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Note

Synthetic mucin fragments: 3-*O*-[2-Acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide and *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside*

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Simultaneous with the synthesis of some sugar halides that are primarily intended for use as glycosyl donors in higher oligosaccharide syntheses, we adopted a trend of coupling the preliminary test of their glycosylating capability with a synthetically useful goal, namely, the synthesis of the *p*-nitrophenyl glycosides derived therefrom[2]. Thus, in congruence with this trend, we now describe the synthesis of a trisaccharide bromide, 3-*O*-[2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**5**), and illustrate its utility as a glycosylating agent by the synthesis of *p*-nitrophenyl 3-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**7**).

Phenyl and nitrophenyl glycosides have proved to be excellent tools in specificity studies of a number of enzymes. For example, the use of α-L-Fuc-(1→2)-β-Gal-1→$OC_6H_4NO_2$-*p* as a novel chromogenic substrate was instrumental in developing a rapid assay-procedure[3] for the α-L-fucosidases that hydrolyze the glycosidic linkage α-Fuc-(1→2)-Gal.

By using other, appropriate phenyl or nitrophenyl glycosides, or both, modified assay-procedures have been developed for the detection and determination of GDP-L-fucose: galactoside 2′-fucosyltransferase[4], and GDP-L-fucose: 2-acetamido-2-deoxy-β-D-glucopyranoside-(1→4)-α-L-fucosyltransferase[5]. The utility of similar glycosides in studies related to some *endo*- and *exo*-glycosidases has also been demonstrated [6].

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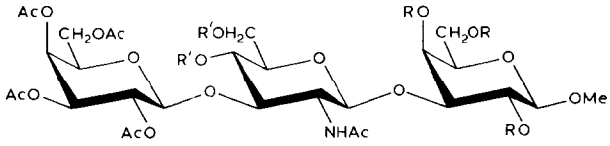
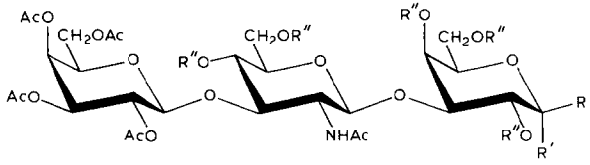
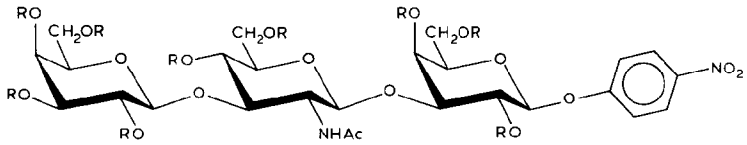
Moreover, on reduction of their nitro groups, and subsequent attachment of the resulting amino groups to suitable solid supports, *p*-nitrophenyl glycosides have proved to be useful in affinity chromatography[7], and as synthetic antigens[8].



1 R = Bn, R′, R′ = C_6H_5CH



2 R = OMe, R′ = R″ = H
3 R = OMe, R′ = H, R″ = Ac
4 R, R′ = OAc(H), R″ = Ac (α,β)
5 R = H, R′ = Br, R″ = Ac



6 R = Ac
7 R = H

For these reasons, it seemed advantageous to adhere to our adopted trend, and thus serve the dual purpose of ascertaining the ability of our synthetic halides to act as glycosyl donors, and in the meantime ensure the availability of a variety of compounds that are increasingly in demand. Additionally, the ease with which *p*-nitrophenyl glycosides can be obtained by reaction of glycosyl halides with resin-bound *p*-nitrophenoxide[9] renders this approach particularly attractive.

Hydrogenolytic cleavage of the acetal and benzyl groups of methyl 3-*O*-[2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[1] (**1**) in glacial acetic acid, in the presence of 10% palladium-on-carbon, afforded crystalline **2**, which, on acetylation with 1:2 acetic anhydride–pyridine furnished perace-

TABLE I

PROPOSED ^{13}C-N M R CHEMICAL SHIFTS FOR TRISACCHARIDE (7)[a,b]

Residues	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NAc or OCH$_3$*
p-Nitrophenyl β-Gal	[c]	99.90	68.80	81.55	66.88	75.25	60.03	—
β-GlcNAc		101.89	56.17	74.01	70.25	76.56	60.57	22.96, 169.70
Methyl β-Gal	[d]	103.87	69.28	82.19	67.12	74.73	60.28	55.70
β-GlcNAc		101.50	54.81	84.53	68.43	76.09	60.46	23.07, 170.37
β-Gal		103.64	70.47	72.64	68.10	75.60	60.46	—
p-Nitrophenyl β-Gal	**7**	99.94	68.81	81.63	67.00	75.30	60.08	—
β-GlcNAc		101.52	54.79	84.42	68.41	76.08	60.43	23.07, 170.28
β-Gal		103.65	70.42	72.61	68.05	75.56	60.43	—

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Aromatic carbon resonances are not shown. [c]*p*-Nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[2]. [d]Methyl 3-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside[1].

tate **3**. Acetolysis of compound **3** gave 3-*O*-[2-acetamido-4,6-di-*O*-acetyl-2-deoxy-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**4**), the ^{1}H-n.m.r. spectrum of which (see Experimental section) contained a lower-field doublet at δ 6.30 (~1 H, *J* 4 Hz), strongly suggesting that it existed almost exclusively as the α anomer. Treatment of **4** in dichloromethane with hydrogen bromide in dichloromethane afforded, in good yield, 3-*O*-[2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**5**). In the ^{1}H-n.m.r. spectrum of **5**, a doublet at δ 6.66 (0.95* H, *J* 4 Hz) was indicative of a predominantly α configuration of the anomeric carbon atom; this was also evidenced by a relatively high, positive, specific rotation for **5**.

Bromide **5** was allowed to react with Amberlyst A-26-*p*-nitrophenoxide[9] in 1:4 dichloromethane–2-propanol for 20 h at room temperature, to give, after column-chromatographic purification, *p*-nitrophenyl 3-*O*-[2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**6**), which was sufficiently pure to be utilized in the next step, regardless of the presence of a marginally faster-migrating contaminant (presumably due to decomposition of bromide **5**).

O-Deacetylation of **6** in 0.5M methanolic sodium methoxide furnished the title trisaccharide (**7**), the ^{13}C-n.m.r. spectrum of which (see Table I) was in accord with the structure assigned. In the ^{13}C-n.m.r. spectrum of **7**, the signals for C-1 (99.94 p.p.m.), C-1′ (101.52 p.p.m.), and C-1″ (103.65 p.p.m.) were all in agreement with β configurations at the glycosidic linkages. The signals for C-3 and C-3′ were observed at low field (δ 81.63 and 84.42, respectively), which was a clear indication that both O-3 and O-3′ were the sites of glycosylation.

*Compared to the acetyl-group methyl protons. An equivalent doublet at δ 6.66 (0.85 H, *J* 4 Hz) was also observed in the ^{1}H-n.m.r. spectrum of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranosyl bromide[2].

EXPERIMENTAL

General methods. — These were the same as those already described[1], except that solvent *A* used for chromatography was 2:1 (v/v) chloroform–acetone.

Methyl 3-O-[2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (**2**). — A mixture of the 4′,6′-*O*-benzylidene derivative (**1**; 3.26 g) and 10% palladium-on-carbon (3.26 g) in glacial acetic acid (90 mL) was shaken under hydrogen at 344.5 kPa for 6 days. The suspension was then filtered (Celite bed), the solid thoroughly washed with 1:1 (v/v) methanol–water, and the filtrate and washings combined and evaporated under diminished pressure to give a solid residue, which was recrystallized from absolute alcohol to afford compound **2** (1.6 g, 73.3%); m.p. 222–225°, $[\alpha]_D$ −8.4° (*c* 0.5, methanol).

Anal. Calc. for $C_{29}H_{45}NO_{26} \cdot 0.5\,H_2O$: C, 47.28; H, 6.29; N, 1.90. Found: C, 46.95; H, 6.52; N, 1.74.

Methyl 3-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,3,4-tri-O-acetyl-β-D-galactopyranoside (**3**). — Compound **2** (1.52 g) was acetylated overnight in 1:2 acetic anhydride–pyridine (24 mL), to give, after the usual processing, a solid residue which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **3** (1.86 g, 94.9%), amorphous; $[\alpha]_D$ +17.2° (*c* 0.6, chloroform): ^{1}H-n.m.r. data ($CDCl_3$): δ 5.97 (d, 1 H, *J* ~7 Hz, exchangeable in D_2O, NH), 3.52 (s, 3 H, OMe), 2.20–1.90 (cluster of singlets, 30 H, 9 AcO, and NAc), and 5.60–3.60 (unresolved signals, 21 H).

Anal. Calc. for $C_{39}H_{55}NO_{25}$: C, 49.94; H, 5.91; N, 1.49. Found: C, 49.68; H, 5.93; N, 1.32.

3-O-[2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-1,2,4,6-tetra-O-acetyl-D-galactopyranose (**4**). — A solution of compound **3** (1.76 g) in acetic anhydride (30 mL) containing ~0.8% by volume of concentrated sulfuric acid was stirred for 6 h at room temperature. The mixture was then diluted with dichloromethane (200 mL), successively washed with water, saturated sodium hydrogencarbonate, and water, dried, and evaporated to a syrup which was dissolved in a little dichloromethane. Addition of ether–hexane caused the precipitation of **4** (1.62 g, 89.4%) as a white, amorphous material; $[\alpha]_D$ +51.7° (*c* 0.6, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 6.30 (d, ~1 H*, *J* 5 Hz, H-1), 5.70 (d, 1 H, *J* ~7 Hz, exchangeable in D_2O, NH), 2.20–1.90 (cluster of singlets, 33 H, 10 AcO and NAc), and 5.65–3.30 (unresolved signals, 21 H).

Anal. Calc. for $C_{40}H_{55}NO_{26}$: C, 49.74; H, 5.74; N, 1.45. Found: C, 49.66; H, 6.00; N, 1.30.

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*Compared to the acetyl-group methyl protons.

3-O-[2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-O-acetyl-α-D-galactopyranosyl bromide (**5**). — To a cold (~0°, bath), stirred solution of the peracetate **4** (1.22 g) in dry dichloromethane (50 mL) was added dropwise, during 0.5 h, a saturated solution of hydrogen bromide in dry dichloromethane (50 mL), and stirring was continued for a total of 1.5 h at ~0°. The solution was then diluted with an equal volume of dichloromethane, and successively washed with cold water, cold saturated sodium hydrogencarbonate, and cold water, dried, and evaporated to a syrup, which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **5** (1.06 g, 85%), a white powder; m.p. 115–118°, $[\alpha]_D$ +99.5° (*c* 0.95, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 6.66 (d, ~0.95 H, *J* 4 Hz, H-1), 2.30–1.90 (cluster of singlets, 30 H, 27 AcO and 1 NAc).

p-Nitrophenyl 3-O-[2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**6**). — A mixture of bromide **5** (0.3 g) and Amberlyst A-26-*p*-nitrophenoxide resin (1.5 g) in 1:4 dichloromethane–2-propanol (10 mL) was stirred for 20 h at room temperature. After dilution with dichloromethane (10 mL), the resin was filtered off, and thoroughly washed with dichloromethane, and the filtrate and washings were combined and evaporated. T.l.c. (solvent *A*) then revealed the disappearance of **5** and the presence of a major product, slightly less mobile than **5**; some slower- and some faster-migrating contaminants that were not detectable under u.v. light (presumably resulting from decomposition of **5**) were also revealed by t.l.c. The crude product was subjected to column chromatography on silica gel by using 4:1 (v/v) chloroform–acetone as the eluant, to afford compound **6** (0.17 g) as an amorphous material that was slightly contaminated (t.l.c. solvent *A*) with a marginally faster-migrating impurity (undetectable under u.v. light). This material was utilized, without purification, in the next step.

p-Nitrophenyl 3-O-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**7**). — A suspension of peracetate **6** (0.14 g) in 0.5M methanolic sodium methoxide (10 mL) was stirred at room temperature. The suspended **6** gradually dissolved, and, in ~0.5 h, crystallization ensued. The mixture was then stirred overnight at room temperature, the base neutralized with a few drops of glacial acetic acid, and an equal volume of ethanol added to the mixture, which was then refrigerated for 5 h. The crystalline material was filtered off, and thoroughly washed with cold ethanol, to give a crystalline solid which was recrystallized from aqueous ethanol to furnish trisaccharide **7** (83 mg, 93%); m.p. 273–274°, $[\alpha]_D$ −6.5° (*c* 0.3, dimethyl sulfoxide); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{26}H_{38}N_2O_{18} \cdot 2\,H_2O$: C, 44.44; H, 6.03; N, 3.99. Found: C, 44.17; H, 6.01; N, 3.61.

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