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SYNTHESIS OF PHENYL *O*-α-L-FUCOPYRANOSYL-(1→2)-*O*-β-D-GALACTOPYRANOSYL-(1→3)-2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSIDE*

SURJIT S. RANA, JOSEPH J. BARLOW, AND KHUSHI L. MATTA**
Department of Gynecology, Roswell Park Memorial Institute, Buffalo, N.Y. 14263 (U.S.A.)
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ABSTRACT

Phenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-[4,6-*O*-(*p*-methoxybenzylidene)-β-D-galactopyranosyl]-α-D-galactopyranoside (**3**) was prepared from phenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside by Zemplén deacetylation, followed by reaction with *p*-methoxybenzaldehyde in the presence of anhydrous zinc chloride. The selective benzoylation of **3** gave the 3′-benzoate which, on condensation with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide under catalysis by halide ion, afforded a crystalline trisaccharide from which the title trisaccharide was obtained by debenzoylation followed by catalytic hydrogenolysis.

INTRODUCTION

The endoglycosidases are the enzymes that specifically remove an oligosaccharide portion from glycoproteins and complex saccharides. According to Huang and Aminoff[2], the culture filtrate of *Clostridium perfringens* contains an oligosaccharidase that catalyzes the release of oligosaccharide from glycoproteins having 2-acetamido-2-deoxy-α-D-galactose at the reducing terminus. Desialated, porcine submaxillary mucin (H^+) has been employed as the substrate for the enzyme, which has a pH optimum of 6.5. Recently, we reported[3] that the culture fluid of *Clostridium perfringens* hydrolyzes the synthetic, chromogenic substrates β-Gal-(1→3)-α-GalNAc-1→OR (R = Ph and C_6H_4-NO_2-*o* or -*p*) to β-Gal-(1→3)-GalNAc and the aglycon. We also reported that the partially purified enzyme fraction showed activity over a broad range of pH, with an optimum at pH 9.0, but that less-pure material had two pH optima, at 4.0 and 9.0. For achieving further specificity of our enzyme preparation, we have attempted a chemical synthesis of the title compound.

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**To whom correspondence should be directed

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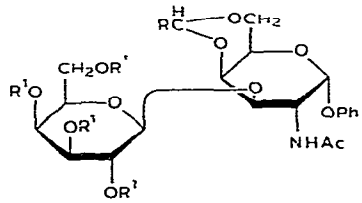
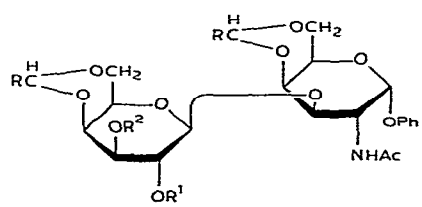
RESULTS AND DISCUSSION

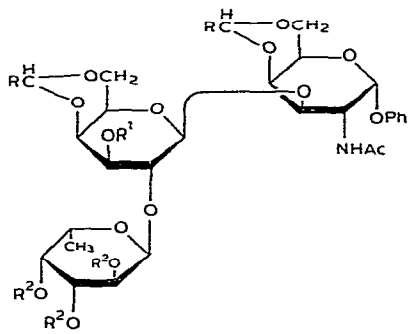
Easily accessible phenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside[4] (**1**) was chosen as a suitable starting-material for synthesis of the title compound. Deacetylation of **1** in methanol in the presence of a catalytic amount of sodium methoxide provided **2** in 90% yield. Treatment of compound **2** with *p*-methoxybenzaldehyde in the presence of anhydrous zinc chloride gave crystalline **3** in 82% yield.



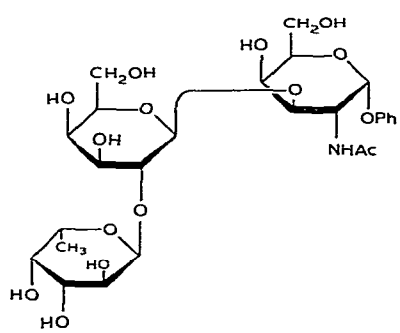
1 R = C_6H_4OMe-*p*, R^1 = Ac
2 R = C_6H_4OMe-*p*, R^1 = H



3 R = C_6H_4OMe-*p*, $R^1 = R^2$ = H
4 R = C_6H_4OMe-*p*, R^1 = H, R^2 = Bz



5 R = C_6H_4OMe-*p*, R^1 = Bz, R^2 = CH_2Ph
6 R = C_6H_4OMe-*p*, R^1 = H, R^2 = CH_2Ph



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Bz = —COPh

For introduction of an α-L-fucopyranosyl group at O-2′ of compound **3**, it is essential that the 3′-hydroxyl group be selectively protected. Selective benzoylation of 4,6-*O*-benzylidene-D-galactopyranosides warrants mention here. For example, treatment of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside with one equivalent of benzoyl chloride in pyridine–dichloromethane[5] at 0° gives the 3-benzoate as the main product. Thin-layer chromatography (t.l.c.) of the crude product showed the presence of unreacted starting-diol, the 2,3-dibenzoate, and the 2-benzoate also. The same reaction with 1-benzoylimidazole as the selective, acylating agent provided the 3-benzoate in 89–93% yield[6]. Interestingly, on selective benzoylation with benzoyl chloride in pyridine, phenyl 4,6-*O*-benzylidene-β-D-galactopyranoside gave the 3-*O*-benzoyl derivative as the main product[7]. In our laboratory, we have observed that,

under similar reaction-conditions, *p*-nitrophenyl 4,6-*O*-(*p*-methoxybenzylidene)-β-D-galactopyranoside gave exclusively the 3-*O*-substituted derivative, and we did not observe the formation of 2-benzoate under these conditions[4]. As reported recently by Paulsen and Kolář[8], on treatment with benzoyl chloride in absolute pyridine, benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside gives the corresponding 3-*O*-benzoyl derivative in 91% yield, and formation of 2-benzoate did not occur under these conditions. We attempted the selective benzoylation of compound **3**, and obtained **4** as crystalline material. The i.r. spectrum of compound **4** showed the presence of hydroxyl and ester groups, and the n.m.r. spectrum showed a double doublet at δ 5.10 ($J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz) for H-3', confirming thereby that benzoylation of **3** occurred at the 3'-hydroxyl group.

Exposure of **4** to 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide under catalysis by halide ion[9] for 4 days, followed by the usual processing, gave **5** in 74% yield. The presence of an α-linked L-fucosyl group in **5** was established by its n.m.r. spectrum, which showed a clear doublet for an anomeric proton (H-2") at δ 6.38 (J 3.5 Hz). On treatment with a catalytic amount of sodium methoxide in methanol and benzene, compound **5** gave phenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-[4,6-*O*-(*p*-methoxybenzylidene)-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-α-D-galactopyranoside (**6**), which was isolated crystalline in 87% yield. Catalytic hydrogenolysis of **6** produced the title trisaccharide **7** as an amorphous material. The absolute purity of compound **7** was established by t.l.c. and paper chromatography.

Extracts from *Aspergillus niger* have been found to contain a highly specific, (1→2)-α-L-fucosidase which releases α-L-fucose[3] linked at O-2 of galactosyl residues in complex saccharides and glycoproteins[10]. Our enzyme preparation did not release L-fucose from such disaccharides as α-L-Fuc-(1→3)-D-Gal and α-L-Fuc-(1→6)-D-Gal

TABLE I

VALUES OF R OF SUGARS, RELATIVE TO FUCOSE (R_{Fuc})

No.	*Compound*	*Chromatographic solvent*[a]	
		A	B
1	Fucose	1.00	1.00
2	β-D-Gal-(1→3)-α-D-GalNAc-1→OPh	1.02	1.27
3	α-L-Fuc-(1→2)-β-D-Gal-(1→3)-α-D-GalNAc-1→OPh	0.52	1.09
4	D-Galactose	0.44	0.68
5	2-Acetamido-2-deoxy-D-galactose	0.77	1.06
6	Hydrolysis of α-L-Fuc-(1→2)-β-D-Gal-(1→3)-α-D-GalNAc-1→OPh with (1→2)-α-L-fucosidase from *Aspergillus niger*	1.01	1.02, 1.28

[a]Solvent *A*, 4:1:1 (v/v) 1-butanol–ethanol–water (72 h); solvent *B*, 3:2:1 (v/v) butyl acetate–acetic acid–water (16 h).

Incubation of synthetic trisaccharide **7** with (1→2)-α-L-fucosidase gave L-fucose and the disaccharide phenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside as indicated by paper chromatography in two solvent-systems (see Table I). We have recently observed that the disaccharide β-Gal-(1→3)-α-GalNAc-1→OPh acts as an acceptor for a (1→2)-α-L-fucosyltransferase present in human serum[11]. The radio-labelled product of this reaction co-chromatographed with our synthetic trisaccharide. Furthermore, treatment of the radiolabelled product with (1→2)-α-L-fucosidase from *Aspergillus niger* completely released α-L-[^{14}C]fucose. Thus, our synthetic trisaccharide is a valuable reference compound for assaying (1→2)-α-L-fucosyltransferase.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. Ascending t.l.c. was conducted on plates coated with a 0.25-mm layer of silica gel CC-7 (Mallinckrodt); the components were located by exposure to u.v. light, or spraying the plate with 5% sulfuric acid in ethanol and heating. Descending p.c. was performed on Whatman No. 1 paper, and spots were detected with periodate, followed by silver nitrate reagent[12]. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey, U.S.A. I.r. spectra were recorded with a Perkin–Elmer 297 spectrophotometer, and n.m.r. spectra with a Varian XL-100 instrument at 100 MHz, with Me_4Si as the internal standard.

Phenyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-4,6-O-(p-methoxybenzylidene)-α-D-galactopyranoside (**2**). — A molar solution of sodium methoxide in methanol (2 mL) was added to a solution of phenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**1**: 2.5 g) in methanol (25 mL), and the mixture was kept overnight at room temperature, made neutral with acetic acid, and evaporated, followed by a few additions and evaporations of dry toluene. The residue crystallized from methanol–ether, to give **2** (1.74 g) in 90% yield, m.p. 147–148° $[\alpha]_D$ +117.5° (*c* 1, MeOH); ν_{max}^{KBr} 3450 (OH), 3300, 1650 (amide), and 760, 700 cm^{-1} (aromatic); n.m.r. data (CD_3OD): δ 2.0 (s, 3 H, NHAc), 3.82 (s, 3 H, OMe), 5.60 (s, 1 H, benzylic proton), 5.70 (d, 1 H, *J* 3.5 Hz, H-1), and 6.8–7.7 (m, 9 H, aromatic protons).

Anal. Calc. for $C_{28}H_{35}NO_{12}$: C, 58.22; H, 6.11; N, 2.43. Found: C, 58.15; H, 6.26; N, 2.35.

Phenyl 2-acetamido-2-deoxy-4,6-O-(p-methoxybenzylidene)-3-O-[4,6-O-(p-methoxybenzylidene)-β-D-galactopyranosyl]-α-D-galactopyranoside (**3**). — A mixture of compound **2** (2.5 g), anhydrous zinc chloride (2.5 g), and *p*-methoxybenzaldehyde (25 mL) was stirred for three days at room temperature, and then poured into cold water with stirring. The solid residue was filtered off, washed with hexane and water, and recrystallized from $HCONMe_2$–water, to afford crystalline compound **3** in 82% yield (2.47 g), m.p. 260–261°, $[\alpha]_D$ +91.2° (*c* 1, Me_2SO): n.m.r. data (Me_2SO-d_6):

δ 1.86 (s, 3 H, NHAc), 3.76 (s, 6 H, 2 OMe), 5.54 and 5 62 (each s, 2 H, benzylic H), and 6.8–7.9 (m, 13 H, aromatic protons).

Anal. Calc. for $C_{36}H_{41}NO_{13}$: C, 62.15; H, 5.94: N, 2.01. Found: C, 62.40: H, 6.00: N, 2.01.

*Phenyl 2-acetamido-3-*O*-[3-*O*-benzoyl-4,6-*O*-(p-methoxybenzylidene)-β-*D*-galactopyranosyl]-2-deoxy-4,6-*O*-(p-methoxybenzylidene)-α-*D*-galactopyranoside* (**4**). — A solution of compound **3** (1.6 g, 2.3 mmol) in absolute pyridine was stirred for 2 h at room temperature, then cooled to −5°, and benzoyl chloride (325 mg, 2.3 mmol) was added with stirring. Stirring was continued for 2 days, during which, additional benzoyl chloride (486 mg, 3.4 mmol) was added, and the reaction was monitored by t.l.c. in 3:1 CH_2Cl_2–ethyl acetate. After completion of the reaction, methanol (1 mL) was added, and the solution was evaporated under diminished pressure. A solution of the solid residue in chloroform (100 mL) was successively washed with cold 5% HCl (2 × 10 mL) and cold water (3 × 20 mL), dried (anhydrous Na_2SO_4), and evaporated. The solid mass was purified by chromatography on a column of silica gel, with elution with 3:1 (v/v) dichloromethane–ethyl acetate, to give **4** (1.51 g, 82%), m.p. 287–288°, $[\alpha]_D$ +159.5° (*c* 1, chloroform); ν_{max}^{KBr} 3400 (OH), 3310, 1650 (amide), 1710 (ester), and 830, 780, 760, 710, and 690 cm^{-1} (aromatic); n.m.r. data ($CDCl_3$): δ 2.02 (s, 3 H, NHAc), 3.78 and 3.82 (s, 2 × 3 H, 2 OMe), 4.64 (d, 1 H, *J* 8.5 Hz, H-1′), 5.10 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz, H-3′), 5.50 and 5.64 (s each, 2 H, benzylic protons), 5.80 (d, 1 H, *J* 3.5 Hz, H-1), and 6.7–8.2 (m, 18 H, aromatic protons).

*Phenyl 2-acetamido-3-*O*-[3-*O*-benzoyl-4,6-*O*-(p-methoxybenzylidene)-2-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl)-β-*D*-galactopyranosyl]-2-deoxy-4,6-*O*-(p-methoxybenzylidene)-α-*D*-galactopyranoside* (**5**). — A suspension of compound **4** (1.06 g, 1 33 mmol) in dichloromethane (25 mL) was stirred for 2 h at room temperature in the presence of tetraethylammonium bromide (0 555 g, 2.65 mmol) and molecular sieves (4A; 5 g). A solution of freshly prepared 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (1.32 g, 2.66 mmol) in dichloromethane (25 mL) and dry $HCONMe_2$ (30 mL) was added, and the mixture was stirred under dry nitrogen for 4 days at room temperature. Methanol (10 mL) was added, the mixture was stirred for 4 h, the solids were removed by filtration, and the filtrate was evaporated A solution of solid residue in dichloromethane (150 mL) was successively washed with $NaHCO_3$ solution and water, dried (anhydrous Na_2SO_4), and evaporated. The residue was purified by chromatography on a column of silica gel, eluting first with dichloromethane, and then with 9:1 dichloromethane–ethyl acetate to give **5** in 79% yield (1.19 g), m.p. 205–206°, $[\alpha]_D$ +49.0° (*c* 0.5, chloroform); t.l.c. (9·1 dichloromethane–ethyl acetate) R_F 0.52; ν_{max}^{KBr} no OH absorption; n.m.r. data (C_6D_6): δ 1.38 (d, 3 H, *J* 6.5 Hz, CMe), 1.86 (s, 3 H, Ac), 3.28 and 3.32 (s, 2 × 3 H, 2 OMe), 5.44 (d, 1 H, *J* 3.5 Hz, H-1), 5.38 and 5.56 (s each, 2 H, benzylic H), 6.38 (d, 1 H, *J* 3.5 Hz, H-1″), and 6.8–8.4 (m, 33 H, aromatic protons).

Anal. Calc. for $C_{70}H_{73}NO_{18}$: C, 69.12, H, 6.05; N, 1.15. Found: C, 69.32, H, 6.25; N, 1.10.

Phenyl 2-acetamido-2-deoxy-4,6-O-(p-methoxybenzylidene)-3-O-[4,6-O-(p-methoxybenzylidene)-2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-α-D-galactopyranoside (**6**). — A solution of crystalline **5** (310 mg) in absolute methanol (21 mL) and dry benzene (7 mL) containing 3M sodium methoxide solution (2.5 mL) was kept overnight at room temperature, and then made neutral with acetic acid, and evaporated. A solution of the solid residue in chloroform (50 mL) was washed with water (2 × 10 mL), dried (anhydrous sodium sulfate), and evaporated to dryness. Crystallization of the residue from ethyl acetate–ether–hexane gave **6** (247 mg, 87%), m.p. 127–128°, $[\alpha]_D$ +14.4° (*c* 0.5, chloroform); ν_{max}^{KBr} 3410 (OH), 3310 (NH), 1660 (amide), and 755 and 700 cm^{-1} (aromatic); n.m.r. data ($CDCl_3$): δ 1.32 (d, 3 H, *J* 6.5 Hz, CMe), 1.76 (s, 3 H, Ac), 3.74 and 3.82 (s, 2 × 3 H, OMe), 5.54 and 5.58 (s each, 2 H, benzylic protons), 6.0 (d, 1 H, *J* 3.5 Hz, H-1″), and 6.7–7.7 (28 H, aromatic protons).

Anal. Calc. for $C_{63}H_{69}NO_{17}$: C, 68.03; H, 6.25; N, 1.26. Found: C, 67.89; H, 6.13; N, 1.25.

Phenyl O-*α*-L-*fucopyranosyl*-(1→2)-O-*β*-D-*galactopyranosyl*-(1→3)-*2-acetamido-2-deoxy-α*-D-*galactopyranoside* (**7**). — A solution of **6** (200 mg) in acetic acid (20 mL) was hydrogenolyzed in the presence of Pd/C (10%) for 2 days. The suspension was filtered, and the filtrate evaporated to dryness. Crystallization of the residue from ethanol–ether gave amorphous **7** (87 mg, 80%), $[\alpha]_D$ +49.4° (*c* 0.5, MeOH); ν_{max}^{KBr} 3350 (OH), 1640 (amide), 1600, and 760 and 690 (phenyl); n.m.r. data (CD_3OD): δ 1.37 (d, 3 H, *J* 6.5 Hz, CMe), 2.02 (s, 3 H, Ac), 5.68 (d, 1 H, *J* 3.5 Hz, H-1), and 6.9–7.5 (5 H, phenyl).

Anal. Calc. for $C_{26}H_{39}NO_{15}$: C, 51.56; H, 6.49; N, 2.31. Found: C, 51.42; H, 6.38; N, 2.30.

Assay mixtures for (1→2)-α-L-fucosidase from *Aspergillus niger* contained 0.01M acetate buffer (pH 4.0), 5μM α-L-Fuc-(1→2)-β-D-Gal-(1→3)-α-D-GalNAc-1→OPh, and enzyme. Mixtures containing enzyme, along with controls lacking enzyme or substrate, were incubated in a total volume of 50 μL for 1 h at 37°. Reactions were terminated by cooling to 4°. Assay mixtures and appropriate reference compounds were chromatographed on Whatman No. 1 paper, using either 4:1:1 (v/v) 1-butanol–ethanol–water or 3:2:1 (v/v) butyl acetate–acetic acid–water for 72 and 16 h, respectively. Compounds were detected with silver nitrate reagent[12] following periodate oxidation.

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