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THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-4-*O*-β-D-MANNOPYRANOSYL-D-GLUCOSE*

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ABSTRACT

Condensation of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide with 2-amino-2-*N*,3-*O*-carbonyl-5,6-*O*-isopropylidene-D-glucose diethyl acetal gave, unexpectedly, 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl)-5,6-*O*-isopropylidene-D-glucose diethyl acetal, further transformed, by de-esterification followed by acetylation, into the previously known 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-5,6-*O*-isopropylidene-4-*O*-α-D-mannopyranosyl-D-glucose diethyl acetal and its tetra-*O*-acetyl derivative. Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside was condensed with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl bromide to give benzyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside. Removal of the 2-*O*-acetyl group, followed by oxidation with acetic anhydride–dimethyl sulfoxide, gave a β-D-*arabino*-hexosid-2-ulose (**25**). After reduction with sodium borohydride, removal of the benzyl groups gave crystalline 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose (**27**). The anomeric configuration of the glycosidic linkage was ascertained by comparison with the α-D-linked disaccharide.

INTRODUCTION

The disaccharide 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose (**27**) is a part of the carbohydrate chain of many *N*-glycoproteins[2], and it has been isolated from the urine of patients suffering from mannosidosis, a lysozomal-storage disease[3].

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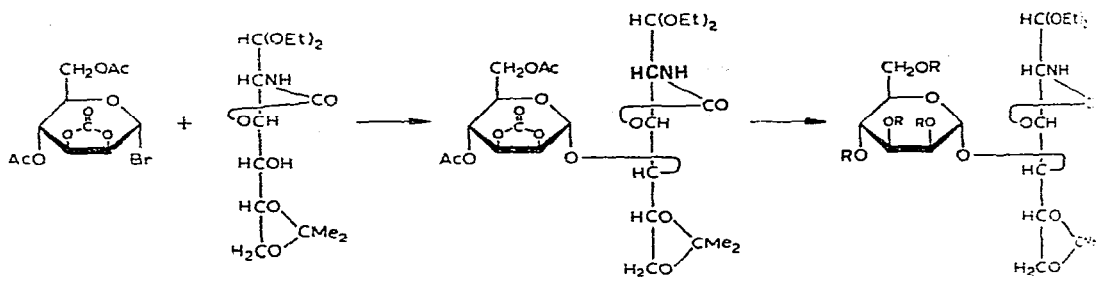
As part of a research program for the synthesis of oligosaccharides[4], glycopeptides[5], and isoprenoid sugar phosphates[6] containing 2-acetamido-2-deoxy-β-D-glucopyranosyl and α- and β-D-mannopyranosyl residues, the chemical synthesis of **27** was undertaken. This compound will be used for the identification of carbohydrate fragments obtained by degradation of glycoproteins and isoprenoid sugar phosphates, for testing the specificity of β-D-mannosidases, for a search for lectins specific for the β-D-mannopyranosyl residue, and as a starting material for the synthesis of larger oligosaccharides[4] to be linked to peptide chains[5] and to polyprenyl phosphates[6]. While this work was in progress, a synthesis of **27** involving the degradation of a naturally occurring disaccharide, followed by elongation of the chain, was reported[7].

RESULTS AND DISCUSSION

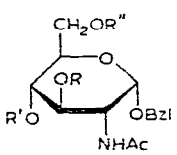
In a first attempt at the synthesis of **27**, 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide[8] (**1**), which has a nonparticipating group at C-2, was condensed with 2-amino-2-*N*,3-*O*-carbonyl-5,6-*O*-isopropylidene-D-glucose diethyl acetal[9] (**2**) in the presence of mercuric cyanide, to give the crystalline disaccharide **3**. This compound showed an optical rotation that indicated an α-D (rather than a β-D) configuration for the interglycosidic linkage. This assignment was verified by deesterification followed by acetylation, to give the known[10] α-D-linked disaccharides **4** and **5**. Although the bromide **1** was reported[8] to give β-D-mannopyranosides, the formation of **4** and **5**, as well as the previously reported synthesis[1] of 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose from **1**, suggests that both α-D- and β-D-glycosides are obtained, starting from bromide **1**.



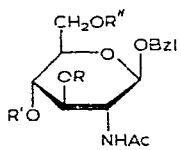
As this approach failed to give the desired compound, the route[11–13] used for the synthesis of the 3-*O*-β-D-mannopyranosyl analog[1] was selected. Although 2-acetamido-3,6-di-*O*-acetyl-D-glucopyranose derivatives show no reactivity of the hydroxyl group at C-4 in the Koenigs–Knorr condensation[4,14], benzyl 2-acetamido-3,6-di-*O*-benzyl-α-D-glucopyranoside[15,16] (**6**) was, recently, successfully condensed to give (1→4)-linked oligosaccharides[17]. When the synthesis of **6** according to the earlier method[16] was repeated, the step in which benzyl 2-acetamido-4-*O*-benzoyl-

3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**7**) is benzylated with benzyl bromide in the presence of silver oxide gave, in our hands, a mixture difficult to resolve by chromatography; this may be due to partial migration of the 4-*O*-benzoyl group under the slightly alkaline conditions of the benzylation reaction.

Consequently, a new route for the synthesis of benzyl 2-acetamido-3,6-di-*O*-benzyl-α-D-glucopyranoside (**6**) and of its β analog **20** was devised with intermediates containing the *O*-allyl protecting group[18,19]. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[20] (**13**) was benzylated with benzyl bromide in the presence of a mixture of *N*,*N*-dimethylformamide, barium hydroxide, and barium oxide, to give the 3-*O*-benzyl derivative **14**. Removal of the 4,6-*O*-benzylidene group by heating with 60% acetic acid gave **15**, which was tritylated to afford **16**. Allylation with allyl bromide and potassium hydroxide in dry benzene, which gave benzyl 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-6-*O*-trityl-β-D-glucopyranoside (**17**), was followed by detritylation with 2M aqueous hydrochloric acid in chloroform–methanol for 4 h at room temperature to give **18** without the formation of by-products, such as are generally formed on detritylation with aqueous acetic acid. Benzylation of **18** gave **19**, the allyl group of which was removed by isomerization with tris(triphenylphosphine)rhodium chloride and 1,4-diazabicyclo[2.2.2]octane[21,22], and hydrolysis of the resulting *O*-propen-1-yl groups with mercuric chloride[23] to give benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**20**). Application of the same reaction sequence to benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[16] (**8**) gave the intermediates **9**, **10**, and **11**, and, finally, benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**6**) having the same properties as those described by Jacquinet *et al.*[16]. After this work had been completed, Jacquinet and Sinaÿ[17] described a direct synthesis of **6** by selective benzylation at O-6 of benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**12**).



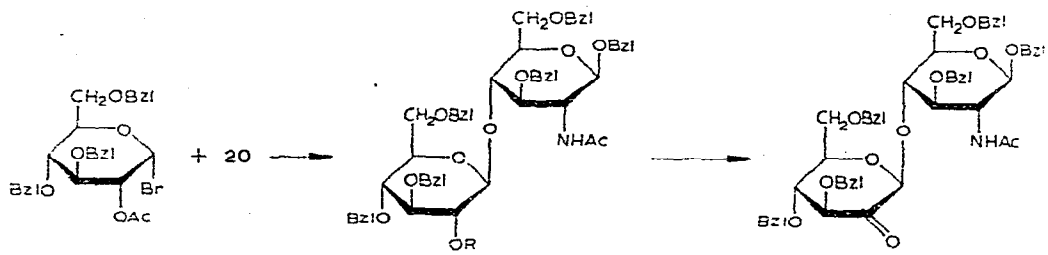
6 R = R'' = Bzl, R' = H
7 R = Bzl, R' = Bz, R'' = H
8 R = Bzl, R' = H, R'' = Tr
9 R = Bzl, R' = CH_2—CH=CH_2, R'' = Tr
10 R = Bzl, R' = CH_2—CH=CH_2, R'' = H
11 R = R'' = Bzl, R' = CH_2—CH=CH_2
12 R = Bzl, R' = R'' = H

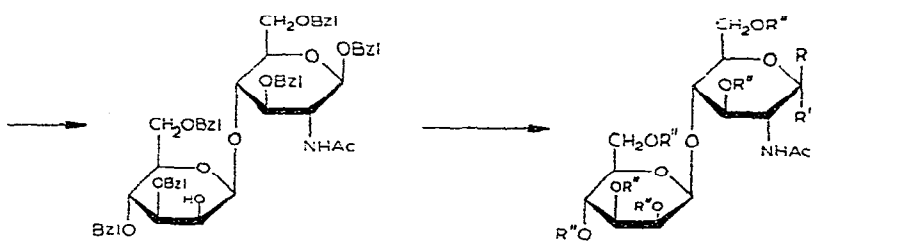


13 R = H, R' = R'' = CHPh
14 R = Bzl, R' = R'' = CHPh
15 R = Bzl, R' = R'' = H
16 R = Bzl, R' = H, R'' = Tr
17 R = Bzl, R' = CH_2—CH=CH_2, R'' = Tr
18 R = Bzl, R' = CH_2—CH=CH_2, R'' = H
19 R = R' = Bzl, R' = CH_2—CH=CH_2
20 R = R'' = Bzl, R' = H

Koenigs–Knorr condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[1,12] (**21**) with **20** in the presence of mercuric cyanide gave, in 67% yield, crystalline benzyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-gluco-

pyranosyl)-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**22**) showing the expected n.m.r.-spectral data. *O*-Deacetylation of **22** gave **23**, which was oxidized with dimethyl sulfoxide–acetic anhydride[24] to give benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-*arabino*-hexopyranosyl-2-ulose)-β-D-glucopyranoside (**25**) and the 2-*O*-(methylthio)methyl derivative **24** in the ratio of 7:2. *O*-(Methylthio)methyl derivatives have frequently been isolated as by-products of this method of oxidation[1,25,26]. The crystalline hexosid-2-ulose **25** showed a characteristic, carbonyl-group absorption at 1740 cm^{-1}. Stereospecific reduction of **25** with sodium borohydride gave crystalline benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-β-D-glucopyranoside (**26**), which showed the absence of the infrared carbonyl-group absorption of the parent hexosid-ulose. Hydrogenolysis of **26** in the presence of palladium-on-charcoal gave crystalline 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose (**27**). Methanolysis of **27**,





followed by per-*O*-(trimethylsilyl)ation showed, in g.l.c., peaks corresponding to those of per(trimethylsilyl)ated methyl α- and β-D-mannopyranoside and methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside, the ratio of the peak areas of the D-mannosides to those of the 2-acetamido-2-deoxy-D-glucosides being 10:8.6; no peaks corresponding to those of methyl D-glucosides could be detected. The oxidation–reduction sequence that transformed the β-D-glucopyranosyl disaccharide **23** into the corresponding β-D-mannopyranosyl disaccharide **26** would not be expected to affect

the β configuration of the glycosidic linkage, and this was verified by comparison of the properties of **27** with those of previously prepared 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose[10]. G.l.c. of the per-*O*-(trimethylsilyl) derivatives showed that the α-D-linked disaccharide is eluted before the β-D-linked analog. In addition, **27** did not depress the melting point of a sample prepared by a different synthetic pathway[7]. Acetylation of **27** gave the amorphous heptaacetate **28**, which showed properties different from those of the α-D-disaccharide heptaacetate[10].

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 hot-stage equipped with a microscope, and correspond to "corrected melting-points". Optical rotations were determined, for solutions in 1-dm, semimicro tubes, with a Perkin–Elmer Model 141 polarimeter. The chloroform used was analytical-reagent grade and contained ~0.75% of ethanol. Infrared spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. Nuclear magnetic resonance spectra were recorded at 60 MHz with a Varian T-60 spectrometer for solutions in chloroform-*d* containing 1% of tetramethylsilane (MSD Isotopic Products, Montreal, Canada) as the internal standard. Gas–liquid chromatography of the per-*O*-(trimethylsilyl) derivatives was performed on a Perkin–Elmer Model 900 gas chromatograph equipped with a flame-ionization detector, with nitrogen as the carrier gas. Column chromatography was performed on Silica Gel Merck (0.05–0.2 cm, 70–325 mesh, E. Merck A.G., Darmstadt, Germany), used without pretreatment; the ratio of the diameter of the column to its length was 1:8 to 1:12. The ratio of weight of substance to weight of silica gel was 1:60 to 1:100. The volume of the fractions eluted was 2–3 ml/g of the substance to be chromatographed. Thin-layer chromatography was performed on precoated Silica Gel G plates (layer thickness 0.25 mm, Merck) used without pretreatment. The distance of solvent-travel was 5 cm, and the spots were detected by spraying the chromatograms with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol[27], followed by heating on a hot plate for a few minutes. All proportions for the solvent systems used for elution of column and t.l.c. chromatograms were v/v. Evaporations were conducted *in vacuo*, with a bath temperature <45°. Solutions (<5 ml) in volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

*2-Amino-2-*N*,3-*O*-carbonyl-2-deoxy-4-*O*-(4,5-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl)-5,6-*O*-isopropylidene-D-glucose diethyl acetal* (**3**). — A solution of 2-amino-2-*N*,3-*O*-carbonyl-5,6-*O*-isopropylidene-D-glucose diethyl acetal[9] (1.06 g) and mercuric cyanide (1.0 g) in 1:1 (v/v) benzene–nitromethane (100 ml) was distilled at atmospheric pressure until the volume of the mixture was ~75 ml. The mixture was cooled to room temperature, and treated with a solution of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide[8] (2.0 g) in benzene (25 ml) during 3 h, while being stirred. After 92 h, the mixture was treated with additional amounts of

mercuric cyanide (1 g) and **1** (1 g), and stirring was continued for a further 72 h. The mixture was diluted with chloroform (400 ml), filtered, and the filtrate successively washed with water (2 × 50 ml), saturated KI solution (3 × 50 ml), saturated $NaHCO_3$ solution (3 × 50 ml), and water (3 × 50 ml), dried (Na_2SO_4), and evaporated, giving a yellowish syrup that was chromatographed on a column of silica gel with 4:1 chloroform–acetone to give 0.74 g (36%) of **2**. Crystallization from acetone–ether gave needles, m.p. 143–144°, $[\alpha]_D^{25}$ −3° (*c* 2.3, chloroform); ν_{max}^{KBr} 3300 (NH), 1805 (five-membered cyclic O–CO–O), 1745 (OAc), and 1725 cm^{-1} (five-membered cyclic N–CO–O); t.l.c. in 4:1 chloroform–acetone: R_F 0.25.

Anal. Calc. For $C_{25}H_{37}NO_{17}$: C, 50.76; H, 6.30; N, 2.37; O, 40.57. Found: C, 50.75; H, 6.30; N, 2.36; O, 40.37.

*2-Amino-2-*N*,3-*O*-carbonyl-2-deoxy-5,6-*O*-isopropylidene-4-*O*-α-*D*-mannopyranosyl-*D*-glucose diethyl acetal* (**4**). — A solution of **3** (590 mg) in methanol (40 ml) was treated with M sodium methoxide solution in methanol (1.5 ml) for 2 h at room temperature. The mixture was cooled to 0°, passed rapidly through a column of Dowex 50 (H^+) cation-exchange resin, and the eluate evaporated. The residue was chromatographed on a column of silica gel with 4:1 chloroform–ethanol, to give 90 mg (83%) of **4**. It crystallized from methanol–acetone–benzene, m.p. and mixed m.p. 188–189° (lit[10] m.p. 188–189°), $[\alpha]_D^{25}$ −3° (*c* 2, methanol) [lit.[10] $[\alpha]_D^{20}$ −2.7° (*c* 1.3, methanol)]; ν_{max}^{KBr} 3350 (broad, OH and NH) and 1730 cm^{-1} (five-membered cyclic N–CO–O).

Anal. Calc. for $C_{20}H_{35}NO_{12}$: C, 49.90; H, 7.33; N, 2.91; O, 39.82. Found: C, 49.77; H, 7.32; N, 2.82; O, 39.66.

*2-Amino-2-*N*,3-*O*-carbonyl-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-*D*-glucose diethyl acetal* (**5**). — A solution of **4** (235 mg) in pyridine (15 ml) was treated with acetic anhydride (10 ml) for 16 h at room temperature. Evaporation of the mixture gave a residue that crystallized from acetone–ether–pentane; 265 mg (81%), m.p. and mixed m.p. 146–148° (lit.[10] m.p. 147–148°), $[\alpha]_D^{25}$ −8° (*c* 2, chloroform); [lit.[10] $[\alpha]_D^{20}$ −7.6° (*c* 1.7, chloroform)]; ν_{max}^{KBr} 3300 (NH), 1775 (five-membered NH–CO–O), and 1750 cm^{-1} (OAc); t.l.c. in 19:1 chloroform–ethanol: R_F 0.28.

Anal. Calc. for $C_{28}H_{43}NO_{16}$: C, 51.76; H, 6.67; N, 2.16; O, 39.41. Found: C, 51.73; H, 6.51; N, 2.15; O, 39.53.

*Benzyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**14**). — A solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[20] (**13**, 6.5 g) in dry *N*,*N*-dimethylformamide (130 ml) was treated with benzyl bromide (4 ml), BaO (12.0 g), and $Ba(OH)_2 \cdot 8H_2O$ (4.0 g), and the mixture was boiled for 3 h under reflux, cooled to room temperature, diluted with chloroform (300 ml), heated to boiling, and filtered, while hot, through a Celite layer. The inorganic residue was washed with hot, 1:1 chloroform–*N*,*N*-dimethylformamide (100 ml), and the combined filtrate and washings were evaporated. The residue was dried by several additions and distillations of 1,4-xylene. Crystallization from 1:1 toluene–methanol gave 7.3 g (88%) of **14**, m.p. 279–281°, $[\alpha]_D^{25}$ −72° (*c* 0.9, pyridine):

ν_{max}^{KBr} 3280 (NH), 1650 (Amide I), 1555 (Amide II), 735, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol, R_F 0.48.

Anal. Calc. for $C_{29}H_{31}NO_6$: C, 71.15; H, 6.38; N, 2.86; O, 19.61. Found: C, 71.14; H, 6.39; N, 2.80; O, 19.54.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**15**). — A suspension of **14** (7.0 g) in 60% acetic acid (200 ml) was heated at 100° for 2 h while being stirred. The solution was evaporated, and the residue was dried by repeated addition and distillation of toluene. Chromatography on a column of silica gel with 9:1 chloroform–ethanol gave 3.5 g (61%) of **15**, which crystallized from methanol–ether–pentane; m.p. 183–184°, $[\alpha]_D^{25}$ −19° (*c* 0.84, methanol); ν_{max}^{KBr} 3350 (OH), 3280 (NH), 1645 (Amide I), 1545 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.36.

Anal. Calc. for $C_{22}H_{27}NO_6$: C, 65.87; H, 6.80; N, 3.49; O, 23.91. Found: C, 65.69; H, 6.79; N, 3.44; O, 23.81.

*Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**16**). — A solution of **15** (3.0 g) in dry pyridine (80 ml) was treated with chlorotriphenylmethane (4.0 g) for 72 h at room temperature. The mixture was then diluted with chloroform (400 ml), successively washed with a saturated solution of $NaHCO_3$ (3 × 100 ml) and water (3 × 100 ml), and dried (K_2CO_3). Evaporation of the solvents and removal of the last traces of pyridine by several additions and distillations of toluene gave a syrup that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol containing 0.3% of triethylamine. Evaporation of the fractions containing **16** gave 3.7 g (76%) of material that crystallized from ethanol as needles, m.p. 185–186° (soft. at 111°), $[\alpha]_D^{25}$ −23° (*c* 1.2, methanol); ν_{max}^{KBr} 3435 (OH), 3325 (NH), 1650 (Amide I), 1545 (Amide II), 760, 740, and 680 cm^{-1} (Ph); t.l.c.: R_F 0.36 (29:1 chloroform–ethanol) and 0.58 (19:1 chloroform–ethanol).

Anal. Calc. for $C_{41}H_{41}NO_6$: C, 76.49; H, 6.42; N, 2.18; O, 14.91. Found: C, 76.29; H, 6.38; N, 2.13; O, 14.93.

*Benzyl 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**17**). — A mixture of **16** (3.0 g), allyl bromide (0.8 ml), and powdered KOH (2.0 g) in absolute benzene (100 ml) was boiled for 4 h under reflux, and then stirred for a further 16 h at room temperature. It was filtered through a Celite layer, and the inorganic residue was washed with dichloromethane (100 ml). The combined filtrate and washings were evaporated, and the residue was chromatographed on a column of silica gel with 29:1 chloroform–ethanol containing 0.3% of triethylamine, to give 2.3 g (72%) of **17**. This crystallized from ethanol as needles, m.p. 188–189°, $[\alpha]_D^{25}$ +11° (*c*, 2.1 chloroform); ν_{max}^{KBr} 3350 (NH), 1655 (Amide I and allyl), 1535 (Amide II), 730, and 680 cm^{-1} (Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.55.

Anal. Calc. for $C_{44}H_{45}NO_6$: C, 77.28; H, 6.63; N, 2.05; O, 14.04. Found: C, 77.26; H, 6.71; N, 2.00; O, 13.86.

*Benzyl 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**18**). — To a solution of **17** (1.35 g) in 5:1 (v/v) methanol–chloroform (60 ml) was added 2M HCl (5 ml), and the mixture was kept for 4 h at room temperature. Evaporation

of the solvents gave a residue that was dissolved in chloroform (300 ml). The solution was successively washed with water (2 × 50 ml), saturated $NaHCO_3$ solution (2 × 50 ml), and water (2 × 50 ml), dried (Na_2SO_4), and evaporated; the residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to give 0.65 g (74%) of **13**. This crystallized from methanol as needles, m.p. 209°, $[\alpha]_D^{22}$ −17° (*c* 2.2, chloroform); ν_{max}^{KBr} 3400 (OH), 3295 (NH), 1645 (Amide I and allyl), 1548 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c.: R_F 0.36 (29:1 chloroform–ethanol) and 0.68 (19:1 chloroform–ethanol).

Anal. Calc. for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.08; N, 3.17; O, 21.74. Found: C, 68.16; H, 7.11; N, 3.04; O, 21.82.

Benzyl 2-acetamido-4-O-allyl-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**19**). — A mixture of **18** (0.88 g), benzyl bromide (1.5 ml), and powdered KOH (2.0 g) in dry benzene (80 ml) was stirred while being boiled for 3 h under reflux. The mixture was cooled, filtered on a Celite layer, and the inorganic residue washed with benzene (100 ml). The combined filtrate and washings were evaporated, and the residue was crystallized from methanol, to give 0.970 g (91%) of **19**, m.p. 166°, $[\alpha]_D^{22}$ +3° (*c* 3.2, chloroform); ν_{max}^{KBr} 3280 (NH), 1650 (Amide I and allyl), 1555 (Amide II), 735, and 680 cm^{-1} (Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.38.

Anal. Calc. for $C_{32}H_{37}NO_6$: C, 72.29; H, 7.01; N, 2.64; O, 18.06. Found: C, 72.30; H, 7.05; N, 2.62; O, 18.14.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (**20**). — A solution of **19** (2.0 g) and 1,4-diazabicyclo[2.2.2]octane (1.0 g) in 90% methanol (50 ml) was heated to boiling and treated, while being stirred, with $(Ph_3P)_3RhCl$ (0.6 g). The mixture was boiled for 4 h under reflux, cooled, and filtered on a layer of Celite. The filtrate was evaporated and the residue was dissolved in chloroform (300 ml). The solution was successively washed with water (2 × 50 ml), saturated $NaHCO_3$ solution (3 × 50 ml), and water (3 × 50 ml), and evaporated without being dried. The residue was dissolved in 90% acetone (100 ml), and treated with $HgCl_2$ (2 g) for 1 h at room temperature. The mixture was evaporated, and the residue dissolved in chloroform (300 ml). The solution was successively washed with a saturated KI solution (4 × 50 ml) and water (2 × 50 ml), dried (Na_2SO_4), and evaporated, giving a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol to afford 1.52 g (82%) of **20**. This crystallized from dichloromethane–pentane; m.p. 181°, $[\alpha]_D^{22}$ −37° (*c* 1.7, chloroform); ν_{max}^{KBr} 3320 (OH and NH), 1650 (Amide I), 1545 (Amide II), 725, and 685 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.34.

Anal. Calc. for $C_{29}H_{33}NO_6$: C, 70.86; H, 6.77; N, 2.85; O, 19.53. Found: C, 70.85; H, 6.82; N, 2.90; O, 19.45.

Benzyl 2-acetamido-4-O-allyl-3-O-benzyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (**9**). — A solution of benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[16] (**8**) (4.0 g) in dry benzene (100 ml) was treated with allyl bromide (1 ml) and powdered KOH (2.5 g), and boiled for 4 h under reflux. The mixture was then processed as just described for the preparation of **17**. Chromatography of the

crude product on a column of silica gel with 29:1 chloroform–ethanol containing 0.3% of triethylamine gave 3.2 g (76%) of **9**. This crystallized from methanol as needles, m.p. 162–163°, $[\alpha]_D^{25}$ +104° (*c* 2.7, chloroform); ν_{max}^{KBr} 3250 (NH), 1650 (Amide I and allyl), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.60.

Anal. Calc. for $C_{44}H_{45}NO_6$: C, 77.28; H, 6.63; N, 2.05; O, 14.04. Found: C, 77.15; H, 6.64; N, 1.95; O, 14.08.

*Benzyl 2-acetamido-4-*O*-allyl-3-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**10**). — Compound **9** (1.5 g) in 5:1 methanol–chloroform (70 ml) was treated with 2M HCl, and processed as just described for the preparation of **18**. Chromatography of the crude product on a column of silica gel with 19:1 chloroform–ethanol gave 0.75 g (78%) of **10**, which crystallized from methanol as needles, m.p. 198°, $[\alpha]_D^{22}$ +156° (*c* 3.3, chloroform); ν_{max}^{KBr} 3450 (OH), 3300 (NH), 1645 (Amide I and allyl), 1550 (Amide II), 725, and 685 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.28.

Anal. Calc. for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.08; N, 3.17; O, 21.74. Found: C, 67.98; H, 7.10; N, 3.10; O, 21.59.

*Benzyl 2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**11**). — A solution of **10** (1.32 g) in a mixture of dry benzene (80 ml) and α-bromotoluene (1.5 ml) was treated with powdered KOH (2.5 g), and boiled for 4 h under reflux while being stirred. The mixture was processed as described for the preparation of **19**, and the resulting compound **11** crystallized from methanol to give 1.44 g (90%) of needles, m.p. 145–146°, $[\alpha]_D^{22}$ +135° (*c* 3.7, chloroform); ν_{max}^{KBr} 3310 (NH), 1645 (Amide I and allyl), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.54.

Anal. Calc. for $C_{32}H_{37}NO_6$: C, 72.29; H, 7.01; N, 2.64; O, 18.06. Found: C, 72.29; H, 7.00; N, 2.66; O, 18.10.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**6**). — Compound **11** (2.0 g) was treated as described for the preparation of **20**. The resulting compound (1.44 g, 78%) crystallized from dichloromethane–pentane as needles, m.p. 145–146° [lit.[16] m.p. 145–145.5°], $[\alpha]_D^{22}$ +115° (*c* 1.5, chloroform) [lit.[16] $[\alpha]_D^{20}$ +115° (*c* 1.5, chloroform)]; ν_{max}^{KBr} 3460 (OH), 3300 (NH), 1650 (Amide I), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.40.

Anal. Calc. for $C_{29}H_{33}NO_6$: C, 70.86; H, 6.77; N, 2.85; O, 19.53. Found: C, 70.64; H, 6.75; N, 2.78; O, 19.53.

*Benzyl 2-acetamido-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**22**). — A mixture of **20** (1.5 g) and $HgCl_2$ (1.0 g) in 1:1 (v/v) benzene–nitromethane (200 ml) was distilled at atmospheric pressure until the volume of the mixture was ~150 ml, cooled to room temperature, treated with a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[1,12] (**21**, 2.5 g), and stirred for 72 h at room temperature. The mixture was then treated with additional amounts of $HgCl_2$ (0.5 g) and **21** (1.0 g), and stirring was continued for a further 48 h. The mixture was filtered though a Celite layer, the inorganic residue was washed with chloroform (50 ml), and the combined filtrate and

washings were combined, and diluted with chloroform (300 ml). The solution was successively washed with water (2 × 50 ml), saturated $NaHCO_3$ solution (3 × 50 ml), saturated KI solution (3 × 50 ml), and water (2 × 50 ml), dried (Na_2SO_4), and evaporated, to give a syrup that was chromatographed on a column of silica gel with 29:1 chloroform–ethanol, affording 1.97 g (67%) of **22**. This crystallized from methanol; m.p. 193–194°, $[\alpha]_D^{22}$ +7° (*c* 2.3, chloroform); ν_{max}^{KBr} 3295 (NH), 1735 (OAc), 1650 (Amide I), 1545 (Amide II), 740, and 680 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 1.87 (S, 3 H, NAc), 1.93 (S, 3 H, OAc), and 7.30 (m, 30 H, 6 Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.34.

Anal. Calc. for $C_{58}H_{63}NO_{12} \cdot H_2O$: C, 70.78; H, 6.66; N, 1.42; O, 21.13. Found: C, 70.71; H, 6.91; N, 1.48; O, 21.30.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**23**). — A solution of **22** (1.93 g) in 1:2 (v/v) dichloromethane–methanol (30 ml) was treated with M sodium methoxide in methanol (2 ml) for 6 h at room temperature. The solution was passed through a column of Dowex 50 (H^+) cation-exchange resin, and the eluate evaporated. The residue crystallized from methanol, to give 1.64 mg (89%) of **23**, m.p. 194–196°, $[\alpha]_D^{22}$ +5° (*c* 3.7, chloroform); ν_{max}^{KBr} 3480 (OH), 3305 (NH), 1650 (Amide I), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): δ 1.87 (S, 3 H, NAc) and 7.37 (m, 30 H, 6 Ph); t.l.c. in 29:1 chloroform–ethanol: R_F 0.28.

Anal. Calc. for $C_{56}H_{61}NO_{11} \cdot H_2O$: C, 71.39; H, 6.74; N, 1.48; O, 20.37. Found: C, 71.40; H, 6.84; N, 1.54; O, 21.42.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-arabino-hexopyranosyl-2-ulose)-β-*D*-glucopyranose* (**25**) *and benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-[3,4,6-tri-*O*-benzyl-2-*O*-(methylthio)methyl-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**24**). — A solution of **23** (1.39 g) in 1:2 (v/v) acetic anhydride–dimethyl sulfoxide (45 ml) was kept for 16 h at room temperature, and evaporated; the residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 0.80 g (56%) of **25** as the slower-moving fraction. This crystallized from methanol, m.p. 172–174°, $[\alpha]_D^{22}$ +6° (*c* 1.5, chloroform); ν_{max}^{KBr} 3450 (OH), 3270 (NH), 1740 (C=O), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.33.

Anal. Calc. for $C_{56}H_{59}NO_{11} \cdot 1.5H_2O$: C, 70.87; H, 6.58; N, 1.48. Found: C, 70.89; H, 6.66; N, 1.54.

Compound **24** was obtained as the faster-moving fraction (0.24 g, 16%), and crystallized from methanol, m.p. 192–194°, $[\alpha]_D^{22}$ +16° (*c* 2.1, chloroform); ν_{max}^{KBr} 3290 (NH), 1650 (Amide I), 1550 (Amide II), 745, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.58.

Anal. Calc. for $C_{58}H_{65}NO_{11}S$: C, 70.78; H, 6.66; N, 1.42; S, 3.26. Found: C, 70.74; H, 6.74; N, 1.40; S, 3.50.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-manno-pyranosyl)-β-*D*-glucopyranoside* (**26**). — A solution of **25** (7.10 mg) in 1:1 (v/v) dichloromethane–methanol (50 ml) was treated with $NaBH_4$ (250 mg) for 4 h at

room temperature while being stirred. The mixture was diluted with chloroform (200 ml), and successively washed with water (2×25 ml), 5% citric acid solution (4×25 ml), water (2×25 ml), saturated $NaHCO_3$ solution (2×25 ml), and water (4×25 ml), dried (Na_2SO_4), and evaporated, to give a residue that was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, affording 490 mg (69%) of **26**. This crystallized from methanol, m.p. 165–167°, $[\alpha]_D^{22}$ +8° (*c* 1, chloroform); ν_{max}^{KBr} 3425 (OH), 3300 (NH), 1645 (Amide I), 1550 (Amide II), 725, and 680 cm^{-1} (Ph); t.l.c. in 19:1 chloroform–ethanol: R_F 0.43.

Anal. Calc. for $C_{56}H_{61}NO_{11}\cdot 1.5H_2O$: C, 70.71; H, 6.78; N, 1.47. Found: C, 70.46; H, 6.69; N, 1.58.

*2-Acetamido-2-deoxy-4-*O*-β-*D*-mannopyranosyl-α-*D*-glucopyranose* (**27**). — A solution of **26** (476 mg) in abs. ethanol (50 ml) was hydrogenolyzed with hydrogen in the presence of 10% Pd/C (200 mg) for 24 h at room temperature and 2.0 atm. The catalyst was filtered off (Celite layer), and the filtrate was hydrogenolyzed twice more under the same conditions. Filtration, and evaporation of the solvent, gave a residue that crystallized from ether–80% ethanol to give 132 mg (69%) of **27**, m.p. and mixed m.p. 169–170° (lit.[7] m.p. 167–169°), $[\alpha]_D^{22}$ +11° (no mutarotation; *c* 4.9, water) lit.[7] $[\alpha]_D^{25}$ +0.4° (*c* 5.4, water); ν_{max}^{KBr} 3350 (broad, OH and NH), 1650 (Amide I), and 1550 cm^{-1} (Amide II).

Anal. Calc. for $C_{14}H_{25}NO_{11}\cdot H_2O$: C, 41.89; H, 6.78; N, 3.49. Found: C, 41.49; H, 6.42; N, 3.47.

The α analog, 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-α-D-glucose[10] showed m.p. 154–156° (dec.), $[\alpha]_D^{20}$ +77 → +66° (equil.; *c* 0.66, 50% methanol).

G.l.c. of the per-*O*-(trimethylsilyl) derivatives of **27** and the α analog[10] was performed on a column (300×0.2 cm) of stainless steel 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°/min from 200 to 300°: $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 2.87 for **27**, and 2.71 for the α analog.

Methanolysis of **27** (~50 μg) with M HCl 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 HCl in methanol (0.5 ml), evaporation, and per-*O*-(trimethylsilyl)ation, gave a mixture of compounds that was chromatographed through the column under the conditions just described. Two major peaks, at 16.40 and 23.92 min, corresponding to methyl α-D-mannopyranoside and methyl 2-acetamido-2-deoxy-α-D-glucopyranoside, respectively, and two minor peaks at 16.72 and 23.52 min, corresponding to methyl β-D-mannopyranoside and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, respectively, were observed. The ratio of the peaks of methyl α- and β-D-mannopyranoside to that of methyl 2-acetamido-2-deoxy-α- and β-D-glucopyranoside was 10:8.6.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-mannopyranosyl)-α-*D*-glucopyranose* (**28**). — A solution of **27** (75 mg) in 1:1 pyridine–acetic anhydride (10 ml) was kept for 16 h at room temperature, and then evaporated. The residue was dried by several additions and distillations of toluene, and then chromato-

graphed on a column of silica gel with 19:1 chloroform–ethanol, to give 91 mg (72%) of **28**. This could not be crystallized, but was obtained as an amorphous powder by precipitation from dichloromethane–ether–pentane; $[\alpha]_D^{22}$ +13° (*c* 1.5, chloroform); ν_{max}^{KBr} 3290 (NH), 1740 (OAc), 1665 (Amide I), and 1550 cm^{-1} (Amide II); t.l.c. in 19:1 chloroform–ethanol: R_F 0.33. The α configuration of the D-glucopyranose residue was indicated on the basis of the optical rotation; this suggests that the major part of **27** crystallized with the α configuration of the D-glucopyranose residue.

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.70; H, 6.12; N, 1.89; O, 42.57.

The analog, 2-acetamido-1,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-glucopyranose[10] had m.p. 113–114°, $[\alpha]_D^{22}$ +1.5° (*c* 1.4, chloroform).

An amorphous compound obtained in trace amounts from the earlier fractions from the column of silica gel, and showing in t.l.c. an R_F value higher than that of **28**, was assumed to be the anomer having the β configuration of the D-glucopyranose residue.

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.54; H, 5.87; N, 2.03; O, 42.47.

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REFERENCES

1 M. A. E. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 52 (1976) 103–114.
2 See J. MONTREUIL, *Pure Appl. Chem.*, 42 (1975) 431–477.
3 N. E. NORDÉN, A. LUNDBLAD, S. SVENSSON, AND S. AUTIO, *Biochemistry*, 13 (1974) 871–874, and previous papers in this series.
4 M. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 19 (1971) 311–318, and references cited therein.
5 H. G. GARG AND R. W. JEANLOZ, *Carbohydr. Res.*, 32 (1974) 37–46; M. A. E. SHABAN AND R. W. JEANLOZ, *ibid.*, 43 (1975) 281–291, and references cited therein.
6 C. D. WARREN, I. Y. LIU, A. HERSCOVICS, AND R. W. JEANLOZ, *J. Biol. Chem.*, 250 (1975) 8069–8078, and references cited therein.
7 G. JOHNSON, R. T. LEE, AND Y. C. LEE, *Carbohydr. Res.*, 39 (1975) 271–281.
8 P. A. J. GORIN AND A. S. PERLIN, *Can J. Chem.*, 39 (1961) 2474–2485; G. M. BEBAULT AND G. G. S. DUTTON, *Carbohydr. Res.*, 36 (1974) 444–454.
9 K. HEYNS, K. PROPP, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 2655–2663.
10 M. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 20 (1971) 17–22.
11 G. EKBORG, B. LINDBERG, AND J. LÖNNGREN, *Acta Chem. Scand.*, 26 (1972) 3287–3292; H. B. BORÉN, G. EKBORG, K. EKLING, P. J. GAREGG, Å. PILOTTI, AND C.-G. SWAHN, *ibid.*, 27 (1973) 2639–2644.
12 N. K. KOCHETKOV, B. A. DMITRIEV, O. S. CHIZHOV, E. M. KLIMOV, N. N. MALYSHEVA, V. I. TORGOV, A. YA. CHERNYAK, AND N. E. BAIRAMOVA, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1974) 1386–1392; *Bull. Acad. Sci USSR, Div. Chem. Sci.*, (1974) 1305–1311; N. K. KOCHETKOV, B. A. DMITRIEV, N. N. MALYSHEVA, A. YA. CHERNYAK, E. M. KLIMOV, N. E. BAIRAMOVA, AND V. I. TORGOV, *Carbohydr. Res.*, 45 (1975) 283–290.
13 M. MILJKOVIĆ, M. GLIGORIJEVIĆ, AND D. MILJKOVIĆ, *J. Org. Chem.*, 39 (1974) 2118–2120.

14 M. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 17 (1971) 411–417; 26 (1973) 315–322.
15 A. LIAV, J. HILDESHEIM, AND N. SHARON, *Chem. Commun.*, (1973) 668–669.
16 J.-C. JACQUINET, J.-M. PETIT, AND P. SINAŸ, *Carbohydr. Res.*, 38 (1974) 305–311.
17 J.-C. JACQUINET AND P. SINAŸ, *Carbohydr. Res.*, 46 (1976) 138–142.
18 P. A. GENT AND R. GIGG, *J. Chem. Soc. Perkin Trans. 1*, (1974) 1446–1455; (1975) 361–363.
19 A. LUBINEAU, A. THIFFERY, AND A. VEYRIÈRES, *Carbohydr. Res.*, 46 (1976) 143–148.
20 P. GROSS AND R. W. JEANLOZ, *J. Org. Chem.*, 32 (1967) 2759–2763.
21 E. J. COREY AND J. W. SUGGS, *J. Org. Chem.*, 38 (1973) 3224.
22 P. A. GENT AND R. GIGG, *Chem. Commun.*, (1974) 277–278.
23 R. GIGG AND C. D. WARREN, *J. Chem. Soc., C*, (1968) 1903–1911.
24 J. D. ALBRIGHT AND L. GOLDMAN, *J. Org. Chem.*, 30 (1965) 1107–1110; *J. Am. Chem. Soc.*, 87 (1965) 4214–4216.
25 J. L. GODMAN AND D. HORTON, *Carbohydr. Res.*, 5 (1967) 149–160; R. F. BUTTERWORTH AND S. HANESSIAN, *Can. J. Chem.*, 49 (1971) 2755–2759; *Synthesis*, 2 (1971) 70–88.
26 M. A. E. SHABAN, D. K. PODOLSKY, AND R. W. JEANLOZ, *Carbohydr. Res.*, 52 (1976) 129–135.
27 P. J. DUNPHY, J. D. KERR, J. F. PENNOCK, AND K. J. WHITTLE, *Chem. Ind. (London)*, (1966) 1549–1550; P. J. DUNPHY, J. D. KERR, J. F. PENNOCK, K. J. WHITTLE, AND J. FEENY, *Biochim. Biophys. Acta*, 136 (1967) 136–147.