Carbohydrate Research, 183 (1988) 163–173
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHETIC MUCIN FRAGMENTS. BENZYL *O*-(2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSYL)-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-[(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→6)]-2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSIDE AND BENZYL *O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-(1→3)-*O*-β-D-GALACTOPYRANOSYL-(1→3)-*O*-[β-D-GALACTOPYRANOSYL-(1→6)]-2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSIDE*

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(Received July 20th, 1987; accepted for publication, November 19th, 1987)

ABSTRACT

Treatment of benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside with 4-methoxybenzaldehyde dimethyl acetal in *N,N*-dimethylformamide in the presence of 4-toluenesulfonic acid afforded the 4,6-*O*-(4-methoxybenzylidene) acetal, which was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**). Reductive ring-opening of the acetal group provided a 6-*O*-(4-methoxybenzyl) derivative (**4**) which was glycosylated with **1**, followed by removal of the 4-methoxybenzyl ether group, to give benzyl 2-acetamido-2-deoxy-3,4-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**7**). The disaccharide diol **5**, obtained from **4**, and benzyl *O*-(2-acetamido-3,4,6-tri-*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 (**11**) were similarly glycosylated with **1** to afford a trisaccharide derivative **9** and a tetrasaccharide derivative **14**, respectively. Diol **11** was also condensed with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline to give a tetrasaccharide derivative **16**. *O*-Deacetylation of trisaccharides **7** and **9**, and tetrasaccharides **14** and **16** furnished trisaccharides **8** and **10**, and the title tetrasaccharides **15** and **17**, respectively. The structures of compounds **8, 10, 15**, and **17** were established by ^{13}C-n.m.r. spectroscopy.

*Synthetic Studies in Carbohydrates, Part LX. For Part LIX, see ref. 1. This investigation was supported by Grants No. CA-36021 and CA-35329, from the National Cancer Institute, U.S. Public Health Service.

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0008-6215/88/$ 03.50

INTRODUCTION

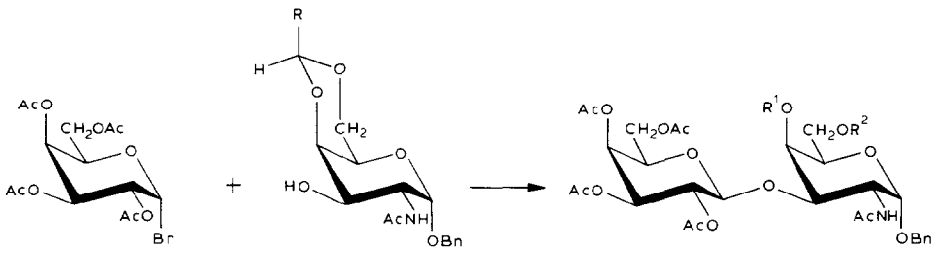
In many glycoproteins, particularly the mucins and blood-group substances, the oligosaccharide chains are *O*-glycosylically linked to a polypeptide backbone, through a 2-acetamido-2-deoxy-α-D-galactopyranosyl residue to serine, or threonine, or both. These oligosaccharides vary greatly in size and complexity, and contain three main regions, *i.e.*, the core, the backbone, and the peripheral regions[2]. The structures of the carbohydrate components of various *O*-glycosylically linked glycoconjugates have been well documented[3]. It has been postulated that the biosynthesis of such glycoconjugates is catalyzed by a variety of glycosyltransferases[3,4]. Moreover, it has become increasingly evident that well-defined substrates (and reference compounds) can provide an insight into these biosynthetic pathways. For example, it was shown that the synthetic trisaccharide, β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn[5], acts as an acceptor for an *N*-acetyl-β-D-glucosaminyltransferase (from porcine gastric mucosa) to give a tetrasaccharide having a 2-acetamido-2-deoxy-β-D-glucopyranosyl group that was, presumably, (1→6)- or (1→3)-linked to the β-D-galactopyranosyl residue[6]. It was, therefore, of interest to obtain both the (1→6)- and (1→3)-linked isomeric tetrasaccharides expected by such an enzymic reaction. For this purpose, we previously described the synthesis of the (1→6)-linked tetrasaccharide[5], and we now describe that of its (1→3)-linked isomer, *i.e.*, β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-α-D-Gal*p*NAcOBn, which was subsequently found to be identical with the aforementioned enzymic product.

More recently, Slomiany *et al.*[7] reported the isolation, from human gastric mucin, of oligosaccharides containing the tetrasaccharide β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→6)]-D-GalNAc. It is noteworthy that the occurrence of oligosaccharides containing the β-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→6)]-D-GalNAc sequence in gastric or other mucin-type glycoproteins was not reported theretofore. Thus it seemed of interest to investigate the biosynthetic pathway leading to this tetrasaccharide. Hence, we synthesized the trisaccharide β-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→6)]-α-D-Gal*p*NAcOBn for use as a substrate for (1→3)-*N*-acetyl-β-D-glucosaminyltransferase, and also the title tetrasaccharide **14**, for use as a reference compound. Additionally, we describe herein the synthesis of the trisaccharide β-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→4)]-α-D-Gal*p*NAcOBn. The parent compound of the latter trisaccharide was previously synthesized with the intention of being employed as an acceptor-substrate for sialyltransferase[8]. However, its benzyl α-D-glycoside we now describe may prove to be a better acceptor for the same enzyme, because of the similarity of configuration with the naturally occurring substrate.

RESULTS AND DISCUSSION

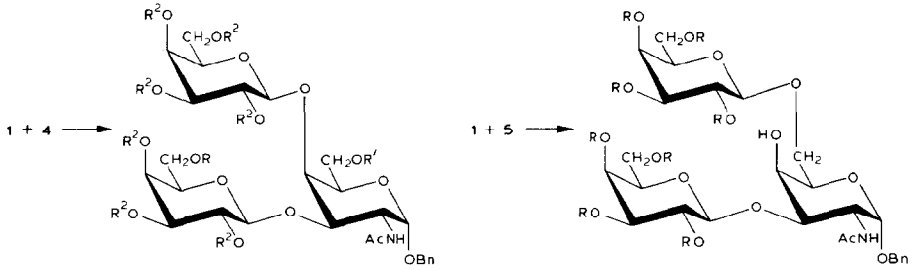
Benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside (**2**) was obtained from known[10] benzyl 2-acetamido-2-deoxy-α-D-

galactopyranoside by treatment with 4-methoxybenzaldehyde dimethyl acetal in *N,N*-dimethylformamide in the presence of 4-toluenesulfonic acid. Glycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) afforded the fully protected disaccharide **3**, which, on reductive ring-opening[11] of its 4,6-acetal group, furnished the 6-*O*-(4-methoxybenzyl) derivative **4**. The ^{1}H-n.m.r. spectra of both **3** and **4** were consistent with their expected overall structures (see Experimental section).



1 2 R = C_6H_4OMe-(4) 3 R^1, R^2 = CHC_6H_4OMe-(4) 4 R^1 = H, R^2 = $CH_2C_6H_4OMe$-(4) 5 R^1 = R^2 = H

Condensation of **4** with bromide **1** in dichloromethane and in the presence of silver trifluoromethanesulfonate and 1,1,3,3-tetramethylurea gave, in 52% yield, a trisaccharide **6**, whose 4-methoxybenzyl group was readily cleaved by treatment with ceric ammonium nitrate in aqueous acetonitrile[11] to give alcohol **7**. *O*-Deacetylation of **7** in methanolic sodium methoxide then gave the desired trisaccharide **7**, the ^{13}C-n.m.r. spectrum of which (see Table I) contained three low-field carbon atom resonances (δ 97.97, 104.98, and 106.26), in support of one α-D and two β-D configurations at the anomeric centers. The signals for C-3 and C-4 were both shifted downfield, and were observed at δ 77.62 and 76.35, respectively, indicating that both carbon atoms were sites of glycosylation.



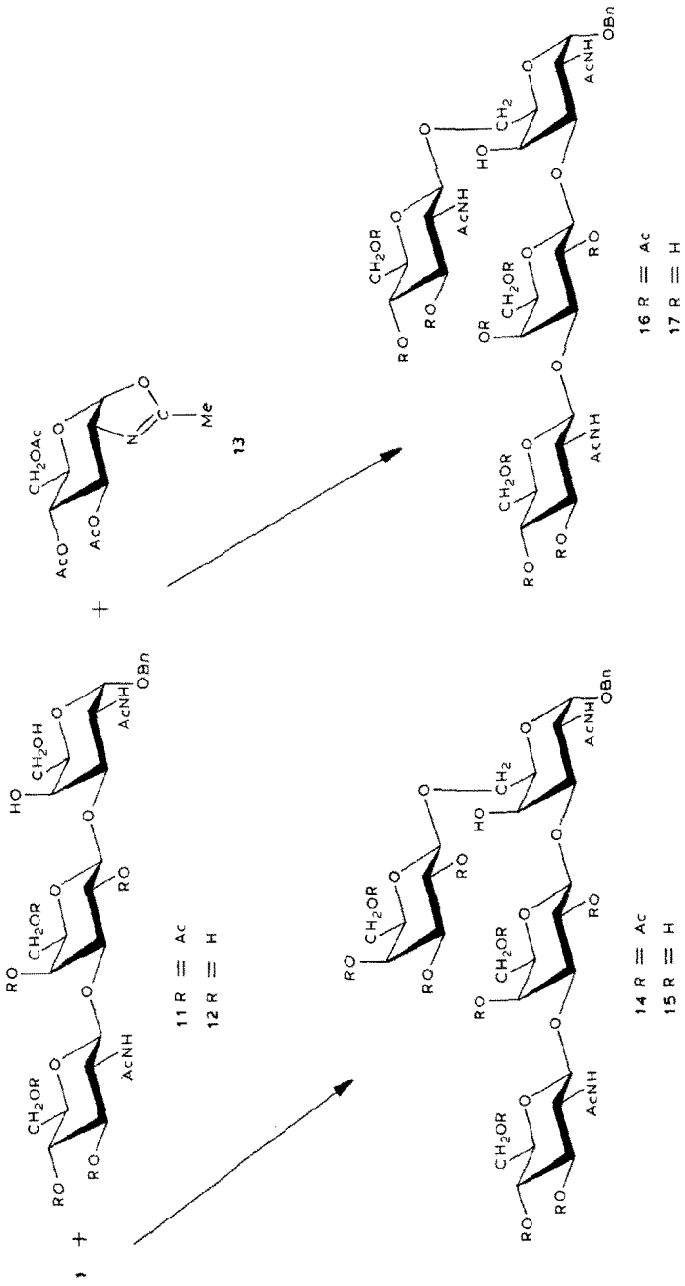
6 R^1 = $CH_2C_6H_4OMe$-(4), R^2 = Ac 7 R^1 = H, R^2 = Ac 8 R^1 = R^2 = H 9 R = Ac 10 R = H

TABLE I

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

Residue or Group	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_3CO	$CH_2C_6H_5$
α-D-Gal*p*NAcOBn	**7**	97.97	50.84	77.62	76.35	71.53	61.40	22.67	71.53
β-D-Gal*p*-(1→3)		104.98	72.82	74.41[b]	70.25	76.59	62.59		
β-D-Gal*p*-(1→4)		106.26	72.21	74.67[b]	70.25	76.59	62.59		
α-D-Gal*p*NAcOBn	**10**	96.10	48.30	75.41	67.48	67.96	69.94	22.54	69.97
β-D-Gal*p*-(1→3)		103.51	70.64	73.23[c]	67.96	75.01	60.31[d]		
β-D-Gal*p*-(1→6)		103.51	70.39	73.30[c]	67.96	75.21	60.24[d]		
α-D-Gal*p*NAcOBn	**12**	96.25	48.31	75.51	67.26	71.33	60.54	22.55	67.86
β-D-Gal*p*-(1→3)		103.12	69.66	81.90	67.01	74.03	60.80		
β-D-Glc*p*NAc-(1→3)		101.66	56.39	74.82	70.36	76.60	60.33	22.98	
α-D-Gal*p*NAcOBn	**14**	96.62	48.51	75.66	67.93	70.77	68.34	22.66	68.34
β-D-Gal*p*-(1→3)		103.13	70.03	82.28	66.78	73.69	60.62		
β-D-Glc*p*NAc-(1→3)		102.13	56.68	74.95	70.77	76.96	61.15	23.09	
β-D-Gal*p*NAc-(1→6)		103.98	70.30	73.32	67.42	74.75	60.62		
α-D-Gal*p*NAcOBn	**17**	96.10	48.30	75.38	67.62	69.53	68.80	22.54	68.80
β-D-Gal*p*-(1→3)		103.12	69.68	81.84	67.20	74.01	60.32		
β-D-Glc*p*NAc-(1→3)		101.65	56.32	74.92	70.39	76.78	60.86	22.94	
β-D-Glc*p*NAc-(1→6)		101.65	55.07	74.01	70.53	76.70	60.86	22.95	

[a]For solutions in di($^2H_3$)methyl sulfoxide, with Me_4Si as the internal reference; except for **7**, where the solvent was CD_3OD. Carbonyl and aromatic resonances not shown. [b,c,d]Values with similar superscripts may be interchanged.


11 R = Ac
12 R = H
13
14 R = Ac
15 R = H
16 R = Ac
17 R = H

Glycosylation of benzyl 2-acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside[5] (**5**) with bromide **1** in 1:1 benzene–nitromethane [catalyzed by $Hg(CN)_2$], and *O*-deacetylation of **9** so obtained, afforded the final trisaccharide **10**, whose ^{13}C-n.m.r. spectrum was, also, in accord with the structure assigned (see Table I). On similar condensation with bromide **1**, benzyl *O*-(2-acetamido-3,4,6-tri-*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[12] (**11**) gave, in ~74% yield, a tetrasaccharide derivative **14**. Diol **11** was also condensed with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**13**) in 1,2-dichloroethane and in the presence of 4-toluenesulfonic acid to afford, in 65% yield, tetrasaccharide **16**. *O*-Deacetylation of **14** and **16** furnished the title tetrasaccharides **15** and **17**, respectively, whose ^{13}C-n.m.r. spectra were in full accord with the structures assigned (see Table I).

^{13}C-N.m.r. assignments. — The assignments of the ^{13}C-n.m.r. resonances for compounds **14** and **17** were made by comparison of their spectra with each other, as well as with that of benzyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside[12] (**12**). In the ^{13}C-n.m.r. spectrum of **14**, the presence of four anomeric carbon-atom resonances at δ 96.62, 102.13, 103.13, and 103.98 was indicative of one α-D and three β-D configurations at the glycosidic linkages. The ^{13}C-n.m.r. spectrum of **16** also showed one α-D and three β-D anomeric carbon-atom resonances; but, by contrast to that of **14**, two of the β-D anomeric carbon-atoms co-resonated at δ 101.65 (see Table I). The signals for C-6 in the ^{13}C-n.m.r. spectra of both **14** and **17** were shifted downfield, and occurred at δ 68.34 and 68.80, respectively, a clear indication that both carbon atoms were glycosidated. By the same token, both C-3 of the β-D-Gal*p*-(1→3) residues of compounds **14** and **17** were, also, observed at low-field at δ 82.28 and 81.84, respectively.

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 thin-layer chromatography (t.l.c.) was conducted on aluminum sheets precoated with a 0.2-mm layer of Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany); the components were located either by exposure to u.v. light or by spraying the plate 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*) 6:1 chloroform–acetone and (*B*) 20:1 chloroform–methanol. N.m.r. spectra were recorded at ~25°, ^1H-n.m.r. spectra with a Varian EM-390 instrument operating at 90 MHz, and ^{13}C-n.m.r. spectra with a Varian XL-100 instrument operating at 25.2 MHz in the F.t. mode; the positions of the peaks (δ) are indicated from the Me_4Si signal. Nitromethane was distilled from P_2O_5 immediately before being used, and benzene was

dried with Na. 4-Toluenesulfonic acid, employed as a catalyst in glycosylation, was an ~0.02M solution in 1,2-dichloroethane. $Ce(NH_4)(NO_3)_3$ was purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, 29 Samson Avenue, Madison, New Jersey, U.S.A.

Benzyl 2-acetamido-2-deoxy-4,6-O-(4-methoxybenzylidene)-α-D-galactopyranoside (**2**). — A mixture of benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside[10] (4 g), 4-methoxybenzaldehyde dimethyl acetal (4 mL), and 4-toluenesulfonic acid (40 mg) in dry *N,N*-dimethylformamide (30 mL) was stirred for 4 h at ~40°. The acid was neutralized with a few drops of triethylamine and the solution was evaporated to give a solid, which was dissolved in a fresh portion of *N,N*-dimethylformamide, and the solution slowly poured into water. The precipitate was filtered off and thoroughly washed with water to afford **2** (4.5 g, 83%), $[\alpha]_D^{26}$ +130° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–6.85 (2 d, *J* 9 Hz, and s, 9 H, arom.), 5.45 (s, 3 H, C*H*C$_6$H$_4$OMe), 3.75 (s, 3 H, OMe), and 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{23}H_{27}NO_7$: C, 64.31; H, 6.35; N, 3.26. Found: C, 64.05; H, 6.13; N, 3.16.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-O-(4-methoxybenzylidene)-α-D-galactopyranoside (**3**). — A stirred solution of **2** (3.2 g, 7.4 mmol) in 1:1 (v/v) benzene–nitromethane (150 mL) was boiled until ~50 mL of the solvent had been distilled. It was then cooled to room temperature, and $Hg(CN)_2$ (2.5 g, 9.7 mmol) and bromide **1** (4.0 g, 9.7 mmol) were added, and stirring continued for 18 h at room temperature. After processing in the usual manner[13], the residue was purified in a column of silica gel with 10:1 (v/v) chloroform–acetone as the eluent to give a white solid, which was dissolved in ethyl acetate. Addition of ether–hexane precipitated amorphous **3** (4.7 g, 83%) $[\alpha]_D^{26}$ +95.5° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–6.70 (m, 9 H, arom.), 5.65 (d, 1 H, NH), 5.35 (d, 1 H, *J* 3 Hz, H-1), 5.15 (d, 1 H, *J* 7 Hz, H-1′), 3.75 (s, 3 H, OMe), and 2.1–1.85 (s, 15 H, 4 OAc and NAc).

Anal. Calc. for $C_{37}H_{45}NO_{16}$: C, 58.49; H, 5.97; N, 1.84. Found: C, 58.23; H, 5.87; N, 1.80.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-O-(4-methoxybenzyl)-α-D-galactopyranoside (**4**). — A mixture of **3** (1.0 g, 1.3 mmol) and sodium cyanoborohydride (0.4 g, 6.6 mmol) in anhydrous *N,N*-dimethylformamide (8 mL) containing crushed 3A molecular sieves (1.0 g) was stirred for 0.5 h at room temperature. Trifluoroacetic acid (1 mL) in anhydrous *N,N*-dimethylformamide (6 mL) was added, and the mixture was stirred for ~40 h at room temperature. T.l.c. (4:1, v/v, chloroform–acetone) then showed the presence of a major product, slower-migrating than **3**; some unchanged **3** was also revealed by t.l.c. The acid was neutralized with triethylamine (1 mL), and the mixture was diluted with chloroform (100 mL) and successively washed with ice-cold water, cold saturated $NaHCO_3$, and water, dried, and concentrated to a small volume, and the concentrate was applied to a column of silica gel. Elution with

10:1 (v/v) chloroform–acetone, and evaporation of the fractions corresponding to the starting material, afforded **3** (0.3 g). On elution with solvent *A*, evaporation of the fraction corresponding to the major product gave a solid residue, which was dissolved in ethyl acetate. Addition of ether–hexane caused the crystallization of **4** (0.6 g, 60%); m.p. 146–147°, $[\alpha]_D^{26}$ +63° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–6.80 (m, 9 H, arom.), 5.45 (d, 1 H, NH), 5.3 (d, 1 H, *J* 3 Hz, H-1), 3.75 (s, 3 H, OMe), and 2.10–1.90 (s, 15 H, 4 OAc, NAc).

Anal. Calc. for $C_{37}H_{47}NO_{16}$: C, 58.34; H, 6.22; N, 1.84. Found: C, 58.46; H, 6.33; N, 1.80.

*Benzyl 2-acetamido-2-deoxy-6-O-(4-methoxybenzyl)-3,4-di-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**6**). — A mixture of **4** (0.2 g, 0.26 mmol), silver trifluoromethanesulfonate (0.15 g), 1,1,3,3-tetramethyl urea (0.16 mL), and crushed 3A molecular sieves (0.5 g) in dichloromethane (20 mL), protected from light and moisture, was stirred for 1 h at room temperature in an atmosphere of N_2. Bromide **1** (0.22 g, 0.53 mmol) was then added, and the mixture was stirred overnight at room temperature. T.l.c. [3:1 (v/v) chloroform–acetone] revealed the disappearance of **1** and the presence of a major product, faster-migrating than **4**; some unchanged **4**, as well as some slower-migrating contaminants (presumably resulting from the decomposition of **1**) were also revealed. More portions of **1** (0.22 g), 1,1,3,3-tetramethylurea (0.16 mL), and silver trifluoromethanesulfonate (0.15 g) were added and the stirring continued for 24 h at room temperature. After customary processing, crude **6** was purified in a column of silica gel with 10:1 and then 6:1 (v/v) chloroform–acetone as the eluent to give a solid residue, which was dissolved in a small volume of ethyl acetate. Addition of ether caused the crystallization of **6** (0.15 g, 52%), m.p. 183–185°, $[\alpha]_D^{26}$ +36° (*c* 0.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–6.80 (m, 9 H, arom.), 3.75 (s, 3 H, OMe), and 2.10–1.90 (s, 27 H, 8 OAc and NAc).

Anal. Calc. for $C_{51}H_{65}NO_{25}$: C, 56.45; H, 6.06; N, 1.37. Found: C, 56.09; H, 5.96; N, 1.28.

*Benzyl 2-acetamido-2-deoxy-3,4-di-O-(2,3,4,6-tetra-O-acetyl-β-*D*-galacto-pyranosyl)-α-*D*-galactopyranoside* (**7**). — Compound **6** (0.12 g, 0.11 mmol) in 9:1 (v/v) acetonitrile–water (5 mL) was treated with $Ce(NH_4)(NO_3)_3$ (0.12 g, 0.22 mmol), in 9:1 (v/v) acetonitrile–water (5 mL), and the mixture stirred for 3 h at room temperature. It was then diluted with dichloromethane (75 mL), and washed with water, $NaHSO_3$, water, $NaHCO_3$, and water, and evaporated, and the residue was dissolved in a small volume of ethyl acetate. Addition of ether caused the crystallization of **7** (80 mg, 74%), m.p. 196–199°, $[\alpha]_D^{26}$ +50° (*c* 0.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (s, 5 H, arom.), and 2.20–1.85 (s, 27 H, 8 OAc and NAc).

Anal. Calc. for $C_{43}H_{57}NO_{24}\cdot 2\,H_2O$: C, 51.24; H, 6.11; N, 1.39. Found: C, 51.10; H, 5.98; N, 1.38.

*Benzyl 2-acetamido-2-deoxy-3,4-di-O-β-*D*-galactopyranosyl-α-*D*-galacto-pyranoside* (**8**). — Compound **7** (50 mg) in 0.1M methanolic sodium methoxide (30

mL) was stirred for 2 h at room temperature. After de-ionization with Amberlite IR-120 (H^+) cation-exchange resin, the solvent was concentrated to a small volume, and the concentrate applied to a column of silica gel. On elution with 3:1 (v/v) chloroform–methanol, evaporation of the fractions corresponding to the product afforded amorphous **8** (25 mg, 77%), $[\alpha]_D^{26}$ +53° (*c* 0.9, methanol); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{27}H_{41}NO_{16}$: C, 51.02; H, 6.50; N, 2.20. Found: C, 50.95; H, 6.28; N, 1.99.

*Benzyl 2-acetamido-2-deoxy-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-α-*D*-galactopyranoside* (**9**). — A stirred solution of diol **5** (ref. 5), (1.25 g, 1.95 mmol) in 1:1 (v/v) nitromethane–benzene (150 mL) was boiled until ~50 mL of the solvent mixture had distilled off. After cooling to room temperature, bromide **1** (1.1 g, 2.6 mmol) and powdered $Hg(CN)_2$ (0.35 g) were added, and the stirring continued for 24 h at room temperature. After customary processing, the crude product mixture was subjected to column chromatography on silica gel using 4:1 (v/v) chloroform–acetone as the eluent. On evaporation, the fractions corresponding to the major product gave a solid material, which was dissolved in a small volume of ethyl acetate. Addition of ether–hexane precipitated **9** (1.5 g, 80%), amorphous, $[\alpha]_D^{26}$ +41° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (m, 5 H, arom.), 2.20–2.00 (s, 24 H, 8 OAc), and 1.70 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{57}NO_{24}$: C, 53.09; H, 5.91; N, 1.44. Found: C, 52.75; H, 6.06; N, 1.28.

*Benzyl 2-acetamido-2-deoxy-3,6-di-*O*-β-*D*-galactopyranosyl-α-*D*-galacto-pyranoside* (**10**). — Compound **9** (1.5 g, 1.6 mmol) was suspended in 0.1M methanolic sodium methoxide (80 mL) and stirred at room temperature. The suspended **9** rapidly dissolved and, in ~0.5 h, crystallization ensued. The stirring was continued overnight at room temperature, the base neutralized by the addition of a few drops of glacial acetic acid, and the mixture refrigated for 1 h. The crystalline material was filtered off, and thoroughly washed with cold ethanol to afford **10** (0.85 g, 91%), m.p. 234–238°, $[\alpha]_D^{26}$ +81° [*c* 1.3, 1:1 (v/v) methanol–water]; for ^{13}C-n.m.r., data see Table I.

Anal. Calc. for $C_{27}H_{41}NO_{16}\cdot 2.5\ H_2O$: C, 47.64; H, 6.81; N, 2.06. Found: C, 47.71; H, 6.58; N, 2.13.

Benzyl O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→3)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→6)]-2-acetamido-2-deoxy-α-*D*-galactopyranoside* (**14**). — A solution of diol[12] **11** (0.5 g, 0.54 mmol) in 1:1 (v/v) benzene–nitromethane (100 mL) was boiled until 50 mL of the solvent had been distilled off. The temperature was then adjusted to 40°, and $Hg(CN)_2$ (0.18 g, 0.73 mmol) and bromide **1** (0.30 g, 0.73 mmol) were added. After the usual processing[13], the crude product was purified, in a column of silica gel, by use of 20:1 (v/v) chloroform–methanol as eluent to afford **14** (0.5 g, 74%), amorphous, $[\alpha]_D^{26}$ +45° (*c* 1.3, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40 (m, 5 H, arom.), 5.70 (d, 1 H, NH), 2.15–1.95 (s, 30 H, 10 OAc), 1.90 and 1.70 (s, 6 H, 2 NAc).

Anal. Calc. for $C_{55}H_{74}N_2O_{31}$: C, 52.46; H, 5.92; N, 2.23. Found: C, 52.50; H, 6.13; N, 2.05.

Benzyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→3)-O-[β-D-galactopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranoside (**15**). — A suspension of **14** (0.29 g, 0.23 mmol) in 0.1M methanolic sodium methoxide (20 mL) was stirred at room temperature. The suspended **14** rapidly dissolved, and, within 5 min, crystallization ensued. The mixture was stirred for a further 48 h at room temperature, and the base was neutralized by addition of a few drops of glacial acetic acid. The mixture was refrigerated for 1 h, and the crystalline material filtered off and washed thoroughly with cold ethanol to afford pure **15** (0.17 g, 88%), m.p. 276–279°, $[\alpha]_D^{26}$ +7° (*c* 1.0, dimethyl sulfoxide); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{35}H_{54}N_2O_{21}\cdot H_2O$: C, 48.44; H, 6.47; N, 3.32. Found: C, 48.72; H, 6.59; N, 3.12.

Benzyl O-(2-acetamido3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→3)-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranoside (**16**). — A mixture of trisaccharide diol **11** (0.86 g, 1.0 mmol), oxazoline **13**, (1.3 g), and 4-toluenesulfonic acid (15.2 mg) in 1,2-dichloroethane (20 mL), protected from moisture, was heated for 24 h at ~70°, in an atmosphere of N_2. The mixture was cooled, the acid neutralized by the addition of a few drops of pyridine, and the solution evaporated to dryness. The crude mixture was purified by column chromatography on silica gel with 19:1 (v/v) chloroform–methanol as eluent to afford **16**, amorphous (0.84 g, 65%), $[\alpha]_D^{26}$ +28° (*c* 1.1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (m, 5 H, arom.) and 2.15–1.80 (s, 36 H, 9 OAc and 3 NAc).

Anal. Calc. for $C_{55}H_{25}N_3O_{30}$: C, 52.49; H, 6.02; N, 3.34. Found: C, 52.69; H, 6.29; N, 3.14.

Benzyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-β-D-galactopyranosyl-(1→3)-O-[(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranoside (**17**). — A suspension of **16** (0.4 g) in 0.5M sodium methoxide in methanol (20 mL) was stirred at room temperature. The suspended **16** gradually dissolved with concomitant precipitation of the *O*-deacetylated product. The stirring was continued for 24 h at room temperature, the base neutralized with a few drops of glacial acetic acid, and the mixture refrigerated for 0.5 h. The crystalline material was filtered off, and thoroughly washed with cold ethanol, to furnish **16** (0.1 g, 75%), m.p. >300°, $[\alpha]_D^{26}$ +37° (*c* 0.4, dimethyl sulfoxide); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{37}H_{57}N_3O_{21}\cdot H_2O$: C, 49.49; H, 6.62; N, 4.68. Found: C, 49.41; H, 6.52; N, 4.30.

ACKNOWLEDGMENTS

The authors thank C. F. Piskorz for his technical assistance, and Mrs. Onda D. Simmons for recording the ^{13}C-n.m.r. spectra. The n.m.r. studies were supported by National Cancer Institute Core Grant CA-16056.

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