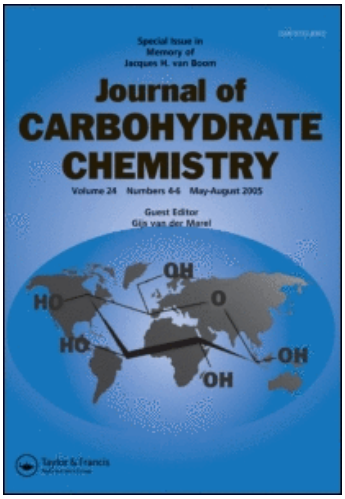

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SYNTHESIS OF GD_3 AS A 4-METHYL-3-PENTENYL GLYCOSIDE AND SUBSEQUENT CONJUGATION TO HSA

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ABSTRACT

The total synthesis of the tetrasaccharide sequence of the ganglioside GD_3 (α-D-Neu*p*5Ac-(2→8)-α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-) as the 4-methyl-3-pentenyl glycoside (**13**) has been accomplished in a relatively straightforward manner. This derivative displays reasonable ^1H NMR characteristics in D_2O and has subsequently been coupled to a carrier protein by employing the ozonolysis-reductive amination procedure.

INTRODUCTION

Over the past two decades, several key developments have led to the present interest in tumor-associated antigens, several of which have turned out to be oligosaccharide determinants.[1] Indisputably, one such development has been the widespread use of monoclonal antibodies (mAbs) as molecular tools

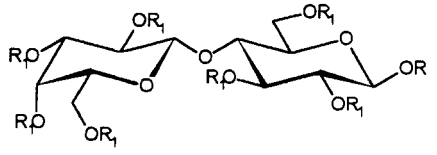
for the detection of these cell-surface antigens on tumor cells. For example, mAbs directed against sialic acid-containing glycolipids, more commonly referred to as gangliosides, have identified these components as significant antigens on the surface of human melanomas. Subsequently, it has been shown that the GM_3:GD_3[2] ratio undergoes a dramatic change upon neoplastic transformation resulting in increased levels of GD_3 expression.[3] As well, preparations containing the gangliosides GD_3 and 9-*O*-acetyl GD_3 have been formulated into vaccines for active specific immunotherapy in melanoma patients.[4] These recent applications have prompted interest in structural information regarding antibody-ganglioside complexes, and since it has been shown that it is possible to study peptide-Fab (antigen-binding antibody fragment) complexes by NMR spectroscopy,[5] it may be possible to extend this approach to carbohydrate antigens.

One difficulty in the application of this approach to gangliosides is that these compounds readily form liposomes in water thereby complicating NMR studies of these carbohydrates in the natural aqueous environment. Thus, it was our goal to synthesize a GD_3 analog which displays suitable NMR characteristics in D_2O. The synthesis of natural GD_3-ceramide,[6,7] and GD_3 trisaccharide as a propyl glycoside[8] have previously been reported, and in view of the fact that alkenyl glycosides can be coupled to carrier proteins under the mild ozonolysis-reductive amination conditions described by Bernstein and Hall,[9] we report herein, the synthesis of GD_3 as its corresponding 4-methyl-3-pentenyl glycoside (4M3P) (**13**). In turn, application of this methodology to **13** has led to the subsequent covalent attachment of this tetrasaccharide to human serum albumin (HSA).

RESULTS AND DISCUSSION

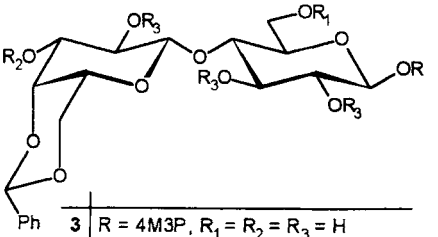
The most obvious dissection of the tetrasaccharide sequence of GD_3 reveals two disaccharide units, one composed of a lactose sialyl acceptor, and the other consisting of a di-NeuAc synthon. Ideally, the blocking scheme of the lactose unit should be such that the protecting groups provide minimum interference to the incoming sialyl donor while providing for a versatile overall blocking map that allows for further extension into other members of the ganglioside series such as GD_2.[2]

With these considerations in mind, we selected lactose-4M3P derivative **6** as our target acceptor. Thus, addition of acetobromolactose to a mixture of 4-methyl-3-penten-1-ol (1.12 equiv) and freshly prepared silver-sieves (generated by the reaction of powdered activated 4Å molecular sieves and silver trifluoromethanesulfonate in anhydrous toluene) in toluene/dichloromethane produced the acetylated lactose-4M3P derivative **1** which was then treated with sodium methoxide in dry methanol to give lactose as its β-4M3P glycoside, namely **2**. Simultaneous protection of the 4- and 6- hydroxyl groups of the galactose moiety of **2** by reaction with α,α-dimethoxytoluene and catalytic *p*-toluenesulfonic acid in dry acetonitrile at 60 °C proceeded as anticipated to give the benzylidene compound **3**. This derivative was then allowed to react with benzoyl chloride (2 equiv) in 2:1 chloroform-pyridine at -70 °C to provide the triol **4** in 50% yield along with a mixture of products at various stages of acylation which could be recycled back to **3** by *O*-deacylation. The ^{1}H NMR spectrum of compound **4** displayed a doublet of doublets centered at δ 5.12 (H-3') as well as a pair of doublet of doublets centered at δ 4.96 (H-6a) and at 4.40 (H-6b) This dibenzoate was further characterized as its fully acetylated derivative **5**. Subsequently, a methanolic solution of triol **4** was treated with magnesium methoxide at 5 °C, neutralized, and chromatography of the resultant reaction mixture over silica gel gave starting dibenzoate **4** (trace), followed by the monobenzoate **6** (85%). The chemical shifts for H-6a and H-6b, at δ 4.91 and 4.44 respectively, showed little deviation from the corresponding signals in the precursor dibenzoate. Per-*O*-acetylation to give fully protected lactose derivative **7**, assisted in further confirmation of the location of the benzoate ester in tetrol **6**.



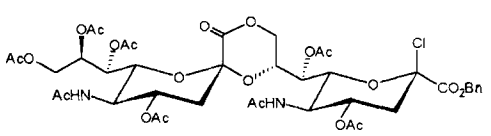
1	R = 4M3P, R_1 = OAc
2	R = 4M3P, R_1 = H

4M3P = $OCH_2CH_2CH{=}C(CH_3)_2$

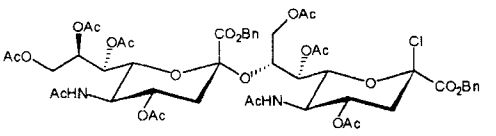


3	R = 4M3P, $R_1 = R_2 = R_3$ = H
4	R = 4M3P, $R_1 = R_2$ = Bz, R_3 = H
5	R = 4M3P, $R_1 = R_2$ = Bz, R_3 = Ac
6	R = 4M3P, R_1 = Bz, $R_2 = R_3$ = H
7	R = 4M3P, R_1 = Bz, $R_2 = R_3$ = Ac

With the acceptor **6** in hand, it was now possible to compare the sialylation reaction using di-NeuAc donors **8** and **9**. These in turn, were prepared as described earlier[10] from the now readily available sialic acid dimer, *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid) -(2→8)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (di-Neu5Ac).[11] Since lactone formation upon acetylation of di-Neu5Ac is quite facile, we first explored the utility of **8**, which was simpler to prepare than **9**. Thus, addition of a solution of lactone chloride **8** in 1:1 toluene-tetrahydrofuran to a cooled (-45 °C) solution of tetrol **6** (1.2 equiv) in tetrahydrofuran containing silver salts yielded a mixture containing **10**, which could not readily be purified to homogeneity. This crude mixture was therefore treated with 2:1 pyridine-acetic anhydride to furnish the totally protected tetrasaccharide **11** (≈ 6%) after purification by chromatography over silica gel. Although the isolated yield of **11** was extremely low, the ^{1}H NMR data for this derivative was consistent with the proposed structure. Along with the presence of the expected 11 acetate singlets, decoupling experiments revealed that acetylation subsequent to sialylation had occured at the 2-,3-, and 2'- hydroxyl moieties of the lactose backbone, strongly suggesting that the regiochemistry of the glycosidation reaction was indeed correct.



8



9

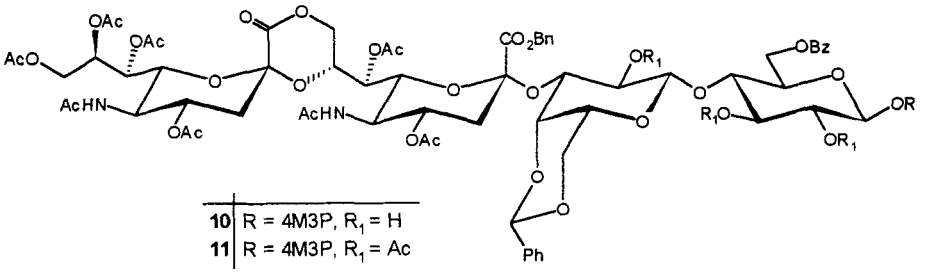
Deprotection of **11** was carried out in two stages; first, *O*-deacylation with 0.5 M potassium hydroxide in 4:1 methanol-water provided the 4',6'-*O*-benzylidene compound **12** in 79% yield, and then hydrolysis of the acetal functionality of **12** by treatment with 4:1 acetic acid-water at 60 °C resulted in a crude mixture containing fully deprotected GD_3-4M3P (**13**). This mixture was again subjected to hydrolysis due to the slow formation of a faster migrating component as detected by TLC (possibly lactone formation) and the resulting tetrasaccharide **13** was then purified to homogeniety by chromatography over silica gel. The mass spectrum along with the NMR spectral data for this

compound were uniformly consistent with the proposed structure, and the downfield shift of the H-3 of galactose to δ 4.10 lent further credence to the assigned regiochemistry while the shifts for the H-3 equatorial protons located at δ 2.79 and 2.67 corroborated the α-stereochemical assignments for both of sialic acid units.

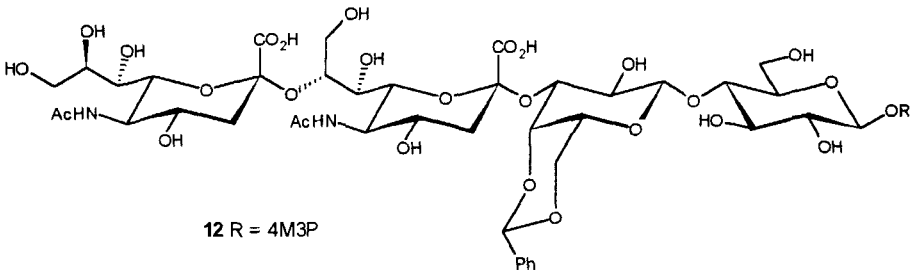
Attention was next shifted to application of dibenzyl di-Neu5Ac donor **9** in order to compare its donor properties with **8**. Thus, reaction of this donor with tetrol **6** (1 equiv) under silver-catalyzed initiation employing essentially the same conditions as for lactone chloride **8** gave a fully protected tetrasaccharide **15**, after acetylation of crude **14**, in approximately double the yield (12%) obtained with the lactone donor. The regiochemical portrait of the sialylation reaction was again established by homonuclear decoupling experiments as before; the acetylation pattern deduced from the proton spectrum of **15** strongly suggested that hydroxyl moieties at positions 2, 3 and 2' had been acetylated thus implying that sialylation had occurred at the desired H-3' position.

Removal of the *O*-benzylidene protection from blocked tetrasaccharide **15** using 4:1 acetic acid-water at 60 °C gave recovered **15** (15%) followed by the 4',6'-diol **16** in 62% yield after purification by chromatography over silica gel. It can be noted that simple protection of the primary hydroxyl group of the galactose unit of this derivative would lead to an intermediate suitable for the synthesis of the important neuroblastoma-associated ganglioside GD_2. In any case, *O*-deacylation of **16** with 0.5 M potassium hydroxide in 4:1 methanol-water proceded smoothly providing the target deblocked GD_3-4M3P derivative **13** in 93% yield.

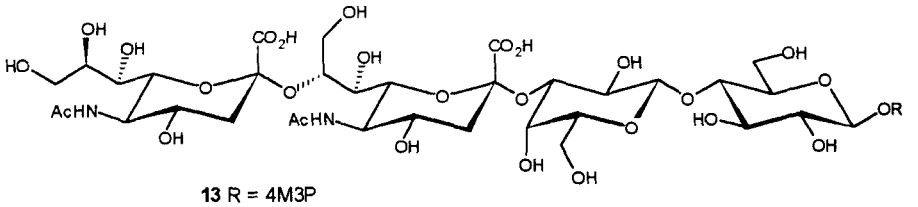
In an effort to unequivocally establish both the stereochemistry and regiochemistry of the novel synthetic GD_3-4M3P derivative **13**, a methanolic solution of GD_3-ceramide **17** obtained from natural sources was first treated with ozone at -15 °C and then stirred in aqueous 0.01 M sodium carbonate at room temperature overnight as described by Schwarzmann and Sandhoff.[12] This procedure served to effect in a sequential manner, ozonide formation, reduction, rearrangement, and subsequent α,β-elimination to provide the reducing sugar **18**, that was subsequently analyzed by mass spectroscopy (negative FAB) and by ^1H NMR spectroscopy in D_2O. The molecular weight for compound **18** was calculated to be 924.8 (FAB, *m/z* 923.87 M-1$^-$) and the ^1H NMR spectrum of the equilibrated solution of the reducing sugar displayed the anticipated 2:1 β/α anomeric mixture. Likewise, when synthetic derivative **13** was subjected to the



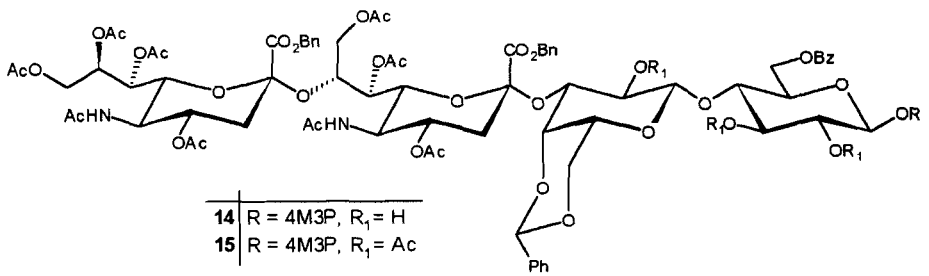
10	R = 4M3P, R_1 = H
11	R = 4M3P, R_1 = Ac



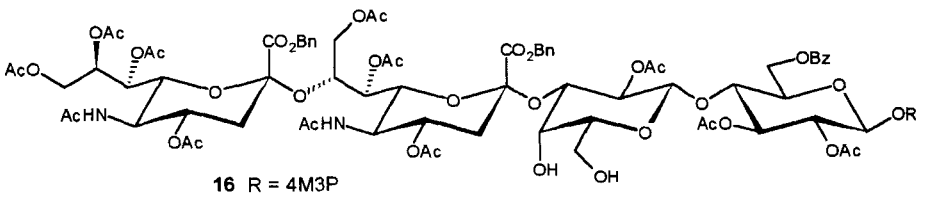
12 R = 4M3P



13 R = 4M3P



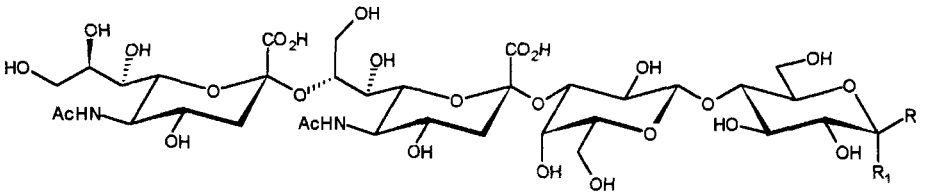
14	R = 4M3P, R_1 = H
15	R = 4M3P, R_1 = Ac



16 R = 4M3P

same sequence of events as the natural source material, a product was obtained which displayed the same TLC profile along with mass and ^1H NMR spectra identical to those of **18** obtained from the natural source material. This unequivocally demonstrates that the structure of GD_3-4M3P derivative **13** is assigned correctly.

The conjugation of GD_3-4M3P was accomplished using the established protocol.[13] Thus, ozonolysis of **13** at -70 °C followed by subsequent reduction of the resulting ozonide to the aldehyde with methyl sulfide[14] gave intermediate aldehyde **19**, presumably as a dimethyl acetal. This product was not purified or further characterized due to its unstable nature. Conjugation of **19** to human serum albumin by reductive amination in phosphate buffer at pH 5.9 or in acetate buffer at pH 4.65 using sodium cyanoborohydride as the reducing agent yielded the desired sialoglycoconjugate **20**. Analysis for sialic acid content using the resorcinol-HCl method[15] as modified by Miettinen and Takki-Luukkainen[16] implied conjugation ratios of N (GD_3/HSA) = 9 and 11 for the reactions at pH 5.9 and 4.65 respectively.



17	R = O-Ceramide, R_1 = H
18	R, R_1 = OH, H
19	R = OCH_2CH_2CHO, R_1 = H
20	R = $O(CH_2)_3NH$-HSA, R_1 = H

In summary, the stereocontrolled synthesis of GD_3 as a 4-methyl-3-pentenyl glycoside **13** was accomplished. This derivative displayed favorable ^1H NMR characteristics in D_2O and could subsequently be attached to carrier proteins. Also, intermediate **16** was suitable for further manipulation for the synthesis of the carbohydrate sequence of neuroblastoma-associated ganglioside GD_2.

EXPERIMENTAL

General Procedures. Specific rotations were determined with an Optical Activity LTD AA-100 polarimeter at 23 °C. ^1H NMR spectra were recorded with a Bruker AM 400 or AM 500 spectrometer in $CDCl_3$ solutions unless otherwise noted and the shift values are expressed in ppm downfield from the internal signal for Me_4Si, or in the case of D_2O, using the signal of HOD at δ 4.8 at ambient temperature as a secondary reference. ^{13}C NMR spectra were recorded at 75.47 MHz and assignments were aided by the J-MOD technique.[17,18] Fast atom bombardment (FAB) mass spectra were obtained with a Kratos AE1 MS9 mass spectrometer in the negative ion mode using TEA matrix. Analytical TLC was performed on Silica Gel F_{254} plates (Merck, Darmstadt) with detection by UV light or by charring with ethanolic sulfuric acid and preparative chromatography was carried out using Kieselgel 60H (Merck, Darmstadt) packed in Michel-Miller HPLPLC columns (Ace Glass, NJ). Ozone was generated using a Welsbach T-408 Ozonator. Concentrations were performed *in vacuo*. GD_3 ganglioside from bovine milk was purchased from Genzyme Corporation.

4 - Methyl - 3 - pentenyl *O* - (2,3,4,6 - tetra - *O* - acetyl - β - D - galactopyranosyl) - (1→4) - 2,3,6 - tri - *O* - acetyl - β - D - glucopyranoside (1). To a rapidly stirred slurry of powdered activated molecular sieves 4Å (25 g) and dry toluene (75 mL) was added dropwise, a solution of silver trifluoromethanesulfonate (20.6 g, 80 mmol) in dry toluene (100 mL) and the mixture was then stirred in the dark at room temperature for 5 h. The solvent was decanted under a stream of argon and a solution of 4-methyl-3-penten-1-ol (2.25 g, 22.5 mmol) in dry dichloromethane (16 mL) was added. After stirring at room temperature for 1 h to ensure dryness, the reaction mixture was cooled to -70 °C under argon and a solution of acetobromolactose (14 g, 20 mmol) in dry dichloromethane (25 mL) was added via syringe. The reaction mixture was allowed to slowly warm to -5 °C and stirred at this temperature overnight. After this time, the mixture was filtered through Celite® and the solvents were removed. The resulting oil was dissolved in dichloromethane (500 mL) and the organic solution was washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), and concentrated. Column chromatography over silica gel using first 40:10:1, then 35:10:1 hexane-ethyl acetate-ethanol gave 6.5 g (45%) of **1** as a white foam: R_f 0.14 (30:10:1 hexane-ethyl acetate-ethanol); $[\alpha]_D$ -15.2°

(*c* 1, chloroform); ^{1}H NMR δ 5.28 (dd, 1H, $J_{4',3'}$ = 3.5 Hz, $J_{4',5'}$ < 1.0 Hz, H-4'), 5.20 (t, 1H, J_t = 9.5 Hz, H-3), 5.16 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.13 (tt, 1H, J_t = 7.0 Hz, J_t < 1.0 Hz, HC=C), 4.88 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.84 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.44 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.44 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 2.0 Hz, H-6a), 4.43 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.07 (dd, 1H, $J_{6'a,6'b}$ = 11.0 Hz, $J_{6'a,5'}$ = 6.5 Hz, H-6'a), 4.05 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.0 Hz, H-6b), 4.04 (dd, 1H, $J_{6'b,6'a}$ = 11.0 Hz, $J_{6'b,5'}$ = 7.5 Hz, H-6'b), 3.84 (dt, 1H, J_t = 7.5 Hz, $J_{5',4'}$ < 1.0 Hz, H-5'), 3.80 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.79 (t, 1H, J_t = 9.5 Hz, H-4), 3.60 (ddd, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.0 Hz, H-5), 3.52 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 2.25 (q, 2H, J_q = 7.0 Hz, $OCH_2C\underline{H}_2$), 2.16 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 6H, 2 × Ac), 2.04 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.68 (bs, 3H, CH_3), 1.60 (bs, 3H, CH_3).

Anal. Calcd for $C_{32}H_{46}O_{18}$: C, 53.48; H, 6.45. Found: C, 53.48; H, 6.54.

4 - Methyl - 3 - pentenyl *O* - (β - D - galactopyranosyl) - (1→4) - β - D - glucopyranoside (2). To a solution of **1** (5 g, 7 mmol) in dry methanol (75 mL) was added a solution of sodium metal (230 mg, 10 mmol) in dry methanol (10 mL) and the resulting solution was stirred at room temperature overnight. The reaction mixture was then deionized with Amberlite® IR 120 (H^+) resin, the mixture was filtered, and the solvents were evaporated to give **2** (2.9 g) in quantitative yield. A small amount of this material (120 mg) was purified over silica gel (7 g) using 5:1 chloroform-methanol as eluant to give pure **2** (104 mg) as a white solid after lyophilization from water: R_f 0.08 (5:1 chloroform-methanol); $[\alpha]_D$ -16.1° (*c* 1, methanol); ^{1}H NMR (D_2O, HOD) δ 5.23 (tm, J_t = 6.5 Hz, J_m = 1.5 Hz, HC=C), 4.50 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.46 (d, 1H, J_d = 7.5 Hz, H-1 or H-1'), 3.98 (dd, 1H, J_d = 12.5 Hz, J_d = 2.0 Hz, H-6a or H-6'a), 3.93 (m, 1H), 3.90 (dt, 1H, J_d = 10.0 Hz, J_t = 7.0 Hz, OCH_2), 3.83 - 3.58 (m, 9H), 3.54 (dd, 1H, J_d = 10.0 Hz, J_d = 8.0 Hz, H-2 or H-2'), 3.30(m, 1H), 2.35 (q, 2H, J_q = 7.0 Hz, $OCH_2C\underline{H}_2$), 1.70 (bs, 3H, CH_3), 1.65 (bs, 3H, CH_3); ^{13}C NMR δ 136.68 [$(CH_3)_2\underline{C}$=C], 120.73 (H$\underline{C}$=C), 103.80 (anomeric C), 102.87 (anomeric C), 79.30, 76.20, 75.61, 75.28, 73.67, 73.40, 71.82, 70.94, 69.41, 61.87, 60.98, 28.76 ($OCH_2\underline{C}H_2$), 25.69 (CH_3), 17.92 (CH_3). Molecular weight for $C_{18}H_{32}O_{11}$: calcd 424.44, found (FAB, positive ion, Cleland matrix): *m/z* 425 $(M+1)^+$.

Anal. Calcd for $C_{18}H_{32}O_{11}$: C, 50.93; H, 7.60. Found: C, 50.45; H, 7.69.

4 - Methyl - 3 - pentenyl *O* - (4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - β - D - glucopyranoside (3). A mixture of **2** (2.8 g, 6.6 mmol) and benzaldehyde dimethyl acetal (2.0 g, 13.2 mmol) in dry

acetonitrile containing a catalytic portion of *p*-toluenesulfonic acid (63 mg, 0.33 mmol) was heated to 60 °C for 30 min. Upon cooling to room temperature, the acid catalyst was quenched by the addition of excess triethylamine and the solvents were evaporated. The resulting solid was washed with ether and then dried *in vacuo* to give crude **3** (3.3 g): R_f 0.50 (6:1 chloroform-methanol).

4 - Methyl - 3 - pentenyl *O* - (3 - *O* - benzoyl - 4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 6 - *O* - benzoyl - β - D - glucopyranoside (4). A solution of benzylidene compound **3** (3.38 g, 6.6 mmol) in 2:1 chloroform-pyridine was cooled to -70 °C under argon and a solution of benzoyl chloride (1.7 mL, 14.5 mmol) in chloroform (5 mL) was added dropwise over 5-10 min. Stirring was continued at this temperature for 1 h. The solution was allowed to slowly warm to -50 °C, quenched with excess methanol, and finally allowed to warm to room temperature. The solvents were evaporated and the residue was coevaporated with toluene. The resulting foam was dissolved in chloroform (350 mL) and the organic solution was washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), and concentrated. Column chromatography over silica gel (250 g) using 20:10:1 hexane-ethyl acetate-ethanol as eluant gave a mixture of benzoates (1.54 g, 28%, which can be recycled to **3** by de-*O*-acylation), followed by pure **4** (2.33 g, 49%): R_f 0.14 (30:1 chloroform-methanol); $[\alpha]_D$ +50.1° (*c* 1, chloroform); ^{1}H NMR δ 8.09 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 8.04 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 7.58 (m, 2H, aromatic), 7.48 - 7.42 (m, 6H, aromatic), 7.38 - 7.33 (m, 3H, aromatic), 5.51 (s, 1H, benzylidene), 5.12 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.10 (tm, 1H, J_t = 7.0 Hz, J_m = 1.0 Hz, HC=C), 4.96 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 1.0 Hz, H-6a), 4.54 (d, 1H, J_d = 7.5 Hz, H-1 or H-1'), 4.49 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 4.40 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.0 Hz, H-6b), 4.37 (m, 1H), 4.35 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.32 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{6'a,5'}$ = 1.0 Hz, H-6'a), 4.28 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 4.10 (dd, 1H, $J_{6'b,6'a}$ = 12.5 Hz, $J_{6'b,5}$ = 1.0 Hz, H-6'b), 3.88 (m, 1H), 3.73 (t, 1H, J_t = 9.0 Hz, H-4), 3.70 (m, 1H), 3.69 (m, 1H, H-5), 3.58 (d, 1H, J_d = 3.5 Hz), 3.55 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.52 - 3.45 (m, 2H), 2.54 (d, 1H, J_d < 1.0 Hz), 2.36 (m, 2H, $OCH_2C\underline{H}_2$), 1.68 (bs, 3H, CH_3), 1.60 (bs, 3H, CH_3).

Anal. Calcd for $C_{39}H_{44}O_{13}$: C, 64.98; H, 6.15. Found: C, 64.44; H, 6.31.

4 - Methyl - 3 - pentenyl *O* - (2 - *O* - acetyl - 3 - *O* - benzoyl - 4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 2,3 - di - *O* - acetyl - 6 - *O* - benzoyl - β - D - glucopyranoside (5). A solution of triol **4** (25 mg, 35 μmol) in 2:1 pyridine-acetic anhydride (4.5 mL) was stirred at room temperature

overnight. The solvents were evaporated and the residue was coevaporated with toluene. Column chromatography over silica gel (4 g) using 70:1 chloroform-methanol gave a quantitative yield of **5** (30 mg) as a white amorphous solid after lyophilization from benzene: R_f 0.41 (30:1 chloroform-methanol); $[\alpha]_D$ +70.9° (*c* 1, chloroform); ^1H NMR δ 8.09 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 8.04 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 7.60 - 7.30 (m, 11H, aromatic), 5.46 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.44 (s, 1H, benzylidene), 5.28 (t, 1H, J_t = 9.5 Hz, H-3), 5.05 (tm, 1H, J_t = 7.0 Hz, J_m = 1.0 Hz, HC=C), 4.98 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.96 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.72 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 2.0 Hz, H-6a), 4.57 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.53 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.45 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 4.44 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.0 Hz, H-6b), 4.32 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{6'a,5'}$ = 1.5 Hz, H-6'a), 4.02 (dd, 1H, $J_{6'b,6'a}$ = 12.5 Hz, $J_{6'b,5'}$ = 1.5 Hz, H-6'b), 3.96 (t, 1H, J_t = 9.5 Hz, H-4), 3.81 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.76 (ddd, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.0 Hz, H-5), 3.45 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.40 (bs, 1H, H-5'), 2.25 (m, 2H, $OCH_2C\underline{H}_2$), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.66 (bs, 3H, CH_3), 1.58 (bs, 3H, CH_3).

Anal. Calcd for $C_{45}H_{50}O_{16}$: C, 63.82; H, 5.95. Found: C, 63.58; H, 6.19.

4 - Methyl - 3 - pentenyl *O* - (4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 6 - *O* - benzoyl - β - D - glucopyranoside (6). A solution of triol **4** (2.25 g, 3.12 mmol) in dry methanol (30 mL) was cooled to -15 °C under argon and magnesium methoxide solution (5 mL, 0.25 mmol) was added. The reaction mixture was stirred at this temperature for 30 min then a second portion of $Mg(OCH_3)_2$ (5 mL) was added. The solution was stirred at 5 ° C for an additional 30 min, then neutralized with excess acetic acid and concentrated. The residue was taken up in chloroform (300 mL) and the solution was then washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), and concentrated. Column chromatography over silica gel (125 g) using 30:1 chloroform-methanol as eluant gave **6** (1.63 g, 85%) as a white solid: R_f 0.50 (8:5:1 chloroform-methanol); $[\alpha]_D$ +25.5° (*c* 1, chloroform); ^1H NMR δ 8.05 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 7.58 (m, 1H, aromatic), 7.49 - 7.42 (m, 4H, aromatic), 7.38 - 7.35 (m, 3H, aromatic), 5.53 (s, 1H, benzylidene), 5.10 (tm, 1H, J_t = 7.0 Hz, J_m = 1.0 Hz, HC=C), 4.91 (dd, 1H, $J_{6a,6b}$ = 12.5 Hz, $J_{6a,5}$ = 1.5 Hz, H-6a), 4.44 (dd, 1H, $J_{6b,6a}$ = 12.5 Hz, $J_{6b,5}$ = 6.0 Hz, H-6b), 4.38 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.35 (d, 1H, J_d = 7.5 Hz, H-1 or H-1'), 4.29 (dd, 1H, $J_{6'a,6'b}$ = 13.0 Hz, $J_{6'a,5'}$ = 1.5 Hz, H-6'a), 4.19 (dd, 1H, $J_{4',3'}$ = 3.5 Hz, $J_{4',5'}$ <

1.0 Hz, H-4'), 4.06 (dd, 1H, $J_{6'b,6'a}$ = 12.5 Hz, $J_{6'b,5'}$ = 2.0 Hz, H-6'b), 3.89 - 3.81 (m, 2H), 3.72 (t, 1H, J_t = 9.5 Hz, H-4), 3.70 - 3.68 (m, 1H, H-5), 3.65 - 3.61 (m, 1H), 3.56 - 3.45 (m, 4H), 2.35 (m, 2H, J_m = 7.0 Hz, $OCH_2C\underline{H}_2$), 1.69 (bs, 3H, CH_3), 1.61 (bs, 3H, CH_3).

Anal. Calcd for $C_{32}H_{40}O_{12}$: C, 62.32; H, 6.54. Found: C, 62.45; H, 6.37.

4 - Methyl - 3 - pentenyl *O* - (2,3 - di - *O* - acetyl - 4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 2,3 - di - *O* - acetyl - 6 - *O* - benzoyl - β - D - glucopyranoside (7). A solution of **6** (32 mg, 52 μmol) in 2:1 pyridine-acetic anhydride (3 mL) was stirred at room temperature overnight. The solvents were evaporated and the residue was coevaporated with toluene. Column chromatography over silica gel (4.5 g) using 75:1 chloroform-methanol gave **7** (38 mg, 93%) as a white amorphous solid after lyophilization from benzene: R_f 0.39 (15:10:1 hexane-ethyl acetate-ethanol); $[\alpha]_D$ +37.0° (*c* 1, chloroform); ^1H NMR δ 8.05 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 7.60 (m, 1H, aromatic), 7.50 - 7.42 (m, 4H, aromatic), 7.40 - 7.35 (m, 3H, aromatic), 5.44 (s, 1H, benzylidene), 5.26 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.26 (t, 1H, J_t = 9.5 Hz, H-3), 5.04 (tm, 1H, J_t = 7.0 Hz, J_m = 1.0 Hz, HC=C), 4.96 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.77 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.69 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 2.0 Hz, H-6a), 4.52 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.41 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.0 Hz, H-6b), 4.28 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{6'a,5}$ = 1.0 Hz, H-6'a), 4.28 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 3.99 (dd, 1H, $J_{6'b,6'a}$ = 12.5 Hz, $J_{6'b,5'}$ = 1.5 Hz, H-6'b), 3.92 (t, 1H, J_t = 9.5 Hz, H-4), 3.80 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.73 (ddd, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,6b}$ = 5.0 Hz, $J_{5,6a}$ = 2.0 Hz, H-5), 3.44 (dt, 1H, J_d = 9.5 Hz, J_t = 7.5 Hz, OCH_2), 3.32 (d, 1H, J_d < 1.0 Hz, H-5'), 2.25 (m, 2H, $OCH_2C\underline{H}_2$), 2.06 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.66 (bs, 3H, CH_3), 1.57 (bs, 3H, CH_3).

Anal. Calcd for $C_{40}H_{48}O_{16}$: C, 61.16; H, 6.16. Found: C, 60.97; H, 6.25.

4 - Methyl - 3 - pentenyl *O* - [benzyl 5 - acetamido - 8 - *O* - (5 - acetamido - 4,7,8,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylono - 1',9 - lactone) - 4,7 - di - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate] - (2→3) - *O* - (4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 6 - *O* - benzoyl - β - D - glucopyranoside (10). A mixture of tetrol **6** (152 mg, 0.25 mmol), silver trifluoromethanesulfonate (67 mg, 0.26 mmol), 2,6-di-*t*-butylpyridine (67 mg, 0.35 mmol), powdered Drierite® (1.9 g), and dry tetrahydrofuran (3 mL) was strirred at room temperature under argon

atmosphere for 4.5 h to ensure dryness. The reaction mixture was then cooled to -45 °C under argon and a solution of chloride **8**[10] (197 mg, 0.21 mmol) in 1:1 dry toluene-tetrahydrofuran (1 mL) was then added dropwise. Upon completion of the addition, the reaction mixture was stirred for an additional 30 min at this temperature and then at 0 °C overnight. The reaction was then diluted with chloroform, filtered through Celite® and the organic filtrate was washed with saturated aqueous sodium bicarbonate, dried (sodium sulfate), and concentrated. The resulting foam was subjected to flash column chromatography over silica gel (10 g) using chloroform, then 60:1 chloroform-methanol as eluant to give crude **10**: R_f 0.06 (4:4:1 hexane-ethyl acetate-ethanol).

4 - Methyl - 3 - pentenyl *O* - [benzyl 5 - acetamido - 8 - *O* - (5 - acetamido - 4,7,8,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylono - 1',9 - lactone) - 4,7 - di - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate] - (2→3) - *O* - (2 - *O* - acetyl - 4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 2,3 - di - *O* - acetyl - 6 - *O* - benzoyl - β - D - glucopyranoside (11). A solution of crude triol **10** was acetylated in 2:1 pyridine-acetic anhydride (9 mL) overnight, after which time the solvents were evaporated and the residue was coevaporated with toluene. Column chromatography over silica gel (4 g) using 6:6:1 hexane-ethyl acetate-ethanol as eluant gave 22 mg of **11** as a white solid after lyophilization from benzene: R_f 0.25 (4:4:1 hexane-ethyl acetate-ethanol, 2 developments); ^{1}H NMR δ 8.05 (dm, 2H, J_d = 8.0 Hz, J_m < 1.0 Hz, aromatic), 7.60 (m, 1H, aromatic), 7.50 (m, 2H, aromatic), 7.40 - 7.24 (m, 10H, aromatic), 5.59 (d, 1H, J_d = 10.0 Hz, NH or NH'), 5.37 (td, 1H, $J_t \approx$ 11.0 Hz, $J_{4'',3''e}$ = 4.5 Hz, H-4''), 5.34 (dd, 1H, $J_{7'',8''}$ = 9.5 Hz, $J_{7'',6''}$ = 2.0 Hz, H-7''), 5.28 (d, 1H, J_d = 10.0 Hz, NH or NH'), 5.25 (d, 1H, J_d = 11.5 Hz, $OC\underline{H}_2C_6H_5$), 5.22 (t, 1H, J_t = 9.5 Hz, H-3), 5.22 (dd, 1H, $J_{7''',8'''}$ = 8.0 Hz, $J_{7''',6'''}$ = 1.5 Hz, H-7'''), 5.20 (ddd, 1H, $J_{8''',7'''}$ = 8.0 Hz, $J_{8''',9'''b}$ = 5.0 Hz, $J_{8''',9'''a}$ = 3.0 Hz, H-8'''), 5.14 (d, 1H, J_d = 11.5 Hz, $OC\underline{H}_2C_6H_5$), 5.06 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.06 (tm, 1H, J_t = 7.0 Hz, J_m = 1.0 Hz, HC=C), 4.99 (td, 1H, $J_t \approx$ 11.0 Hz, $J_{4''',3'''e}$ = 5.0 Hz, H-4'''), 4.98 (s, 1H, benzylidene), 4.94 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.65 (dd, 1H, $J_{6a,6b}$ = 11.5 Hz, $J_{6a,5}$ < 1.0 Hz, H-6a), 4.56 - 4.55 (m, 2H), 4.51 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.43 (dd, 1H, $J_{6b,6a}$ = 11.5 Hz, $J_{6b,5}$ < 1.0 Hz, H-6b), 4.38 (m, 2H), 4.36 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.28 (dd, 1H, J_d = 13.0 Hz, J_d = 3.0 Hz, H-9''a or H-9'''a), 4.22 (q, 1H, J_q = 10.5 Hz, H-5'' or H-5'''), 4.10 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.08 (dd, 1H, $J_{6'a,6'b}$ = 12.0 Hz, $J_{6'a,5'}$ < 1.0 Hz, H-6'a), 4.02 (dd, 1H, J_d = 13.0 Hz, J_d = 5.0 Hz, H-9''b or H-9'''b), 4.00 (m, 1H),

3.96 (dd, 1H, J_d = 10.5 Hz, J_d = 1.5 Hz, H-6" or H-6'"), 3.82 (m, 1H), 3.81 (t, 1H, J_t = 9.5 Hz, H-4), 3.80 (m, 1H, OCH_2), 3.74 (m, 1H, H-5), 3.65 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, $J_{6'b,5'}$ < 1.0 Hz, H-6'b), 3.50 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 3.46 (dt, 1H, J_d = 9.5 Hz, J_t = 6.5 Hz, OCH_2), 2.95 (bs, 1H, H-5'), 2.69 (dd, 1H, $J_{3'''e,3'''a}$ = 13.0 Hz, $J_{3'''e,4'''}$ = 4.5 Hz, H-3'''e), 2.49 (dd, 1H, $J_{3''e,3''a}$ = 13.5 Hz, $J_{3''e,4''}$ = 5.0 Hz, H-3"e), 2.26 (q, 2H, J_q = 7.0 Hz, $OCH_2C\underline{H}_2$), 2.14 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.05 (s, 6H, 2 × Ac), 2.03 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (m, 1H, H-3"a), 1.92 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.82 (t, 1H, J_t = 13.0 Hz, H-3'"a), 1.67 (s, 3H, CH_3), 1.57 (s, 3H, CH_3). Some of the spectral assignments are tentative.

4 - Methyl - 3 - pentenyl *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→8) - *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→3) - *O* - (4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - β - D - glucopyranoside (12). To a solution of **11** (21 mg, 13 μmol) in methanol (2 mL), was added 500 μL of 0.5 M potassium hydroxide in 4:1 methanol-water and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then neutralized with 500 μL of acetic acid and the solvents were removed. The resulting product was purified by column chromatography over silica gel (1.1 g) using 65:35:10, then 60:40:10 chloroform-methanol-water as eluant to give **12** (11 mg): R_f 0.2 (60:40:10 chloroform-methanol-water); ^1H NMR (D_2O, HOD) δ 7.58 (m, 2H, aromatic), 7.48 (m, 3H, aromatic), 5.73 (s, 1H, benzylidene), 5.23 (tm, 1H, J_t = 7.0 Hz, J_m < 1.0 Hz, HC=C), 4.65 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.51 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.41 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 4.28 (dd, 1H, $J_{3',2'}$ = 10.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.25 (bs, 1H, H-5'), 4.24 (dd, 1H, J_d = 12.5 Hz, J_d = 3.5 Hz), 4.16 (m. 1H), 4.05 (dd, 1H, $J_{6'a,6'b}$ = 12.5 Hz, $J_{6'a,5'}$ = 1.0 Hz, H-6'a), 3.94 - 3.59 (m, 20H), 3.58 (ddd, 1H, J_d = 12.5 Hz, J_d = 10.0 Hz, J_d = 4.5 Hz, H-4" or H-4'"), 3.32 (m, 1H, H-2), 2.79 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.66 (dd, 1H, J_d = 12.0 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.36 (q, 2H, J_q = 7.0 Hz, $OCH_2C\underline{H}_2$), 2.07 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.76 (t, 1H, J_t = 12.5 Hz, H-3"a or H-3'"a), 1.73 (s, 3H, CH_3), 1.68 (t, 1H, J_t = 12.0 Hz, H-3"a or H-3'"a), 1.66 (s, 3H, CH_3).

4 - Methyl - 3 - pentenyl *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→8) - *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→3) - *O* - (β - D - galactopyranosyl) - (1→4) -

β - D - glucopyranoside (13). A solution of **12** (11 mg, 10 μmol) in 3:1 acetic acid-water (2.4 mL) was heated to 60 °C for 90 min and the solvents were evaporated. The residue was dissolved in methanol (2 mL) containing 500 μL of 0.5 M potassium hydroxide in 4:1 methanol-water and the reaction mixture was stirred at room temperature overnight, then neutralized with 500 μL of acetic acid and concentrated. The residue was purified by column chromatography over silica gel (1.2 g) using 65:35:2, then 6:4:1 chloroform-methanol-water as eluant to yield 5 mg of deblocked GD_3-4M3P **13**: R_f 0.14 (60:40:10 chloroform-methanol-water); $[\alpha]_D$ -5.7° (*c* 0.5, methanol); ^1H NMR (D_2O, HOD) δ 5.23 (tm, 1H, J_t = 7.0 Hz, J_m < 1.0 Hz, HC=C), 4.53 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.49 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.17 (m, 1H), 4.14 (m, 1H), 4.10 (dd, 1H, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.00 (dd, 1H, J_d = 12.5 Hz, J_d = 2.0 Hz), 3.98 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 3.93 - 3.59 (m, 20H), 3.58 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 3.31 (t, 1H, $J_t \approx$ 8.5 Hz, H-2), 2.79 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.67 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.35 (q, 2H, J_q = 7.0 Hz, $OCH_2\underline{C}H_2$), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.76 (t, 1H, J_t = 12.5 Hz, H-3"a or H-3'"a), 1.74 (t, 1H, J_t = 12.0 Hz, H-3"a or H-3'"a), 1.72 (s, 3H, CH_3), 1.65 (s, 3H, CH_3); ^{13}C NMR δ 175.86 (2 × C=O), 174.56 (C=O), 174.12 (C=O), 136.67 [$(CH_3)_2\underline{C}$=C], 120.73 (H$\underline{C}$=C), 103.54 (anomeric C), 102.68 (anomeric C), 101.43 (sialic acid C-2), 101.21 (sialic acid C-2), 79.01, 78.85, 76.26, 75.98, 75.63, 75.21, 74.91, 73.70, 73.48, 72.63, 70.92, 70.16 (2C), 69.31, 69.05, 68.81, 68.61, 63.46, 62.40, 61.93, 60.92, 53.13 (sialic acid C-5), 52.62 (sialic acid C-5), 41.36 (sialic acid C-3), 40.29 (sialic acid C-3), 28.75 ($OCH_2\underline{C}H_2$), 25.68 (CH_3), 23.15 (CH_3C=O), 22.90 (CH_3C=O), 17.91 (CH_3). Molecular weight for $C_{40}H_{66}O_{27}N_2$: calcd 1006.9, found (FAB, negative ion, TEA matrix): *m/z* 1005.2 $(M-1)^-$.

4 - Methyl - 3 - pentenyl *O* - (benzyl 5 - acetamido - 4,7,8,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→8) - (benzyl 5 - acetamido - 2,4,7,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→3) - *O* - (4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 6 - *O* - benzoyl - β - D - glucopyranoside (14). A mixture of tetrol **6** (373 mg, 0.60 mmol), silver trifluoromethanesulfonate (193 mg, 0.75 mmol), 2,6-di-*t*-butylpyridine (172 mg, 0.9 mmol), powdered Drierite® (2 g), and dry tetrahydrofuran (4.5 mL) was stirred at room temperature under an argon atmosphere for 6 h. The reaction mixture was then cooled to -45 °C under argon and a solution of chloride **9**[10] (649 mg, 0.59 mmol) in dry toluene (1.5 mL)

was added dropwise. Upon completion of the addition, the reaction mixture was stirred for an additional 30 min at this temperature and then at 0 °C overnight. After this time, the reaction mixture was diluted with chloroform and the solution was filtered through Celite®. The filtrate was washed successively with saturated aqueous sodium bicarbonate, water, dried (sodium sulfate), then concentrated to give a yellowish foam. This foam was subjected to flash column chromatography over silica gel (10 g) using 70:1 chloroform-methanol as eluant to provide a crude mixture of **14**, **6**, and by-products that was directly acetylated as follows.

4 - Methyl - 3 - pentenyl *O* - (benzyl 5 - acetamido - 4,7,8,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→8) - (benzyl 5 - acetamido - 2,4,7,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→3) - *O* - (2 - *O* - acetyl - 4,6 - *O* - benzylidene - β - D - galactopyranosyl) - (1→4) - 2,3 - di - *O* - acetyl - 6 - *O* - benzoyl - β - D - glucopyranoside (15). A solution of crude **14** (459 mg) was treated with 2:1 pyridine-acetic anhydride (24 mL) at room temperature overnight and then the mixture was coevaporated with toluene. Column chromatography over silica gel (10 g) using chloroform then 70:1 chloroform-methanol as eluant gave 128 mg of **15** (12% overall yield from chloride **9**) as a fluffy white solid upon lyophilization from benzene: R_f 0.25 (19:1 chloroform-methanol); $[\alpha]_D$ +40.4° (*c* 1, chloroform); ^{1}H NMR δ 8.03 (dd, 2H, J_d = 8.0 Hz, J_d = 1.0 Hz, aromatic), 7.58 (tm, 1H, J_d = 7.5 Hz, J_m < 1.0 Hz, aromatic), 7.45 (bt, 2H, J_t = 8.0 Hz, aromatic), 7.41 - 7.34 (m, 6H, aromatic), 7.32 - 7.28 (m, 9H, aromatic), 6.25 (d, 1H, $J_{NH'',5''}$ = 9.5 Hz, NH''), 5.44 (ddd, 1H, $J_{8''',7'''}$ = 9.5 Hz, $J_{8''',9'''a}$ = 5.5 Hz, $J_{8''',9'''b}$ = 2.5 Hz, H-8'''), 5.36 (bs, 1H, H-7''), 5.33 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.29 (dd, 1H, $J_{7''',8'''}$ = 9.5 Hz, $J_{7''',6'''}$ = 1.5 Hz, H-7'''), 5.25 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.22 (t, 1H, J_t = 9.5 Hz, H-3), 5.20 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.19 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.10 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.07 (d, 1H, $J_{NH''',5'''}$ = 10.5 Hz, NH'''), 5.03 (tm, 1H, J_t = 7.0 Hz, J_m < 1.0 Hz, HC=C), 4.96 (m, 1H, H-4'' or H-4'''), 4.95 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.90 - 4.84 (m, 3H), 4.86 (s, 1H, benzylidene), 4.72 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{6a,5}$ = 2.0 Hz, H-6a), 4.62 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.57 (dd, 1H, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.50 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.43 (dd, 1H, $J_{6b,6a}$ = 12.0 Hz, $J_{6b,5}$ = 5.5 Hz, H-6b), 4.23 (dd, 1H, $J_{9'''a,9'''b}$ = 12.5 Hz, $J_{9'''a,8'''}$ = 5.5 Hz, H-9'''a), 4.16 (dd, 1H, $J_{9'''b,9'''a}$ = 12.5 Hz, $J_{9'''b,8'''}$ = 2.5 Hz, H-9'''b), 4.12 (dd, 1H, $J_{6'a,6'b}$ = 12.0 Hz, $J_{6'a,5'}$ < 1.0 Hz, H-6'a), 4.05 (q, 1H, J_q = 10.5 Hz, H-5'''), 3.95

(q, 1H, J_q = 10.0 Hz, H-5''), 3.92 (dd, 1H, $J_{6'',5''}$ = 10.0 Hz, $J_{6'',7''}$ = 1.5 Hz, H-6''), 3.85 (dd, 1H, $J_{6''',5'''}$ = 10.5 Hz, $J_{6''',7'''}$ = 1.5 Hz, H-6'''), 3.82 (t, 1H, J_t = 9.5 Hz, H-4), 3.78 (m, 1H), 3.77 (dt, 1H, J_d = 9.5 Hz, $J_t \approx$ 7.0 Hz, OCH_2), 3.71 (ddd, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,6b}$ = 5.5 Hz, $J_{5,6a}$ = 2.5 Hz, H-5), 3.67 (dd, 1H, $J_{6'b,6'a}$ = 12.0 Hz, $J_{6'b,5'}$ < 1.0 Hz, H-6'b), 3.46 (d, 1H, $J_{4',3'}$ = 3.5 Hz, H-4'), 3.44 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.30 (bs, 1H, H-5'), 2.81 (dd, 1H, J_d = 12.5 Hz, J_d = 5.0 Hz, H-3''e or H-3'''e), 2.80 (dd, 1H, J_d = 12.5 Hz, J_d = 5.0 Hz, H-3''e or H-3'''e), 2.23 (m, 2H, $OCH_2C\underline{H}_2$), 2.19 (s, 3H, Ac), 2.17 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 6H, 2 × Ac), 2.00 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.89 (s, 3H, Ac), 1.87 (t, 1H, J_t = 12.5 Hz, H-3''a or H-3'''a), 1.77 (t, 1H, J_t = 12.0 Hz, H-3''a or H-3'''a), 1.65 (s, 3H, CH_3), 1.59 (s, 3H, CH_3). Some of the spectral assignments are tentative.

Anal. Calcd for $C_{88}H_{106}O_{38}N_2$: C, 58.73; H, 5.94; N, 1.56. Found: C, 58.52; H, 6.23; N, 1.55.

4 - Methyl - 3 - pentenyl *O* - (benzyl 5 - acetamido - 4,7,8,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→8) - (benzyl 5 - acetamido - 2,4,7,9 - tetra - *O* - acetyl - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonate) - (2→3) - *O* - (2 - *O* - acetyl - β - D - galactopyranosyl) - (1→4) - 2,3 - di - *O* - acetyl - 6 - *O* - benzoyl - β - D - glucopyranoside (16). A solution of blocked tetrasaccharide **15** (85 mg, 47 μ mol) in 3:1 acetic acid-water (6 mL) was heated to 60 °C and the reaction was monitored by TLC. After 5.5 h, the solvents were removed and the resulting mixture was purified by column chromatography over silica gel (4 g) using 60:1, then 50:1 chloroform-methanol as eluant to give first 13 mg (15%) of recovered **15** followed by 50 mg (62%) of the desired **16** as a solid after lyophilization from benzene: R_f 0.16 (15:1 chloroform-methanol); $[\alpha]_D$ +16.7° (*c* 1, chloroform); ^1H NMR δ 8.03 (dd, 2H, J_d = 8.0 Hz, J_d = 1.0 Hz, aromatic), 7.60 (tm, 1H, J_d = 7.5 Hz, J_m < 1.0 Hz, aromatic), 7.45 (bt, 2H, J_t = 8.0 Hz, aromatic), 7.40 - 7.30 (m, 10H, aromatic), 6.30 (d, 1H, $J_{NH'',5''}$ = 10.0 Hz, NH''), 5.44 (ddd, 1H, $J_{8''',7'''}$ = 9.5 Hz, $J_{8''',9'''}$ = 5.5 Hz, $J_{8''',9'''}$ = 2.5 Hz, H-8'''), 5.37 - 5.31 (m, 3H), 5.27 (dd, 1H, $J_{7''',8'''}$ = 9.5 Hz, $J_{7''',6'''}$ = 2.0 Hz, H-7'''), 5.25 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.21 (t, 1H, J_t = 9.5 Hz, H-3), 5.19 (d, 1H, J_d = 12.0 Hz, $OC\underline{H}_2C_6H_5$), 5.09 (d, 1H, $J_{NH''',5'''}$ = 10.0 Hz, NH'''), 5.05 (dd, 1H, J_d = 12.0 Hz, J_d = 2.0 Hz), 5.01 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{2',1'}$ = 8.0 Hz, H-2'), 5.01(m, 1H, HC=C), 4.96 (ddd, 1H, J_d = 12.5 Hz, J_d = 10.0 Hz, J_d = 4.5 Hz, H-4'' or H-4'''), 4.91 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{2,1}$ = 8.0 Hz, H-2), 4.86 (m, 1H, H-4'' or H-4'''), 4.85 (dd, 1H, J_d = 9.5 Hz, J_d =

2.0 Hz), 4.71 (dd, 1H, J_d = 11.5 Hz, J_d = 2.0 Hz), 4.61 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.49 (d, 1H, J_d = 8.0 Hz, H-1 or H-1'), 4.36 (dd, 1H, $J_{3',2'}$ = 10.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.30 (dd, 1H, J_d = 12.0 Hz, J_d = 2.5 Hz), 4.30 (dd, 1H, J_d = 12.0 Hz, J_d = 5.5 Hz), 4.20 - 4.12 (m, 3H), 4.08 - 3.89 (m, 5H), 3.82 (dd, 1H, J_d = 10.5 Hz, J_d = 1.5 Hz), 3.80 (dd, 1H, J_d = 10.0 Hz, J_d = 2.0 Hz), 3.78 - 3.67 (m, 3H), 3.50 (bm, 1H), 3.42 (dt, 1H, J_d = 9.5 Hz, J_t = 7.0 Hz, OCH_2), 3.35 (bt, 1H, J_t = 7.0 Hz), 3.26 (bd, 1H, J_d = 2.5 Hz), 2.77 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.76 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.31 (m, 2H, $OCH_2C\underline{H}_2$), 2.17 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02 (s, 6H, 2 × Ac), 1.96 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.88 (t, 1H, J_t = 12.5 Hz, H-3"a or H-3'"a), 1.83 (t, 1H, J_t = 12.5 Hz, H-3"a or H-3'"a), 1.66 (s, 3H, CH_3), 1.56 (s, 3H, CH_3). Some of the spectral assignments are tentative.

Synthesis of GD3-4M3P (13) from (16). A solution of **13** (53 mg, 31 μ mol) was dissolved in methanol (2 mL) to which 0.5 M potassium hydroxide in 4:1 methanol-water (1 mL) was added and the mixture was stirred at room temperature overnight. The potassium hydroxide was then neutralized by the addition of excess acetic acid and the solvents were evaporated. Column chromatography over silica gel (4 g) using 65:35:6, then 6:4:1 chloroform-methanol-water as eluant provided 29 mg (93%) of the GD3-4M3P derivative **13**.

***O* - (5 - Acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→8) - *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→3) - *O* - (β - D - galactopyranosyl) - (1→4) - β - D - glucopyranoside and α - anomer (18).** A solution of GD_3-ceramide **17** (8 mg, 5 μmol) from bovine source (Genzyme) in methanol (5 mL) was cooled to ≈ -15 °C (dry ice/ethanol) and ozone was bubbled through the solution for 3 min. After this time, argon was passed through the solution for a few minutes, then the solvents were evaporated. The residue was taken up in 0.01 M aqueous sodium carbonate (2.5 mL) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then neutralized with acetic acid and the solvents were evaporated. Column chromatography over silica gel (1 g) using 6:4:1, then 5:4:1 chloroform-methanol-water as eluant provided 2 mg (46%) of **18**: R_f 0.08 (5:4:1 chloroform-methanol-0.2% aqueous calcium chloride); ^1H NMR (D_2O, HOD, 2:1 β/α mixture) δ 5.23 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1, α-anomer), 4.68 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1, β-anomer), 4.68 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.19 (dm, J_d = 13.0 Hz, J_m < 1.0 Hz), 4.16 (m, 1H), 4.11 (dt, J_d = 9.5 Hz, J_t = 3.0 Hz, possibly H-8'), 4.01 - 3.56 (m,

22H), 3.30 (dd, $J_{2,3}$ = 9.0 Hz, $J_{2,1}$ = 8.0 Hz, H-2, β-anomer), 2.80 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.69 (dd, 1H, J_d = 12.5 Hz, J_d = 4.5 Hz, H-3"e or H-3'"e), 2.09 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.75 (t, 2H, J_t = 12.5 Hz, H-3"a and H-3'"a). Molecular weight for $C_{34}H_{56}O_{27}N_2$: calcd 924.8, found (FAB, negative ion, TEA matrix): *m/z* 923.87 $(M-1)^-$.

Synthesis of GD_3-Reducing sugar (18) from (13). A solution of **13** (4 mg, 4 μmol) in methanol (4 mL) was cooled to -70 °C and ozone was bubbled through the solution for 3 min. After this time, argon was passed through the solution for a few minutes then the solvents were evaporated. The residue was taken up in 0.01 M aqueous sodium carbonate (1.1 mL) and the reaction mixture was stirred at room temperature overnight. The mixture was neutralized with acetic acid and the solvents were evaporated. Column chromatography over silica gel (400 mg) using 6:4:1, then 5:4:1 chloroform-methanol-water as eluant provided 2.9 mg (79%) of **18**. The ^1H NMR and mass spectra for **18** obtained in this manner were identical to that obtained from the reaction using GD_3 from natural source.

3 - Oxopropyl *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→8) - *O* - (5 - acetamido - 3,5 - dideoxy - D - *glycero* - α - D - *galacto* - 2 - nonulopyranosylonic acid) - (2→3) - *O* - (β - D - galactopyranosyl) - (1→4) - β - D - glucopyranoside (19). A solution of **13** (5 mg, 5 μmol) in methanol (5 mL) was cooled to -70 °C and ozone gas was bubbled through the solution for 2 min. Argon was then passed through the solution to remove the excess ozone. The solvents were evaporated and the residue was dissolved in methanol (2 mL), cooled to -70 °C, and excess methyl sulfide (6 μL, 82 μmol) was added. The cooling bath was removed and the reaction was stirred for 5 min, then the solvents were evaporated to give **19** (5 mg) which was used directly in the subsequent conjugation reaction without further purification or characterization: R_f 0.2 (40:35:10 chloroform-methanol-water).

GD_3-HSA Conjugate (20). A solution of the aldehyde **19** (5 mg), and human serum albumin (14 mg) in phosphate buffer (1.4 mL) at pH 5.93 (or in acetate buffer at pH 4.65), was stirred at room temperature for 45 min, then a freshly prepared solution of sodium cyanoborohydride (100 μL, 16 mg/mL, 25 μ mol) in buffer (pH 5.93 or 4.65) was added and stirring was continued for an additional 72 h. Exhaustive dialysis (Amicon, YM 10 ultrafiltration membrane) against water followed by lyophilization provided 13 mg of GD_3-HSA conjugate **20**. The sialic acid content of glycoconjugate **20** was estimated by the resorcinol-

hydrochloric acid assay and the GD_3/HSA ratio corresponded 9 and 11 for conjugations performed at pH 5.93 and 4.65 respectively.

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2. GM_3: α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-Ceramide; GD_3: α-D-Neu*p*5Ac-(2→8)-α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-Ceramide, GD_2: β-D-Gal*p*NAc-(1→4)-[α-D-Neu*p*5Ac-(2→8)-α-D-Neu*p*5Ac-(2→3)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*-Ceramide.

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