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Note

Further use of *O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide as a glycosyl donor. Synthesis of two mucin-type tetrasaccharides*

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In an earlier paper in this series[2], we recently described the synthesis and use, as a glycosyl donor, of the trisaccharide halide *O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**1**). It was envisaged that bromide **1** could prove useful for the synthesis of such polyfucosylated oligosaccharides as those known to accumulate in a variety of human carcinomas. A number of glycolipids having the X {α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-D-GlcNAc} as well as the di- and tri-meric X determinant have been isolated and characterized by Hakomori *et al.*[3–7], who also developed monoclonal antibodies that would specifically recognize the di- and tri-fucosylated Type II chain structures, but not the terminal X determinant[5,6].

However, as Hakomori *et al.*[7] have noted, the increasing size of the compounds of interest presents greater problems for isolating homogeneous carbohydrate components in quantities sufficient for analyses, and subsequent production and screening of monoclonal antibodies. Thus, it seemed appropriate to attempt to provide an alternative source for these compounds. To this end, we have embarked on a program for the synthesis of a variety of glycoconjugate fragments, comprising both Type II and Type I chains, that are primarily intended for use in our immunological studies.

We now describe herein the synthesis of 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**6**), and 4-nitrophenyl *O*-α-

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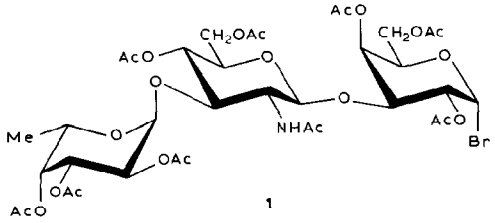
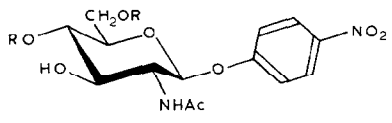
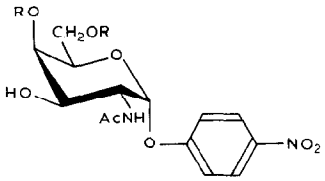
L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy)-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**9**). It is noteworthy that the parent compound of **6** has been found to occur as part of the Le^c-active glycolipid isolated from the plasma of blood-group Le(a$^-$b$^-$) non-secretors[8]. The parent compound of **9**, on the other hand, forms part of one of the complex oligosaccharides isolated from bronchial-mucus glycoproteins of patients suffering from cystic fibrosis[9].



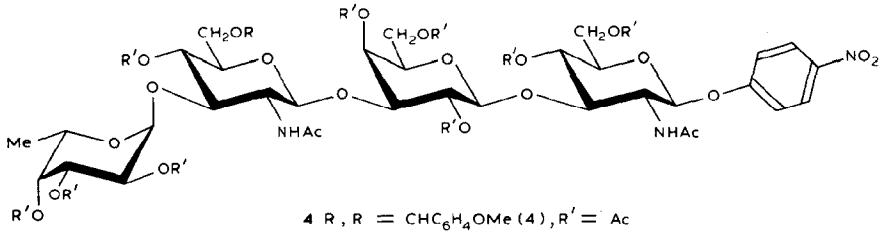
1



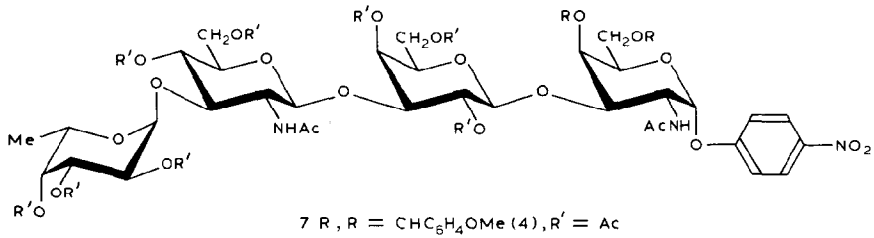
2 R, R = $CHC_6H_4OMe(4)$



3 R, R = $CHC_6H_4OMe(4)$



4 R, R = $CHC_6H_4OMe(4)$, R′ = Ac
5 R = H, R′ = Ac
6 R = R′ = H



7 R, R = $CHC_6H_4OMe(4)$, R′ = Ac
8 R = H, R′ = Ac
9 R = R′ = H

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS FOR COMPOUNDS **6** AND **9**[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3 or CH_3CO*
β-D-Glc*p*NAcO$C_6H_4NO_2$ (4)	**6**	97.96	53.96	81.64	68.29	76.60	60.28	22.90
β-D-Gal*p*		103.61	69.52	81.64	67.05	75.42	60.28	
β-D-Glc*p*NAc		101.59	55.09	83.33	68.75	76.81	60.74	22.90
α-L-Fuc*p*		99.50	68.13	69.78	71.57	66.44	16.33	
α-D-Gal*p*NAcO$C_6H_4NO_2$ (4)	**9**	96.89	48.11	75.79	67.11	72.86	60.43	22.64
β-D-Gal*p*		103.87	69.68	81.89	67.11	75.07	60.23	
β-D-Glc*p*NAc		101.36	55.30	82.15	68.80	76.51	60.79	23.05
α-LFuc*p*		99.56	68.19	69.78	71.63	66.44	16.33	
β-D-GalOMe	[b]	103.83	69.19	82.19	67.00	74.65	60.21	55.64
β-D-Gal*p*		101.85	55.01	81.47	68.59	76.22	60.58	22.96
α-L-Fuc*p*		99.40	67.98	69.63	71.46	66.30	16.26	

[a]For solutions in ($^2H_6$)Me_2SO with Me_4Si as the internal standard. Carbonyl and aromatic resonances are not shown. [b]Methyl 3-*O*-(2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside[2]; the chemical shifts of the last-named compound were recorded for comparison purposes.

On condensation of bromide **1** with 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[10] (**2**) in 1:1 benzene–nitromethane and in the presence of mercury(II) cyanide, examination of the product mixture by t.l.c. (solvent *A*) revealed the presence of a major product, marginally faster-migrating than **2**, which rendered it impractical to attempt any column chromatographic purification. Therefore, the crude mixture was treated with hot, 60% aqueous acetic acid to cleave the acetal group of intermediate **4**. Column-chromatographic separation furnished, in 36% yield, diol **5** as an amorphous solid, the ^{1}H-n.m.r. spectrum of which contained signals supporting its overall structure (see Experimental section). Transesterification of **5** in 0.1M methanolic sodium methoxide afforded the title saccharide **6** in 81% yield. The ^{13}C-n.m.r. spectrum of **6** was consistent with the structure assigned (see Table I).

On similar glycosylation with bromide **1**, the 4-nitrophenyl α-D-galactopyranoside derivative[11] **3** gave a fully protected tetrasaccharide derivative **7** which was not isolated but directly deacetalated as described for **4** (to give **5**) to give, after column chromatographic purification, amorphous diol **8**. Compound **8** was likewise *O*-deacetylated with methanolic sodium methoxide to furnish tetrasaccharide **9**, the ^{13}C-n.m.r. spectrum of which was, also, in conformity with the structure expected.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~26° with a Perkin–Elmer 241 polarimeter. T.l.c. was conducted on aluminum sheets precoated with 0.2-mm layers of Silica Gel 60F-254 (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh), and solvent *A* was 19:1 (v/v) chloroform–methanol. N.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra at 90 MHz with a Varian EM-390, and ^{13}C-n.m.r. spectra at 67.8 MHz with a JEOL FX-270 instrument. The position of the peaks (δ) are expressed from Me_4Si signal. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A.

4-Nitrophenyl O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-O-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**5**). — A stirred solution of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside[10] (**2**, 0.6 g, 1 mmol) in 1:1 benzene–nitromethane (140 mL) was boiled until ~60 mL of the solvent had distilled off. The temperature was then adjusted to ~35° (bath), $Hg(CN)_2$ (0.44 g, 1.2 mmol) and trisaccharide bromide **1** (1.4 g, 1.2 mmol) were added, and the mixture was stirred for 24 h, allowed to cool to room temperature, diluted with benzene, successively washed

with water, M KI solution, aqueous $NaHCO_3$, water, dried, and evaporated. Examination of the crude product mixture by t.l.c. (solvent *A*) revealed the presence of a major product, marginally faster-migrating than **2** and some unchanged **2**. This mixture (1.8 g, containing **4**) was dissolved in 60% aqueous acetic acid (30 mL) and heated for 1 h at 50°. Acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene. The residue was applied to a column of silica gel and eluted with solvent *A* to give **5** (0.55 g, 36%), amorphous, $[\alpha]_D^{26}$ −16° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.20 and 7.05 (d, 2 × 2 H, *J* 11 Hz, arom.), 2.20–1.65 (cluster of s, 30 H, 8 OAc and 2 NAc), and 1.08 (d, 3 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{50}H_{67}N_3O_{29}$: C, 51.14; H, 5.76; N, 3.58. Found: C, 50.91; H, 5.73; N, 3.29.

*4-Nitrophenyl O-α-*L*-fucopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-O-β-*D*-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**6**). — A solution of **5** (0.45 g) in methanol (30 mL) was treated with M sodium methoxide in methanol (3 mL) and the mixture stirred for 24 h at room temperature, whereupon the deacetylation product precipitated as a white solid. The mixture was then diluted with ethanol and chilled, the base neutralized with a few drops of glacial acetic acid, and the solid material filtered and thoroughly washed with cold ethanol to give **6** (0.25 g, 81%), amorphous, $[\alpha]_D^{26}$ −42° (*c* 0.6, dimethyl sulfoxide); ^{13}C-n.m.r.; see Table I.

Anal. Calc. for $C_{31}H_5N_3O_{21}$: C, 46.43; H, 6.42; N, 5.24. Found: C, 46.51; H, 6.30; N, 4.97.

4-Nitrophenyl O-(2,3,4-tri-O-*acetyl-α-*L*-fucopyranosyl-(1→3)-O-(2-acetamido-4,6-di-O-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-*D*-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-*D*-galactopyranoside* (**8**). — Bromide **1** (1.4 g, 1.2 mmol) was condensed with 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside (**3**; 0.6 g, 1 mmol) in 1:1 benzene–nitromethane, in the presence of $Hg(CN)_2$ (0.44 g, 1.2 mmol), exactly as described for **2** (to give **4**). After processing in the usual manner, the crude product mixture (2 g, containing **7**) was heated in 60% aqueous acetic acid (30 mL) for 1 h at 50°. It was then processed as described earlier, and purified in a column of silica gel with solvent *A* as the eluant to give **8** (0.4 g, 26%), amorphous, $[\alpha]_D^{26}$ +64° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.20–7.20 (d, 2 × 2 H, *J* 11 Hz, arom.), 2.20–1.80 (cluster of s, 30 H, 8 OAc and 2 NAc), and 1.05 (d, 3 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{50}H_{67}N_3O_{29}$: C, 51.14; H, 5.76; N, 2.58. Found: C, 50.86; H, 5.61; N, 3.29.

*4-Nitrophenyl O-α-*L*-fucopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-O-β-*D*-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-*D*-galactopyranoside* (**9**). — Compound **8** (0.14 g) was *O*-deacetylated in 0.1M methanolic sodium methoxide, as described for **5** (to give **6**), to afford **9** (80 mg, 84%), amorphous, $[\alpha]_D^{26}$ +57° (*c* 0.5, dimethyl sulfoxide); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{31}H_{51}N_3O_{21}$: C, 46.43; H, 6.42; N, 5.24. Found: C, 46.72; H, 6.43; N, 4.96.

ACKNOWLEDGMENTS

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