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SYNTHESIS OF BENZYL 2-ACETAMIDO-3,6-DI-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-2-DEOXY-α-D-GALACTOPYRANOSIDE*

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ABSTRACT

Glycosylation of benzyl 2-acetamido-2-deoxy-3-*O*-(3,4-6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranoside with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide in dichloromethane, in the presence of silver trifluoromethanesulfonate and 2,4,6-trimethylpyridine, afforded benzyl 2-acetamido-2-deoxy-3,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranoside (**5**). Deacylation of **5** with hot hydrazine hydrate in ethanol, followed by peracetylation, furnished benzyl 2-acetamido-3,6-di-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-acetyl-2-deoxy-α-D-galactopyranoside (**7**). The ^{1}H-n.m.r. spectra of both **5** and **7** were in accord with the structures assigned. Condensation of benzyl 2-acetamido-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside (**4**) with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline, and acetylation of the resulting intermediate, without isolation, also afforded, after column-chromatographic purification, the trisaccharide peracetate **7**. Compound **4** was obtained by deacetylation of its 4,6-*O*-benzylidene derivative in hot, aqueous acetic acid. *O*-Deacetylation of **7** in methanolic sodium methoxide furnished the title trisaccharide (**8**). The structure of **8** was confirmed by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

During studies on oligosaccharides released by reductive, alkaline degradation of blood-group Ii-active, sheep gastric-mucins, a novel type of core structure, namely, β-GlcNAc-(1→3)-[β-GlcNAc-(1→6)]-GalNAc, was identified by Hakomori and co-workers[2,3]. This core structure was designated "Group 4", in order to distinguish it from the "Group 3" core structure, {β-Gal-(1→3)-[β-GlcNAc-(1→6)]-GalNAc}, common to most human and other animal tissues[2].

For some time, we have engaged in a program for the synthesis of oligo-

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saccharides that occur as part of mucinous glycoproteins. Our interest in such compounds was outlined in a preceding paper in this series[4], wherein we also described the synthesis of two related disaccharides.

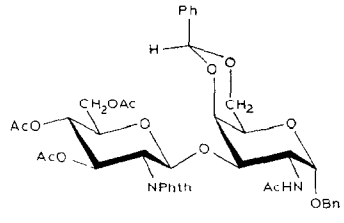
During these investigations, it was observed[5] that a β-N-acetylglucosaminyltransferase, from rat colon, catalyzed the incorporation of GlcNAc into benzyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside[4]. A similar enzyme preparation also catalyzed the incorporation of one, or two, GlcNAc residue(s) into benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside, to give either a disaccharide or a trisaccharide, respectively. The trisaccharide obtained in both cases was, possibly, benzyl 2-acetamido-3,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside. On the other hand, the disaccharide was, presumably, either the β-(1→3)- or the isomeric β-(1→6)-linked compound, already described by us[4]. It is, therefore, needless to stress that the availability of such synthetic oligosaccharides, having well defined structures, will undoubtedly facilitate those endeavors directed towards the elucidation of the biosynthetic pathways of other, more-complex oligosaccharides, by serving both as reference compounds and as acceptor-substrates. We now describe the synthesis of the title trisaccharide (**8**).

RESULTS AND DISCUSSIONS

Condensation of benzyl 2-acetamido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-galactopyranoside[4] (**3**; obtained from its 4,6-*O*-benzylidene derivative **1**) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide in dichloromethane for 3 h at room temperature, in the presence of silver trifluoromethanesulfonate, 2,4,6-trimethylpyridine, and molecular sieves, and purification of the crude product by column chromatography, afforded, in 78.6% yield, the trisaccharide derivative **5**, the ^{1}H-n.m.r. spectrum of which supported the structure proposed: the presence of two interglycosidic β-linkages was evidenced by the occurrence of two doublets, at δ 5.46 and 5.38, with spacings of 8 Hz each, assigned to the two H-1 atoms of the two 2-acylamido-2-deoxy-D-glucosyl groups. Also, two doublets of doublets, at δ 5.85 and 5.68 (*J* 10 and 8 Hz, each), could reasonably be assigned to the two H-2 resonances of the same groups. The signals expected for the aromatic protons and the acetyl methyl protons were also accounted for.

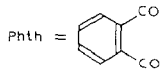
Deacylation of **5** at 70° in 85% hydrazine hydrate in ethanol, as previously described[4,6], and peracetylation of the resulting intermediate, gave the peracetylated derivative **7**, the ^{1}H-n.m.r. spectrum of which contained signals in support of its overall structure.

Condensation of the disaccharide diol **4** (obtained by cleavage of the acetal group of its 4,6-*O*-benzylidene derivative[4] **2**) with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline in the usual way[4], followed by acetylation of the resulting intermediate **6**, gave also, after column-chromatographic purifi-

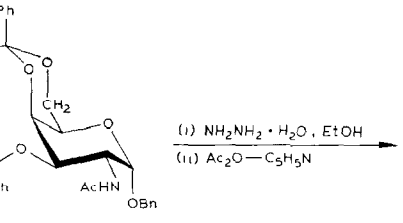


1

Bn = $PhCH_2$

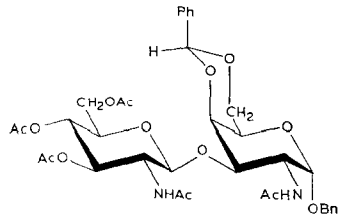


Phth =



(i) $NH_2NH_2 \cdot H_2O$, EtOH

(ii) $Ac_2O-C_5H_5N$



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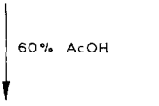
60% AcOH



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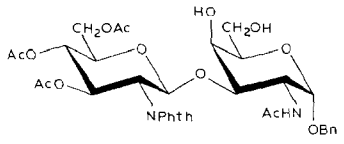

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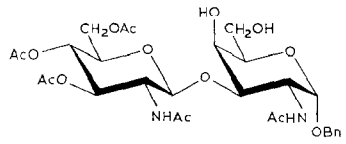

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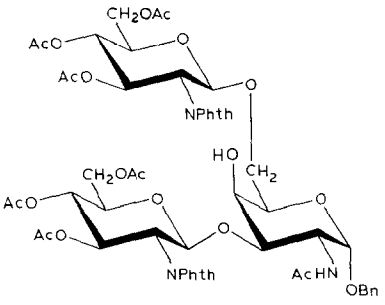

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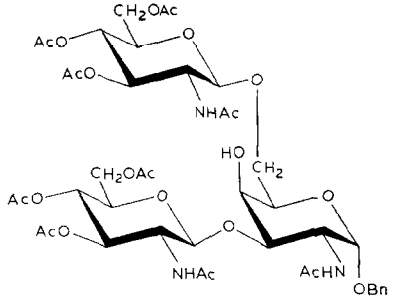

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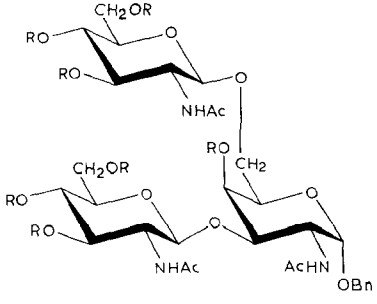

7 R = Ac

8 R = H

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL-SHIFTS FOR TRISACCHARIDE **8**[a,b]

Residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH$_3$CO*	*CH$_2$C$_6$H$_5$*
Benzyl α-GalNAc	95.79	48.01	76.28	67.65	69.45[c]	67.65[c]	22.47	67.65
β-GlcNAc-(1→6)	101.50[d]	55.85	74.75	70.30	76.48[e]	60.86	22.90	-
β-GlcNAc-(1→3)	101.69[d]	55.14	74.06	70.53	76.73[e]	60.86	22.90	-

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Additional assignments: 169.35, 168.90, and 168.71 (C=O); 137.60, 127.87, 127.66, and 127.19 (aromatic carbons). [c,d,e]Assignments having the same superscript may have to be interchanged

cation, the peracetylated derivative **7**. *O*-Deacetylation of **7** in methanolic sodium methoxide furnished the trisaccharide **8**. The ^{13}C-n.m.r. spectrum of **8** (see Table I) was in accord with the structure assigned; the presence of two β-glycosidic linkages was clearly demonstrated by the occurrence of two carbon-atom resonances, at 101.5 and 101.69 p.p.m., and the presence of an α-linked aglycon was evidenced by the higher-field, carbon resonance at 95.79 p.p.m.

Comments on the ^{13}C-n.m.r. assignments. – The availability of the ^{13}C-n.m.r.-spectral data for the β-(1→3)- and the β-(1→6)-linked disaccharides[4] (see Table II) that form parts of the trisaccharide **8** greatly simplified the assignments of the ^{13}C-n.m.r. resonances of this trisaccharide; and, although some of the carbon resonances in the ^{13}C-n.m.r. spectrum of **8** could not be unequivocally assigned (see Table I), this was not detrimental to the conclusions drawn from those assignments. For example, it was not possible to distinguish between the C-1 resonances of the β-(1→3)- and the β-(1→6)-linked GlcNAc groups. However, both resonances were in the range expected for the β configuration of the interglycosidic linkages, whereas the benzyl aglycon, as already mentioned, adopted the α configuration, as evidenced by the presence of a carbon resonance at 95.79 p.p.m. Also, in the absence of additional information, it was not possible to distinguish between the signals for C-5 and C-6 of the GalNAc residue, a similar situation was, likewise, encountered in the case of the C-5 resonances of the two GlcNAc groups (see Table I).

The resonance (76.28 p.p.m.) for C-3 of the GalNAc residue, was close to that (76.41 p.p.m.) of the same carbon atom in the ^{13}C-n.m.r. spectrum of the β-(1→3)-linked disaccharide, and showed a downfield shift of 9.09 p.p.m. in comparison to that of C-3 of the parent benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside. The resonance for C-6 of the GalNAc residue was, also, close to that (68.56 p.p.m.) of the same carbon atom in the spectrum of the β-(1→6)-linked disaccharide, regardless of whether the entry considered in Table I was 69.45 or 67.65 p.p.m., as the difference is +0.89 or −0.91 p.p.m., respectively.

TABLE II

PARTIAL, ^{13}C-N.M.R. CHEMICAL-SHIFTS[a,b] FOR THREE COMPOUNDS

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′*	*CH_3CO*
c	96.08	49.59	67.19	67.55	71.34	60.53	—	—	—	—	—	—	22.49
d	95.94	48.11	76.41	67.12	71.23	60.73	101.50	55.85	74.85	70.18	76.59	60.73	22.49 22.87
e	96.04	49.55	67.00	67.47	69.34	68.56	101.49	55.08	74.06	70.53	76.77	60.92	22.51 22.93

[a]Reproduced from ref. 4. [b]In Me_2SO-d_6, with Me_4Si as the internal standard. [c]Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside. [d]Benzyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside. [e]Benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside.

EXPERIMENTAL

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

*Benzyl 2-acetamido-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-α-*D*-galactopyranoside* (**4**). — The 4,6-benzylidene derivative[4] **2** (0.4 g) in 60% aqueous acetic acid (8 mL) was stirred for 1.5 h at 85°. T.l.c. (solvent *A*) then showed the disappearance of **2**, and the presence of a slower-migrating major product. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene, to afford **4** (0.31 g, 88.6%); amorphous; $[\alpha]_D$ +39° (*c* 0.3, 1:1 v/v, chloroform–methanol).

Anal. Calc. for $C_{29}H_{40}N_2O_{14} \cdot H_2O$: C, 52.87; H, 6.44; N, 4.25. Found: C, 52.72; H, 6.20; N, 4.14.

*Benzyl 2-acetamido-2-deoxy-3,6-di-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-galactopyranoside* (**5**). — A mixture of compound[4] **3** (1.46 g), silver trifluoromethanesulfonate (0.64 g), 2,4,6-trimethylpyridine (0.3 g), and molecular sieves, type 4A (3 g), in dichloromethane (50 mL) was protected from light and moisture, and stirred for 0.5 h at room temperature in an atmosphere of nitrogen. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (1.5 g) in dichloromethane (20 mL) was added dropwise, with stirring, during 0.5 h, and stirring was continued for a further 2.5 h. T.l.c. (solvent *B*) then revealed the presence of a major product, slightly faster-migrating than **3**. A trace of **3**, as well as some faster-migrating contaminants (presumably due to decomposition of the phthalimido bromide), were also revealed by t.l.c. After the customary processing, the mixture was purified in a column of silica gel by using 5:1 (v/v) chloroform–acetone as the eluant. On evaporation, the fractions corresponding to the major product yielded a foam, which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **5** (1.8 g, 78.6%); a fine white powder; $[\alpha]_D$ +61.6° (*c* 0.73, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 8.00–7.00 (m, 13 H, aromatic), 5.85 and 5.68 (2 dd, 1 H, each *J* 10 and 8 Hz, H-2′* and H-2″*), 5.46 and 5.38 (2 d, 1 H each, each *J* 8 Hz, H-1′,1″), 5.25 and 5.05 (AB quartet, 2 H, *J* 9 Hz, OCH_2Ph), 4.43 (d, 1 H, *J* 4 Hz, H-1), 2.52 (broad s, 1 H, exchangeable in D_2O, OH), and 2.14, 2.09, 2.05, 2.03, 1.86, 1.83, and 1.26 (7 s, 21 H, 6 AcO and NAc).

Anal. Calc. for $C_{55}H_{59}N_3O_{24}$: C, 57.63; H, 5.20; N, 3.67. Found: C, 57.41; H, 5.27; N, 3.64.

*Benzyl 2-acetamido-3,6-di-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-4-*O*-acetyl-2-deoxy-α-*D*-galactopyranoside* (**7**). — *Method* (*a*). Compound **5** (0.3 g) was heated for 15 min at 70° in a mixture of ethanol (8 mL) and 85% hydrazine hydrate (4 mL). The mixture was evaporated, and several portions of ethanol were added to, and evaporated from the residue, which was then mixed with

*The single and double primes refer to the GlcNAc groups, and can be interchanged.

1:2 (v/v) acetic anhydride–pyridine (15 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 *B* as the eluant. On evaporation of the solvent, the residue was dissolved in a small volume of dichloromethane. Addition of ether caused the precipitation of **7** (0.21 g, 80.8%); a white powder, homogeneous in t.l.c. (solvent *C*): $[\alpha]_D$ +55.6° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.40 (s, 5 H, aromatic) and 2.10–1.86 (s, 30 H, 10 Ac).

Anal. Calc. for $C_{45}H_{61}N_3O_{23}$: C, 53.40; H, 6.09; N, 4.15. Found: C, 53.16; H, 6.13; N, 4.33.

Method (*b*). A mixture of compound **4** (0.28 g), 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (0.3 g), and *p*-toluenesulfonic acid (5 mg) in dichloroethane (7 mL) was heated for 36 h at ~70°, in an atmosphere of nitrogen, additional amounts of the oxazoline (0.15 g, in dichloroethane 1.5 mL) and *p*-toluenesulfonic acid (2.5 mg, in 1.5 mL of dichloroethane) being added after 16 h. The mixture was cooled, the acid neutralized by the addition of a few drops of pyridine, and the solution evaporated to dryness. The dried residue was mixed with pyridine (8 mL) and acetic anhydride (4 mL), and kept overnight at room temperature. The pyridine and acetic anhydride were then evaporated under diminished pressure, the last traces being removed by co-evaporation with toluene. T.l.c. (solvent *C*) now showed the presence of a compound having a mobility identical to that of **7**; some faster-migrating contaminants were also revealed by t.l.c. The mixture was applied to a column of silica gel, and eluted first with solvent *B*, to remove the faster-migrating contaminants. Elution with solvent *A*, and evaporation of the fractions containing the product, gave a foam which was dissolved in dichloromethane. Addition of ether caused the precipitation of **7** (0.25 g, 62.5%); a white powder, homogeneous in t.l.c. (solvent *C*) and having identical mobility to that of a sample from (*a*); $[\alpha]_D$ +51.7° (*c* 0.3, chloroform).

Benzyl 2-acetamido-3,6-di-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside (**8**). — Compound **7** (0.3 g) was suspended in 0.1M methanolic sodium methoxide, and stirred at room temperature. The suspension gradually dissolved, and, in ~0.5 h, crystallization ensued. The mixture was kept for 2 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** (0.18 g, 85.7%); amorphous; $[\alpha]_D$ +66.3° (*c* 0.3, water); for its ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{31}H_{47}N_3O_{16} \cdot 2\ H_2O$: C, 49.39; H, 6.83; N, 5.58. Found: C, 49.79; H, 6.65; N, 5.20.

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