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Note

Synthetic mucin fragments: benzyl 2-acetamido-3-*O*-[3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-α-D-galactopyranoside*

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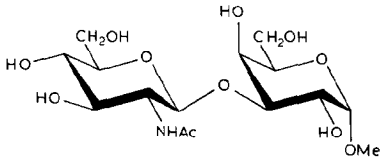
In a continuing program for the synthesis of oligosaccharides that occur as part of mucinous glycoconjugates, we previously described[2] the synthesis of the disaccharide methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-galactopyranoside (**1**). We have also demonstrated that **1** could be readily converted into its per-*O*-acetylated glycosyl bromide (**2**), the glycosylating capability of which was exemplified by the synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-glucopyranosyl-β-D-galactopyranoside[3].



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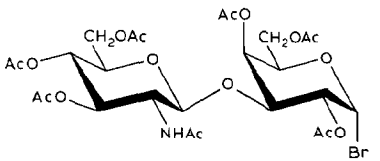

2

Since then, bromide **2** has proved to be a useful and versatile glycosyl donor for the synthesis of a number of mucin-type fragments, and, as an illustration of this utility, we now describe the synthesis of the title trisaccharide benzyl 2-acetamido-3-*O*-[3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranosyl]-2-deoxy-α-D-galactopyranoside (**6**). The parent trisaccharide of **6** represents the terminal, three-sugar unit that is further *O*-glycosylically linked to serine or threonine in mucinous-type glycoproteins[4] (see Fig. 1).

Condensation of bromide **2** with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**3**) in 1:1 benzene–nitromethane, in the presence of powdered mercuric cyanide, and purification of the crude product by column chromatography on silica gel, afforded the fully protected trisaccharide derivative (**4**),

*Synthetic Studies in Carbohydrates, Part XLI, for Part XL, see ref. 1.
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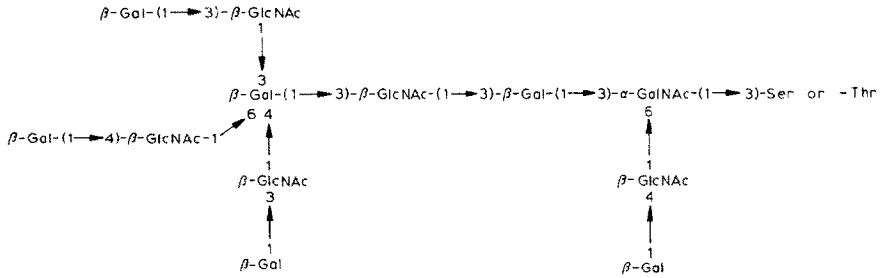
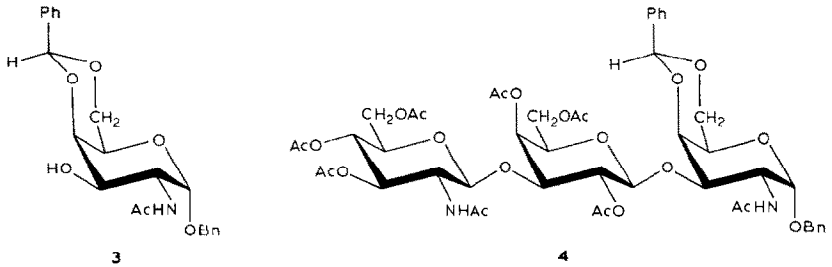
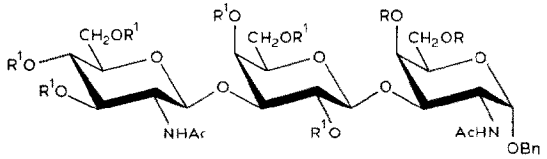


Fig. 1. Overall, composite structure proposed for ovarian-cyst glycoprotein, the I, i precursor, oligosaccharide moiety[4].





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

the ^{1}H-n.m.r. spectrum of which was in agreement with its overall structure (see the Experimental section).

Cleavage of the acetal group of **4** in hot, 80% aqueous acetic acid furnished diol (**5**). It might be pertinent to note that diol **5**, having an exposed primary hydroxyl group, is suitably protected for further attachment of a 2-acetamido-2-deoxy-β-D-glucopyranosyl unit, to give a tetrasaccharide that mimics the branched-chain at the terminal 2-acetamido-2-deoxy-D-galactopyranose moiety, see Fig. 1; the synthesis of this tetrasaccharide has also been accomplished[5].

O-Deacetylation of compound **5** in methanolic sodium methoxide afforded the title trisaccharide (**6**). Interestingly, in contrast to that[2] of **1**, Zemplén transesterification of **5** was complete only after prolonged treatment with M methanolic sodium methoxide*. A similar reluctance to undergo complete transesterification

*When the deacetylation was terminated after 6 h, the ^{13}C-n.m.r. spectrum of the product isolated contained signals that were indicative of a partially acetylated compound. On the other hand, *O*-deacetylation of **1** was virtually complete within 1 h.

TABLE I

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

Residue or group	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH_3CO*	*OCH_3 or $CH_2C_6H_5$*
	[c]	96.08	49.59	67.19	67.55	71.34	60.53	22.49	67.99
	[d]	104.22	70.29	73.17	67.96	74.93	60.28	—	55.61
	[e]	101.56	55.41	74.21	70.44	76.74	60.88	22.96	50.02
Benzyl α-GalNAc	**6**	96.25	48.31	75.51	67.26	71.33	60.54	22.55	67.86
β-Gal-(1→3)		103.12	69.66	81.90	67.01	74.03	60.80	—	—
β-GlcNAc-(1→3)		101.66	56.39	74.82	70.36	76.60	60.33	22.98	—

[a]In Me_2SO_4-d_6, with Me_4Si as the internal standard. [b]Carbonyl and aromatic carbon resonances are not shown. [c]Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside[7]. [d]Methyl β-D-galactopyranoside[8]. [e]Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[7].

was also encountered in the case of other, related, higher oligosaccharides, and it was often necessary to resort to more-drastic conditions to ensure complete de-esterification[5,6].

The ^{13}C-n.m.r. spectrum of compound **6** was in accord with the structure assigned (see Table I). Thus, C-1, carrying the α-benzyloxy group, was accounted for by the signal at δ 96.25, whereas the signals at δ 103.12 and 101.66 accounted for the anomeric carbon atoms of the β-Gal residue and the β-GlcNAc group, respectively. The signal at δ 81.90 could reasonably be assigned to C-3 of the β-Gal residue; the resonance for C-3 of compound **1** was observed[2] at δ 78.97, and that of methyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[6] occurred at δ 82.22. The signals at δ 75.51 and 74.82 for **6** were assigned to C-3 of the benzyl α-GalNAc residue and β-GlcNAc group, respectively.

EXPERIMENTAL

General methods. — These have already been described[1].

Benzyl 2-acetamido-3-O-[3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,4,6-tri-O-acetyl-β-D-galactopyranosyl]-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**4**). — A stirred solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (**3**; 0.4 g, 1 mmol) in 1:1 benzene–nitromethane (40 mL) was boiled until ~20 mL of the solvent mixture had distilled off. After cooling to room temperature, a solution of the disaccharide bromide (**2**; 0.9 g, 1.2 mmol) in 1:1 benzene–nitromethane (5 mL) and powdered mercuric cyanide were added, and stirring was continued for 3 days at room temperature. T.l.c. with 1:1 (v/v) chloroform–acetone then revealed the disappearance of **2**, and the presence of a major product, slower-migrating than both **2** and **3**; some unchanged **3**, as well as some slower-migrating contaminants, presumably resulting from the decomposition of **2**, were also revealed. After processing in the usual

manner, the crude product mixture was applied to a column of silica gel. Elution with 4:1 (v/v) chloroform–acetone, and evaporation of the fractions corresponding to the starting material, afforded **3** (0.1 g). On elution with 2:1 (v/v) chloroform–acetone, evaporation of the fraction corresponding to the major product gave a solid residue which was dissolved in ethyl acetate. Addition of ether–hexane caused the precipitation of amorphous **4** (0.42 g, 41.2%); $[\alpha]_D$ +86.2° (*c* 1.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.70–7.20 (m, 10 H, aromatic), 5.54 (s, 1 H, PhCH), and 2.20–1.80 (cluster of singlets, 24 H, 6 AcO and 2 NAc).

Anal. Calc. for $C_{48}H_{60}N_2O_{22}$: C, 56.68; H, 5.96; N, 2.75. Found: C, 56.47; H, 5.84; N, 2.69.

*Benzyl 2-acetamido-3-*O*-[3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl]-2-deoxy-α-*D*-galactopyranoside* (**5**). — Compound **4** (0.38 g) in 80% aqueous acetic acid (25 mL) was stirred for 40 min at ~98°. The acetic acid was evaporated under diminished pressure, and then several portions of water and toluene were added to, and evaporated from, the residue, which was now dissolved in chloroform. Addition of hexane caused the precipitation of amorphous **5** (0.28 g, 80.9%); $[\alpha]_D$ +69.9° (*c* 0.7, chloroform): ^{1}H-n.m.r. data ($CDCl_3$): δ 7.38 (s, 5 H, aromatic), and 2.20–1.80 (cluster of singlets, 24 H, 6 AcO and 2 NAc).

Anal. Calc. for $C_{41}H_{56}N_2O_{22}$: C, 53.00; H, 6.09; N, 3.02. Found: C, 52.78; H, 6.07; N, 2.89.

*Benzyl 2-acetamido-3-*O*-[3-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-β-*D*-galactopyranosyl]-2-deoxy-α-*D*-galactopyranoside* (**6**). — A suspension of compound **5** (0.2 g) in M sodium methoxide in methanol (6 mL) was stirred at room temperature, whereupon partial dissolution of the compound occurred, followed by concomitant crystallization of the *O*-deacetylation product. Stirring was continued* for 24 h at room temperature, the base was neutralized with a few drops of glacial acetic acid, and the crystalline material was filtered off, and thoroughly washed with cold ethanol, to furnish **6** (0.12 g, 82.8%); decomposes, without melting, at ~300°; $[\alpha]_D$ +68.6° (*c* 0.6, *N,N*-dimethylformamide); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{29}H_{41}N_2O_{16} \cdot H_2O$: C, 50.35; H, 6.28; N, 4.05. Found: C, 50.09; H, 6.35; N, 3.78.

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