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USE OF 3-*O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-2,4,6-TRI-*O*-ACETYL-α-D-GALACTOPYRANOSYL BROMIDE AS A GLYCOSYL DONOR: SYNTHESIS OF *p*-NITROPHENYL 3-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

Acetolysis of methyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranoside afforded 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**2**). Treatment of **2** in dichloromethane with hydrogen bromide in glacial acetic acid gave 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**3**). The α configuration of **3** was indicated by its high, positive, specific rotation, and supported by its ^{1}H-n.m.r. spectrum. Reaction of **3** with Amberlyst A-26–*p*-nitrophenoxide resin in 1:4 dichloromethane–2-propanol furnished *p*-nitrophenyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**7**). Compound **7** was also obtained by the condensation (catalyzed by silver trifluoromethanesulfonate–2,4,6-trimethylpyridine) of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide with *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside, followed by the usual deacylation–peracetylation procedure. *O*-Deacetylation of **7** in methanolic sodium methoxide furnished the title disaccharide (**8**). The structure of **8** was established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Our growing interest in the synthesis of oligosaccharides that occur as part of glycoconjugates stemmed mainly from the fact that they have proved to be excellent tools in specificity studies of glycosidases[2,3] and glycosyltransferases[4,5], by serving both as reference compounds and as acceptor-substrates.

In a preceding paper in this series[1], we recently described the synthesis of

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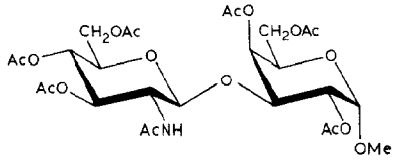
methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside; therein, we indicated that our aim was, primarily, to utilize this disaccharide (in the form of its peracetylatd glycosyl halide) as a glycosyl donor for further oligosaccharide synthesis. Thus, in furtherance of our efforts for the synthesis of such compounds, we now describe the synthesis of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**3**), and exemplify its utilization as a glycosyl donor by the synthesis of *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**8**).

RESULTS AND DISCUSSION

Acetolysis of the readily accessible[1] methyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**1**) in acetic anhydride containing 0.75% by volume of concentrated sulfuric acid afforded 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**2**). In the ^{1}H-n.m.r. spectrum of **2**, a lower-field signal at δ 6.32 (0.86 H*, *J* 4 Hz) was indicative of an anomeric mixture that was rich in the α anomer. Treatment of **2** in dichloromethane at ~0° with hydrogen bromide in glacial acetic acid gave, in fair yield, 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**3**). The high, positive, specific rotation of **3** was evidence of a predominantly α configuration of the anomeric carbon atom; this was supported by its ^{1}H-n.m.r. spectrum, which contained a doublet at δ 6.66 (0.85 H*, *J* 4 Hz). Some contamination with acetate **2** was evidenced by the presence of a low-intensity doublet (δ 6.30, *J* 4 Hz).



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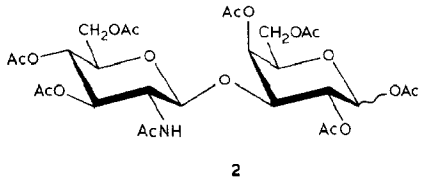

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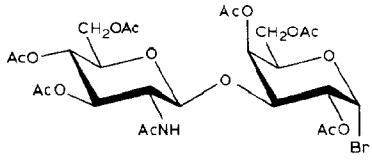

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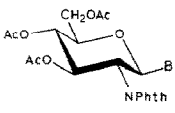
*Compared to the acetyl-group methyl protons.

As a test of its glycosylating capability, bromide **3** was allowed to react with Amberlyst A-26–*p*-nitrophenoxide resin[6] in 1:4 dichloromethane–2-propanol for 16 h at room temperature to give, after column-chromatographic purification, *p*-nitrophenyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**7**). Alternatively, compound **7** was obtained by the condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**4**) with *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**5**) in dichloromethane, in the presence of silver trifluoromethanesulfonate, 2,4,6-trimethylpyridine, and molecular sieves, followed by the customary deacylation–peracetylation sequence[1] of the resulting phthalimido peracetate (**6**). The ^{1}H-n.m.r. spectra of both **6** and **7** were in support of their overall structures assigned. In the spectrum of **6**, the aromatic-ring protons were accounted for by two doublets, at δ 8.16 and 6.97 (2 H each, *J* 10 Hz), for the *p*-nitrophenyl group, and a four-proton multiplet at δ 8.00–6.97 for the phthalimido group. The acetyl-group methyl



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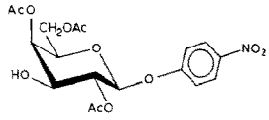

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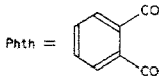

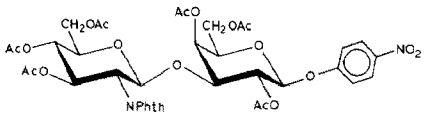

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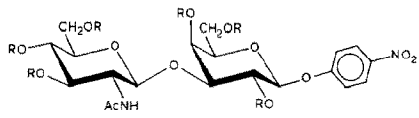

7 R = Ac
8 R = H

protons were observed as six distinct singlets at δ 2.18–1.80. The resonances for H-1, H-1′, and H-2′ were at δ 5.00 (*J* 8 Hz), 5.50 (*J* 8 Hz), and 5.80 (*J* 8 and 10 Hz), respectively. The ^{1}H-n.m.r. spectrum of **7** was, as expected, devoid of a multiplet at δ 8.00–6.97 for the phthalimido group, and the four protons of the *p*-nitrophenyl group were observed as two doublets, at δ 8.22 and 7.10, with spacings of 10 Hz each. The acetyl-group methyl protons occurred as a cluster of singlets, at δ 2.20–1.90, whereas the signals for H-1 and H-1′ were observed as doublets, at δ 5.10 and 5.52, with spacings of 8 Hz each, in accord with a β configuration at both glycosidic linkages.

O-Deacetylation of **7** with M methanolic sodium methoxide furnished the title disaccharide **8**, the ^{13}C-n.m.r. spectrum of which (see Table I) was in accord with the structure assigned. The signals for C-1 (99.90 p.p.m.) and C-1′ (101.89 p.p.m.)

TABLE I

PROPOSED ^{13}C-CHEMICAL SHIFTS[a,b]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*OCH_3*	*NAc*	*C=O*
[c]	100.73	70.25	73.30	68.20	75.90	60.43	—	—	—	—	—	—	—	—	—
[d]	101.56	55.41	74.21	70.44	76.74	60.88	—	—	—	—	—	—	55.02	22.96	168.85
8	99.90	68.80	81.55	66.88	75.25	60.03[e]	101.89	56.17	74.01	70.25	76.56	60.75[e]	—	22 96	169.70

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Aromatic carbon resonances are not shown [c]*p*-Nitrophenyl β-D-galactopyranoside[6]. [d]Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[1]. [e]Assignments having the same superscript may have to be interchanged.

were respectively in agreement with β configurations at the two glycosidic linkages. The downfield shift (8.25 p.p.m.) expected for C-3, compared to that of *p*-nitrophenyl β-D-galactopyranoside, was observed, because of substitution at O-3. The upfield shifts for C-4 and C-2 (1.32 and 1.45 p.p.m., respectively, see Table I), were also in agreement with O-3 as the site of glycosylation.

EXPERIMENTAL

General methods. — These were the same as those already described[1], except that the following solvent systems (v/v) were used for chromatography: *A*, 40:5:1 dichloromethane–ethyl acetate–acetone; *B*, 2:1 chloroform–acetone; and *C*, 4:1 chloroform–acetone.

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,2,4,6-tetra-O-acetyl-D-galactopyranose (**2**). — A solution of methyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-*O*-acetyl-α-D-galactopyranoside (**1**; 5 g) in acetic anhydride (75 mL) containing 0.75% by volume of concentrated sulfuric acid was stirred for 4-6 at room temperature. The mixture was then diluted with dichloromethane (300 mL), successively washed with water, saturated sodium hydrogencarbonate, and water, dried, and evaporated, and the residue dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of **2** (3.5 g, 67%) as a white, amorphous material; $[\alpha]_D$ +79.2° (*c* 0.4, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 6.32 (d, ~0.86 H, *J* 4 Hz, H-1) and 2.80–1.80 (cluster of singlets, 24 H, 7 OAc and 1 NAc).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.62; H, 5.81; N, 2.07. Found: C, 49.53; H, 5.96; N, 2.13.

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-acetyl-α-D-galactopyranosyl bromide (**3**). — To a cold (~0°), stirred solution of **2** (3.5 g) in dichloromethane (300 mL) was added dropwise a 32% solution of hydrogen bromide in glacial acetic acid (30 mL) during 0.5 h, and stirring was continued for a total of 3 h. The solution was then diluted with dichloromethane (200 mL), and successively washed with cold water, cold saturated sodium hydrogencarbonate, and cold water, dried, and evaporated to a syrup, which was stirred in ether–hexane, and the suspension filtered, to afford **3** (2.4 g, 66.5%), amorphous; $[\alpha]_D$ +143° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 6.66 (d, ~0.85 H, *J* 4 Hz, H-1), 6.30 (d, ~0.10 H, *J* 4 Hz, H-1 of residual **2**), and 2.30–1.90 (cluster of singlets, 21 H, 6 OAc and 1 NAc).

p-Nitrophenyl 2,4,6-tri-O-acetyl-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (**6**). — A mixture of **5** (1.29 g), silver trifluoromethanesulfonate (1.28 g), 2,4,6-trimethylpyridine (0.61 g), and molecular sieves type 4A (3 g) in dichloromethane (35 mL), protected from light and moisture, was stirred for 0.5 h at −25° (bath). A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**4**; 2.5 g) in dichloromethane (20 mL) was added dropwise, with stirring, during 0.5 h. The stirred mixture was

allowed to warm up gradually to room temperature, and stirring was continued for a total of 3.5 h. T.l.c. (solvent *A*) then revealed the presence of a major product that was faster-migrating than **5**; a trace of **5**, as well as some slower-migrating contaminants, were also revealed by t.l.c. After the usual processing, the crude product was purified in a column of silica gel, using solvent *A* as the eluant, to give **6** (1.3 g), slightly contaminated (t.l.c., solvent *A*) with a slower-migrating impurity. A portion (0.3 g) of this compound was subjected to preparative-layer chromatography using solvent *A* as the developer, to give pure **6**; amorphous, $[\alpha]_D$ +23.3° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.16 and 6.97 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 8.00–7.70 (m, 4 H, phthalimido group), and 2.18, 2.16, 2.14, 2.05, 1.88, and 1.80 (6 s, 18 H, 6 OAc).

Anal. Calc. for $C_{38}H_{40}N_2O_{20}$: C, 54.02; H, 4.78; N, 3.32. Found: C, 54.29; H, 4.88; N, 3.27.

p-Nitrophenyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**7**). — *Method (a).* A mixture of bromide **3** (0.3 g) and Amberlyst A-26–*p*-nitrophenoxide resin (1 g) in dichloromethane (2 mL) and 2-propanol (8 mL) was stirred for 16 h at room temperature. After dilution with dichloromethane (10 mL), the resin was filtered off, thoroughly washed with dichloromethane, and the filtrate and washings combined and evaporated. The crude product was purified in a column of silica gel by using solvent *C* as the eluant, to give, after recrystallization from ethyl acetate–hexane, compound **7** (0.28 g, 87%); m.p. 220–222°, $[\alpha]_D$ +13.0° (*c* 1.4, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.22 and 7.10 (d, 2 × 2 H, *J* 10 Hz, $C_6H_4NO_2$), 5.52 (d, 1 H, *J* 8 Hz, H-1′), 5.10 (d, 1 H, *J* 8 Hz, H-1), and 2.20–1.90 (cluster of singlets, 21 H, 6 OAc and 1 NAc).

Anal. Calc. for $C_{32}H_{40}N_2O_{19}$: C, 50.79; H, 5.34; N, 3.70. Found: C, 50.51; H, 5.26; N, 3.66.

Method (b). Compound **6** (slightly contaminated with a slower-migrating impurity; 1 g) was heated for 15 min at 70° in a mixture of ethanol (20 mL) and 85% hydrazine hydrate (10 mL). The mixture was evaporated, and several portions of ethanol were added to, and evaporated from, the residue, which was then mixed with 1:2 (v/v) acetic anhydride–pyridine (30 mL), and heated for 20 min at 90°. The acetic anhydride and pyridine were removed under diminished pressure, and the crude product was purified in a column of silica gel by using solvent *C* as the eluant, to afford, after recrystallization from ethyl acetate–hexane, compound **7** (0.7 g, 64.8%), having chromatographic mobility (solvent *B*) identical with that of a sample from (*a*); m.p. 220–222°, undepressed on admixture with a sample from (*a*).

p-Nitrophenyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (**8**). — A suspension of peracetate **7** (0.5 g) in 0.1M methanolic sodium methoxide (40 mL) was stirred at room temperature. The solid gradually dissolved, and within 0.5 h, crystallization ensued. The mixture was kept for 4 h at room temperature, refrigerated overnight, the base neutralized by the addition of a few drops of glacial acetic acid, and the solid material filtered off, and thoroughly

washed with cold methanol, to afford **8** (0.28 g, 93.6%); m.p. 272° (dec.), $[\alpha]_D$ −36.6° (*c* 0.4, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{20}H_{28}N_2O_{13}$: C, 47.61; H, 5.61; N, 5.55. Found: C, 47.54; H, 5.70; N, 5.47.

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