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SYNTHESIS OF *p*-NITROPHENYL 2-ACETAMIDO-2-DEOXY-3-*O*-β-D-GALACTOPYRANOSYL-D-GALACTOPYRANOSIDES*

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ABSTRACT

Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) with *p*-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-galactopyranoside (**3**) under the usual conditions, followed by removal of the *p*-methoxybenzylidene group and *O*-deacylation, gave crystalline *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranoside (**10**). The synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**11**) has also been accomplished by a similar reaction-sequence.

INTRODUCTION

The disaccharide 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose has been isolated from the acid hydrolyzates of A, B, H, and Le^a blood-group substances[2]. It has also been obtained through the partial hydrolysis of human-brain gangliosides[2]. In addition, the carbohydrate structure β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactosyl-(1→3)-L-threonine or -L-serine has been observed to be part of the carbohydrate moieties from mammalian fetuin[3] and porcine submaxillary mucin[4]; it may also be a part of the glycosidically linked carbohydrate units of IgG and IgA immunoglobulins, chorionic gonadotropin, and erythrocyte membrane protein[5]. Recently, the presence of this disaccharide unit has been established in the antifreeze glycoproteins isolated from certain antarctic fish[6]. Interestingly, it has also been observed that these antifreeze glycoproteins function as efficient acceptors for the soluble sialyltransferase enzymes present in rat-liver and rat-mammary adenocarcinoma[7]. However, it has been well documented that the sialyltransferase enzymes from different organs differ in their acceptor specificity[8,9]. Among the simple sugar derivatives used for studying their specificity, the disaccharide *N*-acetyllactosamine [β-D-Gal-*p*-(1→4)-D-GlcNAc] has been found to be the best acceptor for most of these soluble enzymes[8,9]. However, to the best of our

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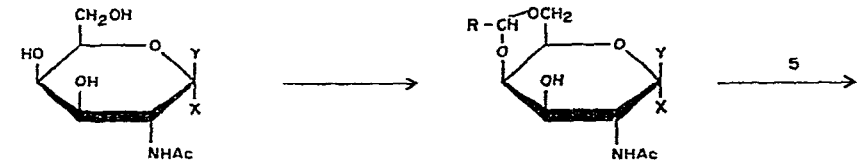
knowledge, 2-acetamido-2-deoxy-*O*-β-D-galactopyranosyl-D-galactose derivatives have not been employed in such studies. As the disaccharide 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactose is the essential part of antifreeze glycoproteins that act as acceptors for sialyltransferase enzymes, the synthetic investigations were directed toward the synthesis of its *p*-nitrophenyl derivative, because, on reduction, this compound may be linked to Sepharose for affinity chromatography of these enzymes. The synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside has been performed for similar reasons.

The complex, sugar derivatives containing the 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose residue have been found to be potential inhibitors for the phytohemagglutinin activities of two lectins isolated from the commercial mushroom *Agaricus bisporus*[10]. These observations suggest that the *p*-nitrophenyl disaccharides described here may prove to be suitable ligands for the purification of these lectins. Bloch and Burger[11] developed a rapid procedure for derivatizing agarose with commercially available *p*-nitrophenyl glycopyranosides for affinity chromatography of various lectins.

RESULTS AND DISCUSSION

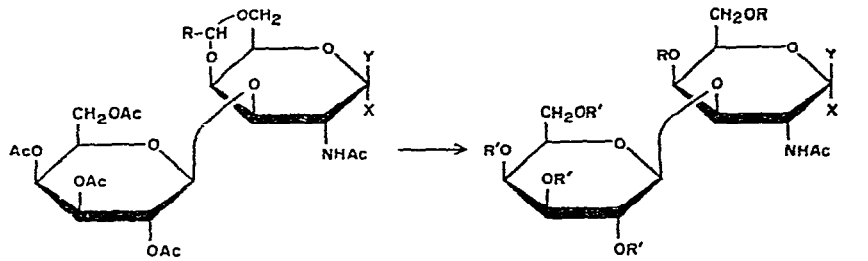
Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside, prepared from benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside, had been employed as one of the "aglycons" for the chemical synthesis of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose[12]. Starting from the *p*-nitrophenyl 2-acetamido-2-deoxy-α- and β-D-galactopyranosides (**1** and **2**), the synthesis of the desired compounds has been achieved by a similar reaction-sequence. However, compounds **1** and **2** were treated with *p*-methoxybenzaldehyde in the presence of anhydrous zinc chloride to give acetals **3** and **4**, respectively; methoxybenzylidene as the protecting group for HO-4 and -6 was preferred over the benzylidene group, because the former can be cleaved under mild conditions[13]. As *p*-nitrophenyl glycosides are known to be quite acid-labile, cleavage of the glycosidic bond could be expected under the reaction conditions generally employed for the removal of the 4,6-*O*-benzylidene group[14]. It may be mentioned that the starting material **2** was prepared by the reaction of crude 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride[15] with the sodium salt of *p*-nitrophenol in the presence of anhydrous *N*,*N*-dimethylformamide[16], followed by *O*-deacylation of the crystalline material thus obtained[17].

Reaction of compound **3** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) was conducted in 1:1 nitromethane–benzene in the presence of mercuric cyanide, to give **6** in 75 percent yield. Under similar conditions, crystalline derivative **7** was isolated from the reaction of compound **4** with **5**. Individual treatment of compounds **6** and **7** with aqueous acetic acid at room temperature provided derivatives **8** and **9**, respectively, which were isolated as crystalline compounds. Upon treatment with triethylamine and water, the partially protected disaccharide derivative **8** in methanol afforded crystalline *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galacto-



1 X = $OC_6H_4NO_2$-*p*, Y = H
2 X = H, Y = $OC_6H_4NO_2$-*p*

3 R = C_6H_4OMe-*p*, X = $OC_6H_4NO_2$-*p*, Y = H
4 R = C_6H_4OMe-*p*, X = H, Y = $OC_6H_4NO_2$-*p*



6 R = C_6H_4OMe-*p*, X = $OC_6H_4NO_2$-*p*, Y = H
7 R = C_6H_4OMe-*p*, X = H, Y = $OC_2H_4NO_2$-*p*

8 R = Y = H, R′ = Ac, X = $OC_6H_4NO_2$-*p*
9 R = X = H, R′ = Ac, Y = $OC_6H_4NO_2$-*p*
10 R = R′ = Y = H, X = $OC_6H_4NO_2$-*p*
11 R = R′ = X = H, Y = $OC_6H_4NO_2$-*p*

pyranosyl-α-D-galactopyranoside (**10**) in a yield of 71.4%. Alternatively, the disaccharide *p*-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**11**) was isolated when compound **9** was exposed to methanolic ammonia.

The optical rotation and t.l.c. behavior clearly distinguished compound **10** from **11**. The n.m.r. spectrum of **10** showed a clear doublet at τ 4.32 (1, H, *J* 3.5 Hz) due to the anomeric proton of the 2-acetamido-2-deoxy-α-D-galactoside residue, whereas the anomeric proton of the interglycosidic linkage was not clearly resolved. However, the n.m.r. spectrum of **11** showed two doublets, at τ 4.74 (1 H, *J* 8 Hz, due to the anomeric proton of the 2-acetamido-2-deoxy-β-D-galactoside residue) and 5.72 (1 H, *J* 6.5 Hz, for the anomeric proton of the intersugar linkage).

EXPERIMENTAL

Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 457 spectrophotometer. N.m.r. spectra were recorded with a Varian HA-100 spectrophotometer; each sample was dissolved in Me_2SO-d_6 (0.4–0.5 ml), deuterium oxide (2 drops) was added, and the solution was kept overnight at room temperature. The purity of the compounds was established by ascending, thin-layer chromatography (t.l.c.) conducted on plates coated with a 250-μm layer of silica gel HF-254 (Merck, Darmstadt). The components were located by exposure to iodine vapor. The solvents for t.l.c. of compounds **3**, **4**, and **6–9** were (*a*) 9:1 benzene–methanol, (*b*) 4:1 benzene–methanol,

and (*c*) 19:1 chloroform–ethanol, whereas t.l.c. of compounds **1**, **2**, **10**, and **11** was performed in solvents (*d*) 3:2 benzene–methanol and (*e*) 7:5:2 propyl alcohol–ethyl acetate–water. The elementary analyses were performed by Robertson Laboratory, Florham Park, New Jersey.

p-*Nitrophenyl 2-acetamido-2-deoxy-4,6*-O-(p-*methoxybenzylidene*)-α-D-*galacto-pyranoside* (**3**). — A mixture of *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-galacto-pyranoside[18] (0.65 g), anhydrous zinc chloride (0.5 g), and *p*-methoxybenzaldehyde (10 ml) was shaken for 60 h at room temperature, diluted with ether (100 ml), and then stirred with ice-cold water (100 ml). The solid material was filtered off, washed several times with cold water and ether, and air-dried; it was then dissolved in *N,N*-dimethylformamide, and the solution poured into an excess of hot water, to give crystalline **3**, yield 0.65 g (74.5%), m.p. 284–286° (dec.), $[\alpha]_D^{23}$ +137.6° (*c* 0.5, *N,N*-dimethylformamide); ν_{max}^{KBr} 3600 (OH), 3280 (NH), 1645 (Amide I), 1600, 1590 (Ph) 1550 (Amide II), 1515, 1435 (NO_2) 750, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{24}N_2O_9$: C, 57.39; H, 5.25; N, 6.08. Found: C, 57.68; H, 5.48; N, 6.17.

p-*Nitrophenyl 2-acetamido-2-deoxy-4,6*-O-(p-*methoxybenzylidene*)-β-D-*galacto-pyranoside* (**4**). — A solution of crude 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride[15] (9.9 g) and sodium *p*-nitrophenoxide (10.0 g) in anhydrous *N,N*-dimethylformamide (60 ml) was stirred for 16 h at room temperature, and then was poured into water (2 liters) and stirred for 2 h. The solid material was filtered off, washed several times with cold water, and air-dried (yield 4.0 g). Without further purification, a solution of the compound in anhydrous methanol (40 ml) was treated with a catalytic amount of sodium methoxide in methanol, to give chromatographically pure **2** (2.8 g), m.p. 206–207°, lit.[17] m.p. 205°.

A suspension of compound **2** (0.85 g), anhydrous zinc chloride (0.5 g), and *p*-methoxybenzaldehyde (10 ml) was shaken for 3 days at room temperature, and the mixture was processed as described for the preparation of **3**. A solution of the air-dried material in pyridine (30 ml) was poured into an excess of water, to give pure **4**, yield 0.7 g (61.2%), m.p. 228–230°, $[\alpha]_D^{23}$ −99.2° (*c* 0.5, *N,N*-dimethylformamide); ν_{max}^{KBr} 3480 (OH), 3240 (NH), 1650 (Amide I), 1605, 1595 (Ph), 1555 (Amide II), 1510, 1350 (NO_2), 750, and 700 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{24}N_2O_9$: C, 57.39; H, 5.25; N, 6.08. Found: C, 57.14; H, 5.25; N, 6.04.

p-*Nitrophenyl 2-acetamido-2-deoxy-4,6*-O-(p-*methoxybenzylidene*)-*3*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-α-D-*galactopyranoside* (**6**). — A solution of **3** (0.46 g, 1 mmole) in 1:1 nitromethane–benzene (120 ml) was boiled under reflux until ~30 ml of the solvent mixture had distilled off; while the temperature was maintained at 60–62°, mercuric cyanide (0.25 g, 1 mmole) and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) (0.41 g, 1 mmole) in anhydrous 1:1 nitromethane–benzene (5 ml) were added, and the mixture was stirred for 20 h. The same amounts of mercuric cyanide and **5** were then introduced, and the mixture was stirred for 40 h at the same temperature, cooled, diluted with benzene (150 ml), and suc-

cessively washed with a cold, saturated solution of sodium hydrogen carbonate and water (until neutral), and dried (sodium sulfate). The sodium sulfate was filtered off, and the filtrate evaporated to a syrup (1.6 g) which was dissolved in warm benzene (20 ml) and the solution diluted with pentane (50 ml), with vigorous stirring, to give a solid material. The suspension was kept for 2 h at room temperature, the solvent was decanted, and the residue was washed with 2:5 benzene–pentane (50 ml), and dried under vacuum, to give **6**, which was purified by recrystallization from ethanol–pentane. The compound was immediately dried under vacuum, yield 0.6 g, (75%), m.p. 143–145°, $[\alpha]_D^{23}$ +104.0° (*c* 1, chloroform); ν_{max}^{KBr} 3390 (NH), 1750 (ester), 1660 (Amide I), 1610, 1595 (Ph), 1545 (Amide II), 1515, 1345 (NO_2), 750, and 700 cm^{-1} (Ph).

Anal. Calc. for $C_{36}H_{42}N_2O_{18} \cdot H_2O$: C, 53.46; H, 5.48; N, 3.46. Found: C, 53.92 H, 5.70; N, 3.64.

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-*galactopyranoside* (**7**). — The reaction of compound **4** (0.69 g, 1.5 mmoles) in 1:1 nitromethane–benzene (80 ml) with bromide **5** (0.63 g, 1.5 mmoles) was performed at 64–68° in the presence of mercuric cyanide (0.37 g, 1.5 mmoles) as described for the preparation of **6**. Additional amounts of mercuric cyanide and bromide **5** were added after 20 h. The reaction was continued for a further 40 h, and the mixture processed as usual. The syrup obtained after evaporation of the solvents was treated with warm benzene (40 ml); on stirring with pentane, a solid material was produced. The solvent was decanted, and the slightly yellow material was recrystallized from ethanol, to give pure **7**, yield 0.7 g (58.3%), m.p. 147–149°, $[\alpha]_D^{23}$ −31.4° (*c* 1, chloroform); ν_{max}^{KBr} 3390 (NH), 1745 (ester), 1660 (Amide II), 1605, 1595 (Ph), 1545 (Amide II), 1510, 1345 (NO_2), 755, and 695 cm^{-1} (Ph).

Anal. Calc. for $C_{36}H_{42}N_2O_{18} \cdot H_2O$: C, 53.46; H, 5.48; N, 3.46; Found: C, 53.55; H, 5.21; N, 3.32.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-*O-(*2,3,4,6-tetra-*O-*acetyl-β-*D-*galacto-pyranosyl)-α-*D-*galactopyranoside* (**8**). — Compound **6** (0.45 g) was stirred with aqueous acetic acid (80%, 60 ml) for 22 h at room temperature. The solvent was evaporated, and traces of acetic acid were removed by repeated codistillation with toluene. The dry residue was triturated with anhydrous ether (50 ml) in order to remove the *p*-methoxybenzaldehyde, and the suspension was filtered to give a crystalline compound; this was recrystallized from acetone–ether–pentane to give pure **8**; yield 0.22 g (57.2%). m.p. 136–138°, $[\alpha]_D^{23}$ +142.8° (*c* 1, chloroform); ν_{max}^{KBr} 3510–3390 (broad, OH and NH), 1750 (ester), 1660 (Amide I), 1610, 1595 (Ph) 1550 (Amide II), 1520, 1350 (NO_2), 755, and 700 cm^{-1} (Ph); n.m.r. data (Me_2SO-d_6–D_2O): τ 1.72–2.72 (m, 4 H, $C_6H_4NO_2$), 4.42 (d, 1 H, *J* 3.5 Hz, H-1), 7.82, 7.93 (with a shoulder), 8.03 and 8.12 (15 H, 4 OAc and 1 NAc).

Anal. Calc. for $C_{28}H_{36}N_2O_{17} \cdot H_2O$: C, 48.69; H, 5.54; N, 4.05. Found: C, 48.85; H, 5.07; N, 4.07.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranoside* (**9**). — For the preparation of **9**, compound **7** (0.5 g) was stirred with aqueous acetic acid for 22 h at room temperature. The compound was isolated as described for **8**. Pure compound **9** was obtained upon recrystallization from acetone–ether; yield 0.26 g (60.8%), m.p. 155–157°, $[\alpha]_D^{23}$ +22.6° (*c* 1, chloroform); ν_{max}^{KBr} 3510–3380 (broad, OH and NH), 1745 (ester), 1665 (Amide I), 1610, 1590 (Ph), 1545 (Amide II), 1510, 1350 (NO_2), 750, and 695 cm^{-1} (Ph); n.m.r. data (Me_2SO-d_6–D_2O): τ 1.76–2.86 (m, 4 H, $C_6H_4NO_2$), 7.84, 7.96, 8.08, and 8.18 (15 H, 4 OAc and 1 NAc).

Anal. Calc. for $C_{28}H_{36}N_2O_{17}\cdot H_2O$: C, 48.69, H, 5.54; N, 4.05. Found: C, 48.81; H, 5.34; N, 4.12.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside* (**10**). — A solution of compound **8** (0.14 g) in methanol (2.5 ml) was treated with triethylamine (0.7 ml), followed by addition of water (0.5 ml); and the clear solution was kept at 4°. Crystals started to appear after 16 h. The mixture was kept for another 4 h at the same temperature, the solvents were distilled off, and toluene was added and evaporated. The white residue was dried under vacuum, and crystallized from methanol–ether, to give chromatographically pure **10**, yield 73 mg (71.4%), m.p. 225–228° (dec.), $[\alpha]_D^{23}$ +202.2° (*c* 0.5, water); ν_{max}^{KBr} 3480–3300 (broad, OH and NH), 1630 (Amide I), 1595 (Ph), 1550 (Amide II), 1510, 1340 (NO_2), 750, and 700 cm^{-1} (Ph); n.m.r. data (Me_2SO-d_6–D_2O): τ 1.72–2.75 (m, 4 H, $C_6H_4NO_2$), 4.32 (d, 1 H, *J* 3.5 Hz, H-1), and 8.15 (3 H, NAc).

Anal. Calc. for $C_{20}H_{28}N_2O_{13}$: C, 47.61; H, 5.66; N, 5.34. Found: C, 47.69; H, 5.90; N, 5.25.

p-*Nitrophenyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranoside* (**11**). — A solution of **9** (0.15 g) in anhydrous methanol (10 ml) was treated with methanolic ammonia (25%, 2 ml) for 24 h at 4°. The mixture (containing a few crystals) was evaporated to dryness, and the residue was recrystallized from methanol, to give chromatographically pure **11**, yield 75 mg (66%), m.p. 212–214°, $[\alpha]_D^{23}$ +10.2° (*c* 0.5, water); ν_{max}^{KBr} 3510–3220 (broad, OH and NH), 1630 (Amide I) 1595 (Ph), 1550 (Amide II), 1510, 1345 (NO_2), 752, and 695 cm^{-1} (Ph); n.m.r. data (Me_2SO-d_6–D_2O): τ 1.74–2.86 (m, 4 H, $C_6H_4NO_2$), 4.74 (d, 1 H, *J* 8 Hz, H-1), 5.72 (d, 1H, *J* 6.5 Hz, H-1), and 8.16 (3 H, NAc).

Anal. Calc. for $C_{20}H_{28}N_2O_{13}\cdot H_2O$: C, 45.97; H, 5.78; N, 5.36. Found: C, 45.83; H, 5.53; N, 5.29.

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