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Note

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 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside*

RAKESH K. JAIN, SAEED A. ABBAS, AND KHUSHI L. MATTA†

Department of Gynecologic Oncology, Roswell Park Memorial Institute, New York State Department of Health, 666 Elm Street, Buffalo, New York 14263 (U.S.A.)

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In a previous paper in this series[2], we outlined our interest in the synthesis of some oligosaccharides containing L-fucose α-(1→3)-linked to 2-acetamido-2-deoxy-D-glucose. Our interest in this class of compounds was, to a large extent, motivated by a desire to obtain reference compounds in studies related to (1→3)-α-L-fucosyltransferase. This interest was, however, enhanced by recent reports associating a variety of such oligosaccharide structures with certain types of human cancers[3–5].

Furthermore, it has been our established practice to test the glycosylating capability of a number of sugar halides that were primarily synthesized for use in higher oligosaccharide syntheses by preparing the nitrophenyl glycosides derived therefrom[6,7]. Such practice has served a dual purpose, because it also coincided with our recently initiated program for the procurement of compounds that are suitable for use as synthetic or artificial antigens. On reduction of their nitro groups, and subsequent attachment to suitable supports, nitrophenyl glycosides have proved useful as synthetic antigens[8,9].

In this context, we describe herein the synthesis of the trisaccharide bromide, *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 (**4**) and, by the synthesis of 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**6**), demonstrate its utility as a glycosyl donor.

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†To whom correspondence should be addressed.

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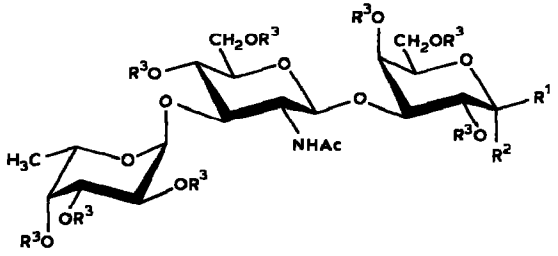

1 R^1 = OMe, R^2 = R^3 = H
2 R^1 = OMe, R^2 = H, R^3 = Ac
3 R^1, R^2 = OAc, H; R^3 = Ac
4 R^1 = H, R^2 = Br, R^3 = Ac
5 R^1 = $C_6H_4NO_2$ (*p*), R^2 = H, R^3 = Ac
6 R^1 = $C_6H_4NO_2$ (*p*), R^2 = R^3 = H

Acetylation of methyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside[2] (**1**) in 1:2 acetic anhydride–pyridine afforded, in excellent yield, the trisaccharide peracetate **2** as an amorphous solid, the ^{1}H-n.m.r. spectrum of which contained signals in support of its overall structure. Acetolysis of **2** in acetic anhydride containing 0.9% (v/v) of concentrated sulfuric acid gave, in ~79% yield, *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)-1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**3**). In the ^{1}H-n.m.r. spectrum of **3**, a lower-field signal at δ 6.20 (~0.9 H*, *J* ~4 Hz) was indicative of an anomeric mixture that was rich in the α anomer. A similar anomeric mixture was previously obtained on acetolysis of two somewhat related compounds[6,7]. Trisaccharide **3** was readily converted, in high yield, into *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 (**4**) by treatment with 30% hydrogen bromide in glacial acetic acid containing a little acetic anhydride. Bromide **4** was obtained as an amorphous powder, the ^{1}H-n.m.r. spectrum of which contained a doublet at δ 6.60 (~1 H, *J* ~4 Hz), indicative of a preponderantly α-D configuration of the anomeric carbon atom; this was also evidenced by a relatively high, positive, specific rotation.

When bromide **4** was allowed to react with Amberlyst A-26-*p*-nitrophenoxide resin[10] in 1:4 dichloromethane–2-propanol, exmination of the product mixture (containing **5**) by t.l.c. (solvent *A*) showed it to be contaminated with some marginally slower- and faster-migrating impurities that were difficult to remove by column chromatography. It was, therefore, directly *O*-deacetylated in methanolic sodium methoxide to give, after an attempted crystallization from aqueous alcohol, amorphous 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**6**), in 46% overall yield from **4**.

*Compared to the acetyl and the L-fucopyranosyl group C-6 methyl protons.

Table I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS (δ)[a]

Residue	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*$NCOCH_3$*	*C=O*
β-D-Gal*p*$OC_6H_4NO_2$ (4)	[b]	99.90	68.80	81.55	66.88	75.25	60.03		
β-D-Glc*p*NAc-		101.89	56.17	74.01	70.25	76.56	60.75	22.96	169.70
β-D-Gal*p*OMe	1	103.83	69.19	82.19	67.00	74.65	60.21	55.64	
β-D-Glc*p*NAc-		101.38	55.01	81.47	68.59	76.22	60.58	22.96	170.29
α-L-Fuc*p*-		99.40	67.98	69.63	71.46	66.30	16.26		
β-D-Gal*p*$OC_6H_4NO_2$ (4)	6	100.23	68.82	81.92	67.08	75.46	60.20		
β-D-Glc*p*NAc-		101.72	55.17	81.63	68.98	74.46	60.79	23.06	170.70
α-L-Fuc*p*-		99.70	68.18	69.83	71.66	66.48	16.32		

[a]For solutions in ($^2H_3$)Me_2SO with Me_4Si as the internal standard. Aromatic resonances are not shown. [b]4-Nitrophenyl 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[6]; the chemical shifts for this compound and those for compound 1 (ref. 2) are recorded for comparison.

This material was fairly pure, but showed in t.l.c. (solvent *B*) a small proportion of a slower-migrating contaminant that was undetectable under u.v. light. However, a chromatographically homogeneous, analytical sample was obtained after a second precipitation of **6**, from its aqueous solution, by the addition of ethanol. The ^{13}C-n.m.r. spectrum of compound **6** was consistent with the structure assigned (see Table I).

EXPERIMENTAL

General methods. — Melting points were determined with a Fischer–John apparatus and are uncorrected. Optical rotations were measured at 25–27°, with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded at ~25°; ^1H-n.m.r. spectra with a Varian EM-390, and ^{13}C-n.m.r. spectra either with a Varian XL-100 or with a Bruker WP-200 instrument, at 25.2 or 50.3 MHz, respectively. The positions of the peaks are expressed from the tetramethylsilane signal. 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 sheets with 5% H_2SO_4 in ethanol and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: *A*, 3:1 chloroform–acetone; *B*, 3:1:1 ethyl acetate–ethanol–water. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A., or by Galbraith Laboratories, Inc., Knoxville, Tennessee, U.S.A.

Methyl 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-β-*D-*galactopyranoside* (**2**). — Compound **1** (ref. 2; 1.8 g) was stirred overnight at room temperature in a 1:2 mixture of acetic anhydride-pyridine. The pyridine and acetic anhydride were evaporated under diminished pressure, and several portions of toluene added to, and evaporated from the residue, which was then dissolved in ethyl acetate. Addition of hexane caused the precipitation of **2** (2.8 g, 96%), amorphous, $[\alpha]_D^{26}$ −31° (*c* 1.1, chloroform); ^1H-n.m.r. ($CDCl_3$): δ 3.45 (s, 3 H, OMe), 2.10–1.80 (cluster of s, 27 H, 8 OAc and NAc), and 1.05 (d, 3 H, *J* ~6 Hz, CMe).

Anal. Calc. for $C_{37}H_{53}NO_{23}$: C, 50.50; H, 6.08; N, 1.59. Found: C, 50.34; H, 6.18; N, 1.39.

*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)-1,2,4,6-tetra-*O-*acetyl-*D-*galactopyranose* (**3**). — A solution of **2** (2.7 g) in acetic anhydride (100 mL) containing 0.9% by volume of conc. H_2SO_4 was stirred for 6 h at room temperature. The mixture was then diluted with dichloromethane (400 mL), and successively washed with water, saturated $NaHCO_3$, and water, dried, and evaporated to dryness to give a solid that was slightly contaminated (t.l.c., 19:1 chloroform–methanol) with faster- and slower-migrating impurities. The crude product was applied to a column of silica

gel and eluted with 2% methanol in chloroform. Evaporation of the fractions corresponding to the product gave a residue which was dissolved in ethyl acetate. Addition of petroleum ether caused the precipitation of **3** (2.2 g, 79%), white solid, $[\alpha]_D^{26}$ +17.5° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.20 (d, 0.9 H, *J* ~4 Hz, H-1), 2.20–1.80 (cluster of s, 30 H, 9 OAc and NAc), and 1.05 (d, 3 H, *J* ~6 Hz, CMe).

Anal. Calc. for $C_{38}H_{53}NO_{24}$: C, 50.27; H, 5.90; N, 1.54. Found: C, 50.00; H, 5.73; N, 1.29.

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 (**4**). — Peracetate **3** (2.2 g) in acetic anhydride (2 mL) was cooled (0°, bath) and treated with 31% HBr in glacial acetic acid (20 mL), and the mixture stirred for 2 h at 0°. It was then allowed to gradually warm up to room temperature and the stirring continued for a total of 3 h. The mixture was diluted with dichloromethane (250 mL), and successively washed with cold water, cold saturated $NaHCO_3$, and cold water, dried, and evaporated. The residue was dissolved in dichloromethane, and addition of ether–hexane caused the precipitation of **4** (2.17 g, 97%), a white powder; $[\alpha]_D^{26}$ +105° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 6.60 (d, 1 H, *J* ~4 Hz, H-1), 2.20–1.80 (cluster of s, 27 H, 8 OAc and NAc), and 1.05 (d, 3 H, *J* ~6 Hz, CMe).

4-Nitrophenyl O-α-L-fucopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**6**). — A mixture of **4** (2 g) and Amberlyst A-26-*p*-nitrophenoxide resin (5 g) in 1:4 dichloromethane–2-propanol (50 mL) was stirred for 20 h at room temperature. T.l.c. (solvent *A*) revealed the presence of an intensely u.v.-visible product, which was marginally slower-migrating than **4**. Some slower- and some faster-migrating contaminants were also revealed in t.l.c. After dilution with dichloromethane (100 mL), the resin was filtered off, thoroughly washed with dichloromethane, and the filtrate and washings were combined, successively washed with water, aqueous $NaHCO_3$, and water, dried, and evaporated, and the residue dissolved in dichloromethane. Addition of ether–hexane caused the precipitation of a material (2.2 g) that contained (t.l.c., solvent *A*) mainly compound **5** (detectable under u.v. light), together with some faster- and some slower-migrating contaminants. This material was dried *in vacuo* and dissolved in 0.5M methanolic sodium methoxide (100 mL), and stirred for 8 h at room temperature. T.l.c. (solvent *B*) then showed the disappearance of **5** and the presence of a prominently u.v. visible, slower-migrating product; a trace of a faster-migrating impurity (detectable under u.v. light) and a slower-migrating impurity (undetectable under u.v. light) were also revealed by t.l.c. The base was neutralized by the dropwise addition of glacial acetic acid, the methanol evaporated, and the residue dissolved in aqueous methanol. After de-ionization with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off (a bed of Celite), thoroughly washed with aqueous methanol, and the filtrate and washings were combined and evaporated, and the residue was dissolved in a small volume of water. Addition of

ethanol caused the precipitation of **6** (0.65 g, 46%; based on **4**), a white powder which showed in t.l.c. (solvent *B*) a trace of the slower-migrating contaminant. An analytically pure sample, homogeneous in t.l.c. (solvent *B*), was obtained after a second precipitation of **6** from its aqueous solution by the addition of ethanol; $[\alpha]_D^{26}$ −118° (*c* 0.6, water); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{26}H_{38}N_2O_{17}\cdot 2\ H_2O$: C, 45.47; H, 6.18; N, 4.08. Found: C, 45.59; H, 5.83; N, 4.08.

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REFERENCES

1 R. L. THOMAS, R. DUBEY, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 169 (1987) 43–54.
2 R. K. JAIN, K. KOHATA, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 172 (1988) 27–35.
3 S. HAKOMORI, *Annu. Rev. Immunol.*, 2 (1984) 103–126.
4 S. HAKOMORI, E. NUDELMAN, S. B. LEVERY, AND R. KANNAGI, *J. Biol. Chem.*, 259 (1984) 4672–4680.
5 Y. FUKUSHI, S. HAKOMORI, E. NUDELMAN, AND N. COCHRAN, *J. Biol. Chem.*, 259 (1984) 4681–4685.
6 S. A. ABBAS AND K. L. MATTA, *Carbohydr. Res.*, 124 (1983) 115–121.
7 K. KOHATA, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 132 (1984) 345–350.
8 P. Z. ALLEN, I. J. GOLDSTEIN, AND R. N. IYER, *Biochemistry*, 6 (1967) 3029–3036; O. T. AVERY AND W. F. GOEBEL, *J. Exp. Med.*, 50 (1929) 533–550.
9 K. R. DIAKUN, S. YAZAWA, L. VALENZUELA, S. A. ABBAS, AND K. L. MATTA, *Immun. Invest.*, 16 (1987) 1–11; 151–163.
10 I. IVERSEN AND R. JOHANSSON, *Synthesis*, (1979) 823–824.