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SYNTHESIS OF *p*-NITROPHENYL 2-ACETAMIDO-2-DEOXY-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSIDES*

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ABSTRACT

Reaction of *p*-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-β-D-glucopyranoside (**2**) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**3**) under the usual conditions, followed by removal of the *p*-methoxybenzylidene group and *O*-deacylation, produced crystalline *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-glucopyranoside (**6**). Starting from *p*-nitrophenyl 2-acetamido 3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside, the synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-β-D-glucopyranoside was also accomplished.

INTRODUCTION

Recently, we reported the synthesis of various 1-thioaldopyranosides that may be employed as affinity adsorbents for glycosidases[1,2]. We have also initiated a program of obtaining synthetic sugar derivatives that may be useful for affinity column-chromatography of various glycosyltransferases present in human serum or other biological sources.

Two distinct, soluble, L-fucosyltransferases have been found to occur in normal, human serum. Schenkel-Brunner *et al.*[3] have shown the presence therein of an α-L-fucosyltransferase capable of transferring the L-fucosyl group from GDP-L-fucose, by an α-L-(1→3)-linkage, to the D-glucose residue of lactose [β-D-Gal*p*-(1→4)-D-Glc] and 2′-*O*-L-fucosyllactose [α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc], and to the 2-acetamido-2-deoxy-D-glucose residue of *N*-acetyllactosamine [β-D-Gal*p*-(1→4)-D-GlcNAc]; the last compound was found to be the best acceptor. Munro and Schachter[4] failed to demonstrate the presence of this enzyme with the aid of sugar derivatives of low molecular weight; however, they found that the L-fucosyltransferase, in fact, attaches an L-fucosyl group to the terminal 2-acetamido-2-deoxy-D-glucose residue of sialidase–β-D-galactosidase-treated α_1-acid glycoprotein, and

*Carbohydrate Derivatives for Affinity Chromatography. III. For Part II of the series, see Ref. 1.
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further suggested that the discrepancy may be due to the different conditions employed in the two studies. Nevertheless, the presence of a second, soluble, L-fucosyltransferase, whose level of activity is quite high compared to that of the other enzyme, was well documented in both studies. This enzyme attaches an α-L-fucosyl group by an α-L-(1→2)-glycosidic bond to a terminal β-D-galactopyranosyl group of macromolecular glycoproteins and of oligosaccharides of low molecular weight. Among the latter, *N*-acetyllactosamine and lacto-*N*-biose [β-D-Gal*p*-(1→3)-D-GlcNAc] have been found to be quite suitable acceptors for this α-L-fucosyltransferase.

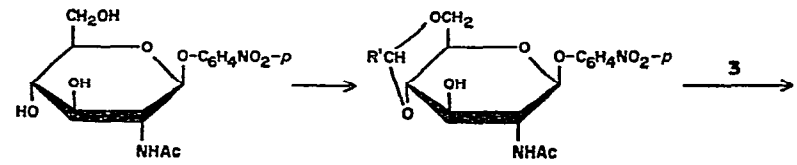
In the present study, a *p*-nitrophenyl lacto-*N*-bioside has been synthesized, as, on reduction, this compound may be linked to Sepharose for affinity chromatography of this enzyme. It may be mentioned that Bloch and Berger[5] reported a rapid procedure for derivatizing agarose with commercially available *p*-nitrophenyl glycosides for the affinity chromatography of various lectins. In the present investigation, the synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-6-*O*-β-D-galactopyranosyl-β-D-glucopyranoside has also been accomplished.

RESULTS AND DISCUSSION

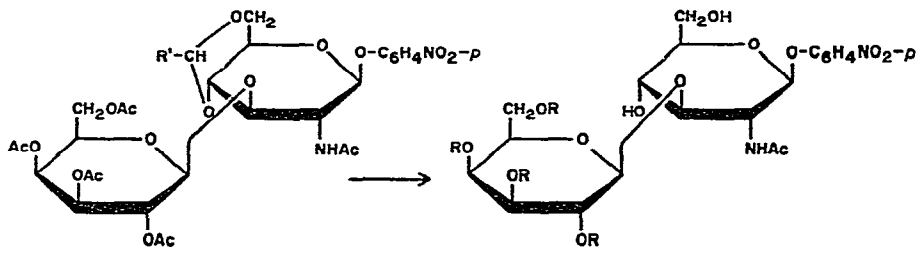
Alkyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranosides have been generally employed for the synthesis of 3-*O*-substituted derivatives of 2-acetamido-2-deoxy-D-glucose. As *p*-nitrophenyl glycosides are known to be quite acid-labile, cleavage of the glycosidic bond can be expected during the removal of the 4,6-*O*-benzylidene group. As a result, use of *p*-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-β-D-glucopyranoside (**2**) was preferred over that of the corresponding 4,6-*O*-benzylidene derivative[6], because the *p*-methoxybenzylidene group can be removed under mild conditions[7]. During our synthetic investigations, Yamamoto[8] reported an elegant synthesis of compound **2** by an acetal-exchange reaction with *p*-methoxybenzaldehyde dimethyl acetal. However, we have found that the desired "aglycon" **2** can be quite conveniently prepared by the direct reaction of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) with *p*-methoxybenzaldehyde in the presence of anhydrous zinc chloride, as already described for the synthesis of benzyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-β-D-glucopyranoside[9].

Condensation of compound **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**3**) was conducted in 1:1 nitromethane–benzene in the presence of mercuric cyanide, to give crystalline **4** in 76% yield. Treatment of **4** with aqueous acetic acid produced *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**5**) as crystalline material in 70% yield. On exposure to anhydrous ammonia in methanol, compound **5** gave the desired compound (**6**) in 65% yield.

The *p*-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside needed as the starting material for the synthesis of the 6-*O*-substituted disaccharide has previously been prepared[10,11] by removal of the trityl group from *p*-nitrophenyl

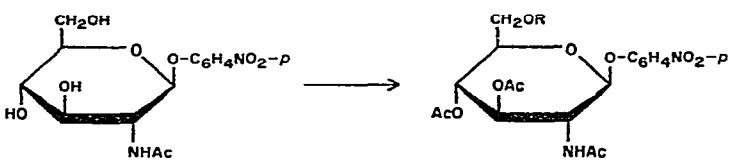


1 2, R' = C_6H_4OMe-*p*

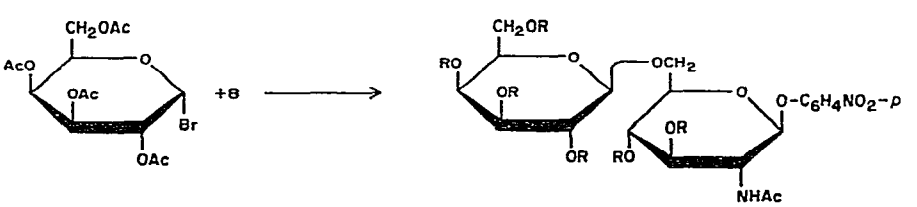


4 5 R = Ac
6 R = H

2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-β-D-glucopyranoside by treatment with aqueous acetic acid for 30 min at 100°. However, in the present study, compound **1** was treated with chloro(*p*-methoxyphenyl)diphenylmethane in pyridine, followed by acylation to give **7**, which, on treatment with aqueous acetic acid for 12 min at 95–100°, afforded the desired compound **8**. On reaction with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**3**) in anhydrous acetonitrile in the presence of mercuric cyanide[12], compound **8** furnished crystalline **9** in 72% yield. *O*-Deacylation of



1 7, R = C(Ph)(Ph)C_6H_4OMe-*p*
8, R = H



3 9, R = Ac
10, R = H

compound **9** gave the desired disaccharide (**10**), which was isolated in crystalline form in 72% yield. The synthesis of **10** is not time-consuming. However, use of mercuric cyanide in acetonitrile for the condensation of **3** with **2** was avoided, because of the low solubility of compound **2** in acetonitrile.

The optical rotation of all of the disaccharide derivatives isolated in the present study supported the β-D configuration for them.

EXPERIMENTAL

General. — Melting points were measured on a Fisher–Johns apparatus and are uncorrected. I.r. spectra were recorded for potassium bromide discs with a Perkin–Elmer Model 457 spectrophotometer. N.m.r. spectra were recorded with a Varian A-60 instrument. Ascending t.l.c. was conducted on plates coated with a 0.25-mm layer of silica gel HF-254 (Merck, Darmstadt); the components were located by exposure to iodine vapor. The solvents for t.l.c. were (*a*) 9:1 benzene–methanol, (*b*) 19:1 chloroform–ethanol, (*c*) 3:2 benzene–methanol, and (*d*) 7:5:2 propyl alcohol–ethyl acetate–water. Optical rotations were measured with a Perkin–Elmer polarimeter Model 141. Elementary analyses were performed by Robertson Laboratory, Florham Park, New Jersey.

p-*Nitrophenyl 2-acetamido-2-deoxy-4,6-*O-(p-*methoxybenzylidene*)-β-D-*glucopyranoside* (**2**). — A mixture of compound **1** (8.5 g), anhydrous zinc chloride (7.5 g), and *p*-methoxybenzaldehyde (100 ml) was shaken for 3 days at room temperature. The mixture was then washed with ether to remove the excess of *p*-methoxybenzaldehyde, filtered, and the solid washed with an excess of cold water and air dried. A solution of the solid in pyridine was poured into an excess of hot water, to give crystalline **2**; alternatively, it may be recrystallized from acetone–toluene; yield 7.0 g (61.2%); m.p. 240–241°, $[\alpha]_D^{24}$ −15.0° (*c* 1, *N,N*-dimethylformamide); lit.[8] m.p. 239–240°, $[\alpha]_D^{25}$ −14.5° (*c* 0.8, *N,N*-dimethylformamide).

p-*Nitrophenyl 2-acetamido-2-deoxy-4,6-*O-(p-*methoxybenzylidene*)-*3-*O-(*2,3,4,6-tetra-*O-*acetyl-β-D-galactopyranosyl*)-β-D-*glucopyranoside* (**4**). — A solution of compound **2** (2.3 g; 5 mmoles) in 1:1 nitromethane–benzene (540 ml) was boiled until ~100 ml of the solvent mixture had distilled off; the temperature was maintained at 50–54°, mercuric cyanide (1.25 g, 5 mmoles) and a solution of **3** (2.05 g, 5 mmoles) in anhydrous benzene (10 ml) were added, and the mixture was stirred for 24 h. The same amounts of mercuric cyanide and **3** were then added, and the mixture was stirred for a further 36 h at the same temperature, cooled, diluted with benzene (200 ml), and successively washed with a cold, saturated solution of sodium hydrogen carbonate and water (until neutral), and dried (sodium sulfate). The suspension was filtered, and the filtrate evaporated to a solid residue which was stirred with hot benzene (100 ml); the suspension was cooled to room temperature, diluted with pentane (100 ml), stirred for 30 min, and the solid collected by filtration and washed with 1:1 benzene–pentane (100 ml). The solid (3.9 g) was dissolved in hot, 1:1 ethanol–methanol (150 ml), and the solution was cooled; the material appeared as a gel, which

was filtered to give **4** (2.5 g). The mother liquor yielded an additional crop (0.5 g) of **4**; total yield, 75.9%; m.p. 196–198°, $[\alpha]_D^{24}$ −4.0° (*c* 1, chloroform); R_F 0.52 (solvent *a*) and 0.90 (solvent *b*); ν_{max}^{KBr} 3380 (NH), 1750 (ester), 1660 (Amide I), 1520 (s, NO_2 and Amide II), 1350 (NO_2), and 1610, 1595, 1500, and 700 cm^{-1} (aromatic); n.m.r. data ($CDCl_3$): τ 1.75–3.0 (m, 8 H, aromatic protons), 3.85 (doublet, *J* 8.0 Hz, NH), and 7.85–8.0 (4 OAc+NAc).

Anal. Calc. for $C_{36}H_{42}N_2O_{18}$: C, 54.68; H, 5.35; N, 3.54. Found: C, 54.42; H, 5.52; N, 3.44.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranoside* (**5**). — A suspension of compound **4** (1.0 g) in 90% acetic acid (100 ml) was stirred at room temperature; the clear solution obtained after 15 min was then heated for 2 min at 100°, allowed to cool to room temperature, and kept for 2 h. The acetic acid was then evaporated off under diminished pressure, and the last traces of acetic acid were removed by repeated co-distillation with toluene, to give a residue which was triturated with ether to remove free *p*-methoxybenzaldehyde. The white residue (0.9 g, 94.0%) thus obtained was crystallized from acetone–ether to give **5** (0.6 g, 70.5%), m.p. 204°, $[\alpha]_D^{26}$ −72° (*c* 1, chloroform); R_F 0.22 (solvent *a*) and 0.25 (solvent *b*); ν_{max}^{KBr} 3480 (OH), 3300 (NH), 1750 (ester), 1655 (Amide I), 1520 (Amide II, NO_2), 1350 (NO_2), and 1605, 1595, and 1500 cm^{-1} (aromatic); n.m.r. data (Me_2SO–D_2O): τ 1.75–2.85 (m, 4 H, $C_6H_4NO_2$) and 7.88–8.12 (4 OAc, NAc).

Anal. Calc. for $C_{28}H_{36}N_2O_{17}$: C, 49.99; H, 5.39; N, 4.16. Found: C, 49.76; H, 5.40; N, 4.10.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-*D*-galactopyranosyl-β-*D*-gluco-pyranoside* (**6**). — A solution of **5** (0.5 g) in anhydrous methanol (10 ml) was treated with methanolic ammonia (25%, 5 ml) for 24 h at 4°. The mixture (containing crystalline material) was evaporated to dryness, and the residue was recrystallized from absolute ethanol, to give **6**, yield 0.25 g (65%); m.p. 184–186°, $[\alpha]_D^{24}$ −14.0° (*c* 0.5, water); R_{Gal} 1.12 (solvent *c*) and 1.28 (solvent *d*); ν_{max}^{KBr} 3400–3230 (OH, NH), 1655 (Amide I), 1560 (Amide II), 1515, 1350 (NO_2), and 1605, 1595, and 1495 cm^{-1} (aromatic).

Anal. Calc. for $C_{20}H_{28}N_2O_{13}\cdot H_2O$: C, 45.97; H, 5.78; N, 5.36. Found: C, 46.35; H, 5.66; N, 5.34.

p-*Nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**8**). — A solution of **1** (10.0 g, 29.2 mmoles) in anhydrous pyridine (100 ml) was stirred with chloro(*p*-methoxyphenyl)diphenylmethane (11.0 g, 35.6 mmoles) for 3 days at room temperature. Acetic anhydride (60 ml) was then added, and the mixture was kept for 24 h at room temperature, and poured into ice-cold water (3 liters) with stirring. The solid was filtered off, washed with water, air dried, and recrystallized from chloro-form–ether to give **7** (20.0 g), m.p. 235–238° (dec.), $[\alpha]_D^{24}$ +12° (*c* 1, chloroform); R_F 0.61 (solvent *a*) and 0.77 (solvent *b*).

Compound **7** (5.0 g) was dissolved in hot acetic acid (80 ml), water (25 ml) was added, and the clear solution was heated for 12 min at 95–100°. The yellow solution was evaporated under diminished pressure, and traces of acetic acid were removed by

co-distillation with toluene. The solid residue was stirred with warm ether, the suspension filtered, and the solid washed with ether and recrystallized from methanol, to give **8** (2.2 g, 70.6% based upon **1**), m.p. 234–236° (dec.) [lit.[10] m.p. 241–242° (dec.); lit.[11] m.p. 235–236° (dec.)].

p-*Nitrophenyl 2-acetamido-3,4-di*-O-*acetyl-2-deoxy-6*-O-(*2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl*)-*β*-D-*glucopyranoside* (**9**). — A suspension of **8** (0.72 g, 1.69 mmoles) and mercuric cyanide (0.4 g) in anhydrous acetonitrile (70 ml, distilled over phosphorus pentaoxide) was stirred with **3** (1.25 g, 3.0 mmoles) for 1 h at 35°; the clear solution thus obtained was stirred for 3 h at room temperature, and evaporated, and the residue was stirred with chloroform (100 ml). The chloroform extract was filtered through glass wool, washed successively with M potassium bromide (3 × 100 ml), saturated sodium hydrogen carbonate (100 ml), and water (3 × 100 ml), and dried (sodium sulfate). The suspension was filtered, and the filtrate was evaporated to a solid which was recrystallized from absolute ethanol to give **9** (0.92 g, 72%), m.p. 177–179°, $[\alpha]_D^{24}$ −22.6° (*c* 1, chloroform); R_F 0.40 (solvent *a*) and 0.65 (solvent *b*); ν_{max}^{KBr} 3380 (NH), 1750 (ester), 1660 (Amide I), 1522 (Amide II and NO_2), 1350 (NO_2), and 1607, 1597, and 1500 cm^{-1} (aromatic); n.m.r. data ($CDCl_3$): τ 1.75, 1.8, 2.68, 2.8 (m, 4 H, $C_6H_4NO_2$), 4.08 (doublet, 1 H, NH), and 7.8–8.15 (21 H, 6 OAc, 1 NAc).

p-Nitrophenyl 2-acetamido-2-deoxy-6-O-*β*-D-*galactopyranosyl-β*-D-*gluco-pyranoside* (**10**). — A solution of compound **9** (0.6 g) in methanolic ammonia (25 ml) was kept overnight at 4°; the mixture (containing a few crystals) was evaporated to dryness, and the residue was recrystallized from ethanol to give **10** (0.3 g, 72.4%), m.p. 158–159°, $[\alpha]_D^{24}$ −27.3° (*c* 1, water), R_{Gal} 0.80 (solvent *c*) and 1.12 (solvent *d*); ν_{max}^{KBr} 3420–3320 (OH, NH), 1655 (Amide I), 1550 (Amide II), 1520, 1350 (NO_2), and 1600, 1597, and 1495 cm^{-1} (aromatic).

Anal. Calc. for $C_{20}H_{28}N_2O_{13}\cdot H_2O$: C, 45.97; H, 5.78; N, 5.36. Found: C, 46.32; H, 5.66; N, 5.34.

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