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

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ABSTRACT

Benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**2**) was obtained by acetalation of its parent disaccharide with 2,2-dimethoxypropane in hot *N*,*N*-dimethylformamide and in the presence of 4-toluenesulfonic acid. Glycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (catalyzed by mercuric cyanide), followed by removal of the protecting groups afforded the title trisaccharide **7**. A second product was also isolated, which was identified as a derivative of **7** having a 2-cyanopropyl group. Glycosylation of diacetal **2** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (under catalysis by bromide ion), followed by systematic removal of the protecting groups furnished the title trisaccharide **13**. The structures of both **7** and **13** were established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

It has been generally accepted[2,3] that the core structures of the complex oligosaccharides that occur as parts of mucinous-type glycoproteins comprise four distinct classes, namely, β-D-Gal*p*-(1→3)-D-GalNAc, β-D-Gal*p*-(1→3)-[β-D-Glc*p*NAc-(1→6)]-D-GalNAc, β-D-Glc*p*NAc-(1→3)-D-GalNAc, and β-D-Glc*p*NAc-(1→3)-[β-D-Glc*p*NAc-(1→6)]-D-GalNAc. However, Slomiany *et al.*[4] reported the isolation, from human gastric mucin, of oligosaccharides containing the carbohydrate sequence β-D-Gal*p*-(1→3)-[β-D-Gal*p*-(1→6)]-α-D-GalNAc, suggesting the

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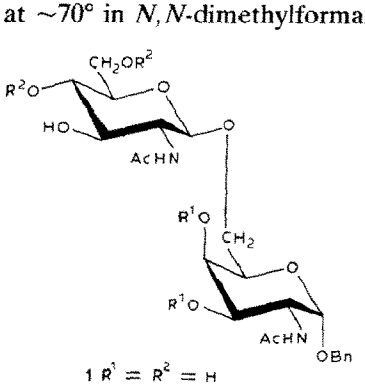
presence of a different core structure which was theretofore not known to occur in this type of glycoconjugates. Also, there are strong suggestions that yet another type of core structure that is devoid of the β-(1→3)-linked glycosyl group (D-Gal or D-GlcNAc) does exist. β-D-Glc*p*NAc-(1→6)-GalNAc is usually found as a branch point in the presence of an existing substituent at C-3. However, this unique sequence has been isolated from a variety of sources, *e.g.*, human seminal plasma mucin[5], human meconium[6], and human K-casein[7]. The possibility thus arises that such a sequence may be specifically synthesized by a glycosyltransferase that does not require the presence of a C-3 substituent, and is hitherto unidentified.

On our part, we have been involved in the chemical synthesis of a variety of mucin-type oligosaccharide fragments[8], some of which occur as parts of the aforementioned core structures[8–10]. In continuation of these efforts, we describe herein the synthesis of the title trisaccharides.

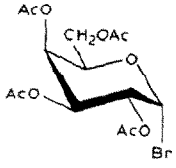
RESULTS AND DISCUSSION

Treatment of benzyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-2-deoxy-α-D-galactopyranoside[9] (**1**) with 2,2-dimethoxypropane for 3 h at ~70° in *N,N*-dimethylformamide and in the presence of 4-toluenesulfonic acid



1 $R^1 = R^2 = H$

2 $R^1, R^1 = R^2$; $R^2 = CMe_2$



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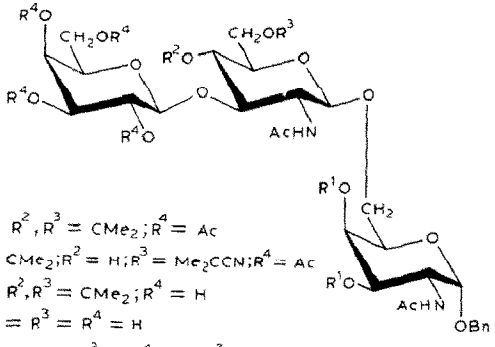


4 $R^1, R^1 = R^2, R^3 = CMe_2; R^4 = Ac$

5 $R^1, R^1 = CMe_2; R^2 = H; R^3 = Me_2CCN; R^4 = Ac$

6 $R^1, R^1 = R^2, R^3 = CMe_2; R^4 = H$

7 $R^1 = R^2 = R^3 = R^4 = H$

8 $R^1, R^1 = CMe_2; R^2 = R^4 = H; R^3 = Me_2CCN$

9 $R^1 = R^2 = R^4 = H; R^3 = Me_2CCN$

afforded, in 68% yield, the 3,4:4′,6′-di-*O*-isopropylidene acetal (**2**), the ^{1}H-n.m.r. spectrum of which contained signals in support of its overall structure (see Experimental section). On glycosylation of **2** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**3**), in 1:1 benzene–nitromethane in the presence of mercuric cyanide, examination of the product mixture by t.l.c. revealed the presence of a major product, faster-migrating than **2**; an appreciable proportion of a compound, migrating slightly slower than the major product, was also revealed by t.l.c. Purification of the reaction mixture in a column of silica gel with solvent *B* as the eluent gave, in 41% yield, a crystalline trisaccharide **4**, the ^{1}H-n.m.r. spectrum of which was in conformity with the overall structure expected. Thus, a low-field signal (δ 7.30) confirmed the presence of the benzyloxy group, whereas the acetyl-group methyl protons were observed as a cluster of singlets at δ 2.15–1.85, and the acetal-group methyl protons occurred as singlets at δ 1.50–1.25. Continued elution of the column with solvent *B* afforded, in 32% yield, another product that was tentatively identified as trisaccharide **5** on the basis of its ^{1}H-n.m.r. spectrum. The ^{1}H-n.m.r. spectra of both **4** and **5** closely resembled each other, except in the region of the acetal-group methyl protons (see Experimental section). Thus, whereas the ^{1}H-n.m.r. spectrum of **4** contained four distinct singlets (δ 1.50–1.20, 3 H each) attributable to the two acetal-groups methyl protons, that of **5** contained two singlets (δ 1.40 and 1.25, 3 H each) and a singlet (δ 1.55, 6 H).

O-Deacetylation of **4** in methanolic sodium methoxide afforded **6**, the ^{13}C-n.m.r. spectrum of which exhibited three carbon-atom resonances at δ 98.05, 103.97, and 104.38, in agreement with one α and two β configurations at the glycosidic linkages. The acetal-group methyl protons occurred as two pairs of signals at δ 29.43 and 19.47 (*i.e.*, separated by 9.96 p.p.m.) and at δ 28.52 and 26.89

TABLE I

PARTIAL ASSIGNMENTS OF SOME ^{13}C-N.M.R. RESONANCES[a]

Residue or group	*Compound*	*C-1*	*C-6*	$(CH_3)_2CO$	$C(CH_3)_2$
3,4-*O*-Isopropylidene-α-D-Gal*p*-NAcOBn	**2**	97.62	68.43	110.72	26.78 28.38
4,6-*O*-Isopropylidene-β-D-Glc*p*-(1→6)		103.80	62.99	100.60	19.36 29.40
3,4-*O*-Isopropylidene-α-D-Gal*p*NAcOBn	**6**	98.05	68.45	110.76	26.89 28.52
4,6-*O*-Isopropylidene-β-D-Glc*p*NAc-(1→6)		103.97	63.24	101.40	19.47 29.43
β-D-Gal*p*-(1→3)		104.38	62.45		
3,4-*O*-Isopropylidene-α-D-Gal*p*NAcOBn	**8**[b]	95.86	66.38	108.09	25.79 27.97
6-*O*-Cyanopropyl-β-D-GlcNAcOBn		101.14	65.05		26.29
β-D-Gal*p*-(1→3)		103.68	60.41		

[a]For solution in CD_3OD, with Me_4Si as the internal standard; except **8** where the solvent was $(CD_3)_2SO$.
[b]Additional assignment: δ 83.71 (C-3′), 70.49 [$(CH_3)_2CCN$]; and 120.60 (CN).

TABLE II

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	**1**[b]	96.04	49.55	67.00	67.47	69.34	68.56	22.51	68.28
β-D-Glc*p*NAc-(1→6)		101.49	55.08	74.06	70.53	76.77	60.92	22.93	
α-D-Gal*p*NAcOBn	**7**	96.15	49.78	67.00	67.65	69.49	68.51	22.52	68.31
β-D-Glc*p*NAc-(1→6)		101.00	54.04	84.22	68.66	76.33	60.46	22.91	
β-D-Gal*p*-(1→3)		103.65	70.51	72.91	68.07	75.67	60.75		
α-D-Gal*p*NAcOBn	**9**[c]	96.49	49.93	67.21	67.89	69.83	68.80	22.58	68.30
6-*O*-Cyanopropyl-β-D-Glc*p*NAc-(1→6)		101.38	54.15	84.24	69.11	74.32	65.30	22.92	
β-D-Gal*p*-(1→3)		104.14	70.63	73.77	68.61	75.93	60.65		
α-D-Gal*p*NAc	**13**	90.86	51.68	67.86	69.64	70.63	68.94	22.47	
β-D-Glc*p*NAc-(1→6)		100.72	54.45	81.72	68.04	76.47	60.82	22.90	
α-L-Fuc*p*-(1→3)		99.64	68.04	69.64	71.51	66.37	16.25		

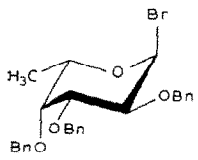
[a]For solution in $(CD_3)_2SO$ with Me_4Si as the internal standard. Carbonyl and aromatic resonances are not shown. [b]The chemical shifts for this compound[9] are included for comparison purposes. [c]Additional assignments: δ 25.85, 26.40 $(CH_3)_2C$, 70.73 $(C_3H_3)_2C(OR)CN$, and 120.67 (CN).

(*i.e.*, separated by 1.63 p.p.m.), in conformity with the presence of 3,4- and 4,6-isopropylidene acetal rings, respectively[11]. This was supported by the occurrence of the acetal carbon-atom resonance at δ 110.76 for the former, and at δ 101.40 for the latter[11] (see Table I).

O-Deacetylation of **5** in methanolic sodium methoxide furnished a partially protected trisaccharide **8**. In the ^{13}C-n.m.r. spectrum of **8**, a low field signal at δ 95.86 (attributable to C-1) supported the presence of the benzyloxy group in α configuration, whereas the signals at δ 101.14 and 103.68 indicated the presence of two β configurations at the interglycosidic linkages. The signal at δ 83.71, which could reasonably be assigned to C-3′, was indicative of O-3′ being a site of glycosylation. A single carbon-atom resonance occurred at δ 60.41, suggesting that the two remaining C-6 atoms were substituted (one of these being a site of glycosylation). The presence of a 3,4-acetal ring was accounted for by two carbon-atom resonances at δ 27.97 and 26.41 for the methyl group, and one carbon-atom resonance at δ 108.09 for the acetal carbon-atom. Interestingly, no carbon-atom resonance in the region of δ 100 (for the acetal carbon-atom) was observed in the ^{13}C-n.m.r. spectrum of **8**. However, two methyl group carbon-atom resonances were observed at δ 26.29 and 25.79, suggesting the presence of two magnetically nonequivalent methyl groups. A low-field carbon-atom resonance (δ 120.60) attested for the presence of a cyano group[12]*. Thus, it could be gathered that the substituent on C-6′ was a 2-cyanopropyl group, apparently resulting from a cyanide-induced ring-opening of the 4,6-*O*-isopropylidene group of trisaccharide **4**.

Treatment of **6** with hot, 60% aqueous acetic acid furnished the desired **7**, the ^{13}C-n.m.r. spectrum of which was in agreement with the structure expected. The three low-field carbon-atom resonances (δ 96.15, 101.00, and 103.65) were evidence of one α and two β configurations. The signals for C-6 and C-3′ were both shifted downfield, and were observed at δ 68.51 and 84.22, respectively, clearly indicating that both O-6 and O-3′ were glycosylated (see Table II). On similar treatment with hot, 60% aqueous acetic acid, **8** furnished a trisaccharide **9**, the ^{13}C-n.m.r. spectrum of which contained three carbon-atom resonances at δ 104.14, 101.38, and 96.49, in accord with two β and one α configurations, respectively. Glycosylation of O-6 and O-3′ was also evidenced by the downfield shifts of the resonances attributable to C-6 (δ 68.80) and C-3′ (δ 84.24). A low-field resonance δ 65.30), tentatively assigned to C-6′, suggested that O-6′ was presumably alkylated. That the alkyl substituent was a 2-cyanopropyl group was indicated by a low-field resonance (δ 120.67) attributable to the CN group, and a high-field resonance (δ 70.73) that could reasonably be assigned to C-2 of the alkyl group. Two methyl group carbon-atom resonances were also observed at δ 26.40 and 25.85 in the ^{13}C-n.m.r. spectrum of **9**, suggesting the presence of two magnetically nonequivalent methyl groups. However, no acetal carbon-atom resonance was ob-

*Also, the ^{13}C-n.m.r. spectrum of 2-cyano-2-propanol (acetone cyanohydrin) in $[(^2H_3)Me]_2SO$ contained the following signals: δ 123.80 (CN), 64.64 (>CMe_2), and 29.23 (Me_2).



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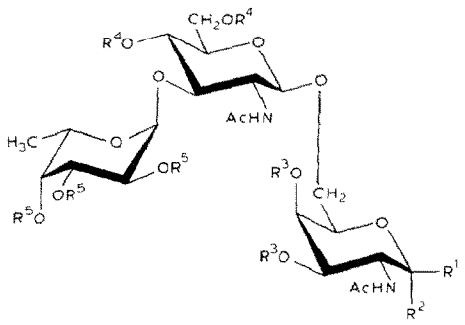

11 $R^1 = H; R^2 = OBn; R^3, R^4 = R^4; R^4 = CMe_2; R^5 = Bn$
12 $R^1 = R^3 = R^4 = H; R^2 = OBn; R^5 = Bn$
13 $R^1, R^2 = H, OH; R^3 = R^4 = R^5 = H$

served in the region of δ ~100, indicating the absence of an isopropylidene group. This was also suggested by the reluctance of the substituent to undergo cleavage, even after prolonged treatment with either hot, 60% aqueous acetic acid, or with 90% trifluoroacetic acid for 3 h at ~50°.

Condensation of **2** (catalyzed by bromide ion) with freshly prepared[13,14] 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide (**10**) afforded the fully protected trisaccharide **11**, the ^{1}H-n.m.r. spectrum of which, also, supported the overall structure expected (see Experimental section). Cleavage of the isopropylidene groups of **11** with hot, 60% aqueous acetic acid, followed by hydrogenolysis of the benzyl groups of intermediate **12** then furnished the title trisaccharide **13**. In the ^{13}C-n.m.r. spectrum of **13** (see Table II), the signal for C-1 (δ 90.86) was evidence of the free 2-acetamido-2-deoxy-D-galactopyranose residue (mainly existing in the α configuration). Signals at δ 100.72 and 99.64 accounted for the anomeric carbon atoms of the β-D-Glc*p*NAc residue and the α-L-Fuc*p* group, respectively. The down-field shift for C-6 and C-3′, which resonated at δ 68.94 and 81.72, respectively, was evidence of glycosylation of O-6 and O-3′, respectively.

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. N.m.r. spectra were recorded at ~25°, ^{1}H-n.m.r. spectra with a Varian EM-390, and ^{13}C-n.m.r. spectra with a Varian XL-100 instrument, at 90 and 25.2 MHz, respectively. The positions of the peaks (δ) are indicated from the Me_4Si signal. T.l.c. was conducted on aluminum sheets precoated with 0.2-mm layer of Silica Gel $60F_{254}$ (E. Merck, Darmstadt, Germany), the components were located either by exposure to u.v. light or by spraying the plates with 5% H_2SO_4 in ethanol and heating. Preparative-layer chromatography (P.l.c.) was conducted on 20 × 20 cm glass sheets, precoated with 1-mm layer of

silica gel (Analtech Inc., New York). Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following solvent systems (v/v) were used for chromatography: (*A*) 2:1 chloroform–acetone, (*B*) 5:1 chloroform–acetone, and (*C*) 13:6:1 chloroform–methanol–water. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, Inc., 29 Samson Avenue, Madison, New Jersey, U.S.A.

Benzyl 2-acetamido-6-O-(2-acetamido-3-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-2-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (**2**). — A mixture of **1** (ref. 9) (1.5 g, 2.5 mmol), 2,2-dimethoxypropane (12 mL), and 4-toluenesulfonic acid monohydrate (0.4 g) in *N,N*-dimethylformamide (40 mL) was stirred for 3 h at ~70°. It was then cooled, made neutral with a little triethylamine, and evaporated under diminished pressure, and the residue applied to a column of silica gel. Elution with solvent *B*, followed by solvent *A*, and evaporation of the fractions corresponding to the major product gave a solid which was dissolved in acetone. Addition of ether caused the precipitation of **2** (1.2 g, 68%), amorphous, $[\alpha]_D^{25}$ +59° (*c* 1.3, 1:1 chloroform–methanol); ^{1}H-n.m.r. [1:1 $(CD_3)_2CO$–CD_3OD]: δ 7.30 (s, 5 H, arom.), 1.90–1.85 (s, 6 H, 2 NAc), and 1.50–1.30 (s, 12 H, 2 CMe_2).

Anal. Calc. for $C_{29}H_{42}N_2O_{11}$: C, 58.57; H, 7.12; N, 4.71. Found: C, 58.67; H, 7.30; N, 4.75.

Benzyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (**4**) *and benzyl O-(2,3,4,6-tetra-O-β-D-galactopyranosyl)-(1→3)-O-[2-acetamido-6-O-(2-cyanopropyl)-2-deoxy-β-D-glucopyranosyl]-(1→6)-2-acetamido-2-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside* (**5**). — A stirred solution of **2** (0.59 g, 1 mmol) in 1:1 benzene–nitromethane (100 mL) was boiled until ~60 mL of the solvent had distilled off. After cooling to room temperature, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**3**; 0.62 g, 1.5 mmol) and $Hg(CN)_2$ (0.4 g, 1.5 mmol) were added, and the stirring continued for 24 h at room temperature. After processing in the usual manner[15], t.l.c. (solvent *A*) showed the absence of **2** and the presence of two compounds, both faster migrating than **2**. The faster of these two compounds appeared to be slightly preponderant. The crude product was applied to a column of silica gel and eluted with solvent *B*. On evaporation, fractions corresponding to the fast-moving product gave a solid which was crystallized from dichloromethane–ether to afford **4** (0.38 g, 41%), m.p. 161–164°, $[\alpha]_D^{25}$ +47° (*c* 0.5, acetone); ^{1}H-n.m.r. [$(CD_3)_2CO$]: δ 7.30–7.25 (m, 5 H, arom.), 2.15–1.85 (s, 18 H, 4 OAc and 2 NAc), and 1.50–1.25 (s, 12 H, 2 CMe_2).

Anal. Calc. for $C_{43}H_{60}N_2O_{20}$: C, 55.87; H, 6.54; N, 3.03. Found: C, 55.71; H, 6.39; N, 3.12.

Continued elution of the column with solvent *B* gave, after recrystallization from dichloromethane–ether, **5** (0.3 g, 32%), m.p. 225–228°, $[\alpha]_D^{25}$ +47° (*c* 0.5, acetone); ^{1}H-n.m.r. [$(CD_3)_2CO$]: δ 7.30–7.25 (arom.), 2.15–1.80 (OAc and NAc), and 3s [1.55 (6 H), and 1.40 and 1.25 (3 H each)].

Anal. Calc. for $C_{44}H_{61}N_3O_{20}$: C, 55.51; H, 6.45; N, 4.41. Found: C, 55.29; H, 6.59; N, 4.22.

Benzyl O-β-D-galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (**6**). — Compound **5** (0.2 g) in 0.1M methanolic sodium methoxide was stirred for 3 h at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid, the solvent evaporated under diminished pressure, and the residue dissolved in a fresh portion of methanol. The solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off and thoroughly washed with methanol, and the filtrate and washings were combined and evaporated. The crude product was then purified by p.l.c. with 5:1 (v/v) chloroform–methanol as the irrigant to give a solid which was dissolved in methanol. Addition of ether caused the precipitation of **6** (0.2 g, 89%), amorphous, $[\alpha]_D^{25}$ +56° (*c* 0.3, 1:1 chloroform–methanol); ^{1}H-n.m.r. (CD_3OD): δ 7.30–7.25 (m, 5 H, arom.), 1.90–1.85 (s, 6 H, 2 NAc), and 1.50–1.30 (s, 12 H, 2 CMe_2).

Anal. Calc. for $C_{35}H_{52}N_2O_{16}\cdot 2.5\ H_2O$: C, 52.43; H, 7.16; N, 3.49. Found: C, 52.33; H, 6.91. N, 3.34.

Benzyl O-β-D-galactopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside (**7**). — Compound **6** (0.1 g) in 60% aqueous acetic acid (15 mL) was stirred for 1 h at ~98°. The acetic acid was evaporated under diminished pressure, and several portions of toluene were added to, and evaporated from the residue, which was dissolved in water (20 mL) and lyophilized to afford **7** (50 mg, 79%), amorphous, $[\alpha]_D^{25}$ +72° (*c* 0.4, water); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 7.30 (center of m, 5 H, arom.) and 1.85–1.80 (s, 6 H, 2 NAc); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{29}H_{44}N_2O_{16}\cdot 2\ H_2O$: C, 50.14; H, 6.67; N, 4.03. Found: C, 49.48; H, 6.47; N, 3.76.

Benzyl O-β-D-galactopyranosyl-(1→3)-O-(2-acetamido-6-O-2-cyanopropyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside (**9**). — Compound **5** (0.2 g) in 0.1M methanolic sodium methoxide (20 mL) was stirred for 3 h at room temperature. The base was neutralized by the dropwise addition of glacial acetic acid, the solvent was evaporated under diminished pressure, and the residue dissolved in a fresh portion of methanol. It was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off and thoroughly washed with methanol, and the filtrate and washings were combined and evaporated. The crude product was purified by p.l.c. with 5:1 (v/v) chloroform–methanol as the irrigant to give a solid, which was dissolved in methanol. Addition of ether precipitated **8** (0.14 g, 83%), amorphous, $[\alpha]_D^{25}$ +62° (*c* 0.25, 1:1 chloroform–methanol); ^{1}H-n.m.r. [$(CD_3)SO$]: δ 7.30–7.25 (m, 5 H, arom.), 1.85–1.80 (s, 6 H, 2 NAc), 1.45 (s, 6 H, CMe_2), and 1.40–1.25 (s, 6 H, CMe_2); ^{13}C-n.m.r., see Table I. Compound **8** (70 mg) in 60% aqueous acetic acid (15 mL) was stirred for 1 h at ~98°. The acetic acid was evaporated under diminished pressure, and several portions of toluene were added and evaporated from the residue. The residue was

purified by p.l.c. with 13:6:1 (v/v) chloroform–methanol–water as the irrigant to give a solid, which was dissolved in water (30 mL) and lyophilized to afford **9** (60 mg, 90%), amorphous, $[\alpha]_D^{25}$ +67° [*c* 0.6, 1:1 (v/v) methanol–water]; ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 7.30 (s, 5 H, arom.), 1.80 (s, 3 H, NAc), 1.70 (s, 3 H, NAc), and 1.50 (s, 6 H, CMe_2); ^{13}C-n.m.r., see Tables I and II.

Anal. Calc. for $C_{33}H_{49}N_3O_{16}\cdot H_2O$: C, 52.03; H, 6.74; N, 5.51. Found: C, 51.70; H, 6.43; N, 5.43.

Benzyl O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene-β*-D-*glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-3,4*-O-*isopropylidene-α*-D-*galactopyranoside* (**11**). — A mixture of acetal **2** (0.45 g, 0.76 mmol), 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide[12,13] [**10**; 0.75 g, 1.5 mmol; freshly prepared from the 1-(4-nitrobenzoate)[13]], tetraethylammonium bromide (0.2 g), ethyldiisopropylamine (0.25 mL), and molecular sieves 3A (1.3 g) in dry dichloromethane (15 mL), protected from light and moisture, was stirred for 3 days at room temperature. The mixture was filtered through Celite, the solids were thoroughly washed with dichloromethane, and the filtrate and washings were combined, successively washed with water, $NaHCO_3$, and water, dried, and evaporated. The residue was applied to a column of silica gel and eluted first with 7:1 hexane–ethyl acetate (to remove some faster-migrating impurities) and then with 1:1 hexane–ethyl acetate to give a solid that was crystallized from dichloromethane–ether to afford **11** (0.5 g, 65%), m.p. 154–156°, $[\alpha]_D^{25}$ +6° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCL_3$): δ 7.30–7.25 (m, 20 H, arom.), 1.90 (s, 3 H, NAc), 1.60–1.30 (s, 15 H, 2 CMe_2 and NAc), and 1.15 (d, 3 H, *J* 6 Hz, Me).

Anal. Calc. for $C_{56}H_{70}N_2O_{15}$: C, 66.52; H, 6.98; N, 2.77. Found: C, 66.45; H, 7.13; N, 3.06.

Benzyl O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α*-D-*galactopyranoside* (**12**). — Trisaccharide acetal **11** (0.45 g) in 60% aqueous acetic acid (20 mL) was stirred at ~98°, and within 15 min a white solid precipitated. The mixture was cooled to room temperature, and the precipitate filtered and thoroughly washed with cold water to afford **12** (0.34 g, 82%), amorphous, $[\alpha]_D^{25}$ +28° (*c* 0.2, dimethyl sulfoxide); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 7.30–7.25 (m, 20 H, arom.), 1.80–1.70 (s, 6 H, 2 NAc), and 1.10 (d, 3 H, *J* 6 Hz, Me).

Anal. Calc. for $C_{50}H_{62}N_2O_{15}$: C, 64.50; H, 6.71; N, 3.01. Found: C, 64.80; H, 6.90; N, 2.84.

O-*α*-L-*Fucopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→6)-2-acetamido-2-deoxy*-D-*galactopyranose* (**13**). — A solution of compound **12** (0.44 g) in glacial acetic acid (15 mL) was shaken under H_2 at ~345 kPa for 48 h at room temperature in the presence of 10% Pd–C (0.44 g). The suspension was filtered through a bed of Celite, the solid was thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and evaporated under diminished pressure, the last traces of acetic acid being removed by coevaporation with several added portions of toluene. The solid residue was dissolved in a little methanol and

applied to a column of silica gel. On elution with solvent *C*, evaporation of fractions corresponding to the debenzylated product afforded **13** (0.18 g, 67%), $[\alpha]_D^{25}$ −33.5° (*c* 1.8, dimethyl sulfoxide); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{22}H_{38}N_2O_{15}$: C, 46.31; H, 6.71; N, 4.91. Found: C, 46.15; H, 6.65; N, 4.64.

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