SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-6-O- β -D-GALACTO-PYRANOSYL-D-GALACTOPYRANOSE AND o-NITROPHENYL 2-ACETAMIDO-2-DEOXY-6-O- β -D-GALACTOPYRANOSYL- α -D-GALAC-TOPYRANOSIDE*

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ABSTRACT

Acetalation of o-nitrophenyl 2-acetamido-2-deoxy- α -D-galactopyranoside with p-methoxybenzaldehyde-zinc chloride complex afforded the 4,6-O-(pmethoxybenzylidene) derivative (1). Acetylation of 1 with pyridine-acetic anhydride gave the crystalline monoacetate (2), the acetal group of which was cleaved by brief treatment with hot, 80% aqueous acetic acid to furnish the diol (3). The ¹Hn.m.r. spectra of both 2 and 3 supported their overall structures. Glycosylation of 3 with 2,3,4,6-tetra-O-acetyl- α -D-galactopyranosyl bromide (5) in 1:1 nitromethane-benzene, in the presence of mercuric cyanide, afforded the disaccharide derivative (6). O-Deacetylation of 6 in methanolic sodium methoxide gave the title disaccharide (8). A similar glycosylation of benzyl 2-acetamido-3-O-acetyl-2deoxy- α -D-galactopyranoside with bromide 5, followed by O-deacetylation of the resulting intermediate, produced disaccharide 9. The structures of 8 and 9 were confirmed by ¹³C-n.m.r. spectroscopy. Hydrogenolysis of the benzyl aglycon of 9 gave the free disaccharide 2-acetamido-2-deoxy-6-O-B-D-galactopyranosyl-Dgalactopyranose (13). The ¹³C-n.m.r. spectrum of 13 is recorded, and discussed in conjunction with those of the β -(1 \rightarrow 3)- and the β -(1 \rightarrow 4)-linked isomers.

INTRODUCTION

For the past few years, our group has been actively involved in the chemical synthesis of oligosaccharides that can be utilized in the study of glycosidases and glycosyltransferases. The availability of a variety of isomeric oligosaccharides enhanced our ability to study substrate specificity of a number of such enzymes. For example, with the aid of the synthetic disaccharides β -Gal-(1 \rightarrow 3)- β -GlcNAc-1 \rightarrow OC₆H₄NO₂(*p*), β -Gal-(1 \rightarrow 4)- β -GlcNAc-1 \rightarrow OC₆H₄NO₂(*p*), β -Gal-(1 \rightarrow 4)- β -GlcNAc-1 \rightarrow OC₆H₄NO₂(*p*), β -Gal-(1 \rightarrow 6)- β -GlcNAc-1 \rightarrow OC₆H₄NO₂(*p*), and β -Gal-(1 \rightarrow 3)- α -GalNAc-1 \rightarrow OC₆H₄NO₂(*p*), we

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were able to develop an assay procedure for study of the specificity of β -D-galactosidase from different sources²; the last-mentioned compound was also shown to act as a chromogenic substrate for endo- α -N-acetylgalactosaminidase³. Furthermore, it was observed that the synthetic disaccharide β -Gal-(1 \rightarrow 3)- α -GalNAc-1 \rightarrow OPh acts as an acceptor for the sialyltransferase present in human serum⁴. Thus, for further examination of the substrate specificity of such glycosidases and glycosyltransferases, we now report the synthesis of the title disaccharides. It is also noteworthy that these synthetic derivatives could be useful in investigations related to galactose-binding lectins. It has been tentatively suggested that the disaccharide β -Gal-(1 \rightarrow 6)-GalNAc unit occurs as part of a glycoprotein isolated from the cervical mucus of the Bonnet monkey⁵.

RESULTS AND DISCUSSION

o-Nitrophenyl 2-acetamido-2-deoxy- α -D-galactopyranoside was allowed to react with *p*-methoxybenzaldehyde–zinc chloride complex in a manner analogous to that already described⁶, to afford the 4,6-acetal (1). Compound 1 was acetylated with acetic anhydride–pyridine to give, in excellent yield, the crystalline monoacetate 2, the ¹H-n.m.r. spectrum of which (see the Experimental section) was in agreement with the structure assigned. Treatment of 2 with 80% aqueous acetic acid for 20 min at ~98° gave, in 90% yield, the diol 3, the ¹H-n.m.r. spectrum of which was, also, in accord with the structure expected.



Condensation of 2,3,4,6-tetra-O-acetyl- α -D-galactopyranosyl bromide (5) with diol 3 in 1:1 nitromethane-benzene, in the presence of powdered mercuric cyanide, and purification of the crude product-mixture in a column of silica gel by using solvent A as the eluant, gave, in 63% yield, amorphous o-nitrophenyl 2-acetamido -3-O-acetyl-2-deoxy-6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)- α -D-galactopyranoside (6). O-Deacetylation of 6 in methanolic sodium methoxide afforded crystalline disaccharide 8.

Glycosylation of benzyl 2-acetamido-3-O-acetyl-2-deoxy- α -D-galactopyranoside (4) with bromide 5, followed by O-deacetylation of the resulting intermediate, as described for 6 (to give 8), furnished, in 30% overall yield based on 4, crystalline disaccharide 9.

Catalytic hydrogenolysis of the benzyl group of **9** in glacial acetic acid, in the presence of 10% palladium-on-carbon, afforded amorphous, free disaccharide **13**, the purity of which was established by paper chromatography and ¹³C-n.m.r. spectroscopy.



TABLE I

¹³C-N.M R CHEMICAL SHIFTS^a

Atom	Compound					
	8	9	10			
C-1	97.43	96.14	96.35			
C-2	49.42	49.65	48.49			
C-3	66.84	67.12	75.79			
C-4	67.49	68.09	67.31			
C-5	68.04	68.38	71.42			
C-6	71.32	70.03	60.67			
NCOCH3	22.64	22.68	22.71			
CH ₂ Ph		68.09	68.11			
C=O	169.78	169.40	169.54			
C-1′	103.46	103.67	103.63			
C-2'	70.37	70.53	70.71			
C-3'	73.37	73.41	73.29			
C-4′	67.78	67.87	68.02			
C-5'	75.11	75.12	75.26			
C-6′	60.31	60.42	60.47			

^{*a*}In Me₂SO- d_6 , with Me₄Si as the internal standard.

Atoms	Compound						
	11α	11 <i>β</i>	12 α	12 β	13α	13 β	
C-1	92.18	96.14	92.08	96.20	91.98	96.30	
C-2	50.01	53.53	51.83	55.15	51.20	54.62	
C-3	78.04	81.01	68.96	72.60	68.17	71.92	
C-4	69.70	69.06	78.53	77.50	69.58	68.87	
C-5	71.19	75.81	70.85	75.25	70.35	74.81	
C-6	62.18	61.98	61.78	61.57	70.35	70.10	
NCOCH ₃	23.12	23.33	23.03	23.25	23.02	23.26	
C=O	175.44	175.44	175.61	175.91	175.81	175.51	
C-1'	105.58	105.76		105.36		104.12	
C-2'		71.64		72.34		71.75	
C-3'		73.55		73.75		73.62	
C-4′		69.61		69.61		69.58	
C-5'		75.95		76.10		76.1 1	
C-6'		61.98		62.01		61.97	

¹³C-N M.R CHEMICAL SHIFTS^a

"In D_2O , with Me₄Si as the external standard.

Comments on the ¹³C-n.m.r. assignments. — The ¹³C-n.m.r. chemical shifts for the $(1\rightarrow3)$ -linked disaccharide **10**, and those for its $(1\rightarrow6)$ -linked isomer **9**, as well as those of the $(1\rightarrow6)$ -linked o-nitrophenyl disaccharide **8** are listed in Table I. Also, it was considered of interest to record and compare the ¹³C-n.m.r. chemi-



cal shifts of the isomeric β -(1 \rightarrow 3)-, β -(1 \rightarrow 4)-, and β -(1 \rightarrow 6)-linked disaccharides 11, 12, and 13, respectively; see Table II.

As is evident from Table I, the resonances for C-1 of 10 (96.35 p.p.m.), 8 (97.43 p.p.m.), and 9 (96.14 p.p.m.) were all in the range expected for an α configuration at the anomeric center. That the site of glycosylation in compound 10 was O-3 was readily revealed by the noticeable, downfield shift for the resonance of C-3, whereas the resonance for C-6 (60.67 p.p.m.) was in the range normally observed for a carbon atom carrying an unsubstituted primary hydroxyl group. On the other hand, the spectra of 8 and 9 contained signals, at δ 71.32 and 70.03, respectively, attributable to C-6.

The resonances for C-1' of 10 (103.63 p.p.m.), of 8 (103.46 p.p.m.), and 9 (103.67 p.p.m.) were all in the range expected for β configurations at the respective interglycosidic linkages.

In the ¹³C-n.m.r. spectra of the free disaccharides 11, 12, and 13 (see Table II), the 2-acetamido-2-deoxy-D-galactopyranose residue exhibited two sets of resonances attributable to the α and β anomers. In the spectra of all three of the compounds, the resonances at higher field were assigned to the carbon atoms of the α anomer, whereas those at lower field were assigned to their counterparts of the β anomer. However, those assignments were reversed for C-4 and C-6 of all three compounds.

Also, with the exception of C-1' of compound 11, which showed two distinct resonances, at δ 105.58 and 105.76, for the α and β anomer, respectively, all of the other carbon atoms of the β -D-galactopyranosyl residue showed only one signal each. The resonances for C-1' of all three of the compounds were in accord with β configurations at the interglycosidic linkages, and the sites of substitution could readily be gleaned from the noticeable, downfield chemical-shifts for C-3, C-4, and C-6 for compounds 11, 12, and 13, respectively.

TABLE III

CHROMATOGRAPHIC MOBILITY OF SOME OF THE COMPOUNDS⁴

Compound	R _F value in solvent ^b				
	A	В	С		
D-Galactose	1.00	1.00	1.00		
2-Acetamido-2-deoxy-D-galactose	1.44	1.61	1.52		
β -D-Gal-(1 \rightarrow 3)-D-GalNAc	0.56	0.55	0.71		
β -D-Gal-(1 \rightarrow 4)-D-GalNAc	0.82	0.67	0.94		
β -D-Gal-(1- \rightarrow 6)-D-GalNAc	0.44	0.36	0.62		
β -D-Gal-(1 \rightarrow 3)-[β -D-Gal-(1 \rightarrow 4)]-D-GalNAc ⁷	0.28	0.20	0.41		

^aOn Whatman No. 1 paper. ^bThe solvent systems (v/v/v) and times were: A, 10:4:3 ethyl acetate-pyridine-water (20 h); B, 4:1:1 1-butanol-ethanol-water (72 h); and C, 3:2:1 butyl acetate-acetic acid-water (20 h).

EXPERIMENTAL

General methods. — These were the same as those already described¹, except that the following solvent systems (v/v) were used for chromatography: A, 4:1 chloroform-acetone, and B, 13:6:1 chloroform-methanol-water. For R_F values, see Table III.

o-Nitrophenyl 2-acetamido-2-deoxy-4,6-O-(p-methoxybenzylidene)- α -Dgalactopyranoside (1). — Compound 1 was prepared from o-nitrophenyl 2acetamido-2-deoxy- α -D-galactopyranoside according to the procedure of Hall⁶, and had m.p. 219-222° (from N,N-dimethylformamide-water), $[\alpha]_D$ +53.2° (c 0.95, chloroform).

Anal. Calc. for $C_{22}H_{24}N_2O_9$: C, 57.38; H, 5.26; N, 6.08. Found: C, 57.13; H, 5.15; N, 5.82.

o-Nitrophenyl 2-acetamido-3-O-acetyl-2-deoxy-4,6-O-(p-methoxybenzylidene)- α -D-galactopyranoside (2). — Conventional acetylation of 1 (0.9 g) with acetic anhydride (10 mL) in pyridine (10 mL) gave crystalline 2 (0.95 g, 97%); m.p. 125–127° (from ethyl acetate–ether–hexane), [α]_D +100.2° (c 1.2, chloroform); ¹H-n.m.r. data (CDCl₃): δ 1.97 (s, 3 H, NAc), 2.08 (s, 3 H, OAc), 3.80 (s, 3 H, OCH₃), 5.31 (dd, 1 H, J 10.5 and 3 Hz, H-3), 5.50 (s, 1 H, PhCH), 5.80 (d, 1 H, J 3 Hz, H-1), 6.20 (d, 1 H, J 9 Hz, NH), and 6.81–7.93 (m, 4 H, aromatic).

Anal. Calc. for $C_{24}H_{26}N_2O_{10}$: C, 57.36; H, 5.22; N, 5.58. Found: C, 57.19; H, 5.24; N, 5.35.

o-Nitrophenyl 2-acetamido-3-O-acetyl-2-deoxy- α -D-galactopyranoside (3). — Compound 2 (0.8 g) in 80% aqueous acetic acid (50 mL) was stirred for 20 min at ~98°. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with water, and finally with toluene, and the residue was dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of 3 (0.55 g, 90%); $[\alpha]_D$ +271.6° (*c* 1.3, dimethyl sulfoxide); ¹H-n.m.r. data (Me₂SO-d₆): δ 1.73 (s, 3 H, NAc), 2.00 (s, 3 H, OAc), 4.95 (dd, 1 H, J 10.5 and 3 Hz, H-3), 5.18 (d, 1 H, J 6 Hz, exchangeable in D₂O, OH-4), 5.80 (d, 1 H, J 3 Hz, H-1), and 7.33–7.90 (m, 5 H, NH and aromatic).

Anal. Calc. for C₁₆H₂₀N₂O₉: C, 50.00; H, 5.25; N, 7.29. Found: C, 49.83; H, 5.42; N, 7.23.

o-Nitrophenyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)- α -D-galactopyranoside (6). — A mixture of diol 3 (0.38 g) and powdered mercuric cyanide (0.38 g) in 1:1 benzene-nitromethane (50 mL) was boiled until 20 mL of the solvent had distilled off. A solution of 2,3,4,6-tetra-Oacetyl- α -D-galactopyranosyl bromide (5; 0.62 g) in dry benzene (5 mL) was rapidly added, and the mixture was stirred for 2 days at ~60°. It was then cooled to room temperature, diluted with benzene, successively washed with 10% aqueous potassium iodide and water, dried, and evaporated to dryness, to give a solid residue which was purified in a column of silica gel with solvent A as the eluant, to afford amorphous 6 (0.45 g, 63%); $[\alpha]_D$ +1.9° (c 1.1, chloroform); ¹H-n.m.r. data (CDCl₃): δ 1.99–2.2 (cluster of singlets, 18 H, 5 OAc and NAc), 5.62 (d, 1 H, J 3 Hz, H-1), 6.42 (d, 1 H, J 9 Hz, NH), and 7.12-7.98 (4 H, aromatic).

Anal. Calc. for C₃₀H₃₈N₂O₁₈: C, 50.42; H, 5.36; N, 3.92. Found: C, 50.63; H, 5.47; N, 3.84.

o-Nitrophenyl 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-α-D-galactopyranoside (8). — Compound 6 (0.25 g) was suspended in methanol (20 mL) containing 0.5M sodium methoxide in methanol (1 mL). The solid gradually dissolved, and crystallization ensued. The crystalline material was filtered off, thoroughly washed with cold ethanol, and recrystallized from methanol, to afford disaccharide 8 (0.15 g, 85%); m.p. 253-254°, $[\alpha]_D$ +205.7° (c 0.5, dimethyl sulfoxide); t.l.c. in solvent B: R_F 0.66; ¹H-n.m.r. data (Me₂SO-d₆): δ 1.85 (s, 3 H, NAc), 5.71 (d, 1 H, J 3 Hz, H-1), and 7.12–7.97 (m, 5 H, NH and aromatic); for ¹³C-n.m.r. data, see Table I.

Anal. Calc. for C₂₀H₂₈N₂O₁₃: C, 47.62; H, 5.59; N, 5.55. Found: C, 47.80; H, 5.55; N, 5.37.

Benzyl 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-α-D-galactopyranoside (9). — Benzyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-galactopyranoside (4) was glycosylated with bromide **5** as described for **3** (to give **6**), to afford the protected disaccharide derivative (7), which was directly O-deacetylated with methanolic sodium methoxide as described for **6** (to give **8**), to furnish disaccharide **9** (0.2 g; 30% yield, based on 4); m.p. 241–242° (from methanol), $[\alpha]_D$ +129.1° (c 1, dimethyl sulfoxide); t.l.c. in solvent B; R_F 0.43; ¹H-n.m.r. data (Me₂SO-d₆): δ 1.85 (s, 3 H, NAc), 4.77 (d, 1 H, J 3 Hz, H-1), 7.39 (m, 5 H, aromatic), and 7.74 (d, 1 H, J 8 Hz, NH).

Anal. Calc. for C₂₁H₃₁NO₁₁: C, 53.27; H, 6.60; N, 2.96. Found: C, 53.08; H, 6.76; N, 3.04.

Benzyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-D-galactopyranose (10). — Compound 10 was prepared from benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside as described by Flowers and Shapiro⁸; ¹H-n.m.r. data (Me₂SO-d₆): δ 1.84 (s, 3 H, NAc), 4.82 (d, 1 H, J 4 Hz, H-1), 7.40 (m, 5 H, aromatic), and 7.68 (d, 1 H, J 8 Hz, NH); for ¹³C-n.m.r. data, see Table I.

2-Acetamido-2-deoxy-3-O- β -D-galactopyranosyl-D-galactopyranose (11). — Disaccharide 11 was obtained from 10 as described by Flowers and Shapiro⁸; ¹H-n.m.r. data (D₂O): δ 2.48 (s, 3 H, NAc), and 5.68 (d, <1 H, J 3 Hz, H-1 α); for ¹³C-n.m.r. data, see Table II.

2-Acetamido-2-deoxy-4-O- β -D-galactopyranosyl-D-galactopyranose (12). — Disaccharide 12 was prepared as described by Rana *et al.*⁹; for ¹³C-n.m.r. data, see Table II.

2-Acetamido-2-deoxy-6-O- β -D-galactopyranosyl-D-galactopyranose (13). — A solution of 9 (0.1 g) in glacial acetic acid (20 mL) was hydrogenolyzed for 2 days in the presence of 10% palladium-on-carbon (0.1 g). The crude product was purified in a column of silica gel by using 65:35:8 (v/v/v) chloroform-methanol-water as the eluant, to give amorphous 13 (75 mg, 85%); [α]_D +43.1° (c 1.1,

water). In paper chromatography in 10:4:3 (v/v/v) ethyl acetate-pyridine-water, it had R_{Gal} 0.44. For ¹³C-n.m.r. data, see Table II.

Anal. Calc. for $C_{14}H_{25}NO_{11} \cdot 2 H_2O$: C, 40.09; H, 6.97; N, 3.34. Found: C, 40.34; H, 6.90; N, 3.09.

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