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Note

A synthesis of 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine)*

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Our recent reports[1–3] on lipopolysaccharide antigens of *Pseudomonas aeruginosa* have shown that 2-acetamido-2,6-dideoxygalactose (*N*-acetylfucosamine) residues are important components of the carbohydrate portion. For structural comparison and enantiomeric assignment, we required authentic, reference samples of 2-amino-2,6-dideoxy-D-galactose derivatives. A synthetic route from 2-amino-2-deoxy-D-galactose was considered the most convenient, as this sugar is commercially available. With this objective in view, the present paper describes the preparation of methyl 2-acetamido-2,6-dideoxy-α-D-galactopyranoside (**5**) and 2-acetamido-2,6-dideoxy-D-galactose (**6**). Although a similar method for preparing 2-amino-2,6-dideoxy-D-galactose has been reported by Zehavi and Sharon[4], various modifications and improvements have been effected in the synthesis described here. Analogous procedures have also been used by Perry and Daoust[5] for the preparation of some methyl ethers of 2-amino-2,6-dideoxy-D-galactopyranose.

Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**1**) was prepared by a minor modification of the literature method[6] from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose, which had been obtained in 93% yield from 2-amino-2-deoxy-β-D-galactopyranose hydrochloride[7] by means of an improved procedure.

Treatment of **1** with one molar equivalent of *p*-toluenesulfonyl chloride in pyridine gave mainly methyl 2-acetamido-2-deoxy-6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (**2**), together with a di-*O*-*p*-tolylsulfonyl derivative and a small proportion of the starting material. Accordingly, 1.2 molar equivalents of the chloride were used in order to decrease the proportion of unchanged starting-material recovered; the yield of **2** was 48%. In an attempt to achieve more selective sulfonylation, 2-mesitylenesulfonyl chloride was evaluated as a possibly more-specific reagent than *p*-toluenesulfonyl chloride, but no noteworthy changes in net yields were observed. In

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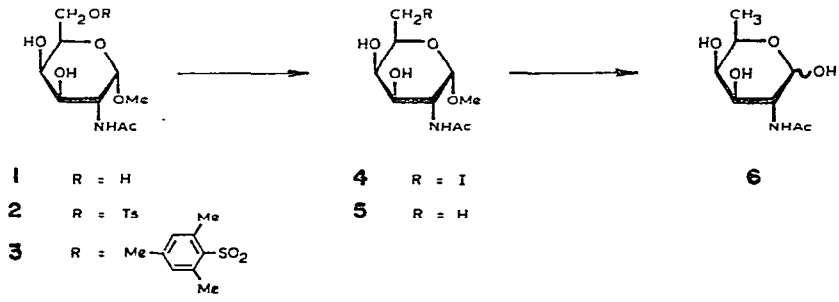

both instances, the desired sulfonates (**2** and **3**) were readily isolated as crystals. The di-*O*-*p*-tolylsulfonyl derivative was obtained in 15% yield as an amorphous powder that was tentatively assigned as the 3,6-di-*p*-toluenesulfonate on the basis of the well known resistance toward sulfonylation of the 4-hydroxyl group of galactopyranosides[8].

The 6-*p*-toluenesulfonate **2** was converted into the 6-deoxy-6-iodo derivative (**4**). It is well known[9,10] that displacement of 6-sulfonyloxy groups of galactopyranosides with sodium iodide is very slow. Consequently, the rather vigorous conditions used for analogous displacement-reactions, namely 13 h at 110° in acetone[4], or 5–6 h at 100° in acetone[5], were kept in mind. In the present instance, compound **2** was treated with an excess of sodium iodide in butanone for 6 h at 100°. The crystalline iodide (**4**) thus produced was hydrogenated in the presence of Raney nickel to give methyl 2-acetamido-2,6-dideoxy-α-D-galactopyranoside (**5**) in 76% yield; the net yield of **5** from 2-amino-2-deoxy-D-galactose hydrochloride was 7.7% in five steps. 2-Acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine, **6**) was then obtained by hydrolysis of the glycoside **5**, and subsequent re-*N*-acetylation of the resultant amino sugar. As already reported[1], the properties of the acetamidodideoxyhexose obtained from the lipid-free antigen of *Pseudomonas aeruginosa* immunotype 2 coincided with those of **6**, except that the former had negligible optical activity, indicating that the natural product was the DL form. The X-ray powder diffraction patterns of both samples were essentially superposable. The same acetamidodideoxy sugar isolated from the lipid-free, type 4 antigen was a 2:1 mixture of the D and L forms[2].

EXPERIMENTAL

General methods. — Solutions were evaporated under diminished pressure. Melting points were measured on a Thomas–Hoover "Unimelt" apparatus and are not corrected. N.m.r. spectra were recorded at 100 MHz on a Varian HA-100 spectrometer. Chemical shifts refer to an internal standard of tetramethylsilane ($\delta = 0.00$). A Perkin–Elmer Model 141 automatic polarimeter and 1-dm tubes were used for measurement of specific rotations. T.l.c. was performed with Silica gel 60G (E. Merck, 7734) as the adsorbent, and spots were made visible by spraying with

dilute sulfuric acid and subsequently heating. Microanalyses were performed by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation (camera diameter 114.59 mm). Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest).

*Improved preparation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-*D*-galactopyranose.* — A mixture of 2-amino-2-deoxy-D-galactopyranose hydrochloride (the pure β anomer[7], 2.156 g, 10 mmol, Pfanstiehl Laboratories, Inc., Waukegan, Ill.), dry pyridine (30 ml), and acetic anhydride (20 ml) was stirred for 18 h at room temperature. The suspended solids were filtered off, washed with water, and dried, giving 3.35 g of crystals, m.p. 235–236° dec. (lit.[6] m.p. 235°, m.p.[4] 234–235° dec.). The filtrate and washings were evaporated to a thin syrup that was diluted with water. The resulting crystals were collected, washed with water, and dried, giving a second crop (235 mg). The total yield was 3.585 g (93%) (lit.[4] yield, 62%).

*Preparation of methyl 2-acetamido-2-deoxy-α-*D*-galactopyranoside*[6] (**1**). — A mixture of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose (3.46 g, 9 mmol) and 2% hydrogen chloride in methanol (100 ml) was boiled under reflux for 2.5 h with stirring. The cooled mixture was made neutral with Dowex-1 X-8 (OH^-) resin (20–50 mesh, ~25 g). Evaporation of the solution gave a crystalline mass that showed 3 spots in t.l.c. [2:3 or 1:4 (v/v) methanol–chloroform]. Recrystallization from ethanol (10 ml) and hexane (5 ml) gave 1.13 g (54%) of pure **1**, m.p. 212–213 dec., $[\alpha]_D^{28}$ +166° (*c* 0.5, methanol) [lit.[6] m.p. 217–218°, $[\alpha]_D^{21}$ +170 (chloroform)].

*Methyl 2-acetamido-2-deoxy-6-*O*-p-tolylsulfonyl-α-*D*-galactopyranoside* (**2**). — To a stirred and cooled suspension of **1** (969 mg, 4.12 mmol) in pyridine (14 ml), freshly recrystallized *p*-toluenesulfonyl chloride (944 mg, 4.94 mmol) was added in two portions, and the mixture was stirred for 2.5 h at room temperature. T.l.c. [1:4 (v/v) methanol–chloroform] showed a small proportion of residual **1**, and two new spots. Water (1.5 ml) was added to the solution, with cooling. The solution was evaporated to a syrup, which was then dissolved in chloroform (50 ml). The solution was washed with saturated, aqueous sodium chloride (10 ml), and the aqueous layer was extracted with chloroform (50 ml). The combined chloroform layers were washed with aqueous sodium chloride (10 ml), dried (anhydrous magnesium sulfate), and evaporated to afford a semicrystalline residue. Recrystallization from ethanol (5 ml) and ether (~5 ml) gave 653 mg (41%) of **2**, m.p. 155–157° dec., $[\alpha]_D^{26}$ +39.3° (*c* 0.7, chloroform); n.m.r. ($CDCl_3$): 7.84 d, 7.38 d (2 H each, *J* 8 Hz, aromatic protons), 6.17 d (1 H, *J* 8 Hz, NH), 4.67 d (1 H, $J_{1,2}$ 4 Hz, H-1), 4.27 d (2 H, H-6,6'), 4.1–3.4 broad m (H-2,3,4,5), 3.34 s (3 H, OCH_3), 2.44 s (3 H, CH_3), and 2.01 s (3 H, NAc).

Anal. Calc. for $C_{16}H_{23}NO_8S$ (389.4): C, 49.35; H, 5.95; N, 3.60; S, 8.23. Found: C, 49.10; H, 6.26; N, 3.26; S, 8.81.

The mother liquor was evaporated to a thick syrup that was chromatographed on a column of silica gel (E. Merck, 7734; 12 g in chloroform) by using 1:19 (v/v) methanol–chloroform (90 ml) and 1:9 (v/v) methanol–chloroform (90 ml) as eluants, to give 343 mg (15%) of a di-*O*-*p*-tolylsulfonyl derivative as an amorphous powder,

$[\alpha]_D^{24}$ +72.4° (*c* 1.3, chloroform); n.m.r. ($CDCl_3$): δ 7.83 d, 7.38 d (4 H each, aromatic protons), 3.32 s (3 H, OCH_3) 2.44 s (6 H, 2 CH_3), 1.83 s (3 H, NAc); and 124 mg (7%) of an additional crop of **2**. The total yield of **2** was 48%. The 3,6-di-*O*-*p*-tolylsulfonyl derivative of **1** has been described[5] as being amorphous and having $[\alpha]_D$ +87° (chloroform).

Methyl 2-acetamido-2-deoxy-6-O-(2-mesitylenesulfonyl)-α-D-galactopyranoside (**3**). — To a stirred suspension of **1** (235 mg, 1 mmol) in pyridine (3.5 ml) was added 2-mesitylenesulfonyl chloride (262 mg, 1.2 mmol). The mixture was stirred for 2 h at room temperature, and then additional chloride (66 mg, 0.3 mmol) was added. The mixture was kept for 1.5 h at room temperature, diluted with water (1 ml), and evaporated. The residue was dissolved in chloroform (30 ml), and the solution was washed with water. The aqueous layer was extracted with chloroform (30 ml), and the combined chloroform layers were washed with aqueous sodium hydrogencarbonate, dried (magnesium sulfate), and evaporated to dryness. The resultant thick syrup, t.l.c. of which showed mainly 2 spots, crystallized on trituration with ether, and gave 191 mg (46%) of **3**, m.p. 170–171° (sintered at ~162°). Recrystallization from ethanol–hexane gave **3** as needles, m.p. 173–174° dec., $[\alpha]_D^{28}$ +44.5° (*c* 0.5, chloroform); n.m.r. ($CDCl_3$): 7.03 s (2 H, aromatic protons), 6.04 d (1 H, *J* 8 Hz, NH), 4.71 d (1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.39 s (3 H, OCH_3), 2.64 s (6 H, 2 CH_3), 2.12 s (3 H, CH_3), and 2.04 s (3 H, NAc).

Anal. Calc. for $C_{18}H_{27}NO_8S$ (317.5): C, 51.79; H, 6.52; N, 3.36; S, 7.68. Found: C, 51.74; H, 6.60; N, 3.26; S, 7.86.

Methyl 2-acetamido-2,6-dideoxy-6-iodo-α-D-galactopyranoside (**4**). — A mixture of **2** (510 mg, 1.31 mmol), sodium iodide (600 mg), and butanone (15 ml) was heated in a sealed flask for 6 h at 100°. T.l.c. [1:5 (v/v) methanol–chloroform] showed that no starting material remained and only a single new component was produced. The mixture was cooled and filtered, and chloroform (100 ml) was added to the filtrate. The resultant solution was washed with 10% aqueous sodium thiosulfate (20 ml), and the aqueous layer was extracted with chloroform (40 ml). The chloroform layers were combined, washed with aqueous sodium chloride (30 ml), dried (sodium sulfate), and evaporated to dryness to give 245 mg (54%) of a crystalline residue which was triturated with chloroform (3 ml) and ether (12 ml), and refrigerated overnight. The solid product (**4**) was collected; yield 190 mg (42%), m.p. 192–193° dec. This compound was used, without further characterization, in the next step.

Methyl 2-acetamido-2,6-dideoxy-α-D-galactopyranoside (**5**). — To a solution of **4** (190 mg, 0.55 mmol) in methanol (20 ml) were added Raney nickel (W-2, in ethanol, 5 ml), ethanol (5 ml), and triethylamine (0.3 ml). The mixture was then shaken for 4 h at ~25° under hydrogen (3.52 $kg.cm^{-2}$). The catalyst was filtered off and washed with methanol. The filtrate and washings were evaporated, and the resultant solid was extracted with hot chloroform. Evaporation of the extract gave 120 mg (quantitative yield) of crystalline residue. Recrystallization from ethanol (2 ml) and ether gave fine needles of pure **5**; yield 92 mg (76%), m.p. 225–226°, $[\alpha]_D^{30}$ +181° (*c* 0.6, methanol) [lit.[5] m.p. 227–228°, $[\alpha]_D$ +179° (*c* 0.11, methanol)]; X-ray powder dif-

fraction data: 10.91 vw, 9.50 s (1), 9.35 s (2), 6.28 vw, 5.76 m (3), 5.54 vw, 4.79 w, 4.35 m, 4.24 m (4,4), 3.96 w, 3.72 w, 3.60 m (4,4), 3.40 m, and 3.14 vw.

Anal. Calc. for $C_9H_{17}NO_5$ (219.2): C, 49.31; H, 7.82; N, 6.39. Found: C, 49.01; H, 7.61; N, 6.18.

*2-Acetamido-2,6-dideoxy-*D*-galactose* (**6**). — A solution of **5** (50 mg, 0.23 mmol) in 2M hydrochloric acid (25 ml) was heated for 2 h at 100°, and evaporated with several intermittent dilutions with water to remove all of the acid. The resulting, semicrystalline 2-amino-2,6-dideoxy-D-galactose hydrochloride (34 mg) was dissolved in water (5 ml), and to the solution were added methanol (1 ml), acetic anhydride (0.5 ml), and Dowex-1 X-8 resin (CO^{2-}, ~2 g). The mixture was stirred for 2 h at ~25°. Additional acetic anhydride (0.5 ml) was then added, and stirring was continued for a further 2 h. The mixture was filtered, and the filtrate passed through a column (5 × 1.5 cm) of Dowex-50W X-8 (H^+) resin (50–100 mesh). The column was washed with water (100 ml), and the combined eluates were evaporated to an oil (28 mg, 59%) that was triturated with ethanol to afford crystals, m.p. 188–190° dec. Recrystallization from ethanol gave prisms (15 mg, 30%), m.p. 201–204° dec., $[\alpha]_D^{20}$ +118→ +95° (*c* 0.8, water) [lit. m.p. 196–197° dec., $[\alpha]_D$ +129→ +92° (water)[4]; m.p. 201–202°, $[\alpha]_D$ +109→ +87° (water)[11]; m.p. 194–196° dec., $[\alpha]_D$ +89° (equil., water)[12]]; X-ray powder diffraction data: 8.77 s (2), 7.46 w, 5.60 m (4,4), 4.77 vs (1), 4.48 m (4,4), 3.97 m (4,4), 3.64 vw, 3.55 m (3), 3.18 w, 3.01 vw, 2.93 w, and 2.69 w.

Anal. Calc. for $C_8H_{15}NO_5$ (205.2): C, 46.82; H, 7.37; N, 6.83. Found: C, 46.86; H, 7.27; N, 6.61.

The sample of 2-acetamido-2,6-dideoxy-DL-galactose obtained[1] from the O-specific chain polysaccharide of *Pseudomonas aeruginosa* immunotype 2 antigen had m.p. 189–191°, $[\alpha]_D^{20}$ −0.5° (*c* 1, pyridine); X-ray powder diffraction data: 8.62 s (2), 7.51 w, 5.58 m (4,4), 4.70 vs (1), 4.38 m (4,4), 3.93 m (4,4), 3.65 vw, 3.54 m (3), 3.33 vw, 3.24 vw, 3.06 vw, 2.96 w, 2.84 w, 2.77 vw, and 2.45 w.

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