. The catalyst was filtered off on a Celite layer, and the filtrate was hydrogenolyzed for a further 24 h under the same conditions. Filtration, evaporation, and drying by several additions and evaporations of toluene gave 217 mg (83%) of **18**. It could not be crystallized, and was obtained as amorphous material from methanol–chloroform, $[\alpha]_D^{25}$ −14° (no mutarotation; *c* 0.4, water); ν_{max}^{KBr} 3350 (broad, NH and OH), 1650 (Amide I), and 1545 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, 43.73; H, 6.54; N, 3.57; O, 45.87.

The α analog, 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucose[10], has m.p. 129–130°, $[\alpha]_D^{20}$ +61 → +58° (*c* 1.5, 60% methanol).

G.l.c. of the per-*O*-(trimethylsilyl) derivatives of **18** and the just-mentioned α analog[10] was performed on a column (300 × 0.2 cm) packed with Gas-Chrom Q (80–100 mesh) coated with 3% of OV-17 (Applied Science Laboratories, Inc., State College, PA. 16801), programmed for a rise of 6.5° per min from 200 to 300°: $t'_{\textit{hexa-O-(trimethylsilyl)-myo-inositol}}$ 3.09 for **18**, and 2.93 for the α analog; and on a column (150 × 0.2 cm) packed with 0.1% of OV-17 on Glc-110 (120–140 mesh) (Column Technology, Inc., Unionville, PA. 19375), programmed for a rise of 10° per min from 80 to 250°: $t'_{\textit{hexa-O-(trimethylsilyl)-myo-inositol}}$ 1.84 for **18**, and 1.77 for the α analog.

Methanolysis of **18** (~50 μg) with M hydrogen chloride in methanol (1 ml) for 20 h at 80°, followed by evaporation, treatment with pyridine (0.1 ml) and acetic anhydride (0.1 ml) for 2 min at room temperature, evaporation, heating with 0.5M methanolic hydrogen chloride (0.5 ml), evaporation, per-*O*-(trimethylsilyl)ation, and injection on a column (300 × 0.3 cm) packed with Gas-Chrom Q (80–100 mesh) coated with 3% of OV-17 showed two major peaks, at 11.68 and 19.2 min, corresponding respectively to methyl α-D-mannopyranoside and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, and two minor peaks at 12.16 and 18.72 min, corresponding respectively to methyl β-D-mannopyranoside and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside. The ratio of the area of the peaks of the methyl α- and β-D-mannopyranosides to that of the methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides was ~1:1.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-mannopyranosyl)-α- and -β-*D*-glucopyranose* (**19** *and* **20**). — A solution of **18** (161 mg) in dry pyridine (5 ml) was treated with acetic anhydride (5 ml) for 20 h at room temperature. Evaporation, followed by drying (repeated addition and distillation of toluene), gave a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol. The β-heptaacetate (**20**) was eluted as the faster-moving fraction, and crystallized from dichloromethane–ether (53 mg, 18%), m.p. 195–196°, $[\alpha]_D^{25}$ −1.2° (*c* 3.03, chloroform); ν_{max}^{KBr} 3275 (NH), 1740 (OAc), 1665 (Amide I), and 1540 cm^{-1} (Amide II); t.l.c.: R_F 0.25 (19:1 chloroform–ethanol) and 0.53 (9:1 chloroform–ethanol).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.51; H, 5.87; N, 2.08; O, 42.41.

The α-heptaacetate **19** (254 mg, 75%) was eluted as the slower-moving fraction, and crystallized from chloromethane–ether, m.p. 197–199°, $[\alpha]_D^{25}$ +19° (*c* 2, chloroform); ν_{max}^{KBr} 3350 (NH), 1750 (OAc), 1660 (Amide I), and 1545 cm^{-1} (Amide II); t.l.c.: R_F 0.17 (19:1 chloroform–ethanol) and 0.45 (9:1 chloroform–ethanol).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.56; H, 5.85; N, 1.94; O, 42.41.

The α analog, 2-acetamido-1,3,4-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucose[10] shows m.p. 188–190°, $[\alpha]_D^{20}$ +65° (*c* 1.3, chloroform).

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