Carbohydrate Research, 228 (1992) 243–253
Elsevier Science Publishers B.V., Amsterdam

A facile synthesis of nitrophenyl oligosaccharides containing the *O*-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl unit at their nonreducing end*,†

Rakesh K. Jain, Conrad F. Piskorz, and Khushi L. Matta‡

Department of Gynecologic Oncology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, New York 14263 (U.S.A.)

(Received March 25, 1991; accepted in revised form October 8th, 1991)

ABSTRACT

A facile approach towards the synthesis of 4-nitrophenyl *O*-α-Lfucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside, 2-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside, 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-α-D-mannopyranoside, and 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-β-D-galactopyranoside has been accomplished through the development and use of methyl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside as the glycosyl donor.

INTRODUCTION

In the past few years, a variety of tumor-associated glycoproteins and glycolipids carrying the X determinant, β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-D-GlcNAc, and the sialylated X determinant structures have been reported[2–4]. Some of these glycoconjugates contain repetitive 3-fucosyllactosamine (X determinant) units. For example, the fucolipid, β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc-(1→4)-β-D-Gal-(1→4)-β-D-Glc*p*-(1→1)-Cer, has been isolated from human colonic and liver adenocarcinoma by Hakomori and associates[5,6]. These investigators have seen the need for the isolation of hybridoma producing a monoclonal antibody which would specifically recognize the internal fucosyl sequence, α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-[α-L-Fuc-(1→3)]-β-D-Glc*p*-NAc-(1→3)-D-Gal, and not the external X determinant, because such an antibody would be unique in detecting structures specific for human cancer. In many of these tumor-associated, fucosyl glycoconjugates, the X determinant is attached at either O-6 of the D-GalNAc, D-Man, and D-Gal; O-2 of D-Man; or O-3 of the D-Gal units. Thus,

* Dedicated to Professor Serge David on the occasion of his 70th birthday.

† Synthetic Studies in Carbohydrates, Part LXXIX; for Part LXXVIII, see ref. 1. This investigation was supported by Grant No. CH 419 awarded by the American Cancer Society.

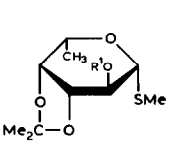
‡ To whom correspondence should be addressed.

0008-6215/92/$ 05.00

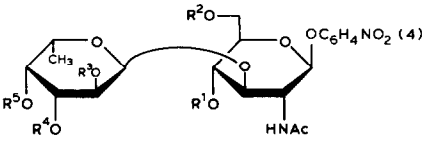
we undertook the chemical synthesis of oligosaccharides containing the α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc sequence linked to O-6 of different sugars in order to produce selected glycoconjugate fragments for immunological studies. We report, herein, a rapid and efficient method for the synthesis of 4-nitrophenyl O-α-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**4**), 2-nitrophenyl *O*-(α-L-fucopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside (**8**), 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-α-D-mannopyranoside (**12**), and 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-β-D-galactopyranoside (**19**). As shown previously[7], these compounds can be employed for the preparation of artificial antigens by reduction of their nitro groups and subsequent covalent linkage to bovine serum albumin through a diazotization reaction.

RESULTS AND DISCUSSION

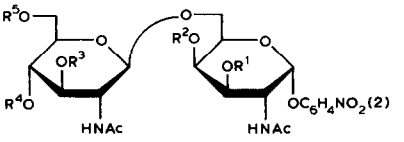
Methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside, 2,3,4-tri-*O*-benzyl-β-L-fucopyranosyl fluoride, and 2,3,4-tri-*O*-benzyl-α,β-L-fucopyranosyl trichloroacetamidate[8–12] have been the glycosylating reagents of choice, but they require hydrogenolysis for removal of the protecting groups. Methyl 3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside[1] (**2**) provides a more efficient route for α-L-fucosylation because removal of the protecting group is straightforward. Compound **2**



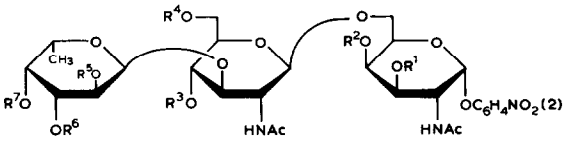
1 R′ = H
2 R′ = (4)MeOC$_6$H$_4$CH$_2$



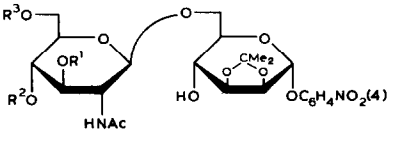
3 R^1, R^2 = R^4, R^5 = CMe$_2$; R^3 = (4)MeOC$_6$H$_4$CH$_2$
4 R^1 = R^2 = R^3 = R^4 = R^5 = H



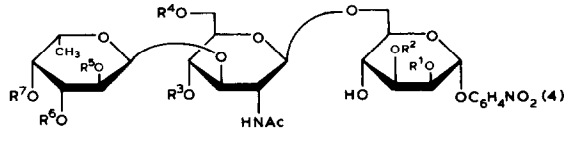
5 R^1 = R^2 = R^3 = R^4 = R^5 = H
6 R^1, R^2 = R^4, R^5 = CMe$_2$; R^3 = H



7 R^1, R^2 = R^3, R^4 = R^6, R^7 = CMe$_2$; R^5 = (4)MeOC$_6$H$_4$CH$_2$
8 R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = H



9 R^1 = R^2 = R^3 = Ac
10 R^1 = H; R^2, R^3 = CMe$_2$



11 R^1, R^2 = R^3, R^4 = R^6, R^7 = CMe$_2$; R^5 = (4)MeOBn
12 R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = H

was obtained through alkylation of methyl 3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside[1] (**1**) with 4-methoxybenzyl chloride–sodium hydride. 4-Nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[13] was treated with glycosyl donor **2** in the presence of cupric bromide–tetrabutyl ammonium bromide[14], to afford, after column chromatography, the protected disaccharide **3**. Removal of both protecting groups was achieved in a single step by treatment with trifluoroacetic acid–water in chloroform to give 4-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**4**), the ^{13}C-n.m.r. spectrum of which was in accord with the structure assigned.

Treatment of 2-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside[13] (**5**) with 2,2-dimethoxypropane–*N,N*-dimethylformamide–4-toluenesulfonic acid monohydrate provided 2-nitrophenyl *O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranostyl)-(1→6)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside (**6**) in 87% yield; its ^{1}H-n.m.r. spectrum was in agreement with the structure proposed. A similar glycosylation of **6** with **2** afforded the trisaccharide derivative **7**, the ^{1}H-n.m.r. spectrum of which showed a doublet at δ 5.38 with spacing of ~ 3 Hz, confirming the α-D configuration of the new interglycosidic bond. The removal of protecting groups from **7**, as described for **3** (to give **4**), afforded in 74% yield amorphous 2-nitrophenyl *O*-α-L-fucopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside (**8**); its ^{13}C-n.m.r. spectrum was in accord with the structure assigned (see Table I).

O-Deacetylation of 4-nitrophenyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2,3-*O*-isopropylidene-α-D-mannopyranoside[15] (**9**) in methanolic sodium methoxide, followed by isopropylidenation with 2,2-dimethoxypropane–*N,N*-dimethylformamide gave the diol derivative **10** in 90% yield. When this was treated with donor **2** under the conditions described earlier, trisaccharide **11** and tetrasaccharide **13** were obtained in a ratio of 8:5. Both the compounds were analytically pure and had ^{1}H-n.m.r. spectra in support to their expected structure (see Experimental section). Treatment of **11** and **13** with trifluoroacetic acid in chloroform furnished trisaccharide **12** and tetrasaccharide **14** in 73 and 90% yield, respectively.

Glycosylation of 4-nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside[16] with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[17] afforded the disaccharide derivative **15**. *O*-Deacetylation of **15** followed by isopropylidenation with 2,2-dimethoxypropane produced the intermediate **16**, the ^{1}H-n.m.r. spectrum of which contained signals in support of the overall structure expected. Regioselective benzoylation[18] of **16** gave **17**, which was glycosylated with donor **2** in a manner analogous to that described earlier, followed by *O*-debenzoylation to afford the trisaccharide derivative **18**. Removal of protecting groups from **18**, as described for the preparation of **4** (from **3**), provided compound **19**; its ^{13}C-n.m.r. spectrum was in accordance with the structure assigned (see Table I).

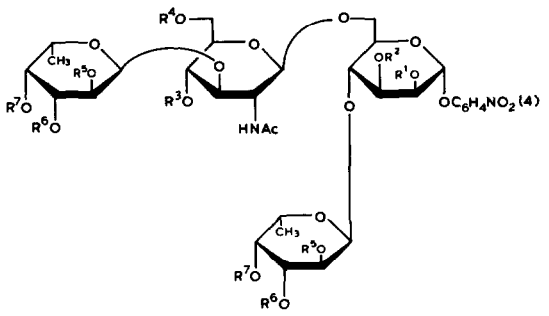
^{13}C-N.m.r. assignments. — In the ^{13}C-n.m.r. spectrum of **17**, the resonance for C-2 showed a downfield shift of 2.19 p.p.m., and that for C-1 an upfield shift of 1.15 p.p.m.,

TABLE I

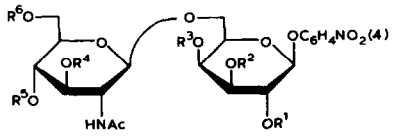
^{13}C-n.m.r. chemical shifts (δ)[a]

Residue or group	*Compound*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_3CO
β-D-Glc*p*NAcOC$_6$H$_4$NO$_2$(4)	**4**	97.86	54.34	80.86	68.46	77.02	60.31	22.89
α-L-Fuc*p*-(1→3)		99.83	68.14	69.67	71.63	66.51	16.34	
α-D-Gal*p*NAcOC$_6$H$_4$NO$_2$(2)	**8**	99.88	49.28	73.82	70.76	74.63	69.73	24.72
β-D-Glc*p*NAc-(1→6)		103.82	57.84	83.40	71.41	78.70	63.63	24.92
α-L-Fuc*p*-(1→3)		102.73	70.95	71.47	72.36	70.04	17.96	
α-D-Man*p*OC$_6$H$_4$NO$_2$(4)	**12**	100.58	72.42	74.68	69.79	75.35	69.52	
β-D-Glc*p*NAc-(1→6)		103.80	57.96	83.59	71.61	78.67	63.76	25.08
α-L-Fuc*p*-(1→3)		102.81	71.28	72.42	73.18	70.85	18.01	
β-D-Gal*p*OC$_6$H$_4$NO$_2$(4)	**19**	102.75	74.64	75.09	71.39	76.77	69.78	
β-D-Glc*p*NAc-(1→6)		103.74	57.96	83.43	71.73	78.73	63.65	24.88
α-L-Fuc*p*-(1→3)		102.75	71.57	72.38	73.04	70.77	17.99	

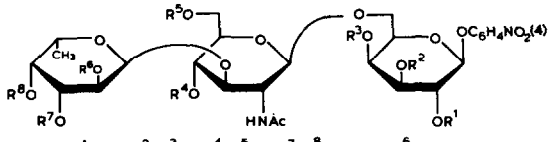
[a] For solutions in D_2O with Me_4Si as the external standard.



13 R^1, $R^2 = R^3$, $R^4 = R^6$, $R^7 = CMe_2$; R^5 = (4)MeOBn
14 $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = H$



15 $R^1 = H; R^2, R^3 = CMe_2; R^4 = R^5 = R^6 = Ac$
16 $R^1 = R^4 = H; R^2, R^3 = R^5, R^6 = CMe_2$
17 R^1 = Bz; $R^2, R^3 = R^5, R^6 = CMe_2; R^4 = H$



18 $R^1 = H; R^2, R^3 = R^4, R^5 = R^7, R^8 = CMe_2; R^6$ = (4)MeOBn
19 $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = R^7 = R^8 = H$

by comparison with those of the parent disaccharide **16**, evidencing that O-2 was the site of benzoylation. In the ^{13}C-n.m.r. spectra of the three trisaccharides, the resonances for C-6 of the reducing residue and for C-3 of the 2-acetamido-2-deoxy-D-glucose residue displayed a downfield shift, confirming the site of glycosylation. On the other hand, the resonances for C-1″ of all three compounds were observed at δ 102.73–102.81, a clear indication of an α-L configuration for the newly introduced L-fucopyranosyl group in compounds **8**, **12**, and **19**.

EXPERIMENTAL

General methods. — Melting points were determined with a Fischer–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 with 5% H_2SO_4 in ethanol (or both) and charring. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). ^{1}H-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 Bruker AM-400 instrument, at 90 and 100.6 MHz, respectively; the chemical shifts (δ) are expressed from the tetramethylsilane signal. Solutions in organic solvents were generally dried with anhydrous Na_2SO_4. 1,2-Dichloroethane and *N,N*-dimethylformamide were dried over 4A molecular sieves.

Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 07940, U.S.A.

Methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-β-L-fucopyranoside (**2**). — To a stirred solution of methyl 3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside[1] (**1**, 2.0 g) in *N,N*-dimethylformamide (20 mL) was added NaH (0.7 g) portionwise, and the stirring continued for 0.5 h at room temperature. The mixture was then cooled (~ 0°, bath), 4-methoxybenzyl chloride (1.8 ml) was added, and stirring continued for 2 h at room temperature. After careful addition of methanol to decompose excess NaH, the solvent was evaporated and the residue dissolved in chloroform. This solution was washed with water, dried, and concentrated under diminished pressure. The residue was applied to a column of silica gel and eluted with 1:9 ethyl acetate–hexane to give **2** (2.4 g, 79%) $[\alpha]_D$ − 1.1° (*c* 1.4, chloroform): ^{1}H-n.m.r. ($CDCl_3$): δ 7.33 (d, 2H, *J* ~ 9 Hz, arom.), 7.17 (d, 2 H, *J* ~ 9 Hz, arom.), 3.43 (s, 3 H, OMe), 2.15 (s, 3 H, SMe), and 1.52–1.35 (cluster of s, 9 H, CMe_2 and CH_3-5).

Anal. Calc. for $C_{18}H_{26}O_5S$: C, 61.00; H, 7.39. Found: C, 60.81; H, 7.32.

4-Nitrophenyl O-[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-α-L-fucopyranosyl]-(1→3)-2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**3**). — A solution of 4-nitrophenyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (0.75 g, 1.96 mmol) and **2** (0.9 g, 2.54 mmol) in 5:1 (v/v) 1,2-dichloroethane–*N,N*-dimethylformamide (60 mL) was stirred for 0.5 h with 4A molecular sieves (5 g) under protection from light and moisture. Tetrabutylammonium bromide (1.3 g, 4.03 mmol) and $CuBr_2$ (0.94 g, 4.03 mmol) were added, and the mixture was stirred for 16 h at room temperature. The mixture was filtered through Celite, the solids were thoroughly washed with chloroform, and the filtrate and washings were combined and then washed with aq. $NaHCO_3$ and water, dried, and concentrated under diminished pressure. The residue was applied to a column of silica gel and eluted with 1:19 acetone–chloroform. On concentration, the fractions corresponding to the product gave **3** (1.3 g, 95%) as an amorphous solid, $[\alpha]_D$ − 58° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.13 (d, 2 H, *J* ~ 9 Hz, arom.), 7.30–6.77 (m, 6 H, arom.), 3.77 (s, 3 H, OMe), 1.64 (s, 3 H, $NCOCH_3$), and 1.46–1.23 (cluster of singlets, 15 H, 2 CMe_2 and CH_3-5′).

Anal. Calc. for $C_{34}H_{44}N_2O_{13}$: C, 59.29; H, 6.44; N, 4.07. Found: C, 59.56; H, 6.37; N, 3.85.

4-Nitrophenyl O-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**4**). — To a solution of **3** (0.5 g) in chloroform (50 mL), were added trifluoroacetic acid (5.0 mL) and water (0.5 mL). After stirring for 2 h at room temperature, the solution was concentrated, and residual acid was removed by several coevaporations with toluene. The residue was purified on a column of silica gel with 1:4 methanol–chloroform as the eluent to afford **4** (0.25 g, 71%) as a solid, $[\alpha]_D$ − 77° (*c* 0.3, methanol); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{20}H_{28}N_2O_{12}\cdot H_2O$: C, 47.43; H, 5.97; N, 5.53. Found: C, 47.49; H, 5.89; N, 5.73.

2-Nitrophenyl 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**). —

To a stirred solution of 2-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-α-D-galactopyranoside[13] (**5**, 0.6 g) in *N,N*-dimethylformamide (30 mL) were added 4-toluenesulfonic acid monohydrate (0.05 g) and 2,2-dimethoxypropane (5 mL). Stirring was continued for 3 h at 70°, after which the acid was neutralized by addition of triethylamine, and the solvent evaporated *in vacuo*. The residue was dissolved in acetone, impurities were filtered off, and addition of ether–hexane afforded **6** (0.6 g; 87%) as an amorphous solid; $[\alpha]_D$ + 87° (*c* 0.6, methanol); ^{1}H-n.m.r. [$CDCl_3$ + $(CD_3)_2SO$]: δ 7.90–7.40 (m, 4 H, arom.), 1.92 and 1.86 (each s, 6 H, 2 $NCOCH_3$), and 1.50–1.32 (cluster of s, 12 H, 2 CMe_2).

Anal. Calc. for $C_{20}H_{39}N_3O_{13}$: C, 53.75; H, 6.28; N, 6.72. Found: C, 53.63; H, 5.99; H, 6.95.

2-Nitrophenyl O-*[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-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* (**7**). — Compound **6** (0.165 g, 0.26 mmol) was treated with **2** (0.12 g, 0.34 mmol) in 5:1 (v/v) 1,2-dichloroethane–*N,N*-dimethylformamide (12 mL) in the presence of tetrabutylammonium bromide (0.165 g, 0.51 mmol), $CuBr_2$ (0.12 g, 0.51 mmol), and 4A molecular sieves (1 g) in a manner analogous to that described for the preparation of **3**. After the aforementioned processing, the crude reaction product was applied to a column of silica gel and eluted with 1:49 methanol–chloroform. On concentration, the fractions corresponding to **7** (0.23 g, 94%) gave an amorphous solid, $[\alpha]_D$ − 23° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.82 (d, 1 H, *J* ~ 9 Hz, arom.), 7.65–7.13 (m, 5 H, arom.), 6.82 (d, 2 H, *J* ~ 9 Hz, arom.), 5.38 (d, *J* ~ 3 Hz, 1 H, H-1″), 3.77 (s, 3 H, OMe), 1.70 (s, 3 H, $NCOCH_3$), 1.53 (s, 3 H, $NCOCH_3$), and 1.38–1.16 (cluster of 21 H, 3 CMe_2 and CH_3-5″).

Anal. Calc. for $C_{45}H_{61}N_3O_{18}$: C, 57.99; H, 6.60; N, 4.51. Found: C, 58.04; H, 6.54; N, 4.29.

2-Nitrophenyl O-α-L-*fucopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-2-acetamido-2-deoxy-β-D-galactopyranoside* (**8**). — A solution of **7** (0.1 g) in chloroform (30 mL) was treated with trifluoroacetic acid (1.5 mL) and water (0.3 mL) for 2 h at room temperature. After processing as described for **3** (to give **4**), the residue was dissolved in methanol. Addition of ether precipitated **8** (0.055 g, 74%), $[\alpha]_D$ − 24° (*c* 0.63, water); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{28}H_{41}N_3O_{17}\cdot H_2O$: C, 47.39; H, 6.11; N, 5.92. Found: C, 47.49; H, 5.89; N, 5.73.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranosyl)-(1→6)-2,3-O-isopropylidene-α-D-mannopyranoside* (**10**). — A solution of 4-nitrophenyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-2,3-*O*-isopropylidene-α-D-mannopyranoside[15] (**9**; 1.4 g) in 0.01M sodium methoxide in methanol (25 mL) was stirred for 3 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate concentrated to give a solid residue. To a stirred solution of this solid in *N,N*-dimethylformamide (20 mL) were added 4-toluenesulfonic acid monohydrate (0.06 g) and 2,2-dimethoxypropane (12 mL). Stirring was continued for 16 h at room

temperature. The acid was neutralized by the addition of a few drops of triethylamine and the solvent evaporated. The residue was applied to a column of silica gel and eluted with 1:9 methanol–chloroform to give **10** (1.1 g, 90%), $[\alpha]_D$ +23° (*c* 1.1, methanol); ^{1}H-n.m.r. ($CDCl_3$): δ 8.17 (d, 2 H, $J \sim 9$ Hz, arom.), 7.14 (d, 2 H, $J \sim 9$ Hz, arom.), 1.95 (s, 3 H, $NCOCH_3$), and 1.51–1.37 (cluster of s. 12 H, 2 CMe_2).

Anal. Calc. for $C_{26}H_{36}N_2O_{13}$: C, 53.42; H, 6.21; N, 4.79. Found: C, 53.21; H, 6.49; N, 5.02.

4-Nitrophenyl O-*[3,4*-O-*isopropylidene-2*-O-*(4-methoxybenzyl)*-α-L-*fucopyranosyl]*-*(1→3)*-O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene*-β-D-*glucopyranosyl)*-*(1→6)-2,3*-O-*isopropylidene*-α-D-*mannopyranoside* (**11**) *and 4-nitrophenyl* O-*[3,4*-O-*isopropylidene-2*-O-*(4-methoxybenzyl)*-α-L-*fucopyranosyl]*-*(1→3)*-O-*(2-acetamido-2-deoxy-4,6*-O-*isopropylidene*-β-D-*glucopyranosyl)*-*(1→6)*-{O-*[3,4*-O-*isopropylidene-2*-O-*(4-methoxybenzyl)*-α-L-*fucopyranosyl]*-*(1→4)*}-*2,3*-O-*isopropylidene*-α-D-*mannopyranoside* (**13**). — Compound **10** (0.46 g, 0.79 mmol) was treated with **2** (0.37 g, 1 mmol) in 5:1 1,2-dichloroethane–*N,N*-dimethylformamide (42 mL) in the presence of tetrabutylammonium bromide (0.51 g, 1.58 mmol), $CuBr_2$ (0.37 g, 1.58 mmol), and 4A molecular sieves (4 g) for 16 h at room temperature. After processing as described above for the preparation of **3**, t.l.c. (4:1 chloroform–acetone) showed the presence of two major products, both faster migrating than **10**. The crude product was chromatographed and eluted with a solvent gradient consisting of 1:19→3:17 acetone–chloroform (300 mL). The earlier fractions contained the faster-migrating tetrasaccharide **13**. On concentration, the fractions corresponding to **13** (0.25 g, 26.5%) gave an amorphous solid, $[\alpha]_D$ −37.5° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.15 (d, 2 H, $J \sim 9$ Hz, arom.), 7.33–6.75 (m, 10 H, arom.), 3.78 (s, 6 H, 2 OMe), 1.55 (s, 3 H, $NCOCH_3$), and 1.45–1.15 (cluster of s, 30 H, 4 CMe_2 and CH_3-5″,5‴).

Anal. Calc. for $C_{60}H_{80}N_2O_{23}$: C, 60.19; H, 6.74; N, 2.34. Found: C, 60.38; H, 6.69; N, 2.18.

The later fractions contained trisaccharide **11** (0.3 g, 43%), $[\alpha]_D$ +12° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$: δ 8.12 (d, 2 H, $J \sim 9$ Hz, arom.), 7.23–7.01 (m, 4 H, arom.), 6.75 (d, 2 H, $J \sim 9$ Hz arom.), 3.72 (s, 3 H, OMe), 1.51 (s, 3 H, $COCH_3$), and 1.43–1.19 (cluster of s, 21 H, 3 CMe_2 and CH_3-5″).

Anal. Calc. for $C_{43}H_{58}N_2O_{18}$: C, 57.97; H, 6.56; N, 3.15. Found: C, 58.11; H, 6.53; N, 2.94.

4-Nitrophenyl O-α-L-*fucopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)*-*(1→6)*-α-D-*mannopyranoside* (**12**). — Compound **11** (0.15 g) in chloroform was treated with trifluoroacetic acid–water, as described for **3** (to give **4**), to afford **12** (0.08 g, 73%), $[\alpha]_D$ −14° (*c* 0.6, water); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{26}H_{38}N_2O_{17}$: C, 47.99; H, 5.89; N, 4.31. Found: C, 47.97; H, 5.85; N, 4.22.

4-Nitrophenyl O-α-L-*fucopyranosyl-(1→3)*-O-*(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)*-*(1→6)*-*[*O-*(*α-L-*fucopyranosyl)*-*(1→4)]*-α-D-*mannopyranoside* (**14**). — Compound **13** (0.2 g) in chloroform (30 mL) was treated with trifluoroacetic acid (1.5 mL) and water (0.3 mL) under stirring for 2 h at room temperature. The mixture was

then processed as described for **7** (to give **8**) to furnish amorphous **14** (0.12 g, 90%), $[\alpha]_D$ −35° (*c* 0.5, water).

Anal. Calc. for $C_{32}H_{48}N_2O_{12}\cdot 1.5\ H_2O$: C, 46.65; H, 6.24; N, 3.40. Found: C, 46.88; H, 6.41; N, 3.12.

4-Nitrophenyl O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β-*D-*glucopyranosyl)-(1→6)-3,4*-O-*isopropylidene-β*-D-*galactopyranoside* (**15**). — A mixture of 4-nitrophenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (1.35 g, 3.96 mmol), 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (1.6 g, 4.86 mmol), and 4-toluenesulfonic acid monohydrate (0.1 g) in 1,2-dichloroethane (100 mL), protected from moisture, was heated for 16 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 concentrated to dryness. Examination of the crude product by t.l.c. in 3:2 chloroform–acetone revealed the presence of a major product migrating slower than the starting materials, and also some slower-migrating contaminants (presumably decomposition products of oxazoline). The crude material was purified by silica gel column chromatography. Elution with a solvent gradient of 1:9→3:17 acetone–chloroform (400 mL) furnished **15** (1.1 g, 41.5%), $[\alpha]_D$ −36° (*c* 0.9, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.27 (d, 2 H, *J* ~ 9 Hz, arom.), 7.12 (d, 2 H, *J* ~ 9 Hz, arom.), 2.07 (s, 3 H, $OCOCH_3$), 2.05 (s, 6 H, 2 $OCOCH_3$), 1.65 (s, 3 H, $NCOCH_3$), 1.52 and 1.33 (each s, 3 H, CMe_2).

Anal. Calc. for $C_{29}H_{38}N_2O_{16}$: C, 51.94; H, 5.71; N, 4.18. Found: C, 51.83; H, 5.67; N, 4.25.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-4,6-*O-*isopropylidene-β-*D-*glucopyranosyl)-(1→6)-3,4*-O-*isopropylidene-β*-D-*galactopyranoside* (**16**). — Compound **15** (0.9 g) was *O*-deacetylated with 0.01M methanolic sodium methoxide, and then treated with 2,2-dimethoxypropane as described for **9** (to give **10**). After processing as described for the preparation of **10**, the residue was purified on a column of silica gel with 1:19 methanol–chloroform as the eluent. Evaporation of the fractions corresponding to **16** (0.7 g, 80.3%) gave an amorphous solid, $[\alpha]_D$ −101° (*c* 0.7, methanol); ^{1}H-n.m.r. [$CDCl_3$ + $(CD_3)_2SO$]: δ 8.19 (d, 2 H, *J* ~ 9 Hz, arom.), 7.12 (d, 2 H, *J* ~ 9 Hz, arom.), 1.64 (s, 3 H, $NCOCH_3$), and 1.45–1.27 (cluster of s, 12 H, 2 CMe_2); ^{13}C-n.m.r. [CD_3OD + $(CD_3)_2SO$]: δ 111.11 (CMe_2), 103.30 (C-1′), 101.34 (C-1), 100.76 (CMe_2), 74.99 (C-3), 73.53 (C-3′ and C-4′), 73.04 (C-2), 69.98 (C-4), 68.62 (C-6), 63.11 (C-6′), and 57.93 (C-2′).

Anal. Calc. for $C_{26}H_{36}N_2O_{13}$: C, 53.42; H, 6.21; N, 4.79. Found: C, 53.63; H, 5.99; N, 4.95.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-4,6-*O-*isopropylidene-β-*D-*glucopyranosyl)-(1→6)-2*-O-*benzoyl-3,4*-O-*isopropylidene-β*-D-*galactopyranoside* (**17**). — A mixture of **16** (0.59 g, 1 mmol) in dichloromethane (25 mL), 5% aq. NaOH (2 mL), benzoyl chloride (0.13 mL, 1.1 mmol), and tetrabutylammonium hydrogen sulfate (0.06 g, 0.18 mmol) was stirred for 1 h at room temperature, and the two layers were separated. The organic layer was washed with water, aq. $NaHCO_3$ solution, dried, and concentrated to dryness. The residue was applied to a column of silica gel and eluted with 1:49 methanol–chloroform to give **17** (0.60 g, 88%), amorphous, $[\alpha]_D$ −43° (*c* 0.8, methanol);

^{1}H-m.n.r. ($CDCl_3$): δ 8.17–7.91 (m, 5 H, arom), 7.40 (d, 2 H, $J \sim 9$ Hz, arom.), 6.95 (d, 2 H, $J \sim 9$ Hz, arom.), 1.68 (s, 3 H, $NCOCH_3$), and 1.59–1.33 (cluster of s, 12 H, 2 CMe_2); ^{13}C-n.m.r. [CD_3OD + $(CD_3)_2SO$]: δ 111.77 (CMe_2), 103.41 (C-1'), 100.78 (CMe_2), 99.19 (C-1), 75.23 (C-2), 74.32 (C-3), 73.83 (C-3'), 73.04 (C-4'), 69.89 (C-4), 68.65 (C-6), 63.12 (C-6'), and 57.89 (C-2').

Anal. Calc. for $C_{33}H_{40}N_2O_{14}$: C, 57.55; H, 5.84; N, 4.07. Found: C, 57.68; H, 5.56; N, 4.22.

4-Nitrophenyl O-*[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-α-*L*-fucopyranosyl]-(1→3)-O-(2-acetamido-2-deoxy-4,6-O-isopropylidene-β-*D*-glucopyranosyl)-(1→6)-3,4-O-isopropylidene-β-*D*-galactopyranoside* (**18**). — Compound **17** (0.55 g, 0.8 mmol) was treated with **2** (0.38 g, 1.07 mmol) in 5:1 (v/v) 1,2-dichloroethane–*N,N*-dimethylformamide (30 mL) in the presence of tetrabutylammonium bromide (0.52 g, 1.6 mmol), $CuBr_2$ (0.38 g, 1.6 mmol), and 4A molecular sieves (3 g) for 16 h at room temperature. After processing as described for the preparation of **3**, the crude mixture was treated with 0.01M sodium methoxide in methanol for 2 h. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate concentrated to dryness. The residue was purified on a column of silica gel with 1:19 acetone–chloroform as the eluent. The fractions corresponding to **18** were concentrated to give an amorphous solid (0.65 g, 91%), $[\alpha]_D$ − 62° (*c* 0.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.21 (d, 2 H, $J \sim 9$ Hz, arom.), 7.33–7.03 (m, 4 H, arom.), 6.80 (d, 2 H, $J \sim 9$ Hz, arom.), 3.75 (s, 3 H, OMe), and 1.55–1.22 (cluster of 24 H, $NCOCH_3$, 3 CMe_2, and CH_3-5'').

Anal. Calc. for $C_{43}H_{58}N_2O_{18}$: C, 57.97; 6.56; N, 3.15. Found: C, 58.04; H, 6.54; N, 3.19.

4-Nitrophenyl O-α-*L*-*fucopyranosyl-(1→3)-O-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1→6)-β-*D*-galactopyranoside* (**19**). — A solution of **18** (0.4 g) in chloroform (75 mL) was treated with trifluoroacetic acid (3 mL) and water (0.3 mL) for 2 h at room temperature. After processing as described for **3** (to give **4**), the crude product was purified in a column of silica gel with 5:4:1 chloroform–methanol–water as the eluent to give **19** (0.19 g, 65%), amorphous, $[\alpha]_D$ − 109.5° (*c* 0.6, water); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{26}H_{38}N_2O_{17}\cdot 2\,H_2O$: C, 45.47; H, 6.18; N, 4.08. Found: C, 45.32; H, 6.32; N, 4.19.

ACKNOWLEDGMENTS

The authors are grateful to Mr. R. Locke, Jr., for technical assistance, and to Mr. Walter Tabaczynski for recording the ^{13}C-n.m.r. spectra.

REFERENCES

1 R. K. Jain and K. L. Matta, *Tetrahedron Lett.*, 31 (1990) 4325–4328.
2 S. Hakomori, *Adv. Cancer Res.*, 52 (1989) 257–331.
3 S. Hakomori, *Chem. Phys. Lipids*, 42 (1986) 209–233.
4 S. Sell, *Hum. Pathol.*, 21(10) (1990) 1003–1019.
5 S. Hakomori, E. Nudelman, S. B. Levery, and R. Kannagi, *J. Biol. Chem.*, 259 (1984) 4672–4680.

6 Y. Fukushi, S. Hakomori, E. Nudelman, and N. Cochran, *J. Biol. Chem.*, 259 (1984) 4681–4685.
7 K. R. Diakun, S. Yazawa, L. Valenzuela, S. A. Abbas, and K. L. Matta, *Immun. Invest.*, 16 (1987) 1–11; 151–163.
8 R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Am. Chem. Soc.*, 97 (1975) 4076–4083.
9 M. Dejter-Juszynski and H. M. Flowers, *Carbohydr. Res.*, 18 (1971) 219–226.
10 S. Sato, Y. Ito, T. Nukada, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 167 (1987) 197–210.
11 R. K. Jain and K. L. Matta, *Carbohydr. Res.*, 208 (1990) 51–58.
12 B. Wegmann and R. R. Schmidt, *Carbohydr. Res.*, 184 (1988) 254–261.
13 C. F. Piskorz and K. L. Matta, unpublished results.
14 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 155 (1986) c6-c10.
15 S. S. Rana, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 96 (1981) 79–85.
16 J. J. Barlow and K. L. Matta, *Carbohydr. Res.*, 101 (1982) 231–244.
17 K. L. Matta and O. P. Bahl, *Carbohydr. Res.*, 21 (1972) 460–464.
18 L. L. Box, V. G. S. Box, and E. V. E. Roberts, *Carbohydr. Res.*, 69 (1979) c1-c3.