SYNTHESIS OF A β-D-LINKED DISACCHARIDE FROM DIMERIC TRI-*O*-ACETYL-2-DEOXY-2-NITROSO-α-D-GLUCOPYRANOSYL CHLORIDE*

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ABSTRACT

Condensation of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride with benzyl alcohol in the presence of *N*,*N*,2,4,6-pentamethylaniline or *N*,*N*,2,6-tetramethylaniline gave an oximino glycoside that was reduced with lithium aluminum hydride to benzyl 2-amino-2-deoxy-α-D-gluco- and -mannopyranoside hydrochloride that were identified as the *N*-acetyl derivatives.

Similar condensation of the nitroso chloride with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (prepared *via* the 6-*O*-trityl derivative) gave an anomeric mixture of benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-oximino-α,β-D-*arabino*-hexopyranosyl)-β-D-galactopyranoside. From the mixture obtained by reduction with lithium aluminum hydride after de-*O*-acetylation, benzyl 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside was obtained in crystalline form; it was found to be identical with the disaccharide obtained by Helferich condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside. Paper chromatography of the mixture resulting from the reduction showed the presence of disaccharides having 2-acetamido-2-deoxy-α-D-glucopyranosyl and 2-acetamido-2-deoxy-α,β-D-mannopyranosyl groups.

INTRODUCTION

The 2-acetamido-2-deoxy-α-D-galactopyranosyl group is the main antigenic determinant of blood-group substances having Type A activity[1]. The synthesis of oligosaccharides containing this group has not, as yet, been reported, since Koenigs–Knorr or Helferich condensations of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl halides give β-D-linked disaccharides[2–4]. Condensation of derivatives of 2-amino-2-deoxy-α-D-galactopyranosyl halide having a nonparticipating

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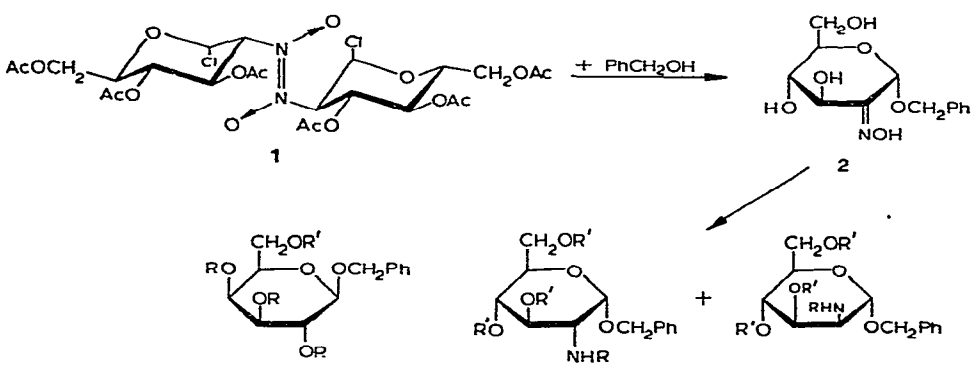
group at C-2, as used in the D-glucosamine series[5,6], might give anomeric mixtures, and the starting material might not be readily available. For these reasons, an advantageous route would be the transformation of the 2-acetamido-2-deoxy-α-D-glucopyranosyl group of an oligosaccharide into the D-galactopyranosyl analog by using the method described in the succeeding publication[4].

The synthesis of disaccharides containing the 2-acetamido-2-deoxy-α-D-glucopyranosyl group has been successfully accomplished by Lloyd and Roberts[5] starting from a 2-acetamido-2-deoxy-α-D-glucopyranosyl halide having at C-2 the nonparticipating 2,4-dinitrophenyl group, and by Heyns, Propp, Harrison, and Paulsen[6] by using the nonparticipating diphenoxyphospho group. These syntheses, however, yielded mixtures of α- and β-D-linked disaccharides. The most promising approach was that of Lemieux and associates[7–9], who have shown that condensation of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride[9,10] (**1**) with simple alcohols gives, exclusively, alkyl 2-deoxy-2-oximino-α-D-*arabino*-hexopyranosides, which, in turn, can be reduced to 2-amino-2-deoxy sugars. Furthermore, the same authors reported, in subsequent preliminary communications[11–13], the synthesis of α-D-linked amino sugar disaccharides from **1**, with practical exclusion of formation of any β-D-linked analog. Application of a similar procedure to a specific disaccharide has not, in our laboratory, been successful; it resulted in the formation of a β-D-linked disaccharide in preponderant proportion, in addition to the α-D-linked analog.

DISCUSSION

Condensation of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (**1**) with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**5**) was selected, because the protective benzyl groups of the resulting disaccharide could be selectively removed, and further transformations of the amino sugar moiety of the disaccharide could be accomplished without involving the D-galactose residue.



1

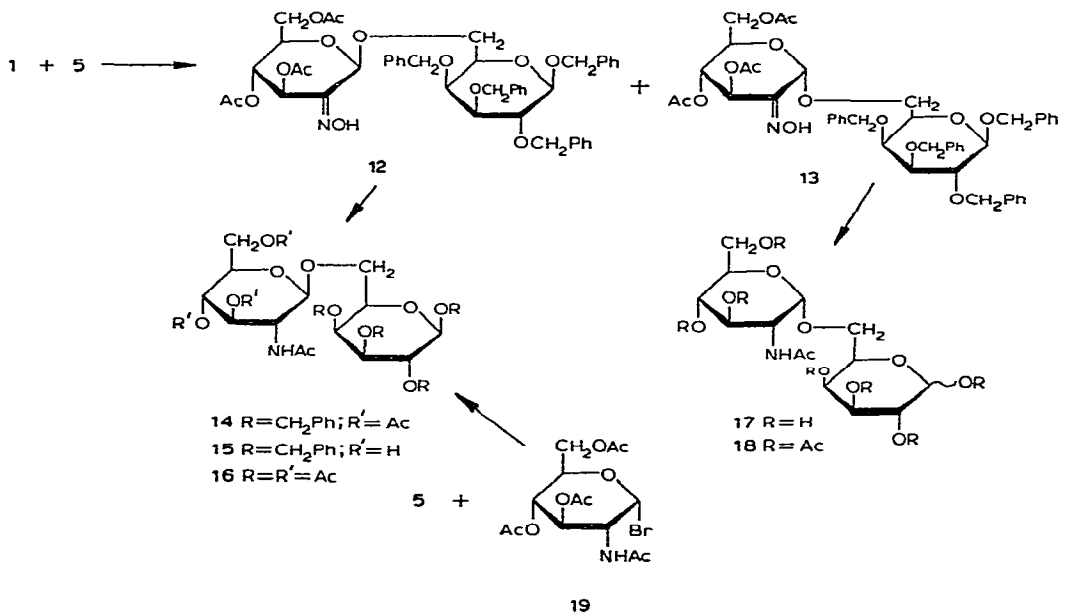
2

3 R = R' = H
4 R = H; R' = Tr
5 R = CH_2Ph; R' = H

6 R = H·HCl; R' = H
7 R = R' = Ac
8 R = Ac; R' = H

9 R = H·HCl; R' = H
10 R = R' = Ac
11 R = Ac; R' = H

In order to establish the conditions of (*a*) the condensation of **1** with **5** to form a disaccharide, and (*b*) the reduction of the oximino group in the presence of benzyl groups, the preparation of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**8**) from **1** was first studied. Dimeric **1** was prepared by a slight modification of the published method[9,10]. The condensation of **1** with benzyl alcohol to give the 3,4,6-triacetate of benzyl 2-deoxy-2-oximino-α-D-*arabino*-hexopyranoside (**2**) proceeded in *N,N*-dimethylformamide at room temperature, the highest yield being obtained when a mild base such as *N,N*,2,4,6-pentamethylaniline or *N,N*,2,6-tetramethylaniline was added to neutralize the hydrogen chloride formed during the reaction. The triacetate was then deacetylated to the oximino glycoside **2**, and this was reduced with lithium aluminum hydride to give an epimeric mixture of 2-amino-2-deoxy glycosides without removal of the anomeric benzyl group. (Treatment with sodium borohydride or lithium borohydride was, however, unsuccessful.) Benzyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (**6**) and benzyl 2-amino-2-deoxy-α-D-mannopyranoside hydrochloride (**9**), formed by the reduction of **2**, were identified by direct comparison with authentic samples, as well as by comparison of their respective *N*-acetyl derivatives **8** and **11**. This result established the α-D configuration of the oximino glycoside **2**. No evidence for the formation of the β-D isomer was found.



Benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**5**) was prepared from benzyl β-D-galactopyranoside[14] (**3**) *via* the trityl ether **4**. In contrast with oximino sugars of monosaccharides obtained in the present study and by Lemieux and associates[8], the mixture of the anomers **12** and **13** was obtained amorphous when the condensation of **1** with **5** was conducted under identical conditions. On de-*O*-acetylation of the

oximino sugar mixture **12** and **13**, followed by reduction with lithium aluminum hydride and reacetylation, only the β-D-linked disaccharide triacetate **14** could be isolated as a well-defined, crystalline compound.

Comparison of the properties of **14** with those of the disaccharide prepared from **5** and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide[15] (**19**) by the Helferich method, and comparison of the optical rotations and melting point of the octaacetate **16** with those reported by Kuhn and Kirschenlohr[16], established that the crystalline disaccharide triacetate **14**, isolated after acetylation of the reduction products of **12** and **13**, had the β-D anomeric configuration. Examination, by paper chromatography, of the reduction products of **12** and **13**, followed by *N*-acetylation, showed the presence of a disaccharide having the same mobility as 6-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-D-galactose (**17**), prepared by Lloyd and Roberts[5]. Combined g.l.c. and paper-chromatographic analyses, and optical rotation data for the reduction products of **12** and **13**, supported the presence of an α-D-linked disaccharide (**13**). Attempts were unsuccessful to resolve (*a*) the anomeric mixture of **12** and **13** or its de-*O*-acetylated product, (*b*) the epimeric and anomeric mixture of the de-*O*-acetylated, or 4,6-*O*-benzylidene, or 3-*O*-acetyl-4,6-*O*-benzylidene derivatives of the 2-acetamido-2-deoxy-D-hexopyranosyl group in the reduction products from **12** and **13**, and (*c*) the epimeric and anomeric mixture of the octaacetyl derivatives **16** and **18** obtained by reduction of **12** and **13** followed by hydrogenolysis and peracetylation.

From the paper-chromatographic data, it was estimated that at least one third of the mixture of **12** and **13** consisted of the β-D linked disaccharide **12**, clearly indicating the nonstereospecificity of the reaction of **1** with such a complex alcohol as **5**. The precise mechanism of glycosidation of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (**1**) is not yet known, and it is possible that the mechanistic pathway followed in the formation of complex disaccharides is different from that obtaining in the formation of simple alkyl glycosides.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 melting-point apparatus, and correspond to corrected melting points. Optical rotations were determined in 1-dm, semimicro tubes with a Perkin–Elmer polarimeter, Model 141; the chloroform used was analytical-reagent grade and contained approximately 0.75% of ethanol. I.r. spectra were recorded, for potassium bromide disks, with a Perkin–Elmer Model 237 spectrophotometer. N.m.r. spectra were recorded with a Japan Electron Optics Laboratory Company, Ltd., Model MH 100, or with a Varian A-60 n.m.r. spectrometer, for a solution in chloroform-*d* or in dimethyl sulfoxide-d_6 with tetramethylsilane as the internal standard. G.l.c. of the per(trimethylsilyl)ated derivatives was performed with a Perkin–Elmer Model 900 gas chromatograph having a column (150 × 0.3 cm) of stainless steel packed with 0.1% of OV 17 on GLC 110, lot 4463 (120–140 mesh) (Supleco, Inc., Bellefonte, Pa.). The compounds were injected at 80°, and the temperature was raised at the rate of 10°/min; the times of

elution were compared with that of hexakis(trimethylsilyl)-*myo*-inositol. 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 adsorbent was 1:60 to 1:80. Ascending t.l.c. was performed on plates coated with Merck Silica Gel F-254 with solvent systems consisting of 0 to 4% of ethanol in chloroform. The components were detected by spraying with anisaldehyde–sulfuric acid, and heating at 125°. All of the compounds reported herein were homogeneous in t.l.c. Descending paper chromatography was performed on Whatman paper No. 1, and components were detected with the silver nitrate reagent. Evaporations were conducted under diminished pressure, the bath temperature being kept below 45°. The microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

Dimeric 3,4,6-tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (**1**). — A solution of tri-*O*-acetyl-D-glucal (14.51 g, 50 mmoles) in ethyl acetate (100 ml) was introduced dropwise into a flask containing liquid nitrosyl chloride (~3 ml) under a nitrogen atmosphere and cooled in Dry Ice–acetone. The solution was kept for 10 min in the acetone–Dry Ice bath with occasional stirring, and then for 10 min at room temperature. The excess of nitrosyl chloride and of ethyl acetate were evaporated off at 20–23° (bath temperature), and the remaining traces of nitrosyl chloride were removed by repeated addition and distillation of ethyl acetate. The crystalline residue was recrystallized from ethyl acetate, to give 13.81 g (82%) of needles, m.p. 129–130°, $[\alpha]_D^{20}$ +163° (*c* 3.1, chloroform); [lit.[9] m.p. 129–130°, $[\alpha]_D^{23}$ +149° (*c* 2.15, chloroform)]; n.m.r. data (chloroform-*d*): τ 3.33 (H-1, doublet, $J_{1,2}$ 3.5 Hz), 3.95 (H-3, triplet, $J_{3,4}$ 9.5 Hz), 4.54 (H-2, quartet, $J_{2,3}$ 9.0 Hz).

Benzyl 2-deoxy-2-oximino-α-D-arabino-hexopyranoside (**2**). — Compound **1** (3.37 g, 5 mmoles), *N,N*,2,4,6,-pentamethylaniline (0.832 g), and dry benzyl alcohol (1.6 ml) were dissolved in dry *N,N*-dimethylformamide (10 ml) under nitrogen, and the solution was stirred for 72 h at room temperature. The solution was diluted with dichloromethane, washed three times with water, dried (sodium sulfate), and evaporated to a syrup; this was dissolved in methanol (75 ml), 0.2M sodium methoxide in methanol (20 ml) was added, and the solution was kept for 20 h at 0°. The sodium ions were removed with Amberlite IR-120 (H^+) ion-exchange resin, and the methanol was evaporated off. The crude product was crystallized from methanol–ether; it was recrystallized from methanol to give 2.35 g (83%) of **2** as needles, m.p. 154–155°; $[\alpha]_D^{20}$ +148.6° (*c* 1.5, pyridine), +163.6° (*c* 0.66, methanol); i.r. data: ν_{max}^{KBr} 1660 (C=N) and 745 cm^{-1} (Ph); n.m.r. data (dimethyl sulfoxide-d_6): τ 4.45 (1 H, NOH) and 6.31 (5 H, Ph).

Anal. Calc. for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.05; N, 4.94. Found: C, 55.05; H, 6.05; N, 4.87.

Compound **2** was also prepared by the procedure just described, but without *N,N*,2,4,6-pentamethylaniline; yield 70%; $[\alpha]_D^{20}$ +164.0° (*c* 0.70, methanol).

Benzyl 6-O-trityl-β-D-galactopyranoside (**4**). — Benzyl β-D-galactopyranoside[14] (**3**, 27.00 g, 100 mmoles) and chlorotriphenylmethane (33.50 g) were dissolved in dry pyridine (250 ml), and the solution was kept for 1.5 h at 100°. After being cooled, it

was poured into ice–water, and the mixture was extracted with chloroform. The extract was washed three times with water, and evaporated, and traces of pyridine were removed by repeated addition and distillation of toluene. The crude product was dissolved in chloroform, and the solution was chromatographed on silica gel. Triphenylmethanol was eluted first with chloroform; the product was then eluted with acetone, and the eluate evaporated to dryness. Compound **4** was crystallized from chloroform–ether–hexane, to give 41.50 g (81%) of long needles, m.p. 125–125.5°; $[\alpha]_D^{20}$ −69.4° (*c* 1.9, pyridine), −50.7° (*c* 2.2, chloroform).

Anal. Calc. for $C_{32}H_{32}O_6$: C, 74.97; H, 6.30; O, 18.73. Found: C, 74.85; H, 6.27; O, 18.59.

*Benzyl 2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**5**). — A solution of compound **4** (25.60 g, 50 mmoles), benzyl bromide (30.80 g), and sodium hydride (4.30 g) in abs. tetrahydrofuran (300 ml) was boiled for 4 h under reflux. The suspension was cooled, and the sodium bromide and excess of sodium hydride were filtered off and washed several times with abs. tetrahydrofuran. The filtrate and washings were combined and evaporated, and the residue was dissolved in chloroform. The solution was washed five times with water, dried (sodium sulfate), and evaporated, to give a syrupy residue which was dissolved in benzene. The solution was chromatographed on silica gel, and the product was eluted with benzene.

The syrupy, benzylated product was dissolved in glacial acetic acid (400 ml), the solution was heated to 80°, and water (40 ml) was added at such a rate that no permanent precipitation occurred. The mixture was kept for 45 min at 100°, cooled, and evaporated, and the residue was treated first with water and then with toluene, each treatment being followed by evaporation. The residue, which was dry and free from acetic acid, was dissolved in chloroform and the solution was chromatographed on silica gel. Triphenylmethanol was first eluted with chloroform, and then the product, with 99:1 chloroform–ethanol. It was crystallized from chloroform–hexane, to give 17.55 g (65%) of long needles, m.p. 96–96.5°; $[\alpha]_D^{20}$ −46.1° (*c* 3.0, chloroform).

Anal. Calc. for $C_{34}H_{36}O_6$: C, 75.52; H, 6.72; O, 17.75. Found: C, 75.52; H, 6.68; O, 17.68.

*Benzyl 2-amino-2-deoxy-α-*D*-glucopyranoside hydrochloride* (**6**). — *A. From compound* **2**. To a solution of compound **2** (1.00 g, 3.5 mmoles) in abs. tetrahydrofuran (5 ml) was added a suspension of lithium aluminum hydride (700 mg) in abs. tetrahydrofuran (35 ml). The mixture was boiled under reflux for 20 h, with stirring, and cooled, and ethyl acetate was added dropwise, followed by ethanol and water. The flocculent precipitate was centrifuged off and washed twice with methanol. The supernatant liquor and washings were combined, and evaporated to a syrup, which was treated with methanol (10 ml) containing conc. hydrochloric acid (0.5 ml). The solution was evaporated, and the residue treated by repeated addition and distillation of abs. ethanol, to give a mixture of **6** and its epimer **9** (0.76 g, 71%). The ratio of **6** to **9** was 2:3 as indicated by g.l.c. analysis of the per(trimethylsilyl)ated derivatives of the mixture. Repeated fractional recrystallizations of the mixture from isopropyl alcohol gave 0.20 g (19%) of **6** as granules, m.p. 233–234° (dec.), $[\alpha]_D^{20}$ +124° (*c* 1.0, water).

Anal. Calc. for $C_{13}H_{20}ClNO_5$: C, 51.07; H, 6.60; Cl, 11.60; N, 4.58; O, 26.17. Found: C, 51.14; H, 6.59; Cl, 11.51; N, 4.52; O, 26.18.

B. From benzyl 2-amino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside. A solution of benzyl 2-amino-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[17] (357 mg, 1 mmole) in methanol (15 ml) containing conc. hydrochloric acid (0.3 ml) was boiled under reflux for 15 min, and then cooled and evaporated. The residue was dried by repeated addition and distillation of abs. ethanol. Crystallization from isopropyl alcohol gave 275 mg (90%) of **6** as granules, m.p. 233–234° (dec.), $[\alpha]_D^{20}$ +124° (*c* 1.0, water).

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**8**). — Compound **6** (153 mg, 0.5 mmole) was treated with pyridine (5 ml) and acetic anhydride (0.5 ml) for 24 h at room temperature, and then methanol was added to decompose the excess of acetic anhydride. Evaporation gave benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**7**), which crystallized from methanol–water as prismatic needles, m.p. 114–114.5°, $[\alpha]_D^{20}$ +109° (*c* 0.77, chloroform); lit.[17] m.p. 111°, $[\alpha]_D^{23}$ +103° (*c* 0.59, chloroform). De-*O*-acetylation with sodium methoxide in methanol in the usual way gave **8** (111 mg, 71% based on **6**) after two recrystallizations from ethanol; m.p. 187–189°, $[\alpha]_D^{20}$ +170° (*c* 1.0, water); lit.[17] m.p. 187–189°; $[\alpha]_D^{23}$ +170° (*c* 0.9, water).

Benzyl 2-acetamido-2-deoxy-α-D-mannopyranoside (**11**). — Isolation of **9** from the mixture of **6** and **9** was unsuccessful. The presence of **9** in the mixture obtained from **2** was ascertained by g.l.c. after per(trimethylsilyl)ation, and by comparison with an authentic sample[18] of **9**.

The mixture (0.61 g) obtained directly by reduction of **2** was treated with acetic anhydride (1 ml) and pyridine (6 ml) for 24 h at room temperature, and then methanol was added to decompose the excess of acetic anhydride. After evaporation, the residual mixture of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**7**) and benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside (**10**) was dissolved in chloroform, and the solution was chromatographed on a column of silica gel. Benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**7**) was first eluted with chloroform, and then benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside (**10**) with 49:1 chloroform–ethanol to give amorphous material (0.44 g), $[\alpha]_D^{20}$ +66° (*c* 2.1, chloroform); lit.[18] $[\alpha]_D$ +67° (*c* 1.5, chloroform).

De-*O*-acetylation of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-mannopyranoside (**10**) with sodium methoxide in methanol in the usual way gave **11** (0.27 g, 43%) as amorphous material, $[\alpha]_D^{20}$ +75° (*c* 1.1, methanol).

Benzyl 2,3,4-tri-O-benzyl-6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-oximino-α,β-D-arabino-hexopyranosyl)-β-D-galactopyranoside (**12** + **13**). — Compound **1** (8.00 g, 11.8 mmoles), compound **5** (8.10 g, 15 mmoles), and *N,N*,2,4,6-pentamethylaniline (2 g) were dissolved in dry *N,N*-dimethylformamide (40 ml) under nitrogen, and the solution was kept for 72 h at room temperature. The solution was diluted with chloroform, washed three times with water, dried (sodium sulfate), and evaporated to a syrup

which was dissolved in chloroform, and the solution chromatographed on a column of silica gel. The product was eluted with chloroform, to give 11.62 g (92%) of **12+13** as amorphous material, $[\alpha]_D^{20}$ +17° (*c* 3.3, chloroform); i.r. data: ν_{max}^{KBr} 3375 (broad, OH), 1650 (C=N), and 730 (Ph) cm^{-1}.

Anal. Calc. for $C_{46}H_{51}NO_{14}$: C, 65.61; H, 6.12; N, 1.67; O, 26.60. Found: C, 65.51; H, 6.11; N, 1.76; O, 26.33.

The mixture was also prepared by the procedure just described, but in the absence of *N,N*,2,4,6-pentamethylaniline; yield 79%, $[\alpha]_D^{20}$ +17° (*c* 3.1, chloroform).

*Benzyl 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**14**). — *A. From* **12+13**. A solution of **12+13** (16.84 g, 20 mmoles) in methanol (200 ml) was treated with 0.2M sodium methoxide in methanol (25 ml) for 24 h at 0°. The sodium ions were removed with Amberlite IR-120 (H^+) ion-exchange resin, the suspension was filtered, and the filtrate was evaporated. Ether was added to the syrupy residue to precipitate the de-*O*-acetylated derivative of **12+13**, which was dried under high vacuum for 16 h at 60°. It was dissolved in abs. tetrahydrofuran (50 ml), a suspension of lithium aluminum hydride (5 g) in abs. tetrahydrofuran (400 ml) was added, and the mixture was boiled under reflux for 24 h, with stirring. The mixture was cooled, and ethyl acetate was added dropwise, followed by ethanol and water. The flocculent precipitate was centrifuged off, and washed twice with methanol. The supernatant liquor and washings were combined, and evaporated to a syrup that was then treated with acetic anhydride (20 ml) in dry pyridine (100 ml) for 24 h at room temperature. The solution was poured into ice–water, and the mixture was extracted with chloroform. The extract was washed three times with water and evaporated, and traces of pyridine were removed by repeated addition and distillation of toluene. The syrup was dissolved in chloroform, and the solution was chromatographed on silica gel. A mixture of acetylated disaccharides was eluted with 99:1 chloroform–ethanol; repeated column chromatography of this mixture failed to resolve it. A solution of the syrup in abs. ethanol gave crystalline compound **14**. Recrystallization from ethanol gave 2.26 g (13% based on **12+13** of **14** as needles, m.p. 195–195.5°; $[\alpha]_D^{20}$ −28° (*c* 2.4, chloroform); i.r. data: ν_{max}^{KBr} 1745 (OAc), 1665 (COHN), and 733 cm^{-1} (Ph); n.m.r. data (chloroform-*d*): τ 2.58, 2.60 (20 H, 4 Ph), 7.96 (9 H, 3 OAc), and 8.14 (3 H, NAc).

Anal. Calc. for $C_{48}H_{55}NO_{14}$: C, 66.26; H, 6.38; N, 1.61; O, 25.75. Found: C, 66.26; H, 6.36; N, 1.60; O, 25.84.

After methanolysis of **14** with 0.5M hydrogen chloride in methanol for 16 h at 65°, followed by per-*O*-(trimethylsilyl)ation, g.l.c. of the ether indicated the presence of methyl 2-acetamido-2-deoxy-D-glucoside in, and the absence of methyl 2-acetamido-2-deoxy-D-mannoside from, the original methanolyzate.

B. From **5** *and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide*[15] (**19**). A chloroform solution (30 ml) of **19**, prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose (3.00 g), was added to a mixture of **5** (0.70 g, 1.29 mmoles), mercuric cyanide (1.5 g), calcium sulfate (0.5 g,) and dry benzene (13 ml), with vigorous stirring. The mixture was stirred for 3 h, and filtered through a Celite

pad, and the salts were washed with chloroform. The filtrate and washing were combined, washed three times with water, dried (sodium sulfate), and evaporated to a syrup which was dissolved in chloroform; the solution was chromatographed on silica gel with 49:1 chloroform–ethanol. The fractions containing a compound having an R_F value identical with that of **14**, previously described, were evaporated. Recrystallization from abs. ethanol gave 0.13 g (11.5%) of **14** as needles; m.p., $[\alpha]_D^{20}$, and i.r. and n.m.r. data identical with those for **14** prepared by method *A*.

Examination of the mother liquor from the crystallization of **14** *obtained by reduction of* **12**+**13** *and acetylation of the product.* — The initial reduction products and residue left after crystallization of **14** were methanolyzed with 0.5M hydrogen chloride in methanol for 16 h at 65°, and then per(trimethylsilyl)ated; g.l.c. analysis indicated the presence of disaccharides containing 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-mannose. The ratio of 2-amino-2-deoxy-D-glucose to 2-amino-2-deoxy-D-mannose was 45 to 50:55 for the initial reduction products, and 30 to 35:70 to 65 for the residue, which showed $[\alpha]_D^{20}$ −7° (*c* 6.7, chloroform). This residue was subjected to de-*O*-benzylation, peracetylation, and de-*O*-acetylation under conditions similar to those used for the preparation of **16** and of 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose[16]. The resulting mixture was resolved by paper chromatography in 10:1:2 butyl alcohol–ethanol–water[19], after 250 h, into 3 components, having $R_{lactose}$ 0.9, 1.2, and 1.4, respectively. The second component had the same mobility as 6-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-D-galactose[5] (**17**) ($R_{lactose}$ 1.2), and the third had the same mobility as 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose[5,16] ($R_{lactose}$ 0.9). The first zone contained disaccharides having a 2-amino-2-deoxymannose residue, as shown by g.l.c.; it presumably consisted of 6-*O*-(2-acetamido-2-deoxy-α,β-D-mannopyranosyl)-D-galactose.

Benzyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**15**). — A solution of compound **14** (4.78 g, 5.5 mmoles) in methanol (250 ml) was treated with 2M sodium methoxide in methanol (7.5 ml) for 1 h at room temperature and 20 h at 0°. The sodium ions were removed with Amberlite IR-120 (H^+) ion-exchange resin, the suspension was filtered, and the filtrate was evaporated; during the evaporation, most of the product crystallized out. It was recrystallized from methanol, to give 3.72 g (91%) of **15** as needles, m.p. 213–215°; $[\alpha]_D^{20}$ +23° (*c* 1.8, pyridine); i.r. datum: ν_{max}^{KBr} 1650 cm^{-1} (CONH).

Anal. Calc. for $C_{42}H_{49}NO_{11}$: C, 67.81; H, 6.65; N, 1.88; O, 23.66. Found: C, 67.55; H, 6.59; N, 1.97; O, 23.46.

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2,3,4-tetra-O-acetyl-β-D-galactopyranose (**16**). — A solution of compound **14** (500 mg, 0.57 mmole) in 1:1 methanol–ethyl acetate (70 ml) was hydrogenolyzed with hydrogen under pressure (2.9 atm.) in the presence of 10% palladium-on-charcoal (200 mg) and 2M hydrochloric acid (1.5 ml) for 72 h. The catalyst was filtered off, the chloride ions were removed with Amberlite IR-45 (OH^-) ion-exchange resin, the suspension was filtered, the filtrate was evaporated to dryness, and the residue was dried by repeated addition and distillation of toluene; it was then treated with a hot solution of anhyd.

sodium acetate (100 mg) in acetic anhydride (4 ml) for 10 min, and poured into ice–water. The mixture was extracted with chloroform, and the extract was washed five times with water, dried (sodium sulfate), and evaporated to a syrup which was treated first with pyridine–methanol and then with toluene, each treatment being followed by evaporation. The residue, which was dry and free from acetic anhydride and pyridine, was dissolved in chloroform, and the solution was chromatographed on silica gel. The product was eluted with 49:1 chloroform–ethanol, the eluate evaporated, and the residue crystallized from abs. ethanol, to give 224 mg (58%) of needles, m.p. 196–197°; $[\alpha]_D^{20}$ +8° (*c* 1.9, chloroform); lit.[16] m.p. 197–198°; $[\alpha]_D$ +6.3° (chloroform).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.62; H, 5.81; N, 2.07; O, 42.50. Found: C, 49.46; H, 5.66; N, 2.12; O, 42.53.

After methanolysis of **16** with 0.5M hydrogen chloride in methanol for 16 h at 65°, followed by per-*O*-(trimethylsilyl)ation, g.l.c. indicated the presence of equimolar proportions of methyl 2-acetamido-2-deoxy-D-glucoside and methyl D-galactoside in the original methanolyzate. De-*O*-acetylation of **16** with sodium methoxide in methanol in the usual way gave 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose as amorphous material, $[\alpha]_D^{20}$ +9° (*c* 0.7, water); lit.[5,16] $[\alpha]_D^{20}$ +9.2° (*c* 0.65, water), $[\alpha]_D^{18}$ +9.9° (*c* 0.1, water).

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REFERENCES

1 W. M. WATKINS, *Science*, 152 (1966) 172.
2 Z. TARASIEJSKA AND R. W. JEANLOZ, *J. Amer. Chem. Soc.*, 80 (1958) 6325.
3 A. J. ACHER AND D. SHAPIRO, *J. Org. Chem.*, 34 (1969) 2652.
4 K. MIYAI AND R. W. JEANLOZ, *Carbohyd. Res.*, 21 (1972) 57.
5 P. F. LLOYD AND G. P. ROBERTS, *J. Chem. Soc.*, (1965) 6910.
6 K. HEYNS, K. PROPP, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 2655.
7 R. U. LEMIEUX AND S. W. GUNNER, *Can. J. Chem.*, 46 (1968) 397.
8 R. U. LEMIEUX, T. L. NAGABHUSHAN, AND S. W. GUNNER, *Can. J. Chem.*, 46 (1968) 405.
9 R. U. LEMIEUX, T. L. NAGABHUSHAN, AND I. K. O'NEIL, *Can. J. Chem.*, 46 (1968) 413.
10 W. J. SERFONTEIN, J. H. JORDAAN, AND J. WHITE, *Tetrahedron Lett.*, 18 (1964) 1069.
11 R. U. LEMIEUX, S. W. GUNNER, Y. ITO, AND M. E. EVANS, *Abstr. Papers Amer. Chem. Soc. Meeting*, 154 (1967) D-18.
12 R. U. LEMIEUX, L. C. N. TUCKER, K. J. CLEMETSON, AND T. L. NAGABHUSHAN, *Abstr. Papers Chem. Inst. Can.–Amer. Chem. Soc. Joint Conf., Toronto (1970)*, MEDI-105.
13 R. U. LEMIEUX, T. L. NAGABHUSHAN, AND K. JAMES, *Abstr. Papers Amer. Chem. Soc. Meeting*, 161 (1971) CARB-8.

14 E. FISCHER AND B. HELFERICH, *Ann. Chem.*, 383 (1911) 68; D. D. REYNOLDS AND W. L. EVANS, *J. Amer. Chem. Soc.*, 60 (1938) 2559.
15 Y. INOUYE, K. ONODERA, S. KITAOKA, AND H. OCHIAI, *J. Amer. Chem. Soc.*, 79 (1957) 4218.
16 R. KUHN AND W. KIRSCHENLOHR, *Chem. Ber.*, 87 (1954) 384.
17 P. H. GROSS AND R. W. JEANLOZ, *J. Org. Chem.*, 32 (1967) 2759.
18 T. CHIU AND P. H. GROSS, unpublished data.
19 R. G. SPIRO, *J. Biol. Chem.*, 237 (1962) 646.