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Note

Synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-galactose*

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The value of purified glycosyltransferases having strict specificities has become apparent in the structural analysis of oligosaccharides and related compounds[2]. As a result, much attention has been focused on the specificity of such enzymes, encouraging the purification of glycosyltransferases present in various biological sources[3,4]. It has been observed that pure sialyltransferase, isolated from porcine submaxillary mucin, transfers *N*-acetylneuraminic acid from cytidine 5′-(*N*-acetylneuraminic acid monophosphate) to the disaccharide β-Gal-(1→3)-GalNAc or to glycoproteins containing this disaccharide unit[5]. The enzyme fails[5] to act on the disaccharide β-Gal-(1→6)-GalNAc. Recently, we have found that our synthetic disaccharide β-Gal-(1→3)-α-GalNAc-1→OPh acts as a suitable acceptor for sialyltransferase present in human serum[6]. The title disaccharide was needed for further specificity testing of this sialyltransferase. To the best of our knowledge, this disaccharide has not been isolated from any biological source. Nevertheless, the presence has been proposed of this disaccharide in the carbohydrate sequence

β-Gal(1→4)-GalNAc
3
↑
1
β-Gal

(having a terminal sialic residue) in one of the blood-group, M-specific glycopentapeptides isolated from human erythrocytes[7].

We now describe a facile synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-galactose (7). It is possible that the availability of compound 7 may lead to the detection of new sialyltransferases that may act specifically on this disaccharide moiety. Disaccharide 7 was also needed in our laboratory for further specificity testing of β-D-galactosidase from different sources[8].

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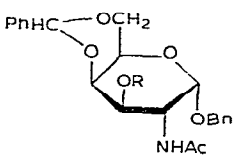
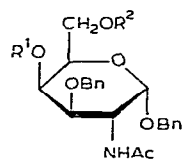
There is no doubt that previous difficulties encountered in attempts to introduce, in good yield, a glycosidic linkage at the 4-hydroxyl group of a glycopyranose *via* chemical synthesis have now been resolved. Various reaction-conditions required for such syntheses have been well established[9–13]. Based upon such reports, we aimed at the preparation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (**4**) as a suitable "aglycon" for the synthesis of the desired disaccharide **7**. Treatment of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[14] (**1**) with α-bromotoluene (benzyl bromide) in *N*,*N*-dimethylformamide in the presence of powdered potassium hydroxide produced crystalline **2** in 70% yield. Removal of the 4,6-*O*-benzylidene group from **2**, to give **3**, was effected with aqueous acetic acid at 100° under the usual conditions.

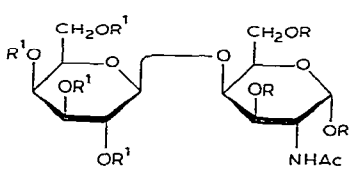
The use of powdered KOH, or a mixture of barium oxide and barium hydroxide, as the base, with an equimolar amount of a benzyl halide in *N*,*N*-dimethylformamide, has frequently been recommended for selective benzylation, such as the preparation of an alkyl 2-acetamido-3-*O*-benzyl-2-deoxy-D-glucopyranoside[13,15]. However, the phase-transfer method of catalysis for selective alkylation of various diols[1,16,17] has been found quite attractive. Under these conditions, diol **3** gave the desired compound **4** in 73% yield. The purity of the product was established by t.l.c., and the n.m.r. spectrum of the compound supported structure **4**, confirming that the product was not the *N*-alkylated derivative.



1 R = H
2 R = Bn



3 R^1 = R^2 = H
4 R^1 = H, R^2 = Bn



5 R = Bn, R^1 = Ac
6 R = Bn, R^1 = H
7 R = R^1 = H

Bn = $PhCH_2$—

Reaction of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (**4**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide was conducted in dichloromethane in the presence of silver triflate and tetra-*N*-methylurea. It must be emphasized that the use of tetra-*N*-methylurea in the coupling reaction of a glycosyl halide with "aglycons" having a 6-*O*-benzyl group seems to be essential. In the

absence of this weak base, the reaction from the condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with the readily accessible compound benzyl 3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside was sluggish, and isolation of the disaccharide product[18] difficult. It seems that the (very strong) trifluoromethanesulfonic acid released in the glycosylation reaction catalyzed by silver triflate is capable of partial or total removal of the labile, 6-*O*-benzyl group from such "aglycons". Nevertheless, the use of an "aglycon" having 3- and 6-*O*-benzyl groups for such preparations is preferred, as deacetylation of the product in the reaction mixture generally occurred, to give the disaccharide derivative and unreacted compound, effectively separated by chromatography on a column of silica gel. The starting material recovered may be successfully re-used for the coupling reaction. On hydrogenolysis, the partially protected derivative **6** afforded the desired compound **7** in 75% yield.

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. I.r. spectra were recorded with a Perkin–Elmer model 297 instrument, and n.m.r. spectra with a Varian XL-100 spectrometer at 100 MHz, with Me_4Si as the internal standard. Ascending t.l.c. was conducted on plates coated with a layer (0.25 mm) of Silica Gel CC-7 (Malinckrodt); the components were located by exposure to u.v. light, and by spraying with 5% sulfuric acid in ethanol and heating.

Benzyl 2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**2**). — To a stirred solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**1**, 3.99 g) and KOH (2.13 g) in *N*,*N*-dimethylformamide (56 mL) was added dropwise benzyl bromide (2.0 mL), and stirring was continued for two days at room temperature. The milky solution was then poured into iced water (500 mL) with stirring; stirring was continued for 30 min, and the white precipitate was filtered off, washed several times with cold water, and recrystallized from $HCONMe_2$–water to give **3** (3.42 g) in 70% yield. An analytical sample was obtained by chromatography on a column of silica gel, with elution by 5:1 chloroform–acetone; m.p. 255–256°, $[\alpha]_D$ +220.4° (*c* 1, $HCONMe_2$); ν_{max}^{KBr} 3300, 1650 (amide), 735, and 700 cm^{-1} (aromatic).

Anal. Calc. for $C_{29}H_{31}NO_6$: C, 71.14; H, 6.38; N, 2.86. Found: C, 70.95; H, 6.39; N, 2.89.

Benzyl 2-acetamido-3-O-benzyl-2-deoxy-α-D-galactopyranoside (**3**). — A mixture of **2** (2.25 g) and 60% acetic acid (30 mL) was stirred for 30 min at 100°. Evaporation, followed by several additions and evaporations of water, and then of dry toluene, gave a pure solid (92%) that was recrystallized from methanol–ether to give compound **3**, m.p. 175–176°, $[\alpha]_D$ +152.5° (*c* 1, $HCONMe_2$); ν_{max}^{KBr} 3400 (OH), 3300, 1645 (amide),

730, and 695 cm^{-1} (aromatic); n.m.r. (CD_3OD): δ 1.94 (s, 3 H, Ac), 4.92 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 7.30–7.50 (m, 10 H, 2 Ph).

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-galactopyranoside* (**4**). — A suspension of compound **3** (1.40 g, 3.5 mmol), 5% sodium hydroxide (5 mL), and benzyl bromide (0.72 mL, 6 mmol) in dichloromethane (60 mL) was boiled under reflux for 3 days in the presence of tetrabutylammonium hydrogensulfate (0.24 g, 0.7 mmol), cooled, washed with water (3 × 20 mL), dried (anhydrous Na_2SO_4), and evaporated. The residue was purified by chromatography on a column of silica gel, first eluting with chloroform, then with 9:1 chloroform–acetone, and finally with 5:1 chloroform–acetone, to give **4** in 73.1% yield (1.25 g); m.p. 123°, $[\alpha]_D$ +121.5° (*c* 1, chloroform); t.l.c. (5:1 chloroform–acetone): R_F 0.69; ν_{max}^{KBr} 3410 (OH), 3300, 1640 (amide), 735, and 695 cm^{-1} (aromatic); n.m.r. data (CD_3OD): δ 1.94 (s, 3 H, Ac), 4.94 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 7.25–7.50 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{29}H_{33}NO_6$: C, 70.85; H, 6.77; N, 2.85. Found: C, 70.77; H, 6.78; N, 2.82.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**5**). — To a stirred solution of **4** (0.75 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.2 g) in dichloromethane (25 mL) was added tetra-*N*-methylurea (1.0 mL dissolved in 25 mL of dichloromethane). The flask was then wrapped in aluminum foil, silver triflate (0.45 g) was added, and stirring was continued for 3 days at room temperature. The suspension was filtered through a Celite pad, and the filtrate was successively washed with a saturated solution of $NaHCO_3$ (twice) and water (3 × 20 mL), dried (anhydrous Na_2SO_4), and evaporated under diminished pressure, giving crude, syrupy disaccharide **5**, which was used as such for the next reaction.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-β-*D*-galactopyranosyl-α-*D*-galactopyranoside* (**6**). — A solution of **5** (2.5 g) in absolute methanol (25 mL) was treated with M sodium methoxide (2.5 mL), and the solution was kept overnight at room temperature with occasional shaking, made neutral with acetic acid, and evaporated; a few additions and evaporations of dry toluene were made, giving a solid mixture containing the glycosyl bromide, monosaccharide **4**, and disaccharide **6**. The residue was dissolved in CH_3OH (4 mL), and $CHCl_3$ (36 mL) was added with stirring. After stirring for 2 h at room temperature, the precipitate was removed by filtration, and the filtrate was evaporated to dryness, giving a solid which was purified by chromatography on a column of silica gel, with elution first with 5:1 chloroform–acetone, to remove unreacted compound **4**, and then with 9:1 chloroform–ethanol, to afford disaccharide **6** in 70% yield; m.p. 174–175°, $[\alpha]_D$ +97.0° (*c* 0.5, CH_3OH); ν_{max}^{KBr} 3400 (OH), 3300, 1650 (amide), 740, and 700 cm^{-1} (aromatic); n.m.r. data (CD_3OD): δ 1.94 (s, 3 H, Ac), 4.92 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), and 7.25–7.5 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{35}H_{43}NO_{11} \cdot H_2O$: C, 62.58; H, 6.75; N, 2.09. Found: C, 62.69; H, 6.74; N, 2.13.

*2-Acetamido-2-deoxy-4-*O*-β-*D*-galactopyranosyl-*D*-galactose* (**7**). — A solution

of **6** (300 mg) in glacial acetic acid (30 mL) was hydrogenolyzed in the presence of Pd–C (10%, 200 mg) for three days, the mixture was filtered, the filtrate evaporated, and the residue recrystallized from ethanol–ether, to give **7** (132 mg, 75%), m.p. 160°, $[\alpha]_D$ +65.6° (*c* 1, CH_3OH); ν_{max}^{KBr} 3350, 1050 (OH), and 1630 cm^{-1} (amide); n.m.r. data (CD_3OD): δ 2.02 (s, 3 H, Ac) and 5.14 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1).

Anal. Calc. for $C_{14}H_{25}NO_{11} \cdot H_2O$: C, 41.89; H, 6.78; N, 3.49. Found: C, 42.02; H, 6.66; N, 3.43.

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