THE SYNTHESIS OF 6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GALACTOPYRANOSYL)-D-GALACTOSE BY INVERSION OF CONFIGURATION*

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ABSTRACT

As a model synthesis for the preparation of oligosaccharides containing the 2-acetamido-2-deoxy-D-galactopyranosyl group, 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactose was prepared by inversion of configuration at C-4 of the amino sugar unit of benzyl 6-*O*-[2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-(methylsulfonyl)-β-D-glucopyranosyl]-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside. This intermediate was obtained by condensation of benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside with benzaldehyde, followed by acetylation, hydrolysis of the 4,6-*O*-benzylidene grouping, and methanesulfonylation. The compound resulting from the inversion of configuration, namely, benzyl 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside, was found to be identical with the product formed by condensation of benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside with 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercuric cyanide. Hydrogenolysis and alkaline treatment gave the title compound, purified through the octaacetate.

INTRODUCTION

Synthesis of oligosaccharides containing the 2-acetamido-2-deoxy-D-galactopyranosyl group is of importance for the preparation of intermediates in the synthesis of gangliosides and of substances possessing blood-group A activity.

Limited availability restricts the use of 2-amino-2-deoxy-D-galactose as the starting material, and no method for preparing disaccharides having a 2-acetamido-2-deoxy-α-D-galactopyranosyl group has yet been developed. Consequently, the method[1–4] used for preparing 2-amino-2-deoxy-D-galactose by inversion of configura-

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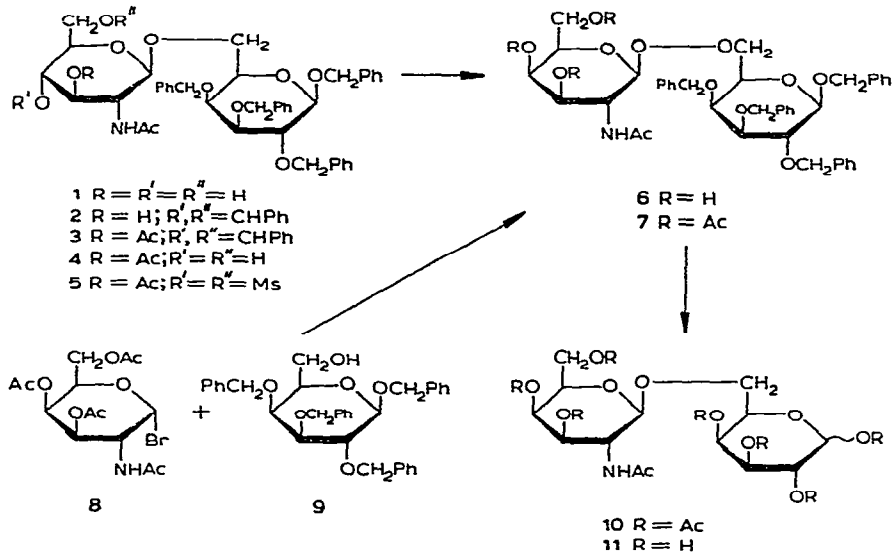
tion at C-4 of 2-amino-2-deoxy-D-glucose was applied to a model disaccharide containing the 2-acetamido-2-deoxy-β-D-glucopyranosyl group.

The resulting disaccharide was found to be identical with that obtained by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-galactopyranosyl bromide[5] (**8**) with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**9**) in the presence of mercuric cyanide.

DISCUSSION

The stable benzyl group was used to protect the D-galactose residue in benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside[6] (**1**), in order to transform the 2-amino-2-deoxy-D-glucosyl group without affecting the D-galactose residue. A procedure similar to that described by Gross, du Bois, and Jeanloz[2] was applied to the 3-*O*-acetyl-4,6-di-*O*-(methylsulfonyl) derivative (**5**) of disaccharide **1**. Compound **5** was obtained in excellent yield *via* the 4,6-*O*-benzyl-



idene derivative (**2**), the 3-*O*-acetyl-4,6-*O*-benzylidene derivative (**3**), and the 3-*O*-acetyl derivative (**4**), all compounds being crystalline. Attempts to obtain selective benzoylation at O-3 and O-6 of **1**, in order to prepare a 4-*O*-(methylsulfonyl) derivative, as described by Horner *et al.*[4], were unsuccessful. Inversion at C-4 of **5** with the benzoate ion in *N*,*N*-dimethylformamide solution proceeded satisfactorily, to afford a good yield. The crude product, which consisted of a mixture of a mono- and a dibenzoate, was de-*O*-acylated to give crystalline **6**, which was then transformed into the crystalline acetate **7**. The overall yield of the preparation of **6** from **1** was 36%. Formation of a disaccharide containing the 2-acetamido-2-deoxy-β-D-gulopyranosyl group was not detected. Alternatively, compound **7** was prepared, in 10% yield, by

condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl bromide[5] (**8**) with benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside[6] (**9**) in the presence of mercuric cyanide. The compounds obtained by the two routes were shown to be identical, by comparison of their m.p., optical rotation, i.r. and n.m.r. spectra, and behavior in thin-layer chromatography. Compound **7** was hydrogenolyzed, and the product was acetylated, to give the amorphous octaacetate **10**; this was saponified to give crystalline 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactose (**11**). The physical constants were different from those observed by Acher and Shapiro[7] for **11** prepared by another synthetic route; these discrepancies may be explained by the presence of a different ratio of the anomers of the D-galactose residue.

The present synthesis demonstrates the feasibility of preparing, in good yield, oligosaccharides containing either the 2-acetamido-2-deoxy-β- or the 2-acetamido-2-deoxy-α-D-galactopyranosyl group by starting from an oligosaccharide containing the 2-acetamido-2-deoxy-β- or the 2-acetamido-2-deoxy-α-D-glucopyranosyl group.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 melting-point apparatus, and correspond to corrected melting point. Optical rotations were determined in 1-dm, semimicro tubes with a Perkin–Elmer polarimeter, Model No. 141; the chloroform used was analytical-reagent grade and contained approximately 0.75% of ethanol. Infrared spectra were recorded, for potassium bromide disks, with a Perkin–Elmer Model 237 i.r. spectrophotometer. N.m.r. spectra were recorded with a Japan Electron Optics Laboratory Company, Ltd., Model MH100 n.m.r. 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 by use of a column (300 × 0.3 cm) of stainless steel packed with 3% of OV-17 on Gas-Chrom A (60–80 mesh) (Applied Science Laboratories, State College, 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-*O*-(trimethylsilyl)-*myo*-inositol. Column chromatography was performed on Silica Gel (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The ratio of wt. of substance to wt. of adsorbent was 1:60 to 1:80. Ascending t.l.c. was performed on plates coated with Merck Silica Gel F-254 in chloroform containing 0 to 4% of ethanol. The components were detected by spraying with *p*-anisaldehyde–sulfuric acid, and heating at 125°. All of the compounds described herein were homogeneous in t.l.c. Evaporations were conducted under diminished pressure, with the bath temperature below 45°. The microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

Benzyl 6-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**2**). — A mixture of benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside[6] (**1**, 3.72 g, 5 mmoles; dried under high vacuum for 12 h at 60°), zinc chloride (3 g), and benzalde-

hyde (40 ml) was stirred for 24 h at room temperature, and the solution was poured into a mixture of ice, water, and hexane. The precipitate was filtered off, washed with cold water, and dried. It was dissolved in chloroform, and the solution chromatographed on silica gel. The product was eluted with 99:1 chloroform–ethanol, and crystallized from chloroform–ether to give 3.49 g (84%) of **2** as needles, m.p. 249–250°; $[\alpha]_D^{20}$ −7.5° (*c* 1.6, pyridine), −57° (*c* 0.9, chloroform).

Anal. Calc. for $C_{49}H_{53}NO_{11}$: C, 70.73; H, 6.43; N, 1.68; O, 21.15. Found: C, 70.67; H, 6.31; N, 1.69; O, 20.80.

Benzyl 6-O-(*2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**3**). — Compound **2** (3.33 g, 4 mmoles) was treated with acetic anhydride (5 ml) in pyridine (20 ml) for 20 h at room temperature. The mixture was poured into ice–water, and the precipitate was filtered off, washed with cold water, and dried. It was dissolved in chloroform, and the solution was chromatographed on silica gel. The product was eluted with 99:1 chloroform–ethanol, and crystallized from chloroform–ethanol, to give 3.22 g (92%) of **3** as needles, m.p. 230–231°; $[\alpha]_D^{20}$ −58° (*c* 1.6, chloroform); i.r. data: ν_{max}^{KBr} 1665 (CONH), 1740 (OAc), and 745 and 733 cm^{-1} (Ph).

Anal. Calc. for $C_{51}H_{55}NO_{12}$: C, 70.08; H, 6.36; N, 1.60; O, 21.97. Found: C, 69.55; H, 6.31; N, 1.58; O, 21.70.

Benzyl 6-O-(*2-acetamido-3-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**4**). — Compound **3** (3.00 g, 3.43 mmoles) was dissolved in glacial acetic acid (80 ml), and the solution was heated to 80°. Water (20 ml), heated to 80–85°, was added to the stirred solution at such a rate that no permanent precipitation occurred. After the solution had been kept for 1 h at 80–85°, the solvent was 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. The product was eluted with 49:1 chloroform–ethanol, and crystallized from methanol–ether–hexane, to give 2.32 g (86%) of **4** as long needles, m.p. 204–205°, $[\alpha]_D^{20}$ −42° (*c* 2.3, chloroform), +7.2° (*c* 1.2, pyridine).

Anal. Calc. for $C_{44}H_{51}NO_{12}$: C, 67.23; H, 6.56; N, 1.78; O, 24.43. Found: C, 66.99; H, 6.44; N, 1.73; O, 24.71.

A side-product, probably the 3,6-diacetate of **1**, was obtained in about 1% yield. Its acetylation gave 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[6], and its de-*O*-acetylation afforded compound **1**. Its R_F in t.l.c. lay between that of **1** and that of the triacetate. This side-product was not further characterized.

Benzyl 6-O-[*2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-(methylsulfonyl)-β-*D*-glucopyranosyl]-2,3,4-tri-*O*-benzyl-β-*D*-galactopyranoside* (**5**). — A solution of compound **4** (3.00 g, 3.81 mmoles; dried under high vacuum for 12 h at 60°) in dry pyridine (15 ml) was cooled to −10° with exclusion of moisture, and methanesulfonyl chloride (0.9 ml) was added slowly with stirring. The mixture was kept for 48 h at −5°, and was then poured into ice–water; the mixture was extracted with chloroform, and the

extract was successively washed with ice-cold water (twice), cold 0.1M hydrochloric acid (thrice), ice–water (twice), cold 0.1M sodium hydrogen carbonate solution, and ice–water (thrice), dried (sodium sulfate), and evaporated to a syrup. This was dissolved in chloroform, and the solution was chromatographed on silica gel. Elution with 99:1 chloroform–ethanol gave a product which was crystallized from chloroform–ether–pentane to give 2.52 g (70%) of **5** as microcrystals, m.p. 180–181° (dec.), $[\alpha]_D^{20}$ −31° (*c* 1.3, chloroform).

Anal. Calc. for $C_{46}H_{55}NO_{16}S_2$: C, 58.64; H, 5.90; N, 1.49; S, 6.80. Found: C, 58.72; H, 5.90; N, 1.43; S, 6.97.

Benzyl 6-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**6**). — A solution of compound **5** (1.88 g, 2 mmoles) and sodium benzoate (1.5 g) in dry *N*,*N*-dimethylformamide (20 ml) was heated for 24 h at 140°, cooled, and diluted with chloroform. The solution was successively washed with water, 0.5M hydrochloric acid, water (twice), 2M sodium hydrogen carbonate, and water (thrice), and evaporated to a syrup which was dissolved in methanol (65 ml) and treated with 0.2M sodium methoxide in methanol (10 ml) for 1 h at room temperature and for 12 h at 5°. The sodium ions were removed with Amberlite IR-120 (H^+) ion-exchange resin, and the solution was evaporated. The resulting solid was recrystallized from methanol, to give 1.15 g (77%) of **6** as short needles, m.p. 195–196°; $[\alpha]_D^{20}$ +19° (*c* 1.0, pyridine).

Anal. Calc. for $C_{42}H_{49}NO_{11}$: C, 67.81; H, 6.65; N, 1.88; O, 23.66. Found: C, 67.69; H, 6.63; N, 1.85; O, 23.72.

After methanolysis of **6** with 0.5M hydrogen chloride in methanol for 16 h at 65°, followed by per(trimethylsilyl)ation, g.l.c. indicated the absence of methyl 2-acetamido-2-deoxy-D-glucoside from, and the presence of methyl 2-acetamido-2-deoxy-D-galactoside in, the methanolyzate.

Benzyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**7**). — *A. From compound* **6**. Compound **6** (1.49 g, 2 mmoles) was treated with acetic anhydride (4 ml) in pyridine (15 ml) for 20 h at room temperature. The mixture was poured into ice–water, and the precipitate was filtered off, washed with cold water, and dried. The crude product was dissolved in chloroform, and the solution was chromatographed on silica gel. Elution with 99:1 chloroform–ethanol gave a product which was crystallized from methanol–isopropyl alcohol, to give 1.6 g (91%) of **7** as short needles, m.p. 165–166°; $[\alpha]_D^{20}$ −32° (*c* 1.7 chloroform); i.r. data: ν_{max}^{KBr} 1745 (OAc), 1665 (CONH), and 733 cm^{-1} (Ph); n.m.r. data: τ 2.58, 2.62 (20 H, 4 Ph), 7.86, 7.96, and 8.15 (12 H: 3 OAc+NAc).

Anal. Calc. for $C_{48}H_{55}NO_{14}$: C, 66.26; H, 6.38; N, 1.61; O, 25.75. Found: C, 66.33; H, 6.39; N, 1.55; O, 25.60.

B. From compounds **8** *and* **9**. A chloroform solution (30 ml) of **8** that had been prepared[5] from 2.3 g of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose was added to a mixture of **9** (ref. 6; 700 mg, 1.29 mmoles), mercuric cyanide (1.5 g), Drierite (500 mg), and dry benzene (13 ml) with vigorous stirring. The mixture was stirred for 3 h, and then filtered through a Celite pad. The solids were washed with

chloroform, and the filtrate and washings 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, and the products were eluted with 99:1 chloroform–ethanol. Fractions that contained a compound having an R_F value identical with that of **7** prepared by method *A* were evaporated, to give 147 mg (13%) of amorphous material, $[\alpha]_D^{20}$ +1° (*c* 3.3, chloroform); this was crystallized and recrystallized from methanol–isopropyl alcohol, and then de-*O*-acetylated catalytically with sodium methoxide in methanol. The product was recrystallized three times from methanol, and then acetylated with acetic anhydride and pyridine in the usual way. Recrystallization from methanol–isopropyl alcohol gave 113 mg (10%) of **7**, having m.p., $[\alpha]_D^{20}$, and i.r. and n.m.r. data identical with those for **7** prepared by method *A*.

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2,3,4-tetra-O-acetyl-β-D-galactopyranose (**10**). — A solution of compound **7** (500 mg, 0.57 mmole) in 1:1 methanol–ethyl acetate (70 ml) was hydrogenolyzed for 72 h with hydrogen under pressure (3 atm.) in the presence of 10% palladium-on-charcoal (200 mg) and 2M hydrochloric acid (1 ml). The catalyst was then filtered off, and the chloride ions were removed by treatment with Amberlite IR-45 (OH^-) ion-exchange resin. The solution was evaporated, and the residue was dried by repeated addition and distillation of toluene, and treated for 10 min with a hot solution of anhydrous sodium acetate (100 mg) in acetic anhydride (4 ml). The mixture was poured into ice–water, and extracted with chloroform, and the extract was washed 5 times with water and then evaporated. The syrupy residue was first treated 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. Elution with 49:1 chloroform–ethanol gave a syrup, which was treated by cooling its solution in dichloromethane–ether–pentane to give 235 mg (61%) of **8** as amorphous material; $[\alpha]_D^{20}$ −12° (*c* 2.4, chloroform).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.62; H, 5.81; N, 2.07; O, 42.50. Found: C, 49.35; H, 5.72; N, 2.07; O, 42.48.

After methanolysis of compound **10** with 0.5M hydrogen chloride in methanol for 16 h at 65°, followed by per(trimethylsilyl)ation, g.l.c. indicated the presence of equimolar proportions of methyl 2-acetamido-2-deoxy-D-galactoside and methyl D-galactoside in the methanolyzate.

6-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactose (**11**). — Compound **10** (200 mg, 0.29 mmole) in methanol (15 ml) was treated with 0.2M sodium methoxide in methanol (1 ml) for 24 h at 0°. The sodium ions were removed with Dowex 50W X8 (H^+) ion-exchange resin, the solution was evaporated, and the residue was crystallized from methanol–ethanol to give 93 mg (83%) of **11**, m.p. 181–184°; $[\alpha]_D^{20}$ +9° (*c* 1.2, water); lit.[7] m.p. 204–205°, $[\alpha]_D^{18}$ +38.5° (*c* 0.9, water). Examination of **11** on Whatman No. 1 paper by descending chromatography in 10:7:3 ethyl acetate–pyridine–water showed the presence of a component having

R_G 0.40; under the same conditions, lactose had R_G 0.53, and 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose[8] had R_G 0.47.

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.85; H, 6.59; N, 3.65; O, 45.91. Found: C, 43.89; H, 6.67; N, 3.42; O, 45.73.

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