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Use of human-milk fucosyltransferase in the chemoenzymic synthesis of analogues of the sialyl Lewis^x and sialyl Lewis^a tetrasaccharides modified at the C-2 position of the reducing unit

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ABSTRACT

Two series of trisaccharides, having the formulas α-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 4)-β-D-GlcZ-OR and α-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 3)-β-D-GlcZ-OR [R = $(CH_2)_8CO_2CH_3$] respectively, in which the 2-deoxy substituent Z is azido, amino, propionamido, or acetamido, were prepared by chemical synthesis. Both types of modified trisaccharides are acceptors for a fucosyltransferase preparation obtained from human milk. Preparative fucosylations using this enzyme provided analogues of the sialyl Lewis^x and sialyl Lewis^a tetrasaccharide structures, which have been proposed to be ligands for cell-adhesion molecules. These syntheses further demonstrate the utility of glycosyltransferases in the preparation of oligosaccharide analogues.

INTRODUCTION

The broad substrate specificity of some glycosyltransferases can be used with advantage in the chemoenzymic synthesis of oligosaccharide analogues[1]. This communication reports the use of a fucosyltransferase preparation extracted from human milk in the synthesis of glycoside analogues of cell-adhesion ligands. The structures of interest were those terminating in the sialyl Lewis^x tetrasaccharide, α-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 4)-[α-L-Fuc-(1 → 3)]-β-D-GlcNAc- (**1**), which are present on leukocytes and have been proposed as ligands for cell-adhesion molecules such as the E- and the P- as well as the L-selectins[2]. The isomeric sialyl Lewis^a, α-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 3)-[α-L-Fuc-(1 → 4)]-β-D-GlcNAc- (**2**), has also been proposed to be a ligand for the E- and the P-selectins[3–6]. ^{1}H NMR data

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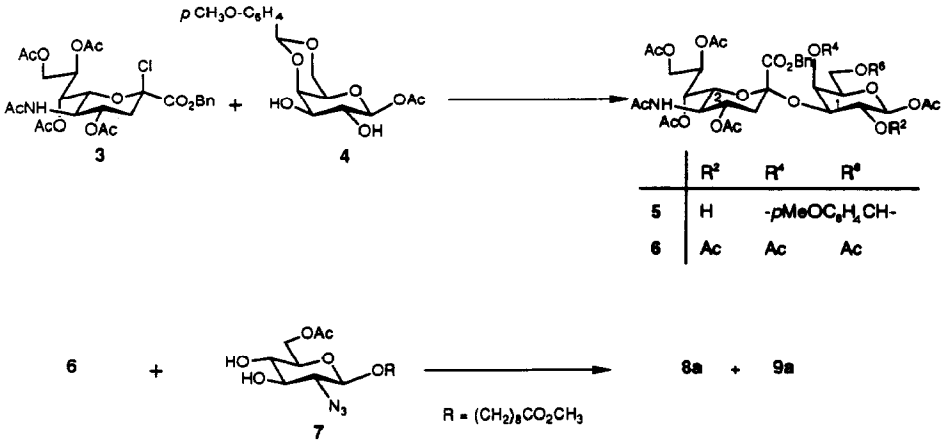
and conformational studies[7–9] indicate that the conformational preferences of the Lewis^x and Lewis^a trisaccharides are maintained in the corresponding sialylated derivatives **1** and **2**. As a result, the orientation of the reducing *N*-acetyl-β-glucosamine relative to the β-galactose seems to provide the main structural difference between the two tetrasaccharides. Consequently, the 2- and 6-substituents of the *N*-acetylglucosamine may not be directly involved in the binding to selectins, which cross-react with the two structures. In keeping with this hypothesis, replacement of the *N*-acetylglucosamine by glucose resulted in an analogue of the sialyl Lewis^x tetrasaccharide with increased binding activity in in vitro assays[10]. It was therefore appropriate to investigate whether other modifications at the periphery of the proposed binding topography could lead to analogues with different binding properties. Glycoside analogues of **1** and **2** modified at the C-2 position of the reducing unit, now described, were synthesized using a chemoenzymic procedure. These results further demonstrate the versatility of glycosyltransferases in oligosaccharide synthesis.

RESULTS AND DISCUSSION

A sequential transfer of Neu5Ac and fucose to β-D-Gal-(1 → 3)-β-D-GlcNAc (type I) and β-D-Gal-(1 → 4)-β-D-GlcNAc (type II) precursors has been used in chemoenzymic syntheses of sialyl Lewis^a (ref 11) and sialyl Lewis^x (refs 8 and 9) structures. To extend this approach to the synthesis of oligosaccharide analogues it is necessary first to consider the specificity of the required glycosyltransferases for modified substrates[1]. For example, the rat liver β-D-Gal-(1 → 3/4)-D-GlcNAc α2,3-sialyltransferase accepts modifications at the C-2 and C-6 positions of the reducing *N*-acetylglucosamine of type I and type II disaccharide acceptors[12], providing modified α-(2 → 3′)-sialylated trisaccharides. A recombinant β-D-Gal-(1 → 3/4)-D-GlcNAc α1,3/4-fucosyltransferase has also been shown to transfer fucose to a variety of modified type II acceptors[13]. A fucosyltransferase preparation easily obtained from human milk[11] contains both the β-D-Gal-(1 → 3/4)-D-GlcNAc α1,3/4-fucosyltransferase (or Lewis enzyme) and the β-D-Gal-(1 → 4)-D-GlcNAc α1,3-fucosyltransferase (serum-type enzyme)[14–16]. A systematic study of the transfer of fucose to modified type I and II acceptors by the milk fucosyltransferase preparation has demonstrated that Lewis^a and Lewis^x derivatives modified at the 2-, the 3- or the 4-position of the β-galactose residue, as well as at the 2- or 6-position of the *N*-acetylglucosamine, could be synthesized *. Therefore, the synthesis of sialyl Lewis^a and sialyl Lewis^x analogues by sequential transfer of Neu5Ac by the rat liver β-D-Gal-(1 → 3/4)-D-GlcNAc α2,3-sialyltransferase, and fucose by the milk fucosyltransferase, to type I and type II disaccharide acceptors modified at the C-2 or the C-6 position of the *N*-acetylglucosamine should be possible.

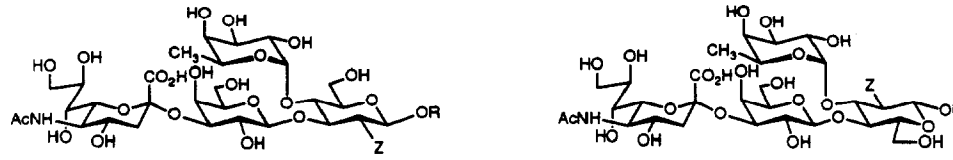
* K.B. Wlasichuk, M.A. Kashem, P.V. Nikrad, P. Bird, and A.P. Venot, unpublished results.



		Z			
R^3	R^6	N_3	NH_2	NHPr	NHAc
Ac	Bn	**8a**	**8b**	**8c**	**8d**
H	H	**10a**	**10b**	**10c**	**10d**

		Z			
R^3	R^6	N_3	NH_2	NHPr	NHAc
Ac	Bn	**9a**	**9b**	**9c**	**9d**
H	H	**11a**	**11b**	**11c**	**11d**

Pr = C(O)CH_2CH_3



12a Z = N_3
12b Z = NH_2
12c Z = NHPr
12d Z = NHAc

13a Z = N_3
13b Z = NH_2
13c Z = NHPr
13d Z = NHAc

In the current work, the sialylated type I trisaccharide **8a** and the corresponding type II trisaccharide **9a** were obtained by chemical synthesis. The glycosyl donor **3** was reacted with diol **4** in the presence of silver trifluoromethanesulfonate and 2,6-di-*tert*-butylpyridine in tetrahydrofuran at −50°C[17]. Disaccharide **5** could be isolated (45–47% relative to **4**) after careful chromatography. However, disaccharide **6**, obtained by mild acidic hydrolysis of partially purified **5**, followed by peracetylation, can also be obtained pure by chromatography. The results of glycosylations by a variety of sialyl glycosyl donors have recently been summarized[18].

TABLE I

Transfer of L-fucose to sialylated type I and type II trisaccharide analogues

Variable substituent (Z, see formulas)	Type I		Type II	
	Compound	Relative rate (%) [a,b]	Compound	Relative rate (%) [a]
N_3	**10a**	154	**11a**	111
NH_2	**10b**	50	**11b**	60
NHPr [c]	**10c**	161	**11c**	141
NHAc	**10d**	154	**11d**	136

[a] Measured at 2 mM acceptor concentration[11], and expressed relative to β-D-Gal-(1 → 4)-β-D-GlcNAc-OR as 100%; R = $(CH_2)_8CO_2CH_3$. [b] Relative rate determined for β-D-Gal-(1 → 3)-β-D-GlcNAc-OR is 130%. [c] Pr = propionyl.

Direct glycosylation of the diol **7** by the glycosyl donor **6**, catalyzed by trimethylsilyl trifluoromethanesulfonate[19,20], provided trisaccharides **8a** (41.5%) and **9a** (23.5%). Examination of the ^1H NMR spectra of the corresponding peracetylated derivatives confirmed the nature of the linkages formed. Thioglycosides[21] and trichloroacetamidate[22,23] derivatives of disaccharide blocks similar to **6** have also been used as donors in glycosylation reactions.

The azido group of trisaccharides **8a** and **9a** was reduced to provide the amino trisaccharides **8b** and **9b**, which were further transformed into the propionamido and the acetamido trisaccharides **8c,d** and **9c,d**, respectively. The benzyl ester groups of **8a** and **9a** were selectively cleaved by reduction in ethyl acetate, providing the corresponding azido trisaccharides. Conventional deprotection gave the type I sialylated trisaccharides **10a–d** and the corresponding type II derivatives **11a–d**. In these compounds, the 2-linked substituents of the reducing unit are azido, amino, propionamido, and acetamido.

The relative rate of transfer of L-fucose from GDP-fucose to the modified sialylated trisaccharides by the milk fucosyltransferase, at 2 mM acceptor concentration, are reported in Table I. The values obtained for the transfer to the 2-azido and 2-propionamido type I (**10a** and **10c**) and type II (**11a** and **11c**) derivatives are similar to those obtained for the respective unmodified compounds **10d** and **11d**. However, both the 2-amino trisaccharides **10b** and **11b** are less active than **10d** and **11d**, respectively. The high activity of the 2-azido trisaccharides is surprising, particularly in the case of the type II derivative **11a**, in which the modification is close to the fucosylation site. Indeed, lactose [β-D-Gal-(1 → 4)-D-Glc] and its α-(2 → 3′)-sialylated derivative are relatively good acceptors for the Lewis enzyme purified from human milk[24], whereas they are very poor acceptors for the serum-type fucosyltransferase[15,25]. However, it is not possible to correlate the results obtained in the present study, using **10a,b** and **11a,b**, with the activity of specific fucosyltransferases from milk. Additionally, consistent with the observations reported earlier for the unmodified structures[11,15,16], the relative rates of transfer to

TABLE II

Selected ^{1}H NMR data for sialylated trisaccharides and their fucosylation products

Sugar unit	Hydrogen atom (coupling)	Chemical shifts in ppm [a] (J values in Hz)							
Type I structures		**10a** [b]	**10b** [b,c]	**10c** [b]	**10d** [b]	**12a** [d]	**12b** [b,c]	**12c** [d]	**12d** [e]
β-Glc-2Z	1 ($J_{1,2}$)	4.55 (8.2)	4.76 (8.5)	4.57 [f] (8.0)	4.55 [f] (8.0)	4.55 (8.2)	4.75 (8.5)	4.56 (8.5)	4.54
	2 ($J_{2,3}$)		3.24 (10.5)				3.31 (10.5)		3.88
	3 ($J_{3,4}$)						4.24 (~ 9.5)	4.12 (9.7)	4.09
	$COCH_2CH_3$			2.29 (7.5)				2.30 (7.5)	
	$COCH_2CH_3$			1.13				1.16	
α-Fuc	1 ($J_{1,2}$)					4.99 (3.5)	5.08 (3.7)	5.01 (3.5)	5.04
	5 ($J_{5,6}$)					4.89 (6.7)	4.75 (6.5)	4.86 (6.5)	4.93
	6					1.18	1.18	1.17	1.19
β-Gal	1 ($J_{1,2}$)	4.72 (7.7)	4.67 (8.0)	4.49 [f] (7.6)	4.49 [f] (7.7)	4.85 (7.7)	4.65 (7.7)	4.52 (7.7)	4.55
	3 ($J_{2,3}$, $J_{3,4}$)	4.13 (10.0;2.7)	4.14 (10.0;3.2)	4.07 (10.0;3.0)	4.09 (10.0;3.0)	4.11 (9.8;2.5)	4.12 (10.0;3.0)	4.03 (10.0;3.0)	4.09
α-Neu5Ac	3*eq* ($J_{3eq,4}$,$J_{3eq,3ax}$)	2.77 (4.5;12.6)	2.77 (4.5;12.6)	2.76 (4.7;12.5)	1.76 (4.7;12.5)	2.77 (4.4;12.5)	2.78 (4.5;12.5)	2.76 (4.5;12.5)	2.79
	3*ax* ($J_{3ax,4}$)	1.81 (12.2)	1.80 (12.0)	1.78 (12.2)	1.78 (12.4)	1.81 (12.0)	1.80 (12.4)	1.76 (12.0)	1.79
Others	$NHCOCH_3$	2.03	2.03	2.03	2.02; 2.03	2.03	2.03	2.03	2.05 (two)
Type II structures		**11a** [b]	**11b** [b,c]	**11c** [b]	**11d** [b]	**13a** [d]	**13b** [b,c]	**13c** [d]	**13d** [g]
β-Glc-2Z	1 ($J_{1,2}$)	4.57 [f] (8.0)	4.76 (8.5)	4.55 [f] (7.7)	4.55 [f] (7.8)	4.60 (8.2)	4.77 (8.5)	4.53 (7.5)	4.520
	2 ($J_{2,3}$)	3.31 (8.5;10.0)	3.05 (8.5;10.5)				3.25 (~ 9.0)		
	$COCH_2CH_3$			2.29 (7.5)				2.29 (7.5)	
	$COCH_2CH_3$			1.12				1.12	
α-Fuc	1 ($J_{1,2}$)					5.40 (4.0)	5.07 (2.0)	5.10 (3.7)	5.101
	5 ($J_{5,6}$)					4.83 (6.5)	4.66 (6.5)	4.82 (6.7)	4.826
	6					1.17	1.21	1.17	1.168
β-Gal	1 ($J_{1,2}$)	4.53 [f] (8.0)	4.56 (7.7)	4.53 [f] (8.0)	4.51 [f] (7.2)	4.50 (7.6)	4.57 (8.0)	4.52 (7.7)	4.520
	3 ($J_{2,3}$,$J_{3,4}$)	4.11 (10.0;3.0)	4.12 (10.0;3.0)	4.11 (10.0;3.0)	4.12 (10.0;3.0)	4.08 (10.0;3.0)	4.11 (10.0;3.2)	4.08 (10.0;3.0)	
α-Neu5Ac	3*eq* ($J_{3eq,4}$,$J_{3eq,3ax}$)	2.76 (4.5;12.6)	2.76 (4.5;12.6)	2.76 (4.5;12.5)	2.76 (4.6;12.5)	2.77 (4.4;12.5)	2.77 (4.5;12.5)	2.76 (4.5;12.5)	2.764
	3*ax* ($J_{3ax,4}$)	1.80 (12.0)	1.81 (12.0)	1.80 (12.0)	1.80 (11.8)	1.80 (12.0)	1.81 (12.2)	1.79 (12.3)	1.794
Others	$NHCOCH_3$	2.03	2.03	2.03	2.03 (two)	2.03	2.03	2.03	2.019; 2.031

[a] In D_2O, with acetone set at 2.225; All derivatives show a singlet at δ 3.69 (CO_2CH_3) and a triplet at δ 2.39 (J 7.5 Hz, CH_2CO_2). [b] At 300 MHz. [c] Hydrochloride form, pD ~ 4.0. [d] At 500 MHz. [e] From ref. 7. [f] Interchangeable. [g] From ref 11.

the α-(2 → 3′)-sialylated modified substrates **10a–c** and **11a–c** are higher than those obtained for the corresponding asialo derivatives *.

Preparative fucosylation of trisaccharides **10a–c** and **11a–c** was performed according to a reported procedure[11], with the exception that, in all cases, the fucosyltransferase employed had been further purified by affinity chromatography on GDP–hexanolamine Sepharose. The products were recovered and purified by sequential chromatography on hydrophobic C_{18} silica gel[11], Iatrobeads, Bio-Gel P-2 and ion-exchange resin. During incubation, some hydrolysis of the methyl ester group of the aglycon could not always be avoided. As a result, the products were sometimes recovered as the methyl ester, and sometimes as the free acid. These were always separated and identified by ^{1}H NMR. Fucosylation of trisaccharides **10a** and **11a** has been scaled up in order to produce 20–38 mg of the corresponding tetrasaccharides **12a** and **13a**, with complete transformation of the starting materials.

Selected ^{1}H NMR data for trisaccharides **10a–c** and **11a–c** and tetrasaccharides **12a–c** and **13a–c** are reported in Table II. Similar data obtained for the unmodified trisaccharides **10d** and **11d** and tetrasaccharides **12d** (ref 7) and **13d** (ref 11) are also reported for comparison. It may be seen that the replacement of the 2-acetamido by a 2-azido substituent in the type I structures **10a** and **12a** results in a deshielding of the H-1 signals of the neighboring β-galactose. Meanwhile, a downfield shift is observed only for the H-1 of the L-fucose of the type II tetrasaccharide **13a**. As expected, in the data reported for the hydrochloride forms of the amino trisaccharides **10b** and **11b** and tetrasaccharides **12b** and **13b**, downfield shifts are observed for the H-1 and H-2 of the modified reducing unit. In this case, some shift changes are also noted for some of the β-D-galactose and α-L-fucose signals. Data for the propionamido trisaccharides **10c** and **11c** and tetrasaccharides **12c** and **13c** are consistent with those of the reference acetamido compounds **10d**, **11d** and **12d**, **13d**, respectively. The results of in vitro binding assays of the above modified tetrasaccharides has been reported elsewhere[26].

EXPERIMENTAL

General.—Reagent and solvent preparation, the workup of reaction mixtures, and chromatographic procedures were carried out as described in the immediately preceding paper. Microanalyses were performed by the Analytical Services of the Department of Chemistry, University of Alberta, Edmonton. To permit the identification of individual H atoms sugar residues are numbered sequentially, beginning with the reducing unit (see formulas).

^{1}H NMR spectra were run at 298 K, at either 300 MHz on a Bruker AM-300, or at 500 MHz on a Varian Unity spectrometer (University of Alberta). Tetramethyl-

* See footnote, p 146.

silane (in $CDCl_3$) and acetone (δ 2.225 in D_2O) were used as internal standards. Only partial NMR data are presented, and coupling constants (observed splittings) are reported as if they were first order. For ^1H NMR purposes, samples of **10b**, **11b**, **12b**, and **13b** in D_2O passed through ion-exchange resin AG 4-X4 (Cl^- form, 100–200 mesh) to give solutions of pD ~ 4.0. The other tetrasaccharide samples were run as sodium salts, and the pD of their solutions was ~ 7.0. All these deprotected materials were freeze-dried twice from 99.96% D_2O prior to dissolution for examination in the spectrometer.

Fucosyltransferase preparation.—The fucosyltransferase was purified from the milk of Lewis positive donors according to the reported procedure[11]. For kinetic studies, as well as for synthetic purposes, the enzyme was further purified by affinity chromatography on GDP–hexanolamine Sepharose and assayed as indicated[11]. One unit of enzyme activity is defined as the amount of enzyme transferring 1 μmol of fucose/min.

Fucosyltransferase kinetics.—Determinations of the relative rates followed the reported procedure[11]. The synthetic substrates β-D-Gal-(1 → 4)-β-D-GlcNAc-OR and β-D-Gal-(1 → 3)-β-D-GlcNAc-OR [R = $(CH_2)_8CO_2CH_3$] and GDP-fucose were obtained from Chembiomed Ltd., Edmonton, Alberta.

Preparative fucosylation.—Preparative enzymic fucosylations were performed as described[11]. For example, trisaccharide **11a** (27 mg, 0.033 mmol), $MnCl_2$ (10 mM), ATP (1.6 mM), NaN_3 (1.6 mM), the fucosyltransferase (88.4 mU), and GDP-fucose (40 mg) in sodium cacodylate buffer (100 mM, pH 6.5, 2.0 mL) were incubated for 40–48 h at 37°C. The final mixtures were diluted with water (10 mL) and applied to a column of C_{18} silica gel (1 × 20 cm). After washing with water (100 mL), MeOH (100 mL) was used to elute the products. The solvent was evaporated, and the residue, dissolved in a small amount of solvent *A*, was applied on a column of Iatrobeads (3.5 g). Successive elution with solvents *B*, *C*, and *D* gave first the product having the aglycon in the ester form [**13a**, 20.2 mg, δ_H (D_2O) 3.69 (s, CO_2CH_3) and 2.39 (t, *J* 7.5 Hz, $CH_2CO_2CH_3$)], then the free acid form [5.0 mg, δ_H (D_2O) 2.17 (t, *J* 7.5 Hz, $CH_2CO_2^-$)]. The products were then chromatographed on Bio-Gel P-2 and eluted with water. After freeze-drying, the recovered materials were dissolved and run through a short column of AG 50 W-X12 ion exchange resin (Bio-Rad, 100-200 mesh, Na^+ form), and the eluates were freeze-dried in vacuo.

*Benzyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-*D*-glycero-β-D-galacto-2-nonulopyranosylonate and the derived glycosyl chloride* (**3**).—Acetylation[27] of the benzyl ester of *N*-acetylneuraminic acid[28] provided the peracetylated β anomer[29]: mp 128°C; $[\alpha]_D$ − 29.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.38 [m, 2 H, incl. N*H* and H-7 (dd, $J_{6,7}$ 2.0, $J_{7,8}$ 5.6 Hz)], 5.26 (m, incl. H-4 and benzylic H), 5.17 (d, 1 H, J_{gem} 12.0 Hz, benzylic H), 5.09 (ddd, 1 H, $J_{8,9a}$ 2.6, $J_{8,9b}$ 6.5 Hz, H-8), 4.45 (dd, 1 H, $J_{9a,9b}$ 12.5 Hz, H-9a), 2.55 (dd, 1 H, $J_{3eq,4}$ 5.0, $J_{3eq,3ax}$ 13.0 Hz, H-3*eq*), 2.13, 2.11, 2.03, and 1.90 (4 s, 19 H, 5 OAc and 1 NAc, overlapping with H-3*ax*). The product was accompanied by a small amount of the α anomer.

Acetyl chloride (12.0 mL) was added to a solution of the above compound (6.0 g, 9.84 mmol) in CH_2Cl_2 (50 mL). Concentrated HCl (1.3 mL) was syringed dropwise into the solution, cooled to 0°C. After stirring overnight at 22°C the mixture was concentrated and the remaining solvents were coevaporated with an excess of toluene. The residue was dried at the vacuum pump, providing crude **3** (5.70 g, 98%): ^{1}H NMR ($CDCl_3$): δ 5.61 (d, 1 H, $J_{5,NH}$ 10.0 Hz, N*H*), 5.48 (dd, 1 H, $J_{6,7}$ 2.4, $J_{7,8}$ 6.4 Hz, H-7), 5.46–5.33 [m, 2 H, incl. H-4 (ddd, $J_{3eq,4}$ 4.8, $J_{3ax,4} \cong J_{4,5} = 10.9$ Hz) and benzylic H (d, J_{gem} 12.2 Hz)], 5.23 (d, 1 H, benzylic), 5.18 (ddd, 1 H, $J_{8,9a}$ 2.6, $J_{8,9b}$ 6.2 Hz, H-8), 2.78 (dd, 1 H, $J_{3eq,3ax}$ 14.0 Hz, H-3*eq*), 2.28 (dd, 1 H, H-3*ax*), 2.12, 2.04 (6 H), 2.02, 1.91 (4 s, 15 H, 4 OAc and 1 NAc).

1-O-Acetyl-4,6-O-p-methoxybenzylidene-β-D-galactopyranose (**4**).—1-*O*-Acetyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose (2.34 g, 56%) was obtained from tetra-*O*-benzyl-D-galactopyranose (4.00 g, 7.41 mmol) following the procedure reported by Tsutsumi et al.[30]: mp 101–102°C (EtOH); $[\alpha]_D$ + 5.3° (*c* 1.0, $CHCl_3$): ^{1}H NMR ($CDCl_3$): δ 5.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 1.99 (s, 3 H, O Ac). Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 73.91, H. 6.64.

A suspension of this material (5.0 g, 8.59 mmol) in acetic acid (100 mL) was hydrogenated for 3 h at atmospheric pressure in the presence of 5% Pd–C (3.0 g). After removal of the catalyst, the acetic acid was evaporated in vacuo (bath temperature 30°C) to give crude 1-*O*-acetylgalactose (~ 2.27 g).

p-Toluenesulfonic acid (0.020 g) was added to a fine suspension of the crude 1-acetate and *p*-methoxy-α,α-dimethoxytoluene (2.53 g, 14 mmol) in MeCN (14 mL). After 0.5 h, some Et_3N was added and the solvent evaporated in vacuo. Chromatography on silica gel using first 7:3 then 1:1 $CHCl_3$–MeCN provided **4** as a solid (2.8 g, 82%): $[\alpha]_D$ + 9.1 (*c* 0.93, acetone); ^{1}H NMR (Me_2SO-d_6): δ 7.38, 6.43 (2 m, 4 H, aromatic), 5.50 (s, 1 H, ArC*H*), 5.41 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.76 (s, 3 H, OCH_3), and 2.10 (s, 3 H, OAc). Anal. Calcd for $C_{16}H_{20}O_8$: C, 56.46; H, 5.92. Found: C, 56.67, H, 6.09.

O-*(Benzyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-1,2,4,6-tetra-O-acetyl-β-D-galactopyranose* (**6**).—Silver trifluoromethanesulfonate (2.0 g, 7.80 mmol) and 2,6-di-*tert*-butylpyridine (1.70 g, 8.90 mmol) were added sequentially to a suspension of the diol **4** (2.50 g, 7.35 mmol) in dry THF (10 mL) under N_2. Dissolution occurred after stirring. Calcium sulfate (2.0 g, crushed) was then added, and stirring was continued for 0.5 h at 22°C. After cooling to −78°C, a portion of the chloride **3** (two thirds of a solution obtained by dissolving **3**, freshly prepared from 6.0 g, 9.84 mmol of the corresponding pentaacetate as described above, in 10 mL of THF) was slowly syringed in over ~ 0.5 h. The mixture was then slowly warmed to −55°C and kept at that temperature for 1 h. More silver trifluoromethanesulfonate (1.0 g, 3.90 mmol) and 2,6-di-*tert*-butylpyridine (0.850 g, 4.50 mmol) in THF (5 mL) were added to the mixture, followed by the remainder of the solution of the chloride. After stirring for 1 h at −55°C, the mixture was slowly warmed to 0°C in ~ 3 h. It was then diluted with CH_2Cl_2 (250 mL) and filtered, and the filtrate was worked

up by the standard procedure. The recovered crude material was flash-chromatographed on silica gel (~ 200 g) using 80:20 then 70:30 $CHCl_3$–acetone as eluant, which provided a fraction of **5** (2.75 g) containing a very small amount of impurities and further, more heavily contaminated fractions (2.40 g, ~ 50% pure).

Both portions of **5** were separately hydrolyzed in 10:1 acetic acid–water (60 mL) for about 1.5 h at 45°C. The mixtures were concentrated, the remaining solvents were coevaporated with toluene, and the residues further dried in vacuo. The crude products were separately acetylated in 10:1 pyridine–Ac_2O (20 mL) for 24 h at 22°C, in the presence of a catalytic amount of 4-dimethylaminopyridine. After addition of MeOH followed by standard workup, the recovered crude acetates were separately chromatographed on silica gel using 50:50:2 hexanes–EtOAc–EtOH as eluants to provide **6** (2.30 g and 0.80 g, respectively, 46% overall based on **4**): $[\alpha]_D + 27.2°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.83 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^1), 5.51 (ddd, 1 H, $J_{7,8}$ 8.3, $J_{8,9a}$ 2.5, $J_{8,9b}$ 6.5 Hz, H-8^2), 5.46 (d, 1 H, J_{gem} 12.0 Hz, benzylic H), 5.31 (dd, 1 H, $J_{6,7}$ 2.5 Hz, H-7^2), 5.17 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2^1), 5.09 [m, 2 H incl. benzylic H and H-4^1 (br d, $J_{3,4}$ 3.5 Hz)], 4.94 (d, 1 H, $J_{5,NH}$ 10.0 Hz, N*H*), 4.86 (ddd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,4}$ 12.5, $J_{4,5}$ 10.0 Hz, H-4^2), 4.79 (dd, 1 H, H-3), 2.63 (dd, 1 H, $J_{3eq,3ax}$ 13.5 Hz, H-3^2eq), 2.21, 2.19, 2.11, 2.09, 2.06 (6 H), 2.04 (6 H), 1.98 (7 s, 27 H, 8 OAc and 1 NAc), 1.69 (t, 1 H, H-3^2ax). Anal. Calcd for $C_{40}H_{51}NO_{22}$: C, 53.50; H, 5.73; N, 1.56; Found: C, 53.27; H, 5.92; N, 1.47.

8-(Methoxycarbonyl)octyl-6-O-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (**7**).—3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide[31] (5g, 0.012 mmol) in CH_2Cl_2 (5 mL) was added dropwise during 0.5 h into a mixture of 8-methoxycarbonyloctanol (5.0 g), 4A molecular sieves (7.5 g, crushed), and dry silver carbonate (4.5 g, 0.053 mmol) in CH_2Cl_2 (5 mL) stirred and cooled at −20°C. The mixture was then stirred for 3–4 h at −10°C, diluted with CH_2Cl_2, and filtered on Celite. The residue obtained after evaporation was dissolved in pyridine (30 mL) and treated with Ac_2O (1.5 mL) at 22°C for 48 h. After work up, the crude product was chromatographed on silica gel using 75:25 hexane–EtOAc as the eluant to give the β-glycoside triacetate (5.5 g, 90%): mp 59–61°C (EtOH); $[\alpha]_D - 13.2°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.00 (m, 2 H, H-3, 4), 4.39 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.45–4.45 [m incl. OCH_3(s, 3.67)], 2.10 and 2.05 (2 s, 6 H, OAc). Anal. Calcd for $C_{22}H_{35}N_3O_{10}$: C, 52.68; H, 7.03; N, 8.38; Found: C, 52.74; H, 6.90; N 8.42.

O-Deacetylation of the triacetate in anhyd MeOH, catalyzed by NaOMe, for 24 h at 22°C followed by deionization with Dowex 50 X-8 resin (H^+ form) provided crude triol, which was used directly in the next step.

Acetyl chloride (0.408 mL, 5.75 mmol) in CH_2Cl_2 (12 mL) was added dropwise (45 min) to a solution of the triol (2.0 g, 4.9 mmol) and pyridine (0.462 mL, 5.75 mmol) in CH_2Cl_2 (80 mL) cooled to −78°C. After 30 min at this temperature the reaction was stopped by the addition of some MeOH. The mixture was diluted with CH_2Cl_2 and washed with water. It was then concentrated in vacuo, the remaining solvents were coevaporated with an excess of toluene, and the residue

chromatographed on silica gel (100 g) with 45:55 hexanes–EtOAc to give **7** (1.71 g, 89%): $[\alpha]_D - 24°$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.53 (dd, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.33 (d, $J_{1,2}$ 7.5 Hz, H-1), 3.68 (s, 3 H, OCH_3), and 2.15 (s, 3 H, OAc). Anal. Calcd for $C_{18}H_{31}N_3O_8$: C, 51.78; H, 7.49; N, 10.07. Found: C, 51.42; H, 7.89; N, 10.56.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)-6*-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranoside* (**8a**) *and 8-(methoxycabonyl)octyl* O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-6*-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranoside* (**9a**). —A solution of trimethylsilyl trifluoromethanesulfonate (0.885 g, 3.75 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a mixture of **6** (1.20 g, 1.34 mmol), the diol **7** (1.20 g, 2.87 mmol), and Drierite (1.0 g) in CH_2Cl_2 (12 mL) stirred at 22°C. After a total stirring time of 1.5 h the reaction was quenched by the addition of Et_3N. Following standard workup evaporation of the solvents left a crude product. Chromatography of this material on silica gel (150 g, Kieselgel 60 H, Merck 7736) using 10:10:0.2 hexane–EtOAc–MeOH provided pure **8a** (0.550 g), some pure **9a** (0.300 g), and a mixed fraction (0.320 g), which was further chromatographed under the same conditions. Final yields of 0.700 g (41.5%) of **8a** and 0.400 g (23.5%) of **9a** were obtained.

Trisaccharide **8a**: $[\alpha]_D + 0.188°$ (*c* 1.0, $CHCl_3$): IR 2115 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.54 (ddd, 1 H, $J_{7,8}$ 9.0, $J_{8,9a}$ 2.7, $J_{8,9b}$ 5.2 Hz, H-8^3), 5.45 (d, 1 H, J_{gem} 12.5 Hz, benzylic H), 5.35 (dd, 1 H, $J_{6,7}$ 2.7 Hz, H-7^3), 5.03–5.13 [m, 2 H, incl. 5.10 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2^2) and 5.03 (d, benzylic H)], 5.00 (br d, 1 H, $J_{3,4}$ 3.5 Hz, H-4^1), 4.88 (m, 2 H, incl. N*H* and H-4^3), 4.66–4.73 [m, 2 H incl. H-1^2 (d) and H-3^2 (dd)], 3.66 (s, 3 H, OCH_3), 2.62 (dd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,3eq}$ 13.5 Hz, H-3^3*eq*) 2.29 (t, 2 H, *J* 7.5 Hz, CH_2CO_2), 2.25, 2.19, 2.09 (6 H), 2.07 (9 H), 1.99, 1.71 (6 s, 27 H, 8 OAc and 1 NAc), 1.57 [m, 5 H, incl. H-3^3*ax* ($J_{3ax,4}$ 13.0 Hz) and 2CH_2], and 1.24 (m, 8 H, CH_2). Anal. Calcd for $C_{56}H_{78}N_4O_{28}$: C, 53.58; H, 6.26; N, 4.46; Found: C, 53.27; H, 6.27; N, 4.50.

Trisaccharide **9a**: $[\alpha]_D + 0.240°$ (*c* 1.0, $CHCl_3$): IR 2113 cm^{-1} (N_3); ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.51 (ddd, 1 H, $J_{7,8}$ 8.5, $J_{8,9a}$ 2.6, $J_{8,9b}$ 5.2 Hz, H-8^3), 5.44 (d, 1 H, J_{gem} 12.0 Hz, benzylic H), 5.34 (dd, 1 H, $J_{6,7}$ 2.7 Hz, H-7^3), 4.78–5.10 [m, 4 H, incl. benzylic H (d), N*H*, H-2^2, and H-4^2), 4.87 (ddd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,4}$ 12.5, $J_{4,5}$ 10.5 Hz, H-4^3), 4.56–4.68 [m, 2 H, incl. H-1^2(d) and H-3^2(dd)], 3.66 (s, 3 H, OCH_3), 3.33 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2^1), 2.64 (dd, 1 H, $J_{3ax,3eq}$ 12.5, H-3^3*eq*), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2CO_2), 2.23, 2.18, 2.14, 2.08 (9 H), 2.06, 1.99, 1.84 (7 s, 27 H, 8 OAc and 1 NAc), 1.66 (t, 1 H, H-3^3*ax*), 1.56, and 1.30 (2 m, 4 H and 8 H, CH_2). Anal. Calcd for $C_{56}H_{78}N_4O_{28}$: C, 53.58; H, 6.26; N, 4.46; Found: C, 53.41; H, 6.30; N, 4.73.

For identification purposes both trisaccharides **8a** and **9a** were acetylated (pyridine, Ac_2O, and DMAP). After the usual work up, the recovered products

were filtered through silica gel using EtOAc as eluent. The appropriate fractions were pooled and evaporated. ^{1}H NMR spectra confirmed the structures of both compounds.

Per-*O*-acetylated trisaccharide **8a**, ^{1}H NMR ($CDCl_3$): δ 7.43 (m, 5 H, aromatic), 5.54 (ddd, 1 H, $J_{7,8}$ 8.5, $J_{8,9a}$ 2.7, $J_{8,9b}$ 5.6 Hz, H-8^3), 5.08 (d, 1 H, J_{gem} 12.0 Hz, benzylic), 5.36 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 8.5, H-7^3), 4.85–5.05 [m, 6 H, incl. H-4^2 (d, $J_{3,4}$ 3.5 Hz), H-2^2 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz), H-1^2(d), and H-4^3(m)], 4.65 (dd, 1 H, H-3^2), 4.28 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^1), 3.66 (s, OCH_3), 3.64 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^1), 3.36 (dd, 1 H, H-2^1), 2.61 (dd, 1 H, $J_{3eq,4}$ 5.0, $J_{3eq,3ax}$ 13.0 Hz, H-3^3*eq*), 2.25, 2.18, 2.08 (9 H), 2.05 (6 H), 2.03, 1.98, 1.83 (7 s, 30 H, 9 OAc and 1 NAc), 1.69 (t, $J_{3ax,4}$ 12.5 Hz, H-3^3*ax*), 1.56, and 1.25 (2 m, 4 H and 8 H, CH_2).

Per-*O*-acetylated trisaccharide **9a**, ^{1}H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.45 [m, 2 H, incl. H-8^3(m) and benzylic H (d, J_{gem} 12.0 Hz)], 5.37 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 8.5 Hz, H-7^3), 5.05 (d, 1 H, benzylic), 5.00 (br d, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 4.80–4.98 [m, 4 H, incl. H-3^1 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz), H-2^2 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz), N*H*(d), and H-4^3(m)], 4.59 (d, 1 H, H-1^2), 4.55 (dd, 1 H, H-3^2), 4.34 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1^1), 3.75 (t, 1 H, $J_{4,5}$ 10.0 Hz, H-4^1), 3.64 (s, 3 H, OCH_3), 3.39 (dd, 1 H, H-2^1), 2.59 (dd, 1 H, $J_{3eq,4}$ 5.0, $J_{3eq,3ax}$ 13.0 Hz, H-3^3*eq*), 2.21, 2.16, 2.11 (6 H), 2.09, 2.07, 2.06, 2.03, 1.98, 1.83 (9 s, 30 H, 9 OAc and 1 NAc), 1.69 (t, $J_{3ax,4}$ 12.5 Hz, H-3^3*ax*), 1.54, and 1.24 (2 m, 4 H and 8 H, CH_2).

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 3)-2-azido-2-deoxy-β*-D-*glucopyranoside* (**10a**).—Trisaccharide **8a** (60.8 mg, 0.048 mmol) was hydrogenated in EtOAc (1.5 mL) at 22°C in the presence of 5% Pd–C (60 mg) for 1 h to obtain the intermediate free acid (57.6 mg, 99%): $[\alpha]_D$ – 14.7° (*c* 0.18, $CHCl_3$); IR ($CHCl_3$): 2120 cm^{-1} (N_3). This intermediate was *O*-deacetylated by using a catalytic amount of NaOMe in MeOH (2 mL) for 16 h at 22°C. After deionization with Bio-Rex 70 (H^+ form) and evaporation of the filtrate, the residue was chromatographed on Bio-Gel P-2 providing **10a** (36.6 mg, 91%): $[\alpha]_D$ – 10.1° (*c* 0.32, H_2O). ^{1}H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)-6*-O-*acetyl-2-amino-2-deoxy-β*-D-*glucopyranoside* (**8b**). —Hydrogen sulfide was bubbled through a solution of trisaccharide **8a** (500 mg, 0.398 mmol) in a mixture of pyridine (40 mL), water (6 mL), and Et_3N (1.5 mL). After 16 h at 22°C, the mixture was concentrated and the remaining solvents coevaporated with toluene to give a crude product (450 mg). Some of this material (105 mg) was chromatographed using 10:1 toluene–EtOH as eluent, providing **8b** (86 mg, 75%): $[\alpha]_D$ – 21.1° (*c* 0.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.32–7.45 (m, 5 H, aromatic), 5.48 (ddd, 1 H, $J_{7,8}$ 8.5, $J_{8,9a}$ 2.5, $J_{8,9b}$ 5.5 Hz, H-8^3), 5.42 (d, 1 H, J_{gem} 12.2 Hz, benzylic), 5.29 (dd, 1 H, $J_{6,7}$ 2.7 Hz, H-7^3), 5.08 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2^2), 5.02 (d, 1 H, benzylic H), 4.98 (br d, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 4.89 (d, 1 H, $J_{5,NH}$ 10.5 Hz, N*H*Ac), 4.83 (ddd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,4}$ 12.5, $J_{4,5}$ 10.5 Hz,

H-4^3), 4.74 (d, 1 H, H-1^2), 4.67 (dd, 1 H, H-3^2), 3.64 (s, 3 H, OCH_3), 2.85 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 9.5 Hz, H-2^1), 2.59 (dd, 1 H, $J_{3ax,3eq}$ 13.0 Hz, H-3^3*eq*), 2.27 (t, 2 H, *J* 7.5 Hz, CH_2CO$_2$), 2.22, 2.15, 2.06, 2.05 (6 H), 2.04, 2.02, 1.96, 1.81 (8 s, 27 H, 8 OAc and 1 NAc), 1.65 (t, 1 H, H-3^3*ax*), 1.56, and 1.30 (2 m, 4 H and 8 H, CH_2). Anal. Calcd for $C_{56}H_{80}N_2O_{28}$: C, 54.71; H, 6.56; N, 2.27. Found: C, 54.87; H, 6.46; N, 2.21.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy-*D-glycero-*α*-D-galacto-2-nonulopyranosylonic acid)-(2 → 3)-*O-*β*-D-galactopyranosyl-(1 → 3)-2-amino-2-deoxy-β-*D-glucopyranoside (**10b**).—A solution of pure **8b** (82 mg, 0.067 mmol) in MeOH (1 mL) was hydrogenated for 1 h at 22°C in the presence of 5% Pd–C (82 mg). Filtration of the catalyst and evaporation left the intermediate free acid (76 mg), $[\alpha]_D$ + 6.0° (*c* 0.4, $CHCl_3$). This compound (72 mg, 0.06 mmol) was O-deacetylated by treatment with a catalytic amount of NaOMe in MeOH (3 mL) for 24 h at 22°C. Evaporation of the solution obtained after neutralization with acetic acid left a residue, which was purified by chromatography on Bio-Gel P-2 to provide **10b** (45.5 mg, 85%): $[\alpha]_D$ − 6.3° (*c* 0.35, H_2O); ^{1}H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra-*O-*acetyl-3,5-dideoxy-*D-glycero-*α*-D-galacto-2-nonulopyranosylonate)-(2 → 3)-*O-*(2,4,6-tri-*O-*acetyl-β-D-galactopyranosyl)-(1 → 3)-6-*O-*acetyl-2-deoxy-2-propionamido-β-*D-*glucopyranoside* (**8c**).—Propionic anhydride (0.5 mL) was added dropwise during 10 min to a solution of the crude amino trisaccharide **8b** (140 mg, 0.114 mmol) in a mixture of pyridine (9.5 mL) and water (0.9 mL). The mixture was stirred overnight at 22°C then concentrated in vacuo, and the remaining solvents were coevaporated with toluene. The residue was chromatographed using 10:1 toluene–EtOH as eluent, providing **8c** (110 mg, 74%, containing a small amount of the 4-*O*-propionyl trisaccharide). ^{1}H NMR ($CDCl_3$): δ 7.31–7.45 (m, 5 H, aromatic), 5.81 (d, 1 H, *J* 8.0 Hz, N*H*Ac), 5.45 [m, incl. H-8^3 and benzylic H (d, J_{gem} 12.5 Hz)], 5.28 (dd, $J_{6,7}$ 2.7, $J_{7,8}$ 8.5 Hz, H-7^3), 5.30 [m, incl. benzylic H (d)], 3.63 (s, 3 H, OCH_3), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.5, $J_{3eq,3ax}$ 13.0 Hz, H-3^3*eq*), 2.26 (t, 2 H, *J* 7.5 Hz, CH_2CO$_2$), 2.20 (q, 2 H, *J* 7.5 Hz, NHCOCH_2), 2.15, 2.06, 2.05 (15 H), 1.96, 1.80 (5 s, 27 H, 8 OAc and 1 NAc), 1.63 (t, 1 H, $J_{3ax,4}$ 13.0 Hz, H-3^3*ax*), 1.57, and 1.24 (2 m, 4 H and 8 H, CH_2).

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy-*D-glycero-*α*-D-galacto-2-nonulopyranosylonic acid)-(2 → 3)-*O-*β*-D-galactopyranosyl-(1 → 3)-2-deoxy-2-propionamido-β-*D-glucopyranoside* (**10c**).—Trisaccharide **8c** (105 mg, 0.081 mmol) was deprotected using the same procedure as indicated for the synthesis of **10b**. Chromatography on Bio-Gel P-2 provided pure **10b** (60.8 mg, 88%): $[\alpha]_D$ − 16.6° (*c* 0.32, H_2O); ^{1}H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy-*D-glycero-*α*-D-galacto-2-nonulopyranosylonic acid)-(2 → 3)-*O-*β*-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D-glucopyranoside* (**10d**).—A solution of the crude amino trisaccharide **8b** (210 mg, 0.171 mmol) in 10:1 pyridine–water (22 mL) was treated with Ac_2O (2

mL) overnight at room temperature. The product recovered after the usual workup was chromatographed on silica gel with 95:5 toluene–EtOH, providing a mixture of trisaccharide **8d** and the corresponding 4-*O*-acetylated product (175 mg). This mixture was deprotected as indicated for the synthesis of **10b** to give the pure trisaccharide **10d** (109 mg, 76% from **8b**): $[\alpha]_D$ −19.8° (*c* 1.0, H_2O); ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-β-D-*galactopyranosyl-(1 → 4)-2-azido-2-deoxy*-β-D-*glucopyranoside* (**11a**).—Trisaccharide **9a** (28.8 mg, 0.023 mmol) was hydrogenated as described for the preparation of **8a** to lead to the intermediate acid; $[\alpha]_D$ −18.6° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$): 2120 cm^{-1} (N_3). This product was O-deacetylated as indicated for the synthesis of **10a** and the recovered material was chromatographed on Bio-Gel P-2, providing **11a** (10.4 mg, 55%): $[\alpha]_D$ −6.5° (*c* 0.17, H_2O). ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-6*-O-acetyl-2-amino-2-deoxy-β-D-*glucopyranoside* (**9b**).—Hydrogen sulfide was bubbled through a solution of trisaccharide **9a** (400 mg, 0.319 mmol) in a mixture of pyridine (32 mL), water (4.8 mL), and Et_3N (1.3 mL) as indicated for the synthesis of **8b**, providing a crude trisaccharide (430 mg). Some of this material (85.9 mg, ~0.063 mmol) was chromatographed using 10:1 toluene–EtOH as eluent to give **9b** (55 mg, 70%): $[\alpha]_D$ +25.9° (*c* 0.22, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.25–7.45 (m, 5 H, aromatic), 5.48 [m, H-8^3, overlapping with 5.45 (d, J_{gem} 12.5 Hz, benzylic H)], 5.34 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 8.5 Hz, H-7^3), 5.05 [(m, incl. benzylic H (d) and H-2^2 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz)], 5.00 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 4.90 (d, 1 H, *J* 10.0 Hz, N*H*Ac), 4.86 (ddd, 1 H, $J_{3eq,4}$ 4.5, $J_{3ax,4}$ 12.5, $J_{4,5}$ 11.0 Hz, H-4^3), 4.64 [m, 2 H, H-1^2 and H-3^2), 3.66 (s, 3 H, OCH_3), 2.78 (dd, $J_{1,2} \cong J_{2,3} = 8.5$ Hz, H-2^1), 2.60 (dd, 1 H, $J_{3eq,3ax}$ 13.0 Hz, H-3^3*eq*), 2.30 (t, *J* 7.5 Hz, CH_2CO_2), 2.26, 2.17, 2.12, 2.08 (9 H), 2.05, 1.99, 1.83 (7 s, 27 H, 8 OAc and 1 NAc), 1.67 (t, 1 H, H-3^3*eq*), 1.60, and 1.24 (2 m, 4 H and 8 H, CH_2). Anal. Calcd for $C_{56}H_{80}N_2O_{28}$: C, 54.71; H, 6.56; N, 2.27. Found: C, 54.66; H, 6.66; N, 2.25.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-β-D-*galactopyranosyl-(1 → 4)-2-amino-2-deoxy*-β-D-*glucopyranoside* (**11b**).—A solution of the pure **9b** (53 mg, 0.043 mmol) in MeOH was hydrogenated as indicated for the synthesis of **10b** to provide the free-acid intermediate (44 mg); $[\alpha]_D$ +11.3° (*c* 0.22, H_2O). This compound was *O*-deacetylated and purified as indicated earlier to provide **11b** (29.5 mg, 86%): $[\alpha]_D$ −5.5° (*c* 0.22, H_2O). ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)-6*-O-*acetyl-2-deoxy-2-propionamido-β-D-glucopyranoside* (**9c**).—The crude amino compound **9b** (98 mg, ~0.080 mmol) was *N*-propionylated as described for the preparation of **8c** to give **9c** (75.4 mg, 72%): $[\alpha[_D$

+10.3° (*c* 0.17, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.54 (d, 1 H, *J* 7.5 Hz, N*H*), 5.48 [m, 1 H, H-8^3, overlapping with 5.44 (d, 1 H, J 12.5 Hz, benzylic H)], 5.34 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 8.5 Hz, H-7^3), 4.49–5.10 [m, 3 H, incl. benzylic H (5.05, d, J_{gem} 12.5 Hz), H-2^2 (5.04, dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz), and N*H* (d, *J* 10.5 Hz)], 4.86 (ddd, 1 h, $J_{3eq,4}$ 4.6, $J_{3ax,4}$ 12.5, $J_{4,5}$ 10.5 Hz, H-4^3), 4.61–4.69 [m, 2 H, incl. H-1^2(d) and H-3^2 (dd)], 3.58–3.70 [m, 4 H, incl. OCH_3 (s, 3.67)], 2.60 (dd, 1 H, $J_{3eq,3ax}$ 12.5 Hz, H-3^3*eq*), 2.15–2.33 [m, 10 H, incl. CH_2CO_2 (t, *J* 7.5 Hz), NHCOCH_2 (q, *J* 7.5 Hz), and Ac (2 s, at 2.26, 2.18)], 2.09 (12 H), 2.07, 1.99, 1.84 (4 s, 21 H, Ac), 1.66 (t, 1 H, H-3^3*ax*), 1.57, 1.24 (2 m, 4 H and 8 H, CH_2), and 1.13 (t, 3 H, CH_2CH_3).

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-β-D-*galactopyranosyl-(1 → 4)-2-deoxy-2-propionamido*-β-D-*glucopyranoside* (**11c**).—Trisaccharide **9c** (71 mg, 0.055 mmol) was hydrogenated as indicated for the synthesis of **10c** to obtain the intermediate acid (64 mg, 97%); $[\alpha]_D$ −22.6° (*c* 0.23, $CHCl_3$). This compound was O-deacetylated as usual and the recovered material chromatographed on Bio-Gel P-2, giving **11c** (39 mg, 83%): $[\alpha]_D$ −8.5° (*c* 0.2, H_2O). ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(benzyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2 → 3)*-O-*(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1 → 4)-6*-O-*acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside* (**9d**).—Acetylation of the crude amino trisaccharide **9b** (250 mg, 0.203 mmol) as indicated for the synthesis of **10d**, followed by chromatography with 100:8 toluene–EtOH, provided trisaccharide **9d** (189 mg, 73%): $[\alpha]_D$ +21.1° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.40 (m, 5 H, aromatic), 5.58 (d, 1 H, *J* 8.0 Hz, N*H*), 5.47 [m, 2 H, incl. H-8^3 and benzylic H (d, J_{gem} 12.5 Hz)], 5.35 (dd, 1 H, $J_{6,7}$ 2.6, $J_{7,8}$ 8.5 Hz, H-7^3), 5.05 [m, 2 H, incl. H-2^2 (dd, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz) and benzylic H (d)], 5.00 (br d, 1 H, $J_{3,4}$ 3.5 Hz, H-4^2), 4.88 [m, 2 H, incl. NH (d, *J* 10.0 Hz) and H-4^3 (m)], 4.78 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^1), 4.65 [m, 2 H, incl. H-1^2 (d) and H-3^2 (dd)], 3.67 (s, 3 H, OCH_3), 3.50 [m, 3 H, incl. H-2^1], 2.61 (dd, 1 H, $J_{3ax,4}$ 4.0, $J_{3ax,3eq}$ 13.0 Hz, H-3^3*eq*), 2.29 [m, 4 H, incl. CH_2CO_2 (t, *J* 7.5 Hz) and OAc (s)], 2.17, 2.08 (15 H), 1.98 (6 H), 1.83 (27 H, 7 OAc and 2 NAc), 1.66 (t, $J_{3ax,4}$ 12.7 Hz, H-3^3*eq*), 1.56, and 1.24 (2 m, 4 H and 8 H, CH_2). Anal. Calcd for $C_{58}H_{82}N_2O_{25}$: C, 54.79; H, 6.50; N, 2.20. Found: C, 54.80; H, 6.50; N, 2.50.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-β-D-*galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**11d**).—Trisaccharide **9d** (187 mg, 0.147 mmol) was deprotected as indicated for the synthesis of **11b** to provide **11d** (119 mg, 95%): $[\alpha]_D$ −8.3° (*c* 1.0, H_2O); ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-β-D-*galactopyranosyl-(1 → 3)*-O-*[α-L-fucopyranosyl-(1 → 4)]-2-azido-2-deoxy*-β-D-*glucopyranoside* (**12a**).—The trisaccharide **10a** (35 mg, 0.042 mmol), GDP-fucose (40 mg), the fucosyltransferase (125 mU), and

calf intestine alkaline phosphatase (40 U) were incubated for 72 h in buffer (5 mL), as described above under *Preparative fucosylation*. Isolation and purification provided **12a** (38.3 mg, 93%): $[\alpha]_D$ −28° (*c* 0.78, H_2O); ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 3)*-O-*[α*-L-*fucopyranosyl-(1 → 4)]-2-amino-2-deoxy-β*-D-*glucopyranoside* (**12b**).—The trisaccharide **10b** (9.5 mg, 0.012 mmol), GDP-fucose (18 mg), the fucosyltransferase (18 mU), and calf intestine alkaline phosphatase (10 U) were incubated for 60 h in the usual buffer (2.8 mL). Isolation and purification provided **12b** (4.94 mg, 43%): $[\alpha]_D$ −25° (*c* 0.35, H_2O) for the hydrochloride form; ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 3)*-O-*[α*-L-*fucopyranosyl-(1 → 4)]-2-propionamido-2-deoxy-β*-D-*glucopyranoside* (**12c**).—Trisaccharide **10c** (8.4 mg, 0.010 mmol), GDP-fucose (18 mg), the fucosyltransferase (19.4 mU), and calf intestine alkaline phosphatase (10 U) were incubated for 72 h in the usual buffer (2 mL). Isolation and purification provided **12c** (5.81 mg, 58%): $[\alpha]_D$ −36° (*c* 0.29, H_2O); ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 4)*-O-*[α*-L-*fucopyranosyl-(1 → 3)]-2-azido-2-deoxy-β*-D-*glucopyranoside* (**13a**).—Trisaccharide **11a** (27 mg, 0.033 mmol), GDP-fucose (40 mg), the fucosyltransferase (88 mU), and calf intestine alkaline phosphatase (40 U) were incubated for 60 h in the usual buffer (5 mL). Isolation and purification provided **13a** (20.2 mg, 62%): $[\alpha]_D$ −22° (*c* 0.78, H_2O); ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 4)*-O-*[α*-L-*fucopyranosyl-(1 → 3)]-2-amino-2-deoxy-β*-D-*glucopyranoside* (**13b**).—Trisaccharide **11b** (8.4 mg, 0.010 mmol), GDP-fucose (18 mg), the fucosyltransferase (20 mU), and calf intestine alkaline phosphatase (10 U) were incubated for 67 h in the usual buffer (2 mL). Isolation and purification provided **13b** (2.46 mg, 26%): $[\alpha]_D$ −24° (*c* 0.25, H_2O) for the hydrochloride form; ^1H NMR data are reported in Table II.

8-(Methoxycarbonyl)octyl O-*(5-acetamido-3,5-dideoxy*-D-glycero-*α*-D-galacto-*2-nonulopyranosylonic acid)-(2 → 3)*-O-*β*-D-*galactopyranosyl-(1 → 4)*-O-*[α*-L-*fucopyranosyl-(1 → 3)]-2-propionamido-2-deoxy-β*-D-*glucopyranoside* (**13c**).—Trisaccharide **11c** (8.3 mg, 0.010 mmol), GDP-fucose (18 mg), the fucosyltransferase (18 mU), and calf intestine alkaline phosphatase (10 U) were incubated for 72 h in the usual buffer (2 mL). Isolation and purification provided **13c** (6.17 mg, 61%): $[\alpha]_D$ −35° (*c* 0.32, H_2O); ^1H NMR data are reported in Table II.

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