Carbohydrate Research, 182 (1988) 290–296
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthetic mucin fragments. Synthesis of a tetra- and two penta-saccharides containing the *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-D-galactopyranose ("lacto-N-triose 1") unit*

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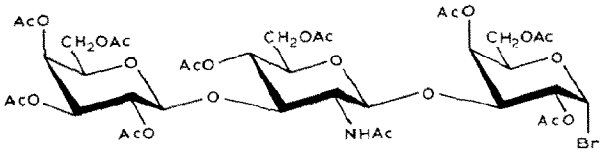
(Received October 13th, 1987; accepted for publication, January 6th, 1988)

We have previously[2] described the synthesis and use, as a glycosyl donor, of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(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 (**1**). Since then, bromide **1** has proved to be a useful and versatile glycosyl donor for the synthesis of a variety of mucin-type, higher oligosaccharide fragments. As an illustration of this use, we herein describe the synthesis of benzyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-



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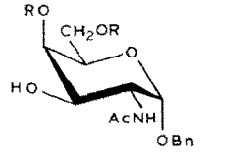
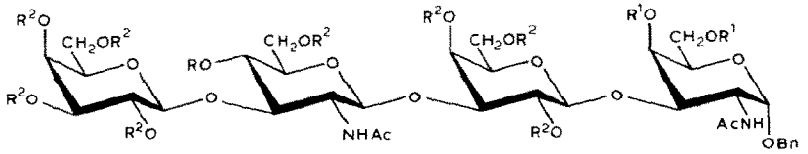


2 R, R = CHC_6H_4OMe-4



3 R^1, R^1 = CHC_6H_4OMe-4, R^1 = Ac
4 R^1 = H, R^2 = Ac
5 R^1 = R^2 = H

*Synthetic Studies in Carbohydrates, Part LXIII. For Part LXII, see ref. 1. This investigation was supported by Grant No. CA-35329 from the National Cancer Institute, U.S. Public Health Service.
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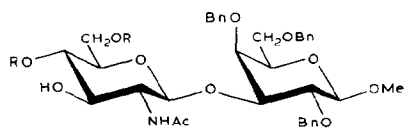
0008-6215/88/$ 03.50

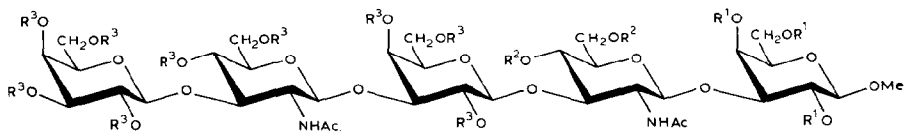
O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**5**), methyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (**9**), and benzyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(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 (**13**). The parent saccharides of **5, 9,** and **13** occur as parts of the oligosaccharide components of mucin-type glycoproteins[3]. Such oligosaccharides can play an important role in specificity studies of glycosidases[4] and glycosyltransferases[5], and also in inhibition studies of antibodies raised against structurally-related oligosaccharides[6].

Glycosylation of benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside[7] (**2**) with bromide **1** in 1:1 benzene–nitromethane at ~35°, and in the presence of powdered mercuric cyanide gave a crude product mixture (containing **3**) which was not separated, but directly treated with hot, 60% aqueous acetic acid to afford, after column-chromatographic purification, tetrasaccharide diol **4** in 76% yield. It is noteworthy that conducting this Helferich-type glycosylation at a lower (~35°) temperature represents a noticeable improvement over procedures previously employed for glycosylations with a related disaccharide bromide[8–10]. Under those conditions [*i.e.*, $Hg(CN)_2$ in boiling benzene, or in 1:1 benzene–nitromethane at 50–55°], relatively rapid decomposition of the glycosyl bromide was observed, resulting in reduced yields of the desired oligosaccharides. *O*-Deacetylation of **4** in 0.25M methanolic sodium methoxide afforded, in 87% yield, the title tetrasaccharide **5** as a trihydrate, the ^{13}C-n.m.r. spectrum of which was in accord with the structure assigned (see Table I).

A similar glycosylation with bromide **1** of methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[11] (**6**) gave a crude product mixture (containing **7**), which was subjected to



6 R, R = PhCH



7 R^1 = Bn; R^2, R^2 = PhCH, R^3 = Ac
8 R^1 = Bn; R^2, R^2 = PhCH, R^3 = H
9 R^1 = R^2 = R^3 = H

TABLE I

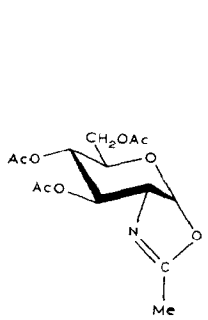
PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a,b]

Residue or group	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NAc*
α-D-Gal*p*NAcOBn	**5**	96.71	48.61	75.91	67.58	71.61	60.68	22.69
β-D-Gal*p*-(1→3)		103.64	69.98	82.29	67.43	75.19	60.85	
β-D-Glc*p*NAc-(1→3)		101.96	55.14	84.59	68.84	76.45	60.68	23.11
β-D-Gal*p*-(1→3)		104.07	70.78	73.01	68.27	75.91	60.85	
α-D-Gal*p*NAcOBn	**13**	96.18	48.29	75.46	67.70	69.54	68.98	22.54
β-D-Gal*p*-(1→3) (*2*)		103.29	69.70	81.97	67.16	74.98	60.43	
β-D-Glc*p*NAc-(1→3) (*3*)		101.44	54.77	84.32	68.52	76.16	60.66	22.96
β-D-Gal*p*-(1→3) (*4*)		103.71	70.47	72.73	68.06	75.62	60.43	
β-D-Glc*p*NAc-(1→6) (*2'*)		101.69	55.13	74.07	76.60	76.83	60.98	22.96
β-D-Gal*p*OMe	**9**	103.97	69.32	82.23	67.15	74.80	60.31	
β-D-Glc*p*NAc-(1→3)		101.53	54.80	84.42	68.47	76.19	60.99	23.06
β-D-Gal*p*-(1→3)		103.25	69.54	81.45	67.15	74.80	60.31	
β-D-Glc*p*NAc-(1→3)		101.64	54.80	84.31	68.13	76.19	60.49	23.06
β-D-Gal*p*-(1→3)		103.73	70.52	72.73	67.27	75.67	60.72	

[a]For solutions in di($^2H_3$)methyl sulfoxide, with Me_4Si as the internal standard. [b]Carbonyl, aromatic, $CH_2C_6H_5$, and OCH_3 resonances are not shown.

Zemplén transesterification, without prior isolation, to afford a pentasaccharide derivative **8**. Compound **8** was hydrogenolyzed in glacial acetic acid in the presence of 10% palladium-on-carbon to give, in 69% yield, the desired pentasaccharide **9**, the ^{13}C-n.m.r. spectrum of which was also in agreement with the structure assigned (see Table I).

Condensation of tetrasaccharide diol **4** with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**10**) in 1,2-dichloroethane in the presence of *p*-toluenesulfonic acid monohydrate, followed by column chromatography on silica gel, gave **11**, which was contaminated (t.l.c. with solvent *B*) with some faster-migrating impurities. Therefore, it was directly acetylated in 1:2 (v/v) acetic anhydride–pyridine to afford analytically pure, amorphous pentasaccharide peracetate **12** in 21% yield (based on **4**). *O*-Deacetylation of **12** in methanolic sodium methoxide then gave, in 89% yield, the title pentasaccharide **13**, the ^{13}C-n.m.r. spectrum of which exhibited all the signals expected in support of its structure (see Table I).



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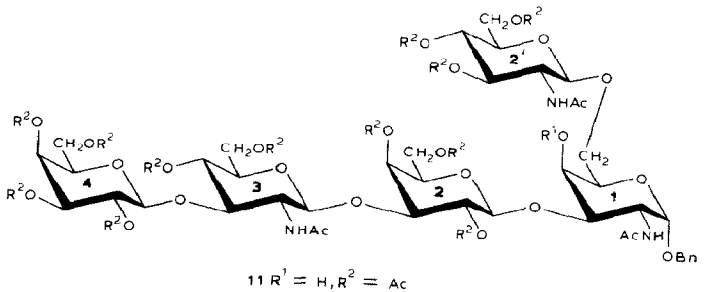

11 $R^1 = H, R^2 = Ac$
12 $R^1 = R^2 = Ac$
13 $R^1 = R^2 = H$

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~25° with a Perkin–Elmer 241 polarimeter. 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). Unless otherwise indicated, the solvent systems used for chromatography were (v/v): (*A*) 3% methanol in chloroform, (*B*) 7% methanol in chloroform. ^{13}C-N.m.r. spectra were recorded, at ~25°, at either 25.2 or 100.6 MHz with a Varian XL-100 or a Bruker AM-400 instrument, respectively. The positions of the peaks (δ) are indicated from the Me_4Si signal. 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 O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-4,6-di*-O-*acetyl-2-deoxy-β*-D-*galactopyranosyl)-(1→3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-α*-D-*galactopyranoside* (**4**). — A stirred mixture of benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside[7] (**2**; 0.7 g, 0.9 mmol) and $Hg(CN)_2$ (0.7 g, 1.2 mmol) in 1:1 benzene–nitromethane (320 mL) was boiled until ~140 mL of the solvent had distilled off. The temperature was then adjusted to ~35° and *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(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[2] (**1**; 3.1 g, 1.2 mmol) was added, and the stirring continued overnight at 35°. The mixture was then cooled, diluted with benzene, and filtered. The filtrate was successively washed with saturated aqueous $NaHCO_3$, 10% aqueous KI solution, and water, dried, and evaporated to a syrup, which showed in t.l.c. (3:2, v/v, chloroform–acetone) the disappearance of **2** and the presence of a major product, slower-migrating than **2**; some marginally slower-migrating contaminants (presumably due to the decomposition of **1**) were also revealed in t.l.c.

The crude product mixture (~3.6 g; containing **3**) was taken up in 60% aqueous acetic acid (50 mL) and heated for 30 min at ~70°. The acetic acid was evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene, and the residue was applied to a column of silica gel. On elution with solvent *A*, evaporation of the fractions corresponding to the product afforded **4** (1.5 g, 76%), a white amorphous solid; $[\alpha]_D^{25}$ +58° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (s, 5 H, arom.), and 2.20–1.80 (cluster of s, 33 H, 9 OAc and 2 NAc).

Anal. Calc. for $C_{53}H_{72}N_2O_{30}$: C, 52.29; H, 5.97; N, 2.30. Found: C, 52.11; H, 6.14; N, 2.12.

Benzyl O-*β*-D-*galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-β*-D-*gluco-*

pyranosyl)-(1→3)-O-β-D-*galactopyranosyl-(1→3)-2-acetamido-2-deoxy*-α-D-*galactopyranoside* (**5**). — Compound **4** (0.5 g) was suspended in 0.25M methanolic sodium methoxide (40 mL) and stirred at room temperature. The suspended **4** gradually dissolved, with concomitant precipitation of the deacetylated product. The stirring was continued for 2 days at room temperature, the base was neutralized with a few drops of glacial acetic acid, and the mixture was refrigerated overnight. The solid material was filtered off and thoroughly washed with cold ethanol to afford **5** (0.3 g, 87%), $[\alpha]_D^{26}$ +65° (*c* 0.8, water); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{35}H_{54}N_2O_{21} \cdot 3\ H_2O$: C, 47.07; H, 6.79; N, 3.14. Found: C, 47.08; H, 6.99; N, 2.84.

Methyl O-β-D-*galactopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1→3)*-O-β-D-*galactopyranosyl-(1→3)*-O-*(2-acetamido-4,6-O-benzylidene-2-deoxy*-β-D-*glucopyranosyl)-(1→3)-2,4,6-tri*-O-*benzyl*-β-D-*galactopyranoside* (**8**). — Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside[11] (**6**; 1.3 g, 1.7 mmol) was condensed with trisaccharide bromide **1** (2.4 g, 2.4 mmol) in a manner analogous to that described for **2** (to give **3**). After being processed in the usual manner, the product mixture (~2.5 g; containing **7**) was taken up in 0.25M methanolic sodium methoxide (100 mL) and stirred for 2 days at room temperature. The base was neutralized with a few drops of glacial acetic acid, the mixture was refrigerated for 2 h, and the crystalline material was filtered off and thoroughly washed with cold methanol to afford **8** (1.7 g, 77%); $[\alpha]_D^{25}$ −27° (*c* 0.3, dimethyl sulfoxide).

Anal. Calc. for $C_{63}H_{82}N_2O_{26} \cdot 1.5\ H_2O$: C, 57.74; H, 6.55; N, 2.14. Found: C, 57.67; H, 6.30; N, 1.87.

Methyl O-β-D-*galactopyranosyl-(1→3)*-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→3)*-β-D-*galactopyranoside* (**9**). — A mixture of **8** (1.4 g) and 10% Pd–C (1.4 g) in glacial acetic acid (50 mL) was shaken under H_2 at ~345 kPa for 3 days at room temperature. The suspension was filtered through a bed of Celite, the solids were thoroughly washed with 1:2 (v/v) methanol–water, and the filtrate and washings were combined and evaporated. The residue so obtained was purified in a column of silica gel with 3:2:2 (v/v) ethyl acetate–2-propanol–water as the eluent to furnish **9** (0.7 g, 69%), $[\alpha]_D^{26}$ −2.4° (*c* 1.1, water); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{35}H_{60}N_2O_{26} \cdot 2\ H_2O$: C, 43.74; H, 6.73; N, 2.92. Found: C, 44.09; H, 6.69; N, 2.64.

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-4,6-di*-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-4*-O-*acetyl-2-deoxy*-α-D-*galactopyranoside* (**12**). — A mixture of **4** (0.6 g, 1 mmol), oxazoline **10** (0.66 g, 2 mmol), and *p*-toluenesulfonic acid monohydrate (38 mg) in 1,2-dichloroethane (21 mL), protected from moisture, was heated with stirring in an atmosphere of N_2 for 24 h at ~72°; additional amounts of **10** (0.33 g, 1 mmol in 7 mL of 1,2-dichloroethane) and *p*-toluenesulfonic

acid (19 mg in 7 mL of 1,2-dichloroethane) being added after 16 h. The mixture was cooled, the acid was neutralized by the addition of a few drops of pyridine, and the solution was evaporated to dryness to give a residue, which was applied to a column of silica gel. Elution with 5% methanol in chloroform, and evaporation of the fractions containing the product gave a material (0.3 g, containing mainly **11**), but contaminated (t.l.c., solvent *B*) with traces of some faster-migrating impurities. This was mixed with 1:2 acetic anhydride–pyridine (15 mL) and stirred overnight at room temperature. The acetic anhydride and pyridine were then evaporated under diminished pressure, the last traces being removed by coevaporation with several portions of toluene. Examination by t.l.c. in 9:1 chloroform–methanol revealed the presence of one major product together with some faster- and some slower-migrating contaminants. The crude product was purified in a column of silica gel with solvent *A* as the eluent to give a solid residue which was dissolved in dichloromethane. Addition of ether–hexane caused the precipitation of **12** (0.16 g, 21%, based on **4**), a white powder, $[\alpha]_D^{26}$ +38° (*c* 1.2, chloroform).

Anal. Calc. for $C_{69}H_{93}N_3O_{39}$: C, 52.16; H, 5.91; N, 2.65. Found: C, 51.85; H, 6.03; N, 2.48.

Benzyl O-β-D-*galactopyranosyl-(1→3)*-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* (**13**). — Compound **12** (0.15 g) was *O*-deacetylated in 0.25M methanolic sodium methoxide, exactly as described for **4** (to give **5**) to afford **13** (90 mg, 89%), amorphous, $[\alpha]_D^{25}$ +39.8° (*c* 1.0, water); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{43}H_{67}N_3O_{26}\cdot 1.5\ H_2O$: C, 48.30; H, 6.61; N, 3.93. Found: C, 48.13; H, 6.49; N, 3.61.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Conrad F. Piskorz for his valuable technical assistance, and to Dr. Krishna Pamidimukkala and Dr. Roger Laine of the Biochemistry Department, Louisiana State University, Baton Rouge, for recording most of the ^{13}C-n.m.r. spectra.

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