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

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ABSTRACT

Condensation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside with 2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl bromide in 1:1 nitromethane–benzene, in the presence of powdered mercuric cyanide, afforded benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4-tri-*O*-acetyl-β-D-fucopyranosyl)-α-D-galactopyranoside (**3**). Cleavage of the benzylidene group of **3** with hot, 60% aqueous acetic acid afforded diol **4**, which, on deacetylation, furnished the disaccharide **5**. Condensation of diol **4** with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline in 1,2-dichloroethane afforded the trisaccharide derivative (**7**). Deacetylation of **7** with Amberlyst A-26 (OH^-) anion-exchange resin in methanol gave the title trisaccharide (**8**). The structures of **5** and **8** were confirmed by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

The use of low-molecular-weight oligosaccharides, having well defined structures, for the study of various glycosyltransferases has been well documented[2]. For example, the availability of such acceptors as β-Gal-(1→3)-GalNAc derivatives has led to the discovery of new glycosyltransferases involved in the biosynthesis of the carbohydrate moiety of mucin[3]. It has also become apparent that studies related to acceptor–substrate specificity that are conducted with the aid of a variety of compounds can elucidate the biosynthetic pathways of such glycoconjugates[4]; and, although it is generally conceded that glycosyltransferases possess a remarkable degree of specificity for their acceptor-substrates[2], yet it is also possible that a particular acceptor-substrate may act as an acceptor for more than one enzyme of the same class that is present in the same source. It would be reasonable to assume, for

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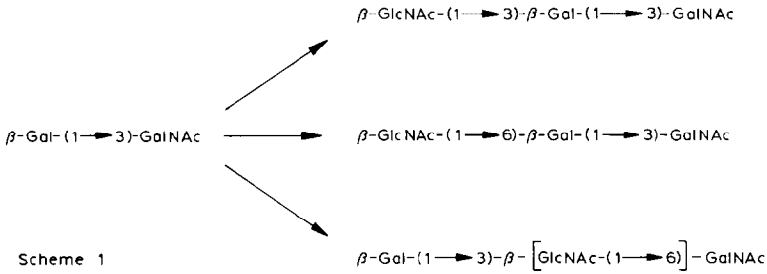
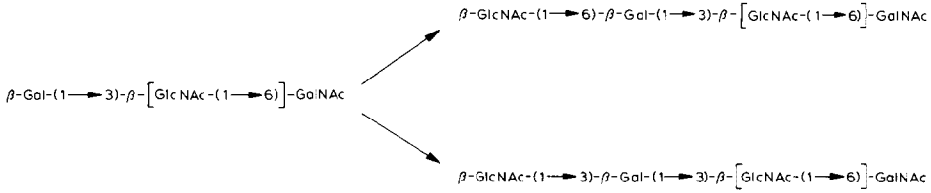
example, that the disaccharides β-Gal-(1→4)-GlcNAc or β-Gal-(1→3)-GalNAc are capable of accepting sialic acid at more than one site in the same molecule. Similarly, the latter disaccharide may also be expected to act as an acceptor for more than one GlcNAc transferase, as depicted in Scheme 1. By the same token,



Scheme 1

our synthetic trisaccharide[5] β-Gal-(1→3)-β-[GlcNAc-(1→6)]-GalNAc would also be expected to act as an acceptor for two different GlcNAc-transferases; see Scheme 2.



Scheme 2

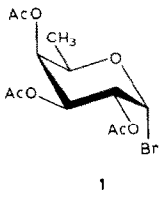
For the past few years, we have been mainly concerned with the synthesis of some oligosaccharides that are required as acceptor-substrates or as reference compounds in studies of glycosyltransferases. In furtherance of these studies, it was desired to obtain certain oligosaccharides that are modified at specific positions of the acceptor molecule. It could be anticipated that such a modified compound would act as an acceptor for a unique and single glycosyltransferase. Specifically, it would be reasonable to predict that the title trisaccharide we now describe would act as an acceptor solely for 3′-GlcNAc transferase, as it lacks a hydroxyl group at C-6 of the D-galactoside residue.

RESULTS AND DISCUSSION

On glycosylation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[6] (**2**) with 2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl bromide[7] (**1**) in 1:1 nitromethane–benzene in the presence of powdered mercuric cyanide, examination of the crude product by thin-layer chromatography (t.l.c.) with solvent *B* revealed the presence of a major product, faster-migrating than **2**, and some faster- and some slower-migrating contaminants. Purification of the crude product in a

column of silica gel, gave, after recrystallization from ethyl acetate–hexane, the disaccharide derivative (**3**), the ^{1}H-n.m.r. spectrum of which supported its overall structure; see Experimental section.

Brief treatment of **3** with hot, 60% aqueous acetic acid cleaved its acetal group to afford, after column-chromatographic purification, diol **4**. The ^{1}H-n.m.r. spectrum of **4** was consistent with the structure expected; the one-proton singlet (δ 5.56) assigned to the benzylidene methine proton in the spectrum of **3** was absent



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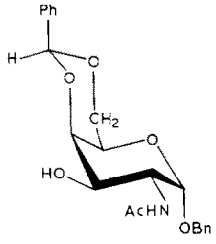

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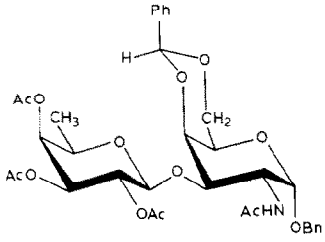

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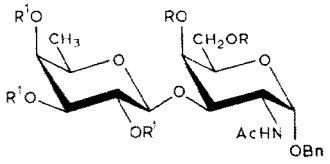
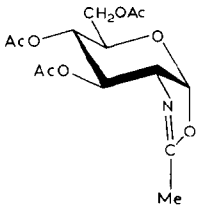
from that of **4**. Additionally, the presence of two multiplets, exchangeable in D_2O, at δ 3.20–2.40, was indicative of the presence of two hydroxyl groups.

Deacetylation of compound **4** with Amberlyst A-26 (OH^-) anion-exchange resin in methanol, furnished, after recrystallization from ethanol, crystalline disaccharide **5**. The ^{13}C-n.m.r. spectrum of **5** was in accord with the structure assigned; the resonance (96.29 p.p.m.) for C-1 and (103.50 p.p.m.) for C-1′ were respectively in agreement with an α and a β configuration at the two glycosidic linkages.



4 R = H, R′ = Ac
5 R = R′ = H

Condensation of the oxazoline **6** with diol **4** in 1,2-dichloroethane, and purification of the crude product by column chromatography, gave the trisaccharide derivative **7**, which, on deacetylation as described for **4** (to give **5**), afforded the trisaccharide **8**, the ^{13}C-n.m.r. spectrum of which was in agreement with the structure proposed. Thus, in the ^{13}C-n.m.r. spectrum of **8**, two carbon-atom resonances were observed, at 96.14 and 103.42 p.p.m. By comparison to the chemical shifts observed for C-1 (96.28 p.p.m.) and for C-1′ (103.50 p.p.m.) in the ^{13}C-n.m.r. spectrum of **5**, the carbon-atom resonance at lower field can reasonably be assigned to the anomeric carbon atom carrying the α-benzyloxy group, whereas that at a higher field can be assigned to the carbon atom at an inter-sugar linkage.



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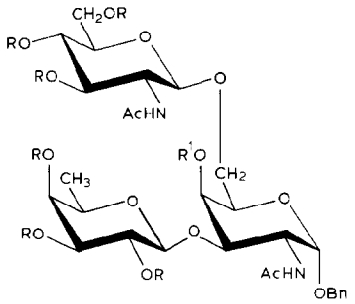

7 R = Ac, R' = H
8 R = R' = H

Moreover, an additional carbon-atom resonance (101.46 p.p.m.) observed in the spectrum of **8** was indicative of the presence of another β-GlcNAc group (*cf.*, the entries for Me β-GlcNAc and for β-GlcNAc-(1→6)-α-GalNAc-1→OBn in Table I). Also, the presence of only one carbon-atom resonance in the range (~60–61 p.p.m.) that is normally observed for a carbon atom carrying an unsubstituted primary hydroxyl group militates against any substitution at O-4, thus confirming the structure proposed for trisaccharide **8** as being β-D-Fuc-(1→3)-[β-D-GlcNAc-(1→6)]-α-D-GalNAc-1→OBn.

EXPERIMENTAL

General methods. — These were those already described[1], except that the following solvent systems (v/v) were used for chromatography: *A*, 2:1 chloroform–acetone; *B*, 4:1 chloroform–acetone; and *C*, 9:1 chloroform–acetone.

TABLE I

^{13}C-N.M.R CHEMICAL SHFTS[a]

Compound	*Residues*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH_3CO*	*OCH_3 or $CH_2C_6H_5$*
[b]		101.56	55.41	74.21	70.44	76.74	60.88	22.96	50.02
[c]		96.08	49.59	67.19	67.55	71.34	60.53	22.49	67.99
[d]		104.06	69.74	73.36	70.83	69.98	16.51	—	55.55
[e]	benzyl α-GalNAc	96.04	49.55	67.00	67.47	69.34	68.56	22.51	68.28
	β-GlcNAc-(1→6)	101.49	55.08	74.06	70.53	76.77	60.92	22.93	—
5	benzyl α-GalNAc	96.28	48.36	75.85	67.16	71.18	60.33	22.56	67.91
	β-D-Fuc-(1→3)	103.50	69.90	73.27	70.78	70.29	16.51	—	—
8	benzyl α-GalNAc	96.14	48.29	75.64	67.64	69.31	68.64	22.57	67.71
	β-D-Fuc-(1→3)	103.42	69.94	73.28	70.60	70.31	16.53	—	—
	β-GlcNAc-(1→6)	101.46	55.10	74.11	70.58	76.78	60.96	22.98	—

[a]In Me_2SO-d_6, with Me_4Si as the internal standard. [b]Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[8]. [c]Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside[8]. [d]Benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside[8]. [e]Methyl β-D-fucopyranoside.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4-tri-O-acetyl-β-D-fucopyranosyl)-α-D-galactopyranoside (**3**). — A stirred solution of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[6] (**2**; 4.17 g) in 1:1 nitromethane–benzene (200 mL) was boiled until 60 mL of the solvent mixture had distilled off. After cooling to room temperature, a solution of 2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl bromide[7] (**1**; 4.8 g) in 1:1 nitromethane–benzene (10 mL), and powdered mercuric cyanide (2.52 g), were added, and stirring was continued for 20 h, whereupon more of bromide **1** (2.9 g, in 1:1 nitromethane–benzene, 6 mL) and mercuric cyanide (1.26 g) were added, and stirring was continued for 40 h. T.l.c. (solvent *B*) then revealed the disappearance of **2**, and the presence of a major product, faster-migrating than **2**; some faster- and some slower-migrating contaminants were also revealed. After processing in the usual manner, the crude product was dissolved in chloroform, and the solution applied to a column of silica gel. Elution with 19:1 (v/v) chloroform–acetone, followed by solvent *C*, removed the faster-migrating contaminants. On elution with solvent *B*, evaporation of the fractions corresponding to the product gave a solid (4.8 g) which was recrystallized from ethyl acetate–hexane, to afford **3** (3.9 g, 58%); m.p. 118–120° $[\alpha]_D$ +109.8° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.70–7.20 (m, 10 H, aromatic), 5.70 (d, 1 H, *J* 9 Hz, NH), 5.56 (s, 1 H, PhCH), 5.08 (d, 1 H, *J* 7 Hz, H-1′), 2.12, 2.04, 1.98, and 1.94 (s, 12 H, 3 OAc and NAc), and 1.22 (d, 3 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{34}H_{41}NO_{13}$: C, 60.79; H, 6.15; N, 2.09. Found: C, 60.57; H, 6.25; N, 1.99.

Benzyl 2-acetamido-2-deoxy-3-O-(2,3,4-tri-O-acetyl-β-D-fucopyranosyl)-α-D-galactopyranoside (**4**). — Compound **3** (3.8 g) in 60% aqueous acetic acid (150 mL) was stirred for 0.5 h at ~98°. The acetic acid was evaporated under diminished pressure, the last traces being removed by co-evaporation with several portions of toluene, and the residue was purified in a column of silica gel by elution with 19:1 (v/v) chloroform–ethanol. The fractions corresponding to the deacetalated disaccharide were evaporated to dryness, and the residue crystallized from ethyl acetate–hexane to give **4** (2.12 g, 64%); m.p. 120–122°, $[\alpha]_D$ +89.8° (*c* 1.0, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.62 (d, 1 H, exchangeable in D_2O, *J* 9 Hz, NH), 5.15 (d, 1 H, *J* 7 Hz, H-1′), 3.20–2.40 (2 m, 2 H, exchangeable in D_2O, 2 OH), 2.18, 2.08, 2.00, and 1.98 (s, 12 H, 3 OAc and NAc), and 1.21 (d, 3 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{27}H_{37}NO_{13}$: C, 55.56; H, 6.40; N, 2.40. Found: C, 55.26; H, 6.18; N, 2.15.

Benzyl 2-acetamido-2-deoxy-3-O-β-D-fucopyranosyl-α-D-galactopyranoside (**5**). — Compound **4** (0.5 g) in methanol (10 mL) was treated with Amberlyst A-26 (OH^-) anion-exchange resin (~150 mg), and the mixture was stirred for 5 h at room temperature. The resin was filtered off, and washed with methanol, the filtrate and washings were combined and evaporated, and the residue crystallized from ethanol, to furnish disaccharide **5** (0.37 g, 94%); m.p. 267–269°, $[\alpha]_D$ +117.2° (*c* 1, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{21}H_{31}NO_{10} \cdot H_2O$: C, 53.04; H, 7.00; N, 2.95. Found: C, 52.97; H, 6.76; N, 2.80.

Benzyl 2-acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-(2,3,4-tri-O-acetyl-β-D-fucopyranosyl)-α-D-galactopyranoside (**7**). — A mixture of compound **4** (0.88 g), oxazoline **6** (1 g), and *p*-toluenesulfonic acid (57 mg) in 1,2-dichloroethane (35 mL), protected from moisture, was heated (~70°) with stirring for 3 h, whereupon more portions of **6** (0.95 g in 9 mL of 1,2-dichloroethane), and *p*-toluenesulfonic acid (34 mg in 9 mL of 1,2-dichloroethane) were added and the stirring and heating were continued overnight. T.l.c. (solvent *A*) then revealed the presence of a major product, marginally slower-migrating than **4**; a trace of **6** and a small proportion of **4** were also revealed. After cooling, a few drops of pyridine were added, the mixture was evaporated to dryness, and the residue dissolved in chloroform. The solution was washed with water, dried, and concentrated, and the concentrate applied to a column of silica gel. Elution with chloroform, followed by solvent *C*, removed some faster-migrating contaminants. On elution with solvent *B*, evaporation of the fractions containing the product gave a solid (1.84 g) that was contaminated (t.l.c., solvent *A*, two developments) with a marginally slower-migrating compound. Recrystallization from hot ethyl acetate afforded compound **7** (0.65 g, 47%); m.p. 268–270°, $[\alpha]_D$ +45.4° (*c* 0.5, methanol).

Anal. Calc. for $C_{41}H_{56}N_2O_{21}$: C, 53.93; H, 6.20; N, 3.07. Found: C, 53.63; H, 5.93; N, 2.85.

Benzyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-3-O-β-D-fucopyranosyl-α-D-galactopyranoside (**8**). — Compound **7** (0.3 g) was deacetylated as described for **4** (to give **5**), and the residue was recrystallized from methanol, to afford the title trisaccharide **8** (0.20 g, 93%); m.p. 282–284° (dec.), $[\alpha]_D$ +66.4° (*c* 1, water); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{29}H_{44}N_2 \cdot H_2O$: C, 51.32; H, 6.83; N, 4.13. Found: C, 51.21; H, 6.57; N, 4.16.

ACKNOWLEDGMENTS

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