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The total synthesis of ganglioside GM3

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Abstract

Previous syntheses of ganglioside GM3 (NeuAcα3Galβ4Glcβ1Cer) are reviewed, and both chemoenzymatic and chemical total synthetic approaches were investigated. In a chemoenzymatic approach, (2*S*,3*R*,4*E*)-5‴-acetyl-α-neuraminyl-(2‴→3″)-β-galactopyranosyl-(1″→4′)-β-glucopyranosyl-(1′↔1)-2-azido-4-octadecene-1,3-diol (azidoGM3) was readily prepared utilizing recombinant β-Gal-(1″→3′/4′)-GlcNAc α-(2‴→3″)-sialyltransferase enzyme, and was evaluated as a synthetic intermediate to ganglioside GM3. The chemical total synthesis of ganglioside GM3 was performed on one of the largest scales yet reported. The highlights of this synthesis include minimizing the steps necessary to prepare the lactosyl acceptor as a useful anomeric mixture, which was present in excess for the highly regioselective and fairly stereoselective sialylation with a known neuraminyl donor to give the protected GM3 trisaccharide. The synthetic methodology maximized convergence by a subsequent glycosidic coupling of the well-characterized GM3 trisaccharide trichloroacetimidate derivative with protected ceramide. The ganglioside GM3 was nearly homogeneous as the two glycosidic couplings utilized preparative HLPC purifications, and variations in the sphingosine base and fatty acyl group were under 0.1 and 0.2%, respectively.

Keywords: Sialyltransferase; Ceramide; Benzyl 4-*O*-(2,6-di-*O*-benzylgalactopyranosyl)-2,3,6-tri-*O*-benzylglucopyranoside; Trichloroacetimidate; Ganglioside GM3

1. Introduction

Ganglioside GM3 (NeuAcα3Galβ4Glcβ1-Cer, **6**) is a ubiquitous metabolite with a variety of cellular activities that have been reviewed and include differentiation [1], the modulation of several receptors and other enzymes [2,3], immunosupression [4], and tumor-association [5]. The preparation of this ganglioside is important for further biophysical and biological studies. There have already been a number of previous syntheses of ganglioside GM3 **6** [6–22], the trisaccharide portion (NeuAcα3Galβ4Glc) [23–28], protected derivatives of this trisaccharide [29–51], and other trisaccharide analogs [4,10,12,16, 43,45,49–57], which have utilized chemoenzymatic as well as chemical total synthetic sequences.

The chemoenzymatic approaches to ganglioside GM3 **6** have utilized one or more enzyme-assisted conversion steps. The obvious advantages of chemoenzymatic approaches are the complete control of regiochemistry and stereochemistry for the glycosidic coupling reactions. The chemoenzymatic routes have generally been demonstrated on smaller scales, until recently [28], and have generally required reversed-phase high-performance liquid chromatography (HPLC) isolations. The low activity of a β-Gal-(1″→3′/4′)-GlcNAc α-

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(2‴ → 3″)-sialyltransferase (α-2,3-NeuAcT) for lactosyl ceramide (Galβ4Glcβ1Cer, LacCer) [58] has required additional steps to synthesize better substrates suitable for chemoenzymatic GM3 **6** syntheses with this enzyme [13–15,19–21,28,34,38,45,49,52,56,57].

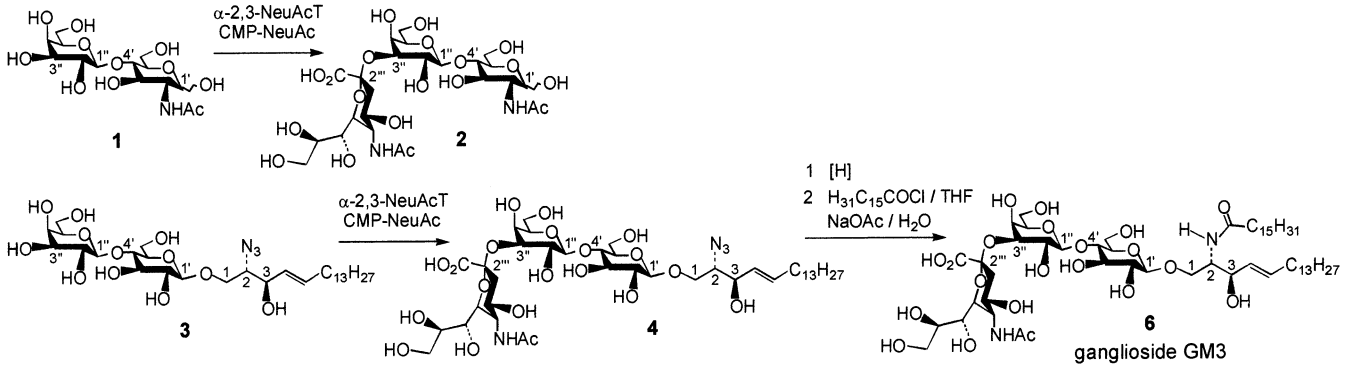
Chemical synthetic methods offer the flexibility to prepare analogs as well as the naturally occuring gangliosides. Chemical neuraminyl glycosidic couplings to 3″,4″-unsubstituted lactose derivatives have already been reported to have very high regioselectivity for the desired 3″-position [6,8,11,22–26,30,31,33,35,36,40–42,44,46,47,50,51]. Control of the α-glycosidic linkage of the neuraminyl residue is a continuing research interest [17,39,42,44,47,50]. Recently, the coupling of activated GM3 trisaccharide derivatives with protected azidosphingosine derivatives [9,10,14,16–18,22,33,41,46] rather than protected ceramide derivatives [6,8,11,13] has become more commonly used when a series of fatty acyl analogs is required.

This report details a practical chemical total synthetic method to obtain a research quantity of ganglioside GM3 **6** with the goal of a more concise and convergent approach. This synthesis proceeds through a minimum of reaction steps to yield ganglioside GM3 **6** that is optically pure and nearly homogeneous with respect to variations in the sphingosine base and the fatty acyl group. This synthesis utilizes an easily prepared anomeric mixture of the lactosyl acceptor [41]. Also, maximum convergence is possible by the subsequent glycosidic coupling of the GM3 trisaccharide trichloroacetimidate with a ceramide derivative rather than an azidosphingosine analog. The ganglioside GM3 (NeuAcα3Galβ4Glcβ1-Cer, **6**) represents the next member of the membrane lipid series under investigation in this laboratory after ceramide (Cer) [59,60], glucosylceramide (Glcβ1Cer) and galactosylceramide (Galβ1Cer) [61], and lactosylceramide (Galβ4Glcβ1Cer) [62]. Similar structural and physical studies of this homogeneous ganglioside GM3 **6** are currently in progress.

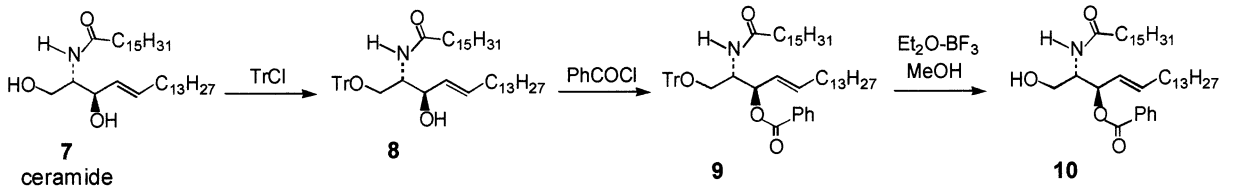
2. Results and discussion

Both chemoenzymatic and chemical total synthetic approaches to ganglioside GM3 **6** were investigated. A chemoenzymatic approach utilizing a sialyl transferase enzyme in the neuraminyl glycosylation step was performed. Alternatively, a chemical total synthesis of ganglioside GM3 **6** was developed, which involved a minimum number of synthetic steps. The purity of synthetic intermediates was assured by HPLC and all compounds were well characterized by NMR spectroscopy in multiple solvent systems.

Several chemoenzymatic synthetic approaches to ganglioside GM3 **6** have previously utilized β-Gal-(1″ → 3′/4′)-GlcNAc α-(2‴ → 3″)-sialyl transferase (α-2,3-NeuAcT, ST3N, EC 2.4.99.6) enzyme [13–15,19,21,34,45,52,56,57]. Recombinant α-2,3-NeuAcT enzyme was obtained from Cytel (Noese) and was first assayed for activity with its natural substrate. *N*-Acetyllactosamine (LacNAc, Galβ4GlcNAc, **1**) was readily converted into the corresponding trisaccharide (α-*N*-acetylneuraminyl-(2‴ → 3″)-*N*-acetyllactos-



Scheme 1.



Scheme 2.

amine, α-NeuAc-(2‴→3″)-LacNAc, NeuAcα-3Galβ4GlcNAc, **2**) (Scheme 1) in the presence of commercially available cytidine 5′-monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) according to the reported method [13,63,64]. The trisaccharide product **2** was chromatographed and demonstrated to be homogeneous in a neutral solvent system (1:3:1 ethyl acetate–*i*-propanol–water), which showed that the product **2** was free of NeuAc. Also, trisaccharide **2** was demonstrated to be homogeneous in an acidic solvent system (40:40:7:3 chloroform–methanol–water–acetic acid), which showed that this product trisaccharide **2** was free of starting disaccharide **1**. The ^{1}H NMR and ^{1}H–^{1}H COSY spectra were consistent with a mixture of the α and β anomers. Electrospray-ionization mass spectral analysis of a permethylated sample gave strong molecular ions for both the monosodium ion at *m*/*z* 879 and the disodium ion at *m*/*z* 451.

(2*S*,3*R*,4*E*)-β-Galactopyranosyl-(1″→4′)-β-glucopyranosyl-(1′↔1)-2-azido-4-octadecene-1,3-diol (lactosylazidosphingosine, **3**) [14,65] was previously demonstrated to be a substrate for the recombinant α-2,3-NeuAcT enzyme [14]. However, this lactoside **3** was found to be a relatively poor substrate for this enzyme. Lactosylazidosphingosine **3** was converted into (2*S*,3*R*,4*E*) - 5‴ - acetyl - α - neuraminyl-(2‴→3″)-β-galactopyranosyl-(1″→4′)-β-glucopyranosyl - (1′↔1) - 2 - azido - 4 - octadecene - 1,3-diol (azidoGM3, **4**) [10,14,18] on a 19 mg scale. The neuraminyl glycosylation was driven nearly to completion by the gradual addition of CMP-NeuAc over 2 weeks. The azidoGM3 **4** was characterized by NMR spectroscopy (^{1}H, ^{13}C, ^{1}H–^{1}H correlation, and ^{1}H–^{13}C heterocorrelation). The α-glycosidic linkage was demonstrated by the characteristic [11,19,22–24,52,66–68] chemical shifts of the 3eq‴ and 4‴ protons. A sample of the permethylated derivative of **4** for analysis by mass spectrometry was prepared with methyl iodide in aqueous alkali. The methyl ester group of the permethylated derivative was quite labile and a sample was completely exchanged with methyl-d_3 iodide in aqueous alkali to give the methyl-d_3 ester labeled analog by mass spectral analysis.

Reduction of azidoGM3 **4** to the known [14,18,21,49,69–72] zwitterionic lysoGM3 **5** was reported to give the amino acid **5** in yields as high as 80% with H_2S [18]. However, the azide reduction of azidoGM3 **4** was found to be slow and gave a mixture of products in accordance with a related report [17]. The subsequent acylation with hexadecanoyl chloride did not give good quality ganglioside GM3 **6**.

Ideally, the multigram preparation of ganglioside GM3 **6** awaits the utilization of cytidine monophospho-*N*-acetylneuraminate: lactosylceramide α-2,3-sialyltransferase (SAT-1, GM3 synthase, EC 2.4.99.9) [73–76] in combination with cytidine monophospho-*N*-acetylneuraminic acid synthetase (CMP-NeuAc synthetase, EC 2.7.7.43) [13,15,28, 34,77–82], which generates the expensive CMP-NeuAc cofactor in situ. The availability of SAT-1 from recombinant technology [78,80–82] would allow conversion of the readily synthesized substrate lactosylceramide [62,65,83–88] directly into ganglioside GM3 **6**. However, enzymatic methods are most useful only for a few endogeneous compounds and closely related analogs. Synthetic methods are critical for analogs with structural variations, which are poor enzyme substrates.

The chemical total synthesis of ganglioside GM3 **6** was performed avoiding the problematic azidoGM3 **4** intermediate by re-investigation of a more convergent approach with the use of a 3-O-protected ceramide in the glyco-

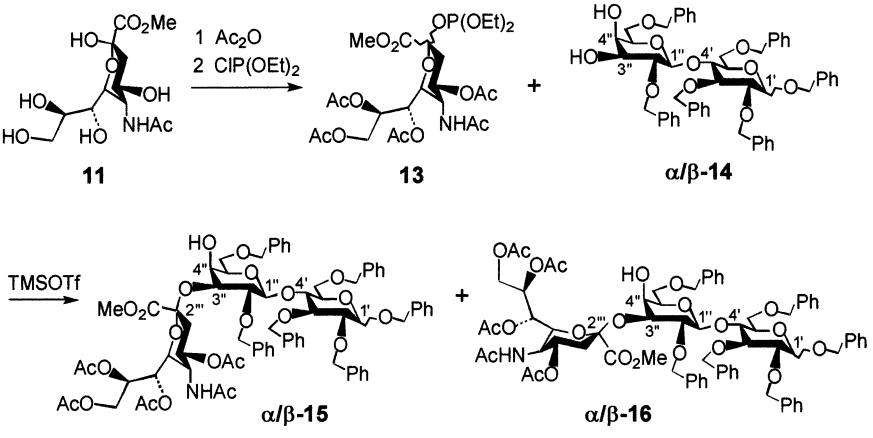
sidic coupling step. This lipid portion was derived (Scheme 2) from synthetic ceramide **7** [59,84,89–91] that was demonstrated to be optically pure, 99.9% as the C18:1 sphingosine base, and the fatty acyl group was demonstrated to be 99.8% hexadecanoyl (C16:0).

The glycosidic coupling of the neuraminyl donor **13** [35,36,41,46,92] with the 3″,4″-unprotected lactose derivative β-**14** [6,8,23–26,35,36,41,47,93–97] was then investigated (Scheme 3). Nearly complete regioselectivity for sialylation at the desired 3″-position of β-**14** has been reported [6,8,23–26,35,36,41,47] to give the ganglioside GM3 trisaccharide derivative (**2**‴α,**1**′β)-**15** [6,23,24,35,36,41,47] and the 2‴β by-product (**2**‴β,**1**′β)-**16** [6,23,24]. However, the easily prepared anomeric mixture of lactosyl acceptor α,β-**14** was recently suggested [41] to be as useful as pure β-**14** for the neuraminyl glycosidic coupling. This has now been demonstrated experimentally.

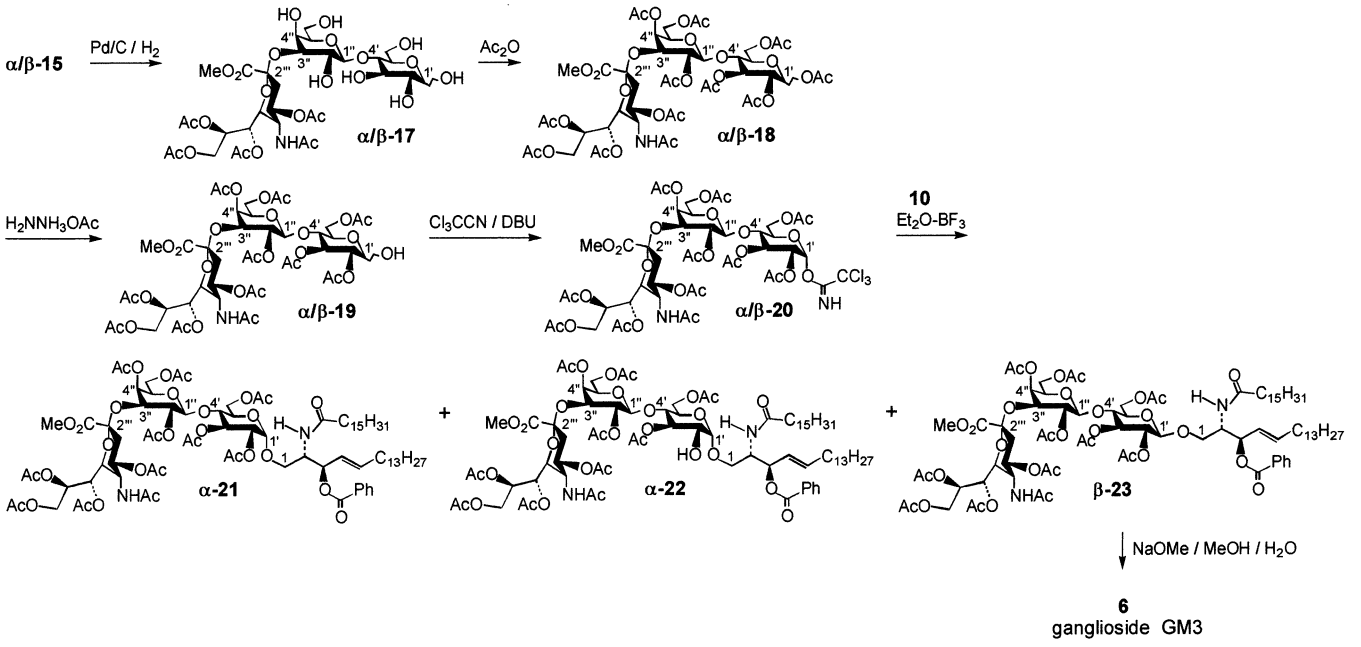
The α,β-hexabenzyllactose (α,β-**14**) synthesized from β-lactose was nearly all β anomer (3:17 α,β), in contrast to the previously reported [41] 6:1 α,β mixture. A satisfactory neuraminyl glycosidic coupling of donor **13** with the anomeric mixture of lactosyl acceptor α,β-**14** gave the ganglioside GM3 oligosaccharide derivative (**2**‴α,**1**′α,β)-**15** as an anomeric mixture, which was readily separated from the anomeric mixture of by-product (**2**‴β,**1**′α,β)-**16**. Extra synthetic steps to improve the α,β ratio of this glycosidic coupling were not performed. The ganglioside GM3 oligosaccharide derivative (**2**‴α,**1**′α,β)-**15** was isolated in 22% (39% based on lactose derivative α,β-**14** consumed) and the by-product (**2**‴β,**1**′α,β)-**16** was isolated in 8.7% (15% yield based on lactose derivative α,β-**14** consumed). The downfield shifts of the 3″ protons for the coupling products and the correlations of the 4″ protons to the 4″-OH groups in anhydrous Me_2SO-d_6 clearly showed the 2‴ → 3″ glycosidic linkages, and the 2‴ configurations were demonstrated by the reported [23,24,36,44] characteristic chemical shift of 4‴ and the upfield shift of the 3ax‴ resonance for (**2**‴β,**1**′β)-**16** in C_6D_6. The synthesis of ganglioside GM3 **6** via α,β-**14** saves four synthetic steps otherwise necessary to fix the C-1′ center, which is epimerized and stereoselectively controlled again later in the synthesis.

The hydrogenolysis of α,β-**15** gave trisaccharide derivative α,β-**17** (Scheme 4) identical to material prepared [36] from the longer sequence using the pure β-glucoside anomer. Peracetylation [6,36] of α,β-**17**, selective deacetylation [6,22] of α,β-**18**, and conversion of α,β-**19** into the mostly 1′α-trichloroacetimidate α,β-**20** [6,22] was then performed.

The glycosidic coupling of the activated GM3 trisaccharide derivative α,β-**20** with a protected ceramide **10** [90,91] was recognized to be the most convergent synthetic approach to GM3 **6**, avoiding later azide reduction and fatty acylation reactions. The use of boron trifluoride etherate catalyst for the coupling rather than the stronger Lewis acid used for the previously discussed neuraminyl glycosidic coupling assured that no cis/trans isomerization of the alkene bond would occur under the reaction and workup conditions. The glyco-



Scheme 3.



Scheme 4.

sidic coupling of trichloroacetimidate donor α,β-**20** with ceramide acceptor **10** gave a 1:1:17 mixture of α-**21**:α-**22**:β-**23** in a high coupling yield based on recovered donor and acceptor. The 2′-hydroxyl by-product α-**22** co-eluted just ahead of the desired coupling product β-**23**. A small amount of this by-product α-**22** from a putative orthoester intermediate was isolated and characterized by NMR spectroscopy in Me_2SO-d_6 and in $CDCl_3$. The identification of this alcohol by-product α-**22** stimulated the ultimate purification strategy. The crude product mixture of α-**21**, α-**22**, and β-**23** was acetylated with acetic anhydride in pyridine, and the resulting mixture of α-**21** and β-**23** was then easily separated by preparative HPLC. The by-product α-**21** was isolated in a coupling yield of 2.6%, and the desired coupling product β-**23** was isolated in a glycosidic coupling yield of 41%. The yield of the GM3 intermediate β-**23** was actually as high as 66% based on trisaccharide, which was recovered by peracetylation to give α,β-**18**. The ester hydrolysis of a sample of β-**23**, which required the presence of water to complete the benzoate ester hydrolysis, gave ganglioside GM3 **6** [18] in quantitative yield.

Homogeneous ganglioside GM3 **6** was readily prepared on a 100 mg scale by chemical total synthesis with a remarkable economy of reaction steps proceeding through well known ganglioside synthetic intermediates. This short sequence utilized the lightly protected and easily prepared anomers of the lactosyl acceptor α,β-**14** to minimize synthetic steps. Also, the highly convergent use of a 3-O-protected ceramide **10** for coupling was performed to save two synthetic steps and avoid an azide intermediate. Azide reductions are extremely clean with H_2S for neutral glycosphingolipids, but give impure lysoganglioside, and hydrogenation with the Lindlar catalyst [22] can be substituted when necessary. Some *N*-acetyl analog in the ceramide portion has been detected as a by-product by mass spectrometry in our ganglioside products when proceeding through acetate-protected lysoganglioside intermediates, and is completely avoided by this glycosidic coupling with a protected ceramide.

The use of multiple solvents for NMR characterizations was also useful. Anhydrous Me_2SO-d_6 assisted with the identification of protons on carbons with free hydroxyl groups. The OH resonances correlated to vicinal protons and ranged from easily assignable multiplets to broadened signals for certain compounds, especially moist compounds where some rapid exchange must occur. Samples should always be run in anhydrous

Me_2SO-d_6 before adding a few percent of D_2O. Strikingly characteristic spectra of benzylated compounds were obtained in C_6D_6 where unique conformations were observed by solvation of the aromatic rings. The NMR spectra of ganglioside GM3 **6** were compared with literature reports [11,19,22,66,68].

The ganglioside GM3 **6** was synthesized in only nine synthetic steps by a highly convergent sequence starting from commercially available NeuAcMe **11** in an overall yield of 2.4% (3.9% based on recovered α,β-**18**). The yields of the two glycosidic coupling steps kept the overall yield low, but there are no scale limitations to any of these synthetic steps. The long-term storage of ganglioside GM3 **6** in dimethyl sulfoxide below 0 °C, or as the fully protected precursor β-**23**, is recommended since GM3 **6** is known to undergo lactonization [22,98].

3. Experimental

General methods.—Reactions were carried out under argon with magnetic stirring at ambient temperature, unless reported as bath temperature (bt). Organic phases were dried over Na_2SO_4, followed by paper filtration and solvent removal by rotary-evaporation under diminished pressure. Silica gel (grade 60, 230–400 mesh, E. Merck) was used for column chromatography and filtrations. Analytical thin-layer chromatography (TLC) also utilized pre-coated silica gel 60 plates, which were visualized by charring [99]. HPLC columns were Lichrosorb Si-60 of 5 μm particles. Reversed-phase HPLC columns were Delta Pak C_{18} of 3 μm particles. Product solutions were microfiltered through 0.5 μm Teflon membranes (Alltech). NMR utilized Me_4Si as an internal reference, except for Me_2SO-d_6, where the solvent peaks at δ 2.50 for ^1H and δ 39.50 for ^{13}C were used. Samples run in D_2O also utilized Me_2SO-d_6 as an internal reference. Chemical shifts were assigned with the assistance of COSY and HMQC spectra, and an asterix (*) indicates a tentative assignment. Couplings (J) for benzylic protons are geminal. Electrospray-ionization mass spectrometry (ESMS) was performed, and the molecular ion and selected major fragments and clusters have been reported. More clusters with sodium ion were seen by adding NaOH to the sample or by using higher cone voltages where more fragmentation was also observed. The notations for fragment ions have been previously reported [100,101]. The primed (′) fragment notation indicates either the loss of AcOH (M_w 60) or the loss of $PhCO_2H$ (M_w 122). All solvent ratios are by volume. Hex is hexanes, FA is fatty acyl, NeuAc is 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid, Gal is D-galactose, Glc is D-Glucose, and for glycosides **14**–**23** α or β, or α,β without a locant refers to C-1′ of the glucosyl residue.

N-*Acetyl*-α-*neuraminyl*-(2‴→3″)-β-*galactopyranosyl*-(1″→4′)-N-*acetylglucosamine* (α-*NeuAc*-(2‴→3″)-*LacNAc*) (**2**).—To a 1.0 mL solution of LacNAc **1** (2.0 mg, 5.2 μmol, Sigma), CMP-NeuAc (4.0 mg, 5.7 μmol, Sigma), calf intestine alkaline phosphatase (1.6 mg, Sigma, assayed to be active with CMP), bovine serum albumin (0.10 mg, Sigma), containing Triton CF-54 (0.5%, Sigma), HEPES (0.10 M), $MgCl_2$ (5.0 mM), $MnCl_2$ (2.0 mM), and $ZnCl_2$ (5.0×10^{-5} M) at pH 7.50 degassed with Ar was added α-2,3-NeuT (0.10 U, as 20 μL of 5 U/mL, Cytel/Noese) and the reaction was stirred gently at ambient temperature for 3 days. The reaction had become slightly heterogeneous, and the white precipitate only partially redissolved when the pH was adjusted from 6.60 to 7.43 with concd aq KOH. The reaction appeared to be complete by analytical TLC (1:3:1 EtOAc–2-propanol–water, R_f of LacNAc **1** 0.30, R_f of α-NeuAc-(2‴→3″)-LacNAc **2** 0.22, R_f of NeuAc 0.12). After adding 4 mL of 2-propanol and 2 mL of EtOAc, the crude reaction mixture was filtered through a short column (1:3:1 EtOAc–2-propanol–water). The crude product was re-chromatographed (1:3:1 EtOAc–2-propanol–water) and microfiltered to give **2** (2.3 mg, 3.4 μmol, 65%) [13,63,64] as an amorphous white solid that was a 3:2 α,β mixture of anomers but was homogeneous by TLC (40:40:7:3 $CHCl_3$–MeOH–water–HOAc, R_f of LacNAc **1** 0.29, R_f of α-NeuAc-(2‴→3″)-LacNAc **2** 0.10, R_f of NeuAc 0.07); α-**2** ^1H NMR (D_2O): δ 4.98 (br

s, 1 H, 1′), 4.33 (d, 1 H, $J_{1'',2''}$ 7.5 Hz, 1″), 3.90 (dd, 1 H, $J_{2'',3''}$ 10, $J_{3'',4''}$ 2 Hz, 3″), 3.40–3.80 (m, 17 H, 2′, 3′, 4′, 5′, 6a′, 6b′, 4″, 5″, 6a″, 6b″, 4‴, 5‴, 6‴, 7‴, 8‴, 9a‴, 9b‴), 3.38 (dd, 1 H, 2″), 2.53 (dd, 1 H, $J_{3ax''',3eq'''}$ 12, $J_{3eq''',4'''}$ 4 Hz, 3eq‴), 1.82 (s, 3 H, 5‴-NAc), 1.58 (dd, 1 H, $J_{3ax''',4'''}$ 12 Hz, 3ax‴); ^{13}C NMR (D_2O): δ 90.39 (1′), 102.47 (1″). β-**2** ^{1}H NMR (D_2O): δ 4.50 (d, 1 H, $J_{1',2'}$ 7.6 Hz, 1′), 1.81 (s, 3 H, 5‴-NAc); ^{13}C NMR (D_2O): δ 94.78 (1′). α,β-**2** ESMS (pos, 25 V) of permethylated derivative m/z 879 $[M + Na]^+$, 451 $[M + 2Na]^{+2}$.

*(2*S*,3*R*,4*E*)-5‴-Acetyl-α-neuraminyl-(2‴→3″)-β-galactopyranosyl-(1″→4′)-β-glucopyranosyl-(1′↔1)-2-azido-4-octadecene-1,3-diol (azidoGM3)* (**4**).—To a 1.0 mL solution of lactosylazidosphingosine **3** (15 mg, 23 μmol) [14,65], CMP-NeuAc (5 mg, 7.1 μmol), calf intestine alkaline phosphatase (40 U, Boehringer Mannheim), bovine serum albumin (0.75 mg) containing Triton CF-54 (0.5%), HEPES (0.10 M), $MgCl_2$ (5.0 mM), $MnCl_2$ (2.0 mM), and $ZnCl_2$ (5.0×10^{-5} M) at pH 7.50 degassed with Ar was added α-2,3-NeuT (0.125 U, as 25 μL of 5 U/mL) and the mixture was stirred gently at ambient temperature. The reaction was checked daily. The pH was maintained above 7.5 with concd aq KOH. Additional 5 mg portions of CMP-NeuAc were added daily, and an additional 0.125 U of enzyme was added on day six. No additional CMP-NeuAc was added after day nine. On day 13, 2-PrOH (4 mL) was added and the mixture filtered through a short column (4:1 2-PrOH–water). Chromatography (3:1 CH_2Cl_2–MeOH to elute starting material **3**, followed by 4:1 2-propanol–water) gave crude **4**. The product **4** was re-chromatographed (5.5 g Pharmacia LH-20 Sephadex 25–100 μm, MeOH), then again re-chromatographed (4:1 2-propanol–water), and microfiltered (3:1 CH_2Cl_2–MeOH) to give azidoGM3 **4** (19 mg, 20 μmol, 87%) [10,14,18] as a colorless solid, which was homogeneous by TLC (4:1 2-propanol–water, R_f of **3** 0.63, R_f of azidoGM3 **4** 0.52, R_f of NeuAc 0.20); ^{1}H NMR (Me_2SO-d_6): δ Glc, 4.19 (d, 1 H, $J_{1',2'}$ 8 Hz, 1′), 3.00 (ddd, 1 H, $J_{2',2'\text{-OH}}$ 4.9, $J_{2',3'}$ 8 Hz, 2′), 3.25–3.46 (m, 3 H, 3′, 4′, 5′), 3.50–3.65 (m, 1 H, 6a′), 3.70–3.75 (m, 1 H, 6b′); Gal, 4.18 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.25–3.46 (m, 3 H, 2″, 5″, 6a″), 3.96 (dd, 1 H, $J_{2'',3''}$ 9.7, $J_{3'',4''}$ 2.0 Hz, 3″), 3.65–3.70 (m, 1 H, 4″), 3.49 (ddd, 1 H, $J_{5'',6b''}$ 6, $J_{6a'',6b''}$ 11, $J_{6b'',6''\text{-OH}}$ 5 Hz, 6b″); Neu, 1.35 (dd, 1 H, $J_{3ax''',3eq'''}$ 11.5, $J_{3ax''',4'''}$ 12 Hz, 3ax‴), 2.74 (dd, 1 H, $J_{3eq''',4'''}$ 4.3 Hz, 3eq‴), 3.50–3.65 (m, 3 H, 4‴, 8‴, 9b‴), 3.25–3.46 (m, 3 H, 5‴, 6‴, 9a‴), 8.16 (d, 1 H, $J_{5''',5'''\text{-NH}}$ 12.0 Hz, 5‴-NH), 1.89 (s, 3 H, 5‴-NAc), 3.19 (br dd, 1 H, $J_{7''',7'''\text{-OH}}$ 2.7, $J_{7''',8'''}$ 8.6 Hz, 7‴); Sph, 3.50–3.65 (m, 2 H, 1a, 2), 3.65–3.72 (m, 1 H, 1b), 4.09 (ddd, 1 H, $J_{2,3}$ 4.1, $J_{3,3\text{-OH}}$ 4.5, $J_{3,4}$ 6.7 Hz, 3), 5.43 (dd, 1 H, $J_{4,5}$ 15.3 Hz, 4), 5.65 (dt, 1 H, $J_{5,6}$ 6.6 Hz, 5), 1.99 (dt, 2 H, $J_{6,7}$ 6.4 Hz, 6), 1.32 (m, 2 H, 7), 1.23 (m, 20 H, 8–17), 0.85 (t, 3 H, $J_{17,18}$ 6.6 Hz, 18); ^{13}C NMR (Me_2SO-d_6): δ Glc, 102.65 (1′), 73.00 (2′), 74.84* (3′), 80.48 (4′), 74.90* (5′), 60.32 (6′); Gal, 104.05 (1″), 68.60 (2″), 75.69 (3″), 66.65 (4″), 75.81 (5″), 60.32 (6″); Neu, 172.20 (1‴-C=O), 99.63 (2‴), 41.97 (3‴), 67.37 (4‴), 52.89 (5‴), 72.40 (6‴), 68.60 (7‴), 71.07 (8‴), 63.31 (9‴), 170.11 (5‴-NC=O), 22.47 (5‴-NAc); Sph, 68.13 (1), 65.70 (2), 71.25 (3), 129.24 (4), 132.68 (5), 31.61 (6), 28.60–28.96 (7–15), 31.23 (16), 22.03 (17), 13.89 (18); ESMS (pos, 25 V) of permethylated derivative m/z 1146 $[M + Na]^+$; ESMS (pos, 25 V) after subsequent CD_3I transesterification m/z 1149 $[M + Na]^+$.

*(2*S*,3*R*,4*E*)-1-(Triphenylmethyl)-2-(hexadecanamido)-4-octadecene-1,3-diol* (**8**).—To a vigorously stirred solution of ceramide **7** (1.09 g, 2.03 mmol) [59,84,89–91] and ethyldiisopropylamine (0.530 mL, 0.393 g, 3.04 mmol) in 20 mL of dry CH_2Cl_2, was added TrCl (0.622 mol, 2.23 mmol) in 5 mL of CH_2Cl_2 over 20 min, while the mixture was maintained below 20 °C with a water bath. After 18 h, the mixture was concentrated and chromatographed (95:5:0.1 CH_2Cl_2–EtOAc–Et_3N, 35 °C) to give 1-tritylceramide **8** (1.34 g, 1.72 mmol, 85%) as an amorphous white solid that was homogeneous by TLC (90:10:0.1 CH_2Cl_2–EtOAc–Et_3N, R_f 0.42): mp 37–38 °C; ^{1}H NMR ($CDCl_3$): δ 7.24–7.41 (m, 15 H, Ar); Sph, 3.29 (dd, 1 H, $J_{1a,1b}$ 9.7, $J_{1a,2}$ 3.9 Hz, 1a), 3.39 (dd, 1 H, $J_{1b,2}$ 3.6 Hz, 1b), 4.05 (dddd, 1 H, $J_{2,2\text{-NH}}$ 7.9, $J_{2,3}$ 3.7 Hz, 2), 6.09 (d, 1 H, 2-NH), 4.18 (m, 1 H, 3), 5.25 (dd, 1 H, $J_{3,4}$ 6.1, $J_{4,5}$ 15.4 Hz, 4), 5.63 (dt, 1 H, $J_{5,6}$ 6.6 Hz, 5), 1.87–1.92 (m, 2 H, 6), 1.25–1.31 (m,

22 H, 7–17), 0.88 (t, 3 H, $J_{17,18}$ 6.9 Hz, 18); FA, 2.21 (t, 2 H, $J_{2,3}$ 7.6 Hz, 2), 1.61–1.65 (m, 2 H, 3), 1.25–1.31 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 6.9 Hz, 16); ^{13}C NMR ($CDCl_3$): δ 87.36 (CPh_3); 143.27 (3 C), 128.43 (6 C), 128.01 (6 C), 127.30 (3 C) [Ar × 18 C]; Sph, 63.07 (1), 53.28 (2), 74.37 (3), 128.59 (4), 133.44 (5), 32.19 (6), 29.02–29.69 (7–15), 31.91 (16), 22.68 (17), 14.12 (18); FA, 173.37 (NC=O), 36.87 (2), 25.84 (3), 29.02–29.69 (4–13), 31.91 (14), 22.68 (15), 14.12 (16).

(2S,3R,4E)-3-Benzoyl-1-(triphenylmethyl)-2-(hexadecanamido)-4-octadecene-1,3-diol (**9**).—To a vigorously stirred solution of 1-tritylceramide **8** (1.29 g, 1.65 mmol), diisopropylethylamine (1.46 mL, 1.08 g, 8.36 mmol) and recrystallized 4-pyrrolidinopyridine (0.124 g, 0.837 mmol) in 10 mL of anhyd PhMe was added PhCOCl (0.47 g, 3.3 mmol) over 5 min. The solvent was removed and the residue chromatographed (97:3:0.1 CH_2Cl_2–EtOAc–Et_3N, 35 °C) to give 3-benzoyl-1-tritylceramide **9** (1.40 g, 1.58 mmol, 96%) as a clear and colorless viscous liquid that was homogeneous by TLC (97:3:0.1 CH_2Cl_2–EtOAc–Et_3N, R_f 0.49); ^{1}H NMR ($CDCl_3$): δ 7.94 (br d, 2 H, J 8.3 Hz, Ar), 7.55 (br t, 1 H, J 7.4 Hz, Ar), 7.38–7.42 (m, 8 H, Ar), 7.15–7.22 (m, 9 H, Ar); Sph, 3.20 (dd, 1 H, $J_{1a,1b}$ 9.5, $J_{1a,2}$ 4.1 Hz, 1a), 3.44 (dd, 1 H, $J_{1b,2}$ 3.5 Hz, 1b), 4.49 (m, 1 H, 2), 5.66 (d, 1 H, $J_{2,2\text{-NH}}$ 9.4 Hz, 2-NH), 5.70 (dd, 1 H, $J_{2,3}$ 7.3, $J_{3,4}$ 7.5 Hz, 3), 5.45 (dd, 1 H, $J_{4,5}$ 15.4 Hz, 4), 5.87 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 1.99 (dt, 2 H, $J_{6,7}$ 7.4 Hz, 6), 1.23–1.31 (m, 22 H, 7–17), 0.88 (t, 3 H, $J_{17,18}$ 6.8 Hz, 18); FA, 2.10 (t, 2 H, $J_{2,3}$ 7.5 Hz, 2), 1.56 (m, 2 H, 3), 1.23–1.31 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 6.8 Hz, 16); ^{13}C NMR ($CDCl_3$): δ 165.32 (OC=O), 86.81 (CPh_3); 143.48 (3 C), 132.88, 130.27, 129.67 (2 C), 128.54 (6 C), 128.30 (2 C), 127.83 (6 C), 127.06 (3 C) [Ar × 24]; Sph, 61.63 (1), 51.07 (2), 74.40 (3), 125.04 (4), 137.16 (5), 32.31 (6), 28.90–29.68 (7–15), 31.91 (16), 22.67 (17), 14.10 (18); FA, 172.42 (NC=O), 36.97 (2), 25.76 (3), 28.90–29.68 (4–13), 31.91 (14), 22.67 (15), 14.10 (16).

(2S,3R,4E)-3-Benzoyl-2-(hexadecanamido)-4-octadecene-1,3-diol (**10**).—To a vigorously stirred solution of 3-benzoyl-1-tritylceramide **9** (0.528 g, 0.597 mmol) in 10 mL of 6:4 PhMe–MeOH was added $F_3B{\cdot}OEt_2$ (81.2 μL, 93.7 mg, 0.660 mmol) dropwise over 5 min. After 4 h, 20 mL of CH_2Cl_2 was added and the mixture washed with 10 mL of aq $NaHCO_3$ (0.25 g, 3.0 mmol), 10 mL of water, then dried, and the solvent removed. Chromatography (19:1 CH_2Cl_2–MeOH) gave 3-benzoylceramide **10** (0.343 g, 0.534 mmol, 89%) [90,91] as a white solid that was homogeneous by TLC (19:1 CH_2Cl_2–MeOH, R_f 0.43): mp 88–89 °C, lit. 155–158 °C [91]; ^{1}H NMR ($CDCl_3$): δ 8.03 (br d, 2 H, J 8.4 Hz, Ar), 7.59 (br t, 1 H, J 7.4 Hz, Ar), 7.46 (dd, 2 H, Ar); Sph, 3.69 (dd, 1 H, $J_{1a,1b}$ 11.9, $J_{1a,2}$ 3.1 Hz, 1a), 3.74 (dd, 1 H, $J_{1b,2}$ 3.7 Hz, 1b), 4.28 (m, 1 H, 2), 6.06 (d, 1 H, $J_{2,2\text{-NH}}$ 8.6 Hz, 2-NH), 5.54 (dd, 1 H, $J_{2,3}$ 7.3, $J_{3,4}$ 7.6 Hz, 3), 5.60 (dd, 1 H, $J_{4,5}$ 15.1 Hz, 4), 5.86 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 2.04 (dt, 2 H, $J_{6,7}$ 7.4 Hz, 6), 1.35 (m, 2 H, 7), 1.20–1.34 (m, 20 H, 8–17), 0.88 (t, 3 H, $J_{17,18}$ 6.9 Hz, 18); FA, 2.17 (dt, 1 H, $J_{2a,2b}$ 14.5, $J_{2a,3}$ 7.5 Hz, 2a), 2.21 (dt, 1 H, $J_{2b,3}$ 7.5 Hz, 2b), 1.61 (quint, 2 H, $J_{3,4}$ 7.5 Hz, 3), 1.20–1.34 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 6.9 Hz, 16); ^{13}C NMR ($CDCl_3$): δ 166.55 (OC=O); 133.45, 129.79 (2 C), 129.64, 128.51 (2 C) [Ar × 6]; Sph, 61.87 (1), 53.42 (2), 74.67 (3), 124.81 (4), 137.52 (5), 32.29 (6), 28.89–29.67 (7–15), 31.91 (16), 22.67 (17), 14.09 (18); FA, 173.35 (NC=O), 36.89 (2), 25.72 (3), 28.89–29.67 (4–13), 31.91 (14), 22.67 (15), 14.09 (16).

4,5,7,8,9-Pentaacetyl-2-(diethylphospho)-α,β-neuraminic acid methyl ester (α,β-**13**).—Phosphite **13** [35,36,92] was readily available in two steps from commercially available *N*-acetylneuraminic acid methyl ester (NeuAcMe, **11**) via NeuAcMe tetraacetate **12** [102–104] in at least 45% yield as a colorless solid, which was a 1:1 α,β mixture and was used without further purification; TLC (3:2 PhMe–Me_2CO, R_f 0.36); ^{1}H NMR ($CDCl_3$): δ 4.16 (q, 4 H, J_{vicinal} 7 Hz, CH_2), 1.37 (t, 6 H, CH_3), 3.80 and 3.88 (s, 3 H, OMe).

Benzyl 2″,6″-dibenzyl-β-galactopyranosyl-(1″→4′)-2′,3′,6′-tribenzyl-β-glucopyranoside (β-**14**).—The lactoside β-**14** was prepared according to the reported [23,93,96,97] method, and the clear and colorless viscous semi-solid was homogeneous by TLC (22:3 CH_2Cl_2–EtOAc, R_f 0.18); ^{1}H NMR (Me_2SO-d_6): δ 7.20–7.45 (m, 30 H, Ar); benzylic, 5.01 (d, 1

H, *J* 10.8 Hz, a), 4.84 (d, 1 H, *J* 12.2 Hz, b), 4.79 (d, 1 H, *J* 12 Hz, c), 4.77 (ABq, 2 H, d, d), 4.65 (d, 1 H, c), 4.63 (d, 1 H, b), 4.62 (d, 1 H, a), 4.49 (d, 1 H, *J* 12.2 Hz, e), 4.48 (d, 1 H, *J* 12.0 Hz, f), 4.40 (d, 1 H, f), 4.35 (d, 1 H, e); Glc, 4.59 (d, 1 H, $J_{1',2'}$ 7.8 Hz, 1′), 3.27 (dd, 1 H, $J_{2',3'}$ 9.1 Hz, 2′), 3.59 (dd, 1 H, $J_{3',4'}$ 9.1 Hz, 3′), 3.86 (dd, 1 H, $J_{4',5'}$ 9.6 Hz, 4′), 3.57 (m, 1 H, 5′), 3.74 (dd, 1 H, $J_{5',6a'}$ 1, $J_{6a',6b'}$ 10.6 Hz, 6a′), 3.79 (dd, 1 H, $J_{5',6b'}$ 4.0 Hz, 6b′); Gal, 4.45 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.43 (dd, 1 H, $J_{2'',3''}$ 9 Hz, 2″), 3.43–3.52 (m, 3 H, 3″, 5″, 6a″), 4.96 (d, 1 H, $J_{3'',3''\text{-OH}}$ 6.1 Hz, 3″-OH), 3.68 (m, 1 H, 4″), 4.74 (d, 1 H, $J_{4'',4''\text{-OH}}$ 4.5 Hz, 4″-OH), 3.65 (dd, 1 H, $J_{5'',6b''}$ 3.7, $J_{6a'',6b''}$ 7.9 Hz, 6b″); ^{13}C NMR (Me_2SO-d_6): δ 139.12, 139.07, 138.67, 138.58, 138.31, 137.69, 127.03–128.21 (30 C) [Ar × 36]; 74.09, 73.98, 73.86, 72.28, 72.05, 70.09 [benzylic × 6]; Glc, 101.74 (1′), 81.22 (2′), 82.07 (3′), 76.03 (4′), 74.17 (5′), 68.02 (6′); Gal, 102.23 (1″), 79.90 (2″), 73.02 (3″), 68.57 (4″), 73.53 (5″), 68.95 (6″); ^{1}H NMR ($CDCl_3$): δ 7.15–7.40 (m, 30 H, Ar); benzylic, 4.98 (d, 1 H, *J* 11.1 Hz, a), 4.95 (d, 1 H, *J* 12.1 Hz, b), 4.91 (d, 1 H, *J* 10.9 Hz, c), 4.79 (d, 1 H, *J* 11.6 Hz, d), 4.77 (d, 1 H, a), 4.72 (d, 1 H, c), 4.66 (d, 1 H, d), 4.65 (d, 1 H, b), 4.61(d, 1 H, *J* 12.1 Hz, e), 4.46 (d, 1 H, e), 4.44 (d, 1 H, *J* 12.0 Hz, f), 4.38 (d, 1 H, f); Glc, 4.49 (d, 1 H, $J_{1',2'}$ 7.7 Hz, 1′), 3.50 (dd, 1 H, $J_{2',3'}$ 9.0 Hz, 2′), 3.58 (dd, 1 H, $J_{3',4'}$ 9.0 Hz, 3′), 4.02 (dd, 1 H, $J_{4',5'}$ 9.7 Hz, 4′), 3.38 (ddd, 1 H, $J_{5',6a'}$ 1.6, $J_{5',6b'}$ 4.0 Hz, 5′), 3.76 (dd, 1 H, $J_{6a',6b'}$ 10.8 Hz, 6a′), 3.83 (dd, 1 H, 6b′); Gal, 4.44 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.40–3.44 (m, 2 H, 2″, 3″), 3.92 (br d, 1 H, $J_{3'',4''}$ 1.4 Hz, 4″), 3.35 (br dd, 1 H, $J_{5'',6a''}$ 4.9, $J_{5'',6b''}$ 6.6 Hz, 5″), 3.49 (dd, 1 H, $J_{6a'',6b''}$ 9.9 Hz, 6a″), 3.61 (dd, 1 H, 6b″); ^{13}C NMR ($CDCl_3$): δ 139.10, 138.51, 138.34, 138.21, 137.96, 137.47, 127.20–128.45 (30 C) [Ar × 36]; 75.21, 74.93, 74.84, 73.43, 73.17, 70.91 [benzylic × 6]; Glc, 102.53 (1′), 81.77 (2′), 82.80 (3′), 76.53 (4′), 75.11 (5′), 68.24 (6′); Gal, 102.45 (1″), 80.00 (2″), 73.47 (3″), 68.74 (4″), 72.85 (5″), 68.64 (6″).

Benzyl 2″,6″-dibenzyl-β-galactopyranosyl-(1″→4′)-2′,3′,6′-tribenzyl-α,β-glucopyranoside (α,β-**14**).—β-Lactose was converted into 3″,4″-*O*-isopropylidene-α,β-lactose in 31% yield according to the reported [105] method. 3″,4″-*O*-Isopropylidene-α,β-lactose was an amorphous white solid that was a 1:1 α,β mixture, which was homogeneous by TLC (3:2:1 EtOAc–2-PrOH–water, R_f 0.44); ^{1}H NMR (Me_2SO-d_6): δ 1.39 (s, 3 H, Me), 1.24 (s, 3 H, Me); ^{13}C NMR (Me_2SO-d_6): δ 28.10 (Me), 26.30 (Me). To a stirred mixture of NaH (9.11 g, 0.380 mol) in 60 mL of DMF was added BnBr (59.04 g, 0.345 mol). A solution of 3″,4″-*O*-isopropylidene-α,β-lactose (8.67 g, 22.7 mmol) in 100 mL of DMF was added dropwise over 30 min. The exothermic reaction was stirred for an additional 1.5 h, then concentrated by kugelrohr distillation. After the cautious addition of 22 mL of MeOH, the mixture was partitioned between 250 mL of water and 200 mL of EtOAc. The aqueous phase was extracted with additional EtOAc (2 × 200 mL), and the aqueous extracts were combined, dried, and concentrated. The residue was chromatographed (23:2 CH_2Cl_2–EtOAc) to give benzyl 2″,6″-di-*O*-benzyl-3″,4″-*O*-isopropylidene-β-galactopyranosyl-(1″→4′)-2′,3′,6′-tri-*O*-benzyl-α,β-glucopyranoside (16.45 g, 17.8 mmol, 78%) [41] as a faint yellow viscous semi-solid that was a 3:17 α,β mixture identified by NMR spectroscopy and was free of other impurities by TLC (23:2 CH_2Cl_2–EtOAc, R_f 0.45); β anomer ^{1}H NMR (Me_2SO-d_6): δ 7.10–7.38 (m, 30 H, Ar); benzylic, 4.88 (d, 1 H, *J* 10.8 Hz, a), 4.84 (d, 1 H, *J* 12.2 Hz, b), 4.79 (d, 1 H, *J* 11.3 Hz, c), 4.74 (d, 1 H, *J* 12.1 Hz, d), 4.70 (d, 1 H, d), 4.64 (d, 1 H, c), 4.63 (d, 1 H, b), 4.62 (d, 1 H, a), 4.53 (d, 1 H, *J* 12.0 Hz, e), 4.52 (d, 1 H, *J* 12.3 Hz, f), 4.43 (d, 1 H, e), 4.29 (d, 1 H, f); 1.31 (s, 3 H, Me), 1.28 (s, 3 H, Me); Glc, 4.60 (d, 1 H, $J_{1',2'}$ 7.8 Hz, 1′), 3.25 (dd, 1 H, $J_{2',3'}$ 9.1 Hz, 2′), 3.60 (dd, 1 H, $J_{3',4'}$ 9.3 Hz, 3′), 3.86 (dd, 1 H, $J_{4',5'}$ 9.7 Hz, 4′), 3.59–3.64 (m, 1 H, 5′), 3.76 (ABq, 2 H, 6a′, 6b′); Gal, 4.57 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, 1″), 3.28 (dd, 1 H, $J_{2'',3''}$ 6.3 Hz, 2″), 4.11 (dd, 1 H, $J_{3'',4''}$ 7.7 Hz, 3″), 4.13 (dd, 1 H, $J_{4'',5''}$ 1.4 Hz, 4″), 3.84 (ddd, 1 H, $J_{5'',6a''}$ 7.1, $J_{5'',6b''}$ 5 Hz, 5″), 3.47 (dd, 1 H, $J_{6a'',6b''}$ 10.3 Hz, 6a″), 3.62 (dd, 1 H, 6b″); ^{13}C NMR (Me_2SO-d_6): δ 138.87, 138.54 (2 C), 138.31, 138.23, 137.66, 127.10–128.20 (30 C) [Ar × 36]; 74.18, 73.86, 72.37, 72.26, 72.17, 70.08 [benzylic × 6]; 108.91 (CMe_2), 26.28 (Me), 27.76 (Me); Glc, 101.71 (1′), 81.29

(2′), 81.90 (3′), 75.58 (4′), 74.01 (5′), 67.95 (6′); Gal, 101.01 (1″), 80.14 (2″), 78.47 (3″), 73.46 (4″), 71.61 (5″), 68.73 (6″). α Anomer ^{1}H NMR (Me_2SO-d_6): δ Glc, 4.99 (d, 1 H, $J_{1',2'}$ 3.5 Hz, 1′), 3.39 (dd, 1 H, $J_{2',3'}$ 9.7 Hz, 2′). A solution of the isopropylidene derivative (6.45 g, 17.8 mmol) in 210 mL of water and 55 mL of AcOH was heated at 115 °C (bt) for 1.5 h, then cooled and concentrated at 50 °C (bt), and given one chase with 100 mL of EtOAc. The crude product was chromatographed (4:1 CH_2Cl_2–EtOAc) to give α,β-**14** (7.58 g, 8.58 mmol, 48%) [41] as a white solid that was homogeneous by TLC (4:1 CH_2Cl_2–EtOAc, R_f 0.40; 45:55 hex–EtOAc, R_f 0.62) with the α anomer eluting just ahead of the β anomer. The product α,β-**14** was a 3:17 α,β mixture identified by NMR spectroscopy. Pure β-**14** was already characterized, and a few α-**14** peaks were assigned; α-**14** ^{1}H NMR (Me_2SO-d_6): δ Glc, 5.00 (d, 1 H, $J_{1',2'}$ 3.7 Hz, 1′), 3.43 (dd, 1 H, $J_{2',3'}$ 9.5 Hz, 2′), 3.76 (dd, 1 H, $J_{3',4'}$ 9.2 Hz, 3′).

Benzyl [methyl (4‴,5‴,7‴,8‴,9‴-penta-O-*acetyl-α-neuramin)ate]yl-(2‴→3″)-2″,6″-di*-O-*benzyl-β-galactopyranosyl-(1″→4′)-2′,3′,6′-tri*-O-*benzyl-α,β-glucopyranoside* (α,β-**15**).— A stirred solution of phosphite donor **13** (1.21 g, 1.98 mmol) [35,36,41,46,92] and hexabenzyllactose acceptor α,β-**14** (2.82 g, 3.19 mmol) [41] in 6 mL of dry CH_3CN was cooled to −41 °C (bt, CH_3CN/N_2) for 15 min, then a solution of Me_3SiOTf (75 mg, 0.34 mmol) in 0.5 mL of CH_3CN was added dropwise over 2 min. The reaction was maintained at −41 °C (bt) for 1 h, then quenched with Et_3N (0.64 mL, 0.46 g, 4.5 mmol). The solvent was removed and the crude product chromatographed (3:2 PhMe–Me_2CO) to recover starting hexabenzyllactose (α,β-**14**, 1.82 g, 2.06 mmol), followed by the crude mixture of products. Due to the presence of impurities just above and below the two coupling products, the products were best isolated by injection of the crude mixture in seven portions onto a preparative HPLC (97:3 PhMe–MeOH). The homogeneous by-product (**2‴**β,**1′**α,β)-**16** (0.235 g, 1.73 mmol, 8.7%) eluted before the homogeneous desired coupling product (**2‴**α,**1′**α,β)-**15** (0.602 g, 0.444 mmol, 22%). The desired trisaccharide analog (**2‴**α,**1′**α,β)-**15** was greater than 90% the β-glycoside [6,23,24,36,41] and was homogeneous by TLC (22:3 PhMe–MeOH, R_f 0.17); (**2‴**α,**1′**β)-**15** ^{1}H NMR (Me_2SO-d_6): δ 2.03, 1.92, 1.90, 1.78, 1.68 [(s, 3 H, CH_3) × 5]; 7.20–7.45 (m, 30 H, Ar); benzylic, 4.98 (d, 1 H, *J* 10.7 Hz, a), 4.81 (d, 2 H, *J* 12 Hz, b, b′), 4.78 (d, 1 H, *J* 11 Hz, c), 4.68 (d, 1 H, b), 4.63 (d, 1 H, c), 4.62 (d, 1 H, a), 4.61 (d, 1 H, b′), 4.46 (d, 1 H, *J* 11.9 Hz, d), 4.44 (d, 1 H, *J* 12.1 Hz, e), 4.41 (d, 1 H, d), 4.33 (d, 1 H, e); Glc, 4.56 (d, 1 H, $J_{1',2'}$ 7.3 Hz, 1′), 3.26 (dd, 1 H, $J_{2',3'}$ 9.0 Hz, 2′), 3.56 (dd, 1 H, $J_{3',4'}$ 9.4 Hz, 3′), 3.82 (dd, 1 H, $J_{4',5'}$ 9.3 Hz, 4′), 3.53 (m, 1 H, 5′), 3.65–3.75 (m, 2 H, 6a′, 6b′); Gal, 4.59 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.48 (dd, 1 H, $J_{2'',3''}$ 8 Hz, 2″), 4.18 (dd, 1 H, $J_{3'',4''}$ 3 Hz, 3″), 3.60 (m, 1 H, 4″), 4.57 (d, 1 H, $J_{4'',4''\text{-OH}}$ 5 Hz, 4″-OH), 3.51 (m, 1 H, 5″), 3.40 (dd, 1 H, $J_{5'',6a''}$ 5.9, $J_{6a'',6b''}$ 9.9 Hz, 6a″), 3.61 (dd, 1 H, $J_{5'',6b''}$ 6.8 Hz, 6b″); Neu, 3.73 (s, 3 H, OMe‴), 1.86 (dd, 1 H, $J_{3ax''',3eq'''}$ 12, $J_{3ax''',4'''}$ 12.4 Hz, 3ax‴), 2.56 (dd, 1 H, $J_{3eq''',4'''}$ 4.4 Hz, 3eq‴), 4.72 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 3.95 (ddd, 1 H, $J_{5''',5\text{-NH}'''}$ 9.2, $J_{5''',6'''}$ 10.5 Hz, 5‴), 7.68 (d, 1 H, 5-NH‴), 3.90 (dd, 1 H, $J_{6''',7'''}$ 1.8 Hz, 6‴), 5.18 (dd, 1 H, $J_{7''',8'''}$ 8.1 Hz, 7‴), 5.42 (ddd, 1 H, $J_{8''',9a'''}$ 5.5, $J_{8''',9b'''}$ 3.0 Hz, 8‴), 3.93 (dd, 1 H, $J_{9a''',9b'''}$ 12.0 Hz, 9a‴), 4.19 (dd, 1 H, 9b‴); ^{13}C NMR (Me_2SO-d_6): δ 169.96, 169.55, 169.36, 169.18, 169.08, 168.31 [C=O × 6]; 22.56, 20.88, 20.55, 20.44, 20.30 [CH_3 × 5]; 139.23, 139.07, 138.56 (2 C), 138.40, 137.68, 126.90–128.30 (30 C) [Ar × 36]; 74.13, 74.02, 73.85, 72.21, 72.00, 70.10 [benzylic × 6]; Glc, 101.68 (1′), 81.28 (2′), 82.02 (3′), 76.05 (4′), 74.10 (5′), 68.15 (6′); Gal, 101.80 (1″), 78.06 (2″), 75.51 (3″), 66.66 (4″), 72.76 (5″), 68.42 (6″); Neu, 52.79 (OMe‴), 97.64 (2‴), 36.88 (3‴), 69.35 (4‴), 47.77 (5‴), 71.62 (6‴), 66.95 (7‴), 68.15 (8‴), 61.66 (9‴); ^{1}H NMR ($CDCl_3$): δ 2.09, 2.01, 1.98, 1.90, 1.87 [(s, 3 H, CH_3) × 5]; 7.10–7.45 (m, 30 H, Ar); benzylic, 4.98 (d, 1 H, *J* 10.8 Hz, a), 4.93 (d, 1 H, *J* 12.0 Hz, b), 4.90 (d, 1 H, *J* 10.8 Hz, c), 4.78 (d, 1 H, *J* 11.7 Hz, d), 4.74 (d, 1 H, a), 4.72 (d, 1 H, c), 4.68 (d, 1 H, d), 4.64 (d, 1 H, b), 4.54 (d, 1 H, *J* 12.3 Hz, e), 4.50 (d, 1 H, e), 4.44 (d, 1 H, *J* 12.0 Hz, f), 4.34 (d, 1 H, f); Glc, 4.46 (d, 1 H, $J_{1',2'}$ 7.7 Hz, 1′), 3.47 (dd, 1 H, $J_{2',3'}$ 9 Hz, 2′), 3.55 (dd, 1 H, $J_{3',4'}$ 9 Hz, 3′),

3.99 (dd, 1 H, $J_{4',5'}$ 9.8 Hz, 4′), 3.35 (ddd, 1 H, $J_{5',6a'}$ 2, $J_{5',6b'}$ 4 Hz, 5′), 3.72–3.78 (m, 2 H, 6a′, 6b′); Gal, 4.58 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, 1″), 3.51 (dd, 1 H, $J_{2'',3''}$ 9.5 Hz, 2″), 4.06 (dd, 1 H, $J_{3'',4''}$ 3.2 Hz, 3″), 3.83 (br d, 1 H, 4″), 3.48–3.51 (m, 2 H, 5″, 6a″), 3.68 (dd, 1 H, $J_{5'',6b''}$ 9.2, $J_{6a'',6b''}$ 11.6 Hz, 6b″); Neu, 3.75 (s, 3 H, OMe‴), 2.06 (dd, 1 H, $J_{3ax''',3eq'''}$ 13, $J_{3ax''',4'''}$ 10 Hz, 3ax‴), 2.50 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.86 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.09 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10.0, $J_{5''',6'''}$ 10 Hz, 5‴), 5.20 (d, 1 H, 5‴-NH), 4.00 (dd, 1 H, $J_{6''',7'''}$ 2.1 Hz, 6‴), 5.31 (dd, 1 H, $J_{7''',8'''}$ 7.9 Hz, 7‴), 5.40 (ddd, 1 H, $J_{8''',9a'''}$ 5.9, $J_{8''',9b'''}$ 2.5 Hz, 8‴), 3.96 (dd, 1 H, $J_{9a''',9b'''}$ 12.4 Hz, 9a‴), 4.31 (dd, 1 H, 9b‴); ^{13}C NMR ($CDCl_3$): δ 170.76, 170.53, 170.26, 169.97, 169.88, 168.34 [C=O × 6]; 23.09, 21.06, 20.76, 20.66, 20.49 [CH_3 × 5]; 139.08, 138.91, 138.59, 138.48, 138.36, 137.53, 127.10–128.30 (30 C) [Ar × 36]; 75.34, 74.91 (2 C), 73.27, 73.01, 70.87 [benzylic × 6]; Glc, 102.39 (1′), 81.82 (2′), 82.93 (3′), 76.42 (4′), 75.10 (5′), 68.41 (6′); Gal, 102.28 (1″), 78.40 (2″), 76.31 (3″), 67.87 (4″), 72.44 (5″), 68.41 (6″); Neu, 52.97 (OMe‴), 98.39 (2‴), 36.42 (3‴), 69.07 (4‴), 49.21 (5‴), 72.71 (6‴), 67.17 (7‴), 68.84 (8‴), 62.23 (9‴); ^{1}H NMR (C_6D_6): δ 2.03, 1.90, 1.71, 1.64, 1.63 [(s, 3 H, CH_3) × 5]; 7.00–7.70 (m, 30 H, Ar); benzylic, 5.28 (d, 1 H, *J* 10.9 Hz, a), 5.01 (d, 1 H, *J* 11.4 Hz, b), 4.94 (d, 1 H, a), 4.91 (d, 1 H, *J* 12 Hz, c), 4.87 (d, 1 H, *J* 13 Hz, d), 4.84 (d, 1 H, d), 4.81 (d, 1 H, b), 4.59 (d, 1 H, *J* 12.1 Hz, e), 4.58 (d, 1 H, c), 4.54 (d, 1 H, e), 4.43 (d, 1 H, *J* 12 Hz, f), 4.30 (d, 1 H, f); Glc, 4.48 (d, 1 H, $J_{1',2'}$ 7.2 Hz, 1′), 3.64 (dd, 1 H, $J_{2',3'}$ 8.5 Hz, 2′), 3.67 (dd, 1 H, $J_{3',4'}$ 9.4 Hz, 3′), 4.36 (dd, 1 H, $J_{4',5'}$ 9.4 Hz, 4′), 3.37 (ddd, 1 H, $J_{5',6a'}$ 1.4, $J_{5',6b'}$ 4.3 Hz, 5′), 3.83 (dd, 1 H, $J_{6a',6b'}$ 10.8 Hz, 6a′), 3.98 (dd, 1 H, 6b′); Gal, 4.90 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.84 (dd, 1 H, $J_{2'',3''}$ 9 Hz, 2″), 4.40 (m, 1 H, 3″), 4.12 (br s, 1 H, 4″), 3.79 (br dd, 1 H, $J_{5'',6a''}$ 5.3, $J_{5'',6b''}$ 7.6 Hz, 5″), 3.62 (dd, 1 H, $J_{6a'',6b''}$ 9.4 Hz, 6a″), 3.96 (dd, 1 H, 6b″); Neu, 3.40 (s, 3 H, OMe‴), 2.14 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.8, $J_{3ax''',4'''}$ 12.1 Hz, 3ax‴), 2.70 (dd, 1 H, $J_{3eq''',4'''}$ 4.5 Hz, 3eq‴), 4.86 (m, 1 H, 4‴), 4.38–4.47 (m, 2 H, 5‴, 5‴-NH), 4.02 (dd, 1 H, $J_{5''',6'''}$ 10.4, $J_{6''',7'''}$ 2.3 Hz, 6‴), 5.47 (dd, 1 H, $J_{7''',8'''}$ 7.5 Hz, 7‴), 5.79 (ddd, 1 H, $J_{8''',9a'''}$ 6.7, $J_{8''',9b'''}$ 2.4 Hz, 8‴), 4.21 (dd, 1 H, $J_{9a''',9b'''}$ 12.3 Hz, 9a‴), 4.70 (dd, 1 H, 9b‴); ^{13}C NMR (C_6D_6): δ 170.26, 170.12, 170.09, 169.98, 169.47, 169.21 [C=O × 6]; 22.93, 21.08, 20.62, 20.34 (2 C) [CH_3 × 5]; 140.15, 139.74, 139.65, 139.31, 139.23, 138.38, 127.10–128.80 (30 C) [Ar × 36]; 75.58, 75.34, 75.01, 73.44, 73.34, 70.78 [benzylic × 6]; Glc, 102.89 (1′), 82.41 (2′), 83.24 (3′), 76.74 (4′), 75.73 (5′), 68.84 (6′); Gal, 102.89 (1″), 79.24 (2″), 76.93 (3″), 68.08 (4″), 73.12 (5″), 68.84 (6″); Neu, 52.55 (OMe‴), 98.81 (2‴), 37.20 (3‴), 69.36 (4‴), 49.00 (5‴), 73.34 (6‴), 67.52 (7‴), 69.91 (8‴), 62.87 (9‴). (**2**‴α,**1**′α,β)-**15** ESMS (pos, 20 V) *m*/*z* 1357 $[M + H]^+$, 816 $[B_2]^+$, 710 $[M + 2Na + water]^{+2}$; (**2**‴α,**1**′α,β)-**15** ESMS (pos, 60 V) *m*/*z* 1379 $[M + Na]^+$, 816 $[B_2]^+$, 474 $[B_1]^+$, 414 $[B'_1]^+$.

Benzyl [methyl (4‴,5‴,7‴,8‴,9‴-penta-O-*acetyl - β - neuramin)ate]yl - (2‴ → 3″) - 2″,6″-dibenzyl-β-galactopyranosyl-(1″ → 4′)-2′,3′,6′-tri*-O-*benzyl-α,β-glucopyranoside* (α,β-**16**).—The trisaccharide by-product (**2**‴β,**1**′α,β)-**16** was also greater than 90% the β-benzyl glucoside [23,24] by NMR spectroscopy and was homogeneous by TLC (22:3 PhMe–MeOH, R_f 0.24); (**2**‴β,**1**′β)-**16** ^{1}H NMR (Me_2SO-d_6): δ 2.03, 1.95, 1.93, 1.79, 1.73 [(s, 3 H, CH_3) × 5]; 7.20–7.50 (m, 30 H, Ar); benzylic, 5.00 (d, 1 H, *J* 11.5 Hz, a), 4.82 (d, 1 H, *J* 12.2 Hz, b), 4.78 (d, 1 H, *J* 11.3 Hz, c), 4.72 (d, 1 H, *J* 11.7 Hz, d), 4.64 (d, 1 H, c), 4.63 (d, 1 H, d), 4.62 (d, 1 H, b), 4.61 (d, 1 H, a), 4.48 (d, 1 H, *J* 11 Hz, e), 4.45 (d, 1 H, *J* 12 Hz, f), 4.43 (d, 1 H, e), 4.32 (d, 1 H, f); Glc, 4.56 (d, 1 H, $J_{1',2'}$ 7.6 Hz, 1′), 3.24 (dd, 1 H, $J_{2',3'}$ 9.2 Hz, 2′), 3.56 (dd, 1 H, $J_{3',4'}$ 8 Hz, 3′), 3.88 (m, 1 H, 4′), 3.49 (m, 1 H, 5′), 3.72 (dd, 1 H, $J_{5',6a'}$ 1, $J_{6a',6b'}$ 11 Hz, 6a′), 3.76 (dd, 1 H, $J_{5',6b'}$ 3 Hz, 6b′); Gal, 4.47 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.61 (dd, 1 H, $J_{2'',3''}$ 9 Hz, 2″), 3.92 (m, 1 H, 3″), 3.84 (br dd, 1 H, $J_{3'',4''}$ 3, $J_{4'',4''\text{-OH}}$ 6.1 Hz, 4″), 4.74 (d, 1 H, 4″-OH), 3.57 (m, 1 H, 5″), 3.52 (dd, 1 H, $J_{5'',6a''}$ 6.6, $J_{6a'',6b''}$ 10 Hz, 6a″), 3.64 (dd, 1 H, $J_{5'',6b''}$ 4.3 Hz, 6b″); Neu, 3.47 (s, 3 H, OMe‴), 1.99 (dd, 1 H, $J_{3ax''',3eq'''}$ 13, $J_{3ax''',4'''}$ 13 Hz, 3ax‴), 2.61 (dd, 1 H, $J_{3eq''',4'''}$ 4.5 Hz, 3eq‴), 5.21 (ddd, 1 H, $J_{4''',5'''}$ 8.8 Hz, 4‴), 3.91 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 9.5, $J_{5''',6'''}$ 10.4 Hz, 5‴), 7.43 (d, 1 H, 5‴-NH), 4.37 (br d, 1 H, 6‴), 5.33 (br s, 1 H, 7‴), 5.16 (br d, 1 H, $J_{8''',9a'''}$ 9.1 Hz, 8‴), 4.01 (dd, 1 H, $J_{9a''',9b'''}$ 12.1 Hz, 9a‴), 5.00 (br d, 1 H, 9b‴); ^{13}C NMR (Me_2SO-d_6): δ 169.95, 169.88,

169.82, 169.38, 169.17, 166.25 [C=O × 6]; 22.66, 20.69, 20.64, 20.56, 20.26 [CH_3 × 5]; 139.13, 138.59 (2 C), 138.25 (2 C), 137.69, 126.90–128.70 (30 C) [Ar × 36]; 74.39, 74.07, 73.90, 72.32, 72.12, 70.13 [benzylic × 6]; Glc, 101.75 (1′), 81.16 (2′), 81.88 (3′), 75.68 (4′), 73.96 (5′), 67.96 (6′); Gal, 101.83 (1″), 77.05 (2″), 76.80 (3″), 68.22 (4″), 73.35 (5″), 69.08 (6″); Neu, 52.17 (OMe‴), 99.34 (2‴), 37.64 (3‴), 68.96 (4‴), 47.96 (5‴), 71.76 (6‴), 68.87 (7‴), 72.36 (8‴), 62.07 (9‴); ^{1}H NMR ($CDCl_3$): δ 2.11, 2.09, 1.99, 1.98, 1.74 [(s, 3 H, CH_3) × 5]; 7.15–7.50 (m, 30 H, Ar); benzylic, 4.99 (d, 1 H, *J* 10.9 Hz, a), 4.94 (d, 1 H, *J* 12.1 Hz, b), 4.90 (d, 1 H, *J* 10.9 Hz, c), 4.89 (d, 1 H, *J* 11.1 Hz, d), 4.77 (d, 1 H, a), 4.72 (d, 1 H, c), 4.67 (d, 1 H, d), 4.64 (d, 1 H, b), 4.61 (d, 1 H, *J* 12 Hz, e), 4.57 (d, 1 H, e), 4.46 (d, 1 H, *J* 12 Hz, f), 4.40 (d, 1 H, f); Glc, 4.47 (d, 1 H, $J_{1',2'}$ 7.5 Hz, 1′), 3.49 (dd, 1 H, $J_{2',3'}$ 9 Hz, 2′), 3.58 (dd, 1 H, $J_{3',4'}$ 9 Hz, 3′), 4.06 (dd, 1 H, $J_{4',5'}$ 9 Hz, 4′), 3.39 (dt, 1 H, $J_{5',6'}$ 2 Hz, 5′), 3.84 (d, 2 H, 6a′, 6b′); Gal, 4.58 (d, 1 H, $J_{1'',2''}$ 8 Hz, 1″), 3.64–3.69 (m, 2 H, 2″, 6b″), 3.81 (dd, 1 H, $J_{2'',3''}$ 9.4, $J_{3'',4''}$ 2.9 Hz, 3″), 3.89 (dd, 1 H, $J_{4'',5''}$ 1 Hz, 4″), 3.42 (ddd, 1 H, $J_{5'',6a''}$ 5.0, $J_{5'',6b''}$ 5 Hz, 5″), 3.54 (dd, 1 H, $J_{6a'',6b''}$ 9.8 Hz, 6a″); Neu, 3.65 (s, 3 H, OMe‴), 2.01 (dd, 1 H, $J_{3ax''',3eq'''}$ 13.5, $J_{3ax''',4'''}$ 11 Hz, 3ax‴), 2.55 (dd, 1 H, $J_{3eq''',4'''}$ 4.2 Hz, 3eq‴), 5.18 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.09 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 9.1, $J_{5''',6'''}$ 10.6 Hz, 5‴), 4.69 (d, 1 H, 5‴-NH), 4.32 (dd, 1 H, $J_{6''',7'''}$ 2.2 Hz, 6‴), 5.26 (dd, 1 H, $J_{7''',8'''}$ 4 Hz, 7‴), 5.20 (ddd, 1 H, $J_{8''',9a'''}$ 7.8, $J_{8''',9b'''}$ 1 Hz, 8‴), 4.02 (dd, 1 H, $J_{9a''',9b'''}$ 12.3 Hz, 9a‴), 4.77 (dd, 1 H, 9b‴); ^{13}C NMR ($CDCl_3$): δ 170.56, 170.47, 170.17, 170.06 (2 C), 167.70 [C=O × 6]; 23.06, 21.03, 20.79, 20.69 (2 C) [CH_3 × 5]; 139.02, 138.54, 138.49, 138.43, 138.08, 137.45, 127.10–128.50 (30 C) [Ar × 36]; 75.22, 75.18, 74.89, 73.39, 72.99, 70.90 [benzylic × 6]; Glc, 102.41 (1′), 81.78 (2′), 82.59 (3′), 76.19 (4′), 75.06 (5′), 68.34 (6′); Gal, 102.33 (1″), 78.38 (2″), 77.84 (3″), 67.81 (4″), 72.57 (5″), 68.87 (6″); Neu, 52.90 (OMe‴), 99.28 (2‴), 35.42 (3‴), 68.65 (4‴), 48.56 (5‴), 71.84 (6‴), 68.38 (7‴), 71.49 (8‴), 62.61 (9‴); ^{1}H NMR (C_6D_6): δ 1.96, 1.89, 1.67, 1.65, 1.59 [(s, 3 H, CH_3) × 5]; 7.00–7.65 (m, 30 H, Ar); benzylic, 5.24 (d, 1 H, *J* 11.2 Hz, a), 5.00 (d, 2 H, *J* 11 Hz, b, c), 4.95 (d, 1 H, a), 4.90 (d, 1 H, *J* 12.1 Hz, d), 4.85 (d, 1 H, c), 4.79 (d, 1 H, b), 4.59 (d, 1 H, d), 4.59 (s, 2 H, e, e), 4.43 (d, 1 H, *J* 12.4 Hz, f), 4.38 (d, 1 H, f); Glc, 4.48 (d, 1 H, $J_{1',2'}$ 7.3 Hz, 1′), 3.64 (dd, 1 H, $J_{2',3'}$ 9 Hz, 2′), 3.65 (dd, 1 H, $J_{3',4'}$ 8 Hz, 3′), 4.37 (dd, 1 H, $J_{4',5'}$ 9 Hz, 4′), 3.32 (ddd, 1 H, $J_{5',6a'}$ 1, $J_{5',6b'}$ 3.9 Hz, 5′), 3.87 (dd, 1 H, $J_{6a',6b'}$ 9 Hz, 6a′), 4.01 (dd, 1 H, 6b′); Gal, 4.86 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, 1″), 3.98 (dd, 1 H, $J_{2'',3''}$ 9.1 Hz, 2″), 4.09 (dd, 1 H, $J_{3'',4''}$ 3 Hz, 3″), 4.24 (br d, 1 H, 4″), 3.59 (br dd, 1 H, $J_{5'',6a''}$ 5.1, $J_{5'',6b''}$ 5.6 Hz, 5″), 3.69 (dd, 1 H, $J_{6a'',6b''}$ 10.0 Hz, 6a″), 3.81 (dd, 1 H, 6b″); Neu, 3.29 (s, 3 H, OMe‴), 1.94 (dd, 1 H, $J_{3ax''',3eq'''}$ 13.5, $J_{3ax''',4'''}$ 12 Hz, 3ax‴), 2.61 (dd, 1 H, $J_{3eq''',4'''}$ 4.2 Hz, 3eq‴), 5.46 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.53 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10, $J_{5''',6'''}$ 10.6 Hz, 5‴), 4.37 (d, 1 H, 5‴-NH), 4.72 (dd, 1 H, $J_{6''',7'''}$ 2.3 Hz, 6‴), 5.67 (m, 1 H, 7‴), 5.69 (m, 1 H, 8‴), 4.35 (dd, 1 H, $J_{8''',9a'''}$ 8.0, $J_{9a''',9b'''}$ 12.2 Hz, 9a‴), 5.36 (dd, 1 H, $J_{8''',9b'''}$ 1.8 Hz, 9b‴); ^{13}C NMR (C_6D_6): δ 170.48, 170.22 (3 C), 169.60, 167.82 [C=O × 6]; 23.06, 20.84, 20.69, 20.43 (2 C) [CH_3 × 5]; 140.09, 139.54, 139.29, 139.19, 138.83, 138.32, 127.30–128.70 (30 C) [Ar × 36)]; 75.71, 75.41, 75.03, 73.76, 73.25, 70.83 [benzylic × 6]; Glc, 103.00 (1′), 82.39 (2′), 83.01 (3′), 76.59 (4′), 75.56 (5′), 68.90 (6′); Gal, 102.94 (1″), 78.96 (2″), 78.75 (3″), 68.94 (4″), 73.34 (5″), 69.74 (6″); Neu, 52.44 (OMe‴), 100.08 (2‴), 36.72 (3‴), 69.06 (4‴), 49.05 (5‴), 72.94 (2 C, 6‴, 8‴), 69.12 (7‴), 63.33 (9‴). (**2**‴β,**1**′α,β)-**16** ESMS (pos, 20 V) *m*/*z* 1357 $[M + H]^+$, 816 $[B_2]^+$, 710 $[M + 2Na + water]^{+2}$; (**2**‴β,**1**′α,β)-**16** ESMS (pos, 60 V) *m*/*z* 1379 $[M + Na]^+$, 816 $[B_2]^+$, 474 $[B_1]^+$, 414 $[B'_1]^+$.

*[Methyl (4‴,5‴,7‴,8‴,9‴-penta-*O*-acetyl-α-neuramin)ate]yl-(2‴→3″)-β-galactopyranosyl-(1″→4′)-α,β-glucopyranoside* (α,β-**17**).—Hydrogenation of α,β-**15** (0.602 g, 0.444 mmol) in 18 mL of 9:1 MeOH–AcOH with 95 mg of 10% Pd–C under H_2 (40 PSI) was performed with mechanical shaking on a Parr apparatus for 19 h. The catalyst was removed by micropore filtration. The solvent was removed to give a quantitative yield of α,β-**17** [36] as an amorphous white solid that was a 1:1 α,β mixture of anomers identified by NMR spectroscopy and was used without further purification; β-**17** ^{1}H NMR (Me_2SO-d_6): δ 7.68 (d, 1 H, $J_{5''',5'''\text{-NH}}$ 8.6 Hz, 5‴-NH), 5.25

(ddd, 1 H, $J_{7''',8'''}$ 7.5, $J_{8''',9a'''}$ 6.2, $J_{8''',9b'''}$ 2.5 Hz, 8‴), 5.14 (br d, 1 H, 7‴), 4.76 (ddd, 1 H, $J_{3ax''',4'''}$ 12, $J_{3eq''',4'''}$ 4.6, $J_{4''',5'''}$ 9.1 Hz, 4‴), 4.25–4.35 (m, 3 H, 1′, 1″, 9b‴), 4.03 (dd, 1 H, $J_{9a''',9b'''}$ 12.3 Hz, 9a‴), 3.91 (br d, 1 H, $J_{2'',3''}$ 9.8 Hz, 3″), 3.80–3.90* (m, 2 H, 5‴, 6‴), 3.74 (s, 3 H, OMe‴), 3.10–3.80 (m, 10 H, 3′, 4′, 5′, 6a′, 6b′, 2″, 4″, 5″, 6a″, 6b″), 2.95 (br dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 9 Hz, 2′), 2.48 (dd, 1 H, $J_{3ax''',3eq'''}$ 12 Hz, 3eq‴), 2.04 (dd, 1 H, 3ax‴), 2.05, 2.01, 1.97, 1.93, 1.68 [(s, 3 H, CH_3) × 5]. α-**17** ^{1}H NMR (Me_2SO-d_6): δ 4.99 (dd, 1 H, $J_{1',2'}$ 4, $J_{1',1'\text{-OH}}$ 5 Hz, 1′).

[Methyl (4‴,5‴,7‴,8‴,9‴-penta-O-*acetyl-α-neuramin)ate]yl - (2‴→3″) - 2″,4″,6″ - tri* - O-*acetyl-β-galactopyranosyl-(1″→4′)-1′,2′,3′,6′-tetraacetyl-α,β-glucopyranose* (α,β-**18**).—To a stirred solution of α,β-**17** (0.362 g, 0.444 mmol) in 6 mL of pyridine was added 4 mL of Ac_2O dropwise over 5 min. After 21 h, the solvent was removed and the residue chromatographed (92.5:7.5 EtOAc–MeOH). The β anomer eluted just ahead of the α anomer. All fractions were combined to give α,β-**18** (0.461 g, 0.415 mmol, 93%) [6,36] as an amorphous white solid that was a 1:1 α,β mixture, which was homogeneous by TLC (92.5:7.5 EtOAc–MeOH, R_f 0.40; 1:1 PhMe–Me_2CO, R_f 0.29): β-**18** mp 120–122 °C; ^{1}H NMR ($CDCl_3$): δ 2.24, 2.16, 2.10, 2.09 (2 Me), 2.08 (2 Me), 2.07, 2.04, 2.03, 2.01, 1.86 [(s, 3 H, CH_3) × 12]; Glc, 5.68 (d, 1 H, $J_{1',2'}$ 8.5 Hz, 1′), 5.05 (dd, 1 H, $J_{2',3'}$ 9.3 Hz, 2′), 5.23 (dd, 1 H, $J_{3',4'}$ 9.3 Hz, 3′), 3.93 (dd, 1 H, $J_{4',5'}$ 9.6 Hz, 4′), 3.76 (ddd, 1 H, $J_{5',6a'}$ 5.0, $J_{5',6b'}$ 2 Hz, 5′), 4.20 (dd, 1 H, $J_{6a',6b'}$ 12 Hz, 6a′), 4.42 (dd, 1 H, 6b′); Gal, 4.65 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.93 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, 2″), 4.51 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, 3″), 4.88 (br d, 1 H, 4″), 3.85 (m, 1 H, 5″), 4.01 (m, 2 H, 6a″, 6b″); Neu, 3.84 (s, 3 H, OMe‴), 1.68 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.6, $J_{3ax''',4'''}$ 12.2 Hz, 3ax‴), 2.58 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.89 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.03 (m, 1 H, 5‴), 5.09 (d, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2 Hz, 5‴-NH), 3.63 (dd, 1 H, $J_{5''',6'''}$ 10.8, $J_{6''',7'''}$ 2.7 Hz, 6‴), 5.41 (dd, 1 H, $J_{7''',8'''}$ 9.4 Hz, 7‴), 5.52 (ddd, 1 H, $J_{8''',9a'''}$ 4.6, $J_{8''',9b'''}$ 2.9 Hz, 8‴), 3.98 (dd, 1 H, $J_{9a''',9b'''}$ 12 Hz, 9a‴), 4.42 (dd, 1 H, 9b‴); ^{13}C NMR ($CDCl_3$): δ 170.89, 170.66, 170.64, 170.42, 170.39, 170.31, 170.26, 169.65 (3 C), 169.48, 168.87, 168.01 [C=O × 13]; 23.20, 21.55, 20.96, 20.82 (3 C), 20.80 (3 C), 20.71, 20.63 (2 C) [CH_3 × 12]; Glc, 91.65 (1′), 70.71 (2′), 73.20 (3′), 75.76 (4′), 73.64 (5′), 61.97 (6′); Gal, 100.99 (1″), 69.87 (2″), 71.43 (3″), 67.32 (4″), 70.56 (5″), 61.60 (6″); Neu, 53.16 (OMe‴), 96.82 (2‴), 37.44 (3‴), 69.34 (4‴), 49.24 (5‴), 72.06 (6‴), 66.88 (7‴), 67.80 (8‴), 62.17 (9‴); β-**18** ESMS (pos, 20 V) m/z 1110 $[M+H]^+$, 1050 $[M+H']^+$, 586.5 $[M+2Na+water]^{+2}$; β-**18** ESMS (pos, 60 V) m/z 1132 $[M+Na]^+$, 1050 $[M+H']^+$, 762 $[B_2]^+$, 474 $[B_1]^+$, 414 $[B_1']^+$. α-**18** ^{1}H NMR ($CDCl_3$): δ 2.25, 2.18, 2.16, 2.10 (2 Me), 2.08, 2.07 (2 Me), 2.05, 2.01 (2 Me), 1.86 [(s, 3 H, CH_3) × 12]; Glc, 6.25 (d, 1 H, $J_{1',2'}$ 3.7 Hz, 1′), 5.02 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, 2′), 5.47 (dd, 1 H, $J_{3',4'}$ 9.2 Hz, 3′), 3.91 (dd, 1 H, $J_{4',5'}$ 10.0 Hz, 4′), 3.95–4.05 (m, 1 H, 5′), 4.22 (dd, 1 H, $J_{5',6a'}$ 4.4, $J_{6a',6b'}$ 12.1 Hz, 6a′), 4.39 (dd, 1 H, $J_{5',6b'}$ 1.7 Hz, 6b′); Gal, 4.66 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.95 (dd, 1 H, $J_{2'',3''}$ 10.1 Hz, 2″), 4.52 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, 3″), 4.89 (br d, 1 H, 4″), 3.87 (m, 1 H, 5″), 3.95–4.05 (m, 2 H, 6a″, 6b″); Neu, 3.85 (s, 3 H, OMe‴), 1.68 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.6, $J_{3ax''',4'''}$ 12.2 Hz, 3ax‴), 2.58 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.90 (ddd, 1 H, $J_{4''',5'''}$ 10.2 Hz, 4‴), 4.02 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2, $J_{5''',6'''}$ 10.7 Hz, 5‴), 5.08 (d, 1 H, 5‴-NH), 3.64 (dd, 1 H, $J_{6''',7'''}$ 2.7 Hz, 6‴), 5.41 (dd, 1 H, $J_{7''',8'''}$ 9.3 Hz, 7‴), 5.51 (ddd, 1 H, $J_{8''',9a'''}$ 4.4, $J_{8''',9b'''}$ 2.8 Hz, 8‴), 3.99 (dd, 1 H, $J_{9a''',9b'''}$ 12.7 Hz, 9a‴), 4.43 (dd, 1 H, 9b‴); ^{13}C NMR ($CDCl_3$): δ 170.80, 170.58 (2 C), 170.34, 170.31, 170.24, 170.18, 169.94, 169.56, 169.51 (2 C), 168.97, 167.91 [C=O × 13]; 23.15, 21.50, 20.93, 20.92, 20.80, 20.73 (5 C), 20.59, 20.49 [CH_3 × 12]; Glc, 89.04 (1′), 69.50 (2′), 69.95 (3′), 75.77 (4′), 70.68 (5′), 61.72 (6′); Gal, 101.07 (1″), 69.90 (2″), 71.37 (3″), 67.22 (4″), 70.47 (5″), 61.52 (6″); Neu, 53.12 (OMe‴), 96.73 (2‴), 37.37 (3‴), 69.27 (4‴), 49.13 (5‴), 71.97 (6‴), 66.73 (7‴), 67.71 (8‴), 62.00 (9‴); α-**18** ESMS (pos, 20 V) m/z 1110 $[M+H]^+$, 1050 $[M+H']^+$, 586.5 $[M+2Na+water]^{+2}$; α-**18** ESMS (pos, 60 V) m/z 1132 $[M+Na]^+$, 1050 $[M+H']^+$, 762 $[B_2]^+$, 474 $[B_1]^+$, 414 $[B_1']^+$.

[Methyl (4‴,5‴,7‴,8‴,9‴-penta-O-*acetyl-α-neuramin)ate]yl - (2‴→3″) - 2″,4″,6″ - tri* - O-*acetyl - β - galactopyranosyl - (1″→4′) - 2′,3′,6′-tri*-O-*acetyl-α,β-glucopyranose* (α,β-**19**).—To a stirred solution of α,β-**18** (0.461 g, 0.415 mmol) in 5 mL of anhyd DMF, was added

hydrazine acetate (0.050 g, 0.54 mmol) and the mixture was heated for 1 h at 50 °C (bt). After adding 10 mL of EtOAc, the organic phase was washed with satd aq NaCl (3 × 10 mL), dried, and the solvent removed to give the crude product, which was chromatographed (23:2 EtOAc–MeOH). After the elution of 7 mg of starting material α,β-**18**, the product α,β-**19** (0.335 g, 0.314 mmol, 76%) [6,22] eluted, followed by 52 mg of over-hydrolysis by-product. The deacetylation product α,β-**19** was an amorphous white solid that was a 13:7 α,β mixture of anomers, which was homogeneous by TLC (23:2 EtOAc–MeOH, R_f 0.39); α-**19** ^{1}H NMR ($CDCl_3$): δ 2.24, 2.16, 2.11, 2.10, 2.09, 2.08, 2.07, 2.06, 2.04, 2.01, 1.86 [(s, 3 H, CH_3) × 11]; Glc, 5.38 (d, 1 H, $J_{1',2'}$ 3.6 Hz, 1′), 4.86 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, 2′), 5.51 (dd, 1 H, $J_{3',4'}$ 9 Hz, 3′), 3.87 (dd, 1 H, $J_{4',5'}$ 10 Hz, 4′), 3.64 (ddd, 1 H, $J_{5',6a'}$ 5.2, $J_{5',6b'}$ 2 Hz, 5′), 4.19 (dd, 1 H, $J_{6a',6b'}$ 12 Hz, 6a′), 4.43 (dd, 1 H, 6b′); Gal, 4.66 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.95 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, 2″), 4.51 (dd, 1 H, $J_{3'',4''}$ 3.2 Hz, 3″), 4.88 (m, 1 H, 4″), 3.86 (m, 1 H, 5″), 3.98–4.06 (m, 2 H, 6a″, 6b″); Neu, 3.85 (s, 3 H, OMe‴), 1.68 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.6, $J_{3ax''',4'''}$ 12.2 Hz, 3ax‴), 2.58 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.89 (m, 1 H, 4‴), 3.98–4.06 (m, 2 H, 5‴, 9a‴), 5.06 (d, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2 Hz, 5‴-NH), 3.63 (dd, 1 H, $J_{5''',6'''}$ 10.7, $J_{6''',7'''}$ 2.6 Hz, 6‴), 5.41 (dd, 1 H, $J_{7''',8'''}$ 9.4 Hz, 7‴), 5.52 (m, 1 H, 8‴), 4.42 (dd, 1 H, $J_{8''',9b'''}$ 3, $J_{9a''',9b'''}$ 12 Hz, 9b‴). β-**19** ^{1}H NMR ($CDCl_3$): δ Glc, 4.72 (d, 1 H, $J_{1',2'}$ 8 Hz, 1′), 4.80 (dd, 1 H, $J_{2',3'}$ 10 Hz, 2′), 5.24 (dd, 1 H, $J_{3',4'}$ 9 Hz, 3′).

[Methyl (4‴,5‴,7‴,8‴,9‴-penta-O-*acetyl-α-neuramin)ate]yl - (2‴ → 3″) - 2″,4″,6″ - tri* - O-*acetyl - β - galactopyranosyl - (1″ → 4′) - 2′,3′,6′-tri*-O-*acetyl-α,β-glucopyranosyl trichloroacetimidate* (α,β-**20**).—To a stirred solution of α,β-**19** (0.335 g, 0.314 mmol) and Cl_3CCN (0.944 mL, 1.36 g, 9.42 mmol) in 4 mL of dry CH_2Cl_2, was added DBU (47 μL, 47.8 mg, 0.314 mmol) dropwise over 1 min. After 1.5 h, the solvent was removed and the residue chromatographed (9:1 EtOAc–Me_2CO) to give trichloroacetimidate α,β-**20** (321 mg, 0.265 mg, 84%) [6,22] as an amorphous white solid that was a 19:1 α,β mixture of anomers identified by NMR spectroscopy but was homogeneous by TLC (9:1 EtOAc–Me_2CO, R_f 0.29): α,β-**20** mp 114 °C (soften) 132–133 °C (melt). α-**20** ^{1}H NMR ($CDCl_3$): δ 2.24, 2.16, 2.09, 2.08, 2.07, 2.06 (2 Me), 2.05, 2.00 (2 Me), 1.85 [(s, 3 H, CH_3) × 11]; Glc, 6.49 (d, 1 H, $J_{1',2'}$ 3.8 Hz, 1′), 5.08 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, 2′), 5.55 (dd, 1 H, $J_{3',4'}$ 9.3 Hz, 3′), 3.95 (dd, 1 H, $J_{4',5'}$ 10.3 Hz, 4′), 4.12 (ddd, 1 H, $J_{5',6a'}$ 4.6, $J_{5',6b'}$ 1.8 Hz, 5′), 4.24 (dd, 1 H, $J_{6a',6b'}$ 12.1 Hz, 6a′), 4.43 (dd, 1 H, 6b′); Gal, 4.67 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.96 (dd, 1 H, $J_{2'',3''}$ 10.1 Hz, 2″), 4.52 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, 3″), 4.90 (br d, 1 H, 4″), 3.86 (m, 1 H, 5″), 4.03 (m, 2 H, 6a″, 6b″); Neu, 3.85 (s, 3 H, OMe‴), 1.68 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.7, $J_{3ax''',4'''}$ 12.1 Hz, 3ax‴), 2.58 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.91 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.02 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2, $J_{5''',6'''}$ 10.8 Hz, 5‴), 5.10 (d, 1 H, 5‴-NH), 3.64 (dd, 1 H, $J_{6''',7'''}$ 2.7 Hz, 6‴), 5.41 (dd, 1 H, $J_{7''',8'''}$ 9.3 Hz, 7‴), 5.50 (ddd, 1 H, $J_{8''',9a'''}$ 4.4, $J_{8''',9b'''}$ 2.8 Hz, 8‴), 4.00 (dd, 1 H, $J_{9a''',9b'''}$ 12.5 Hz, 9a‴), 4.42 (dd, 1 H, 9b‴), 8.65 (s, 1 H, Cl_3C–C=N–H); ^{13}C NMR ($CDCl_3$): δ 170.80, 170.58 (2 C), 170.29 (2 C), 170.23, 170.19, 170.08, 169.50, 169.45, 169.31, 167.92 [C=O × 12]; 23.14, 21.49, 21.00, 20.78, 20.72 (3 C), 20.65 (2 C), 20.58, 20.45 [CH_3 × 11]; 161.10 (C=N), 90.71 (CCl_3); Glc, 93.00 (1′), 70.04 (2′), 69.90 (3′), 75.78 (4′), 70.97 (5′), 61.67 (6′); Gal, 101.12 (1″), 69.90 (2″), 71.51 (3″), 67.25 (4″), 70.50 (5″), 61.54 (6″); Neu, 53.11 (OMe‴), 96.74 (2‴), 37.37 (3‴), 69.27 (4‴), 49.15 (5‴), 71.99 (6‴), 66.74 (7‴), 67.73 (8‴), 61.99 (9‴). β-**20** ^{1}H NMR ($CDCl_3$): δ 5.85 (d, 1 H, $J_{1',2'}$ 7.7 Hz, 1′), 5.20 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, 2′), 5.27 (dd, 1 H, $J_{3',4'}$ 7.6 Hz, 3′).

*(2*S*,3*R*,4*E*)-[Methyl (4‴,5‴,7‴,8‴,9‴-penta - O - acetyl - α - neuramin)ate]yl - (2‴ → 3″) -2″,4″,6″ - tri - O - acetyl - β - galactopyranosyl - (1″ → 4′) - 2′,3′,6′ - tri - O - acetyl - α - glucopyranosyl-(1′ ↔ 1) - 3 - benzoyl - 2 - (hexadecanamido) - 4-octadecene-1,3-diol* (α-**21**).—A solution of trichloroacetimidate α,β-**20** (0.321 g, 0.265 mmol), alcohol **10** (0.326 g, 0.508 mmol), and dried 4 Å molecular sieves (1 g) in 5 mL of dry CH_2Cl_2 was stirred for 2 h, then $F_3B{\cdot}OEt_2$ (65.2 μL, 75.2 mg, 0.530 mmol) was added over 2 min. After 3 h, the reaction was quenched with 5 mL of 0.5 M aq $NaHCO_3$. The organic phase was dried, concentrated, and chromatographed (24:1 EtOAc–MeOH)

eluting 232 mg of ceramide derivatives, which were recovered, then 273 mg of glycosidic coupling products, followed by 40 mg of uncoupled trisaccharide, which was combined with recovered trisaccharide from the synthesis of α,β-**19**, then re-acetylated, and purified to recover α,β-**18** (0.112 g, 0.101 mmol). The crude glycosidic coupling mixture was injected in four portions onto a preparative HPLC column (4:1 PhMe–Me_2CO) to elute some nearly pure α-**21**, followed by 200 mg of an intractable mixture of 3:47 α-**22**, β-**23**. The mixture was peracetylated with Ac_2O–pyridine and chromatographed (2:1 PhMe–Me_2CO). The resulting mixture of α-**21** and β-**23** was then easily separated by HPLC (80.5:19.5 PhMe–Me_2CO) eluting α-**21**, followed by β-**23** (184 mg, 0.109 mmol, 41%). The HPLC fractions, confirmed to be pure by NMR spectroscopy, were combined to give by-product α-**21** (11.5 mg, 6.80 μmol, 2.6%) as an amorphous white solid that was homogeneous by TLC (2:1 PhMe–Me_2CO, R_f 0.23): mp 40–55 °C (soften) 78–80 °C (melt); $[\alpha]_D^{22}$ + 39° (*c* 0.699, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 2.24, 2.15, 2.11, 2.09, 2.08, 2.06 (2 Me), 2.05 (2 Me), 2.01, 1.85 [(s, 3 H, CH_3) × 11]; 7.98 (d, 2 H, *J* 8.2 Hz, Ar), 7.56 (t, 1 H, *J* 7.4 Hz, Ar), 7.43 (dd, 2 H, Ar); Glc, 4.79 (d, 1 H, $J_{1',2'}$ 3.8 Hz, 1′), 4.84 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, 2′), 5.47 (dd, 1 H, $J_{3',4'}$ 9.4 Hz, 3′), 3.78 (dd, 1 H, $J_{4',5'}$ 9.4 Hz, 4′), 3.85 (ddd, 1 H, $J_{5',6a'}$ 5.4, $J_{5',6b'}$ 1.7 Hz, 5′), 4.17 (dd, 1 H, $J_{6a',6b'}$ 11.9 Hz, 6a′), 4.38 (dd, 1 H, 6b′); Gal, 4.63 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.94 (dd, 1 H, $J_{2'',3''}$ 10.1 Hz, 2″), 4.51 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, 3″), 4.88 (m, 1 H, 4″), 3.83 (m, 1 H, 5″), 4.02 (m, 2 H, 6a″, 6b″); Neu, 3.84 (s, 3 H, OMe‴), 1.68 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.7, $J_{3ax''',4'''}$ 12.3 Hz, 3ax‴), 2.58 (dd, 1 H, $J_{3eq''',4'''}$ 4.6 Hz, 3eq‴), 4.89 (m, 1 H, 4‴), 4.02 (ddd, 1 H, $J_{4''',5'''}$ 10, $J_{5''',5'''\text{-NH}}$ 10.2, $J_{5''',6'''}$ 10.7 Hz, 5‴), 5.01 (d, 1 H, 5‴-NH), 3.63 (dd, 1 H, $J_{6''',7'''}$ 2.7 Hz, 6‴), 5.40 (dd, 1 H, $J_{7''',8'''}$ 9.4 Hz, 7‴), 5.52 (m, 1 H, 8‴), 3.98 (dd, 1 H, $J_{8''',9a'''}$ 4.9, $J_{9a''',9b'''}$ 12.7 Hz, 9a‴), 4.42 (dd, 1 H, $J_{8''',9b'''}$ 2.6 Hz, 9b‴); Sph, 3.61 (dd, 1 H, $J_{1a,1b}$ 10.6, $J_{1a,2}$ 2.8 Hz, 1a), 3.77 (dd, 1 H, $J_{1b,2}$ 2.8 Hz, 1b), 4.52 (m, 1 H, 2), 5.81 (d, 1 H, $J_{2,2\text{-NH}}$ 9.6 Hz, 2-NH), 5.54 (dd, 1 H, $J_{2,3}$ 8, $J_{3,4}$ 8 Hz, 3), 5.49 (dd, 1 H, $J_{4,5}$ 14.9 Hz, 4), 5.88 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 2.05 (m, 2 H, 6), 1.19–1.35 (m, 22 H, 7–17), 0.88 (t, 3 H, $J_{17,18}$ 7.0 Hz, 18); FA, 2.15–2.32 (m, 2 H, 2), 1.60–1.71 (m, 2 H, 3), 1.19–1.35 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 7.0 Hz, 16); ^{13}C NMR ($CDCl_3$): δ 172.92, 170.83, 170.61, 170.56, 170.45, 170.34, 170.29, 170.27, 170.18, 169.66, 169.58 (2 C), 167.94, 165.05 [C=O × 14]; 23.17, 21.50, 20.96, 20.75 (4 C), 20.68 (4 C) [CH_3 × 11]; 133.14, 129.93, 129.59 (2 C), 128.48 (2 C) [Ar × 6]; Glc, 96.91 (1′), 70.49 (2 C, 2′, 3′), 77.20 (4′), 68.47 (5′), 62.06 (6′); Gal, 101.26 (1″), 69.89 (2″), 71.42 (3″), 67.30 (4″), 70.49 (5″), 61.54 (6″); Neu, 53.13 (OMe‴), 96.76 (2‴), 37.40 (3‴), 69.27 (4‴), 49.18 (5‴), 72.01 (6‴), 66.83 (7‴), 67.75 (8‴), 62.12 (9‴); Sph, 67.45 (1), 50.63 (2), 73.37 (3), 124.98 (4), 138.39 (5), 32.31 (6), 28.90–29.70 (7–15), 31.91 (16), 22.67 (17), 14.09 (18); FA, 36.90 (2), 25.78 (3), 28.90–29.70 (4–13), 31.91 (14), 22.67 (15), 14.09 (16); ESMS (pos, 20 V) *m*/*z* 1692 $[M + H]^+$, 878 $[M + 2Na + water]^{+2}$, 846.5 $[M + 2 H]^{+2}$; ESMS (pos, 60 V) *m*/*z* 1692 $[M + H]^+$, 1570 $[M + H']^+$, 1097 $[Y_2 + 2 H']^+$, 878 $[M + 2Na + water]^{+2}$, 414 $[B_1']^+$.

*(2*S*,3*R*,4*E*)-[Methyl (4‴,5‴,7‴,8‴,9‴-penta*-O*-acetyl-α-neuramin)ate]yl-(2‴ → 3″)-2″,4″,6″-tri*-O*-acetyl-β-galactopyranosyl-(1″ → 4′)-3′,6′-di*-O*-acetyl-α-glucopyranosyl-(1′ ↔ 1)-3-benzoyl-2-(hexadecanamido)-4-octadecene-1,3-diol* (α-**22**).—A small amount of α-**22** was separated and was an amorphous white solid that was homogeneous by TLC (2:1 PhMe–Me_2CO, R_f 0.19); ^1H NMR (DMSO-d_6): δ 2.14, 2.10, 2.04, 2.01, 1.99 (2 Me), 1.98, 1.95, 1.90, 1.65 [(s, 3 H, CH_3) × 10]; 7.90–8.00 (m, 2 H, Ar), 7.63–7.66 (m, 1 H, Ar), 7.50 (dd, 2 H, *J* 8.4, 7.2 Hz, Ar); Glc, 4.75 (d, 1 H, $J_{1',2'}$ 3.6 Hz, 1′), 3.44 (ddd, 1 H, $J_{2',2'\text{-OH}}$ 8.1, $J_{2',3'}$ 9.4 Hz, 2′), 4.88 (d, 1 H, 2′-OH), 5.02 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, 3′), 3.62 (m, 1 H, 4′), 3.75 (m, 1 H, 5′), 4.03 (dd, 1 H, $J_{5',6a'}$ 4.2, $J_{6a',6b'}$ 11.8 Hz, 6a′), 4.29 (dd, 1 H, $J_{5',6b'}$ 1.0 Hz, 6b′); Gal, 4.52 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.66 (dd, 1 H, $J_{2'',3''}$ 10 Hz, 2″), 4.45 (dd, 1 H, $J_{3'',4''}$ 3.5 Hz, 3″), 4.87 (br d, 1 H, 4″), 3.79 (m, 1 H, 5″), 3.96 (m, 2 H, 6a″, 6b″); Neu, 3.77 (s, 3 H, OMe‴), 1.34 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.2, $J_{3ax''',4'''}$ 12.2 Hz, 3ax‴), 2.45 (dd, 1 H, $J_{3eq''',4'''}$ 4.4 Hz, 3eq‴), 4.73 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 3.83 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10, $J_{5''',6'''}$ 10 Hz, 5‴), 7.62 (d, 1 H, 5‴-NH), 3.64 (m, 1 H, 6‴), 5.24 (dd, 1 H,

$J_{6''',7'''}$ 2.5, $J_{7''',8'''}$ 9.1 Hz, 7‴), 5.36 (ddd, 1 H, $J_{8''',9a'''}$ 4.3, $J_{8''',9b'''}$ 2.6 Hz, 8‴), 3.94 (dd, 1 H, $J_{9a''',9b'''}$ 12.6 Hz, 9a‴), 4.25 (dd, 1 H, 9b‴); Sph, 3.60 (m, 1 H, 1a), 3.68 (m, 1 H, 1b), 4.36 (m, 1 H, 2), 7.95 (d, 1 H, $J_{2,2\text{-NH}}$ 9.0 Hz, 2-NH), 5.46 (dd, 1 H, $J_{2,3}$ 6.3, $J_{3,4}$ 7.2 Hz, 3), 5.53 (dd, 1 H, $J_{4,5}$ 15.3 Hz, 4), 5.75 (dt, 1 H, $J_{5,6}$ 6.5 Hz, 5), 2.05 (m, 2 H, 6), 1.15–1.35 (m, 22 H, 7–17), 0.85 (t, 3 H, $J_{17,18}$ 6.8 Hz, 18); FA, 2.15 (m, 2 H, 2), 1.50 (m, 2 H, 3), 1.15–1.35 (m, 24 H, 4–15), 0.85 (t, 3 H, $J_{15,16}$ 6.8 Hz, 16); ^{1}H NMR ($CDCl_3$): δ 2.25, 2.16, 2.14, 2.10, 2.08, 2.07, 2.05 (2 Me), 2.01, 1.86 [(s, 3 H, CH_3) × 10]; 8.02 (d, 2 H, *J* 8.4 Hz, Ar), 7.57 (t, 1 H, *J* 7.4 Hz, Ar), 7.44 (dd, 2 H, Ar); Glc, 4.71 (d, 1 H, $J_{1',2'}$ 3.7 Hz, 1′), 3.55 (dd, 1 H, $J_{2',3'}$ 9.4 Hz, 2′), 5.29 (dd, 1 H, $J_{3',4'}$ 9.4 Hz, 3′), 3.72 (dd, 1 H, $J_{4',5'}$ 9.7 Hz, 4′), 3.80–3.90 (m, 1 H, 5′), 4.17 (dd, 1 H, $J_{5',6a'}$ 5.7, $J_{6a',6b'}$ 11.0 Hz, 6a′), 4.36 (br d, 1 H, 6b′); Gal, 4.67 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.96 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, 2″), 4.52 (dd, 1 H, $J_{3'',4''}$ 3.0 Hz, 3″), 4.89 (br d, 1 H, 4″), 3.80–3.90 (m, 1 H, 5″), 4.01 (dd, 1 H, $J_{5'',6a''}$ 6.4, $J_{6a'',6b''}$ 11.4 Hz, 6a″), 4.07 (dd, 1 H, $J_{5'',6b''}$ 6.8 Hz, 6b″); Neu, 3.85 (s, 3 H, OMe‴), 1.69 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.6, $J_{3ax''',4'''}$ 12.4 Hz, 3ax‴), 2.59 (dd, 1 H, $J_{3eq''',4'''}$ 4.2 Hz, 3eq‴), 4.90 (ddd, 1 H, $J_{4''',5'''}$ 10 Hz, 4‴), 4.04 (ddd, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2, $J_{5''',6'''}$ 10.7 Hz, 5‴), 5.01 (d, 1 H, 5‴-NH), 3.63 (dd, 1 H, $J_{6''',7'''}$ 2.1 Hz, 6‴), 5.39 (dd, 1 H, $J_{7''',8'''}$ 9.3 Hz, 7‴), 5.54 (m, 1 H, 8‴), 3.96 (dd, 1 H, $J_{8''',9a'''}$ 5.2, $J_{9a''',9b'''}$ 12.7 Hz, 9a‴), 4.42 (dd, 1 H, $J_{8''',9b'''}$ 1.5 Hz, 9b‴); Sph, 3.57 (dd, 1 H, $J_{1a,1b}$ 11, $J_{1a,2}$ 3 Hz, 1a), 3.80–3.90 (m, 1 H, 1b), 4.51 (m, 1 H, 2), 5.83 (d, 1 H, $J_{2,2\text{-NH}}$ 9.1 Hz, 2-NH), 5.71 (dd, 1 H, $J_{2,3}$ 8.1, $J_{3,4}$ 8.1 Hz, 3), 5.53 (dd, 1 H, $J_{4,5}$ 15.3 Hz, 4), 5.92 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 2.05 (m, 2 H, 6), 1.20–1.40 (m, 22 H, 7–17), 0.88 (t, 3 H, $J_{17,18}$ 6.8 Hz, 18); FA, 2.21 (m, 2 H, 2), 1.62 (m, 2 H, 3), 1.20–1.40 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 6.8 Hz, 16); ESMS (pos, 20 V) *m*/*z* 1650 $[M+H]^+$, 825.5 $[M+2\ H]^{+2}$; ESMS (pos, 60 V) *m*/*z* 1650 $[M+H]^+$, 1528 $[M+H']^+$, 1054 $[Y_2+2\ H']^+$, 856 $[M+2Na+\text{water}]^{+2}$, 762 $[B_2]^+$, 414 $[B'_1]^+$.

*(2*S*,3*R*,4*E*)-[Methyl (4‴,5‴,7‴,8‴,9‴-penta-*O*-acetyl-α-neuramin)ate]yl-(2‴→3″)-2″,4″,6″-tri-*O*-acetyl-β-galactopyranosyl-(1″→4′)-2′,3′,6′-tri-*O*-acetyl-β-glucopyranosyl-(1′↔1)-3-benzoyl-2-(hexadecanamido)-4-octadecene-1,3-diol* (β-**23**).—The desired coupling product β-**23** (184 mg, 0.109 mmol, 41%) was an amorphous white solid that was homogeneous by TLC (2:1 PhMe–Me_2CO, R_f 0.18): mp 84–86 °C; $[\alpha]_D^{22}$ 0.00° (*c* 0.701, CH_2Cl_2), $[\alpha]_D^{22}$ 0.00° (*c* 2.41, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 2.22, 2.16, 2.08 (2 Me), 2.07 (2 Me), 2.04, 2.02, 2.00, 1.94, 1.85 [(s, 3 H, CH_3) × 11]; 8.00 (d, 2 H, *J* 8.4 Hz, Ar), 7.56 (t, 1 H, *J* 7.4 Hz, Ar), 7.43 (dd, 2 H, Ar); Glc, 4.44 (d, 1 H, $J_{1',2'}$ 7.9 Hz, 1′), 4.85–4.90 (m, 1 H, 2′), 5.17 (dd, 1 H, $J_{2',3'}$ 9.3, $J_{3',4'}$ 9.3 Hz, 3′), 3.85 (dd, 1 H, $J_{4',5'}$ 9.8 Hz, 4′), 3.56 (ddd, 1 H, $J_{5',6a'}$ 5.5, $J_{5',6b'}$ 1.6 Hz, 5′), 4.05 (dd, 1 H, $J_{6a',6b'}$ 11.6 Hz, 6a′), 4.35 (dd, 1 H, 6b′); Gal, 4.64 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, 1″), 4.91 (dd, 1 H, $J_{2'',3''}$ 10.1 Hz, 2″), 4.52 (dd, 1 H, $J_{3'',4''}$ 3.3 Hz, 3″), 4.85–4.90 (m, 1 H, 4″), 3.84 (m, 1 H, 5″), 3.98–4.05 (m, 2 H, 6a″, 6b″); Neu, 3.84 (s, 3 H, OMe‴), 1.67 (dd, 1 H, $J_{3ax''',3eq'''}$ 12.5, $J_{3ax''',4'''}$ 12.4 Hz, 3ax‴), 2.57 (dd, 1 H, $J_{3eq''',4'''}$ 4.5 Hz, 3eq‴), 4.85–4.90 (m, 1 H, 4‴), 3.98–4.05 (m, 2 H, 5‴, 9a‴), 5.15 (d, 1 H, $J_{5''',5'''\text{-NH}}$ 10.2 Hz, 5‴-NH), 3.64 (dd, 1 H, $J_{5''',6'''}$ 10.7, $J_{6''',7'''}$ 2.7 Hz, 6‴), 5.40 (dd, 1 H, $J_{7''',8'''}$ 9.3 Hz, 7‴), 5.52 (m, 1 H, 8‴), 4.41 (dd, 1 H, $J_{8''',9b'''}$ 2.6, $J_{9a''',9b'''}$ 12.6 Hz, 9b‴); Sph, 3.63 (dd, 1 H, $J_{1a,1b}$ 11, $J_{1a,2}$ 3 Hz, 1a), 3.98–4.05 (m, 1 H, 1b), 4.45 (m, 1 H, 2), 5.77 (d, 1 H, $J_{2,2\text{-NH}}$ 9.2 Hz, 2-NH), 5.54 (dd, 1 H, $J_{2,3}$ 6.7, $J_{3,4}$ 7.5 Hz, 3), 5.46 (dd, 1 H, $J_{4,5}$ 15.2 Hz, 4), 5.86 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 2.05 (m, 2 H, 6), 1.19–1.35 (m, 22 H, 7–17), 0.88 (t, 3 H, $J_{17,18}$ 6.9 Hz, 18); FA, 2.14 (m, 2 H, 2), 1.59 (m, 2 H, 3), 1.19–1.35 (m, 24 H, 4–15), 0.88 (t, 3 H, $J_{15,16}$ 6.9 Hz, 16); ^{13}C NMR ($CDCl_3$): δ 175.19, 172.67, 170.81, 170.56, 170.32, 170.27, 170.23, 170.16, 169.70, 169.67, 169.56, 169.50, 167.90, 165.17 [C=O × 14]; 23.12, 21.47, 20.87, 20.79, 20.71 (3 C), 20.62 (2 C), 20.58, 20.52 [CH_3 × 11]; 132.98, 130.20, 129.56 (2 C), 128.35 (2 C) [Ar × 6]; Glc, 100.35 (1′), 71.81 (2′), 72.99 (3′), 76.04 (4′), 72.79 (5′), 62.06 (6′); Gal, 100.92 (1″), 69.81 (2″), 71.31 (3″), 67.26 (4″), 70.44 (5″), 61.52 (6″); Neu, 53.09 (OMe‴), 96.72 (2‴), 37.35 (3‴), 69.27 (4‴), 49.08 (5‴), 71.99 (6‴), 66.84 (7‴), 67.77 (8‴), 62.15 (9‴); Sph, 67.38 (1), 50.69 (2), 74.11 (3), 124.62 (4), 137.40 (5), 32.29 (6), 28.90–29.63 (7–15), 31.87 (16), 22.63 (17), 14.06 (18); FA, 36.80 (2), 25.68 (3), 28.90–29.63 (4–13), 31.87 (14), 22.63 (15), 14.06 (16); ESMS (pos, 20 V) *m*/*z*

1692 $[M+H]^+$, 846.5 $[M+2\ H]^{+2}$; ESMS (pos, 60 V) m/z 1692 $[M+H]^+$, 1570 $[M+H']^+$, 1097 $[Y_2+2\ H']^+$, 414 $[B_1']^+$.

(2S,3R,4E)-5‴-Acetyl-α-neuraminyl-(2‴→3″)-β-galactopyranosyl-(1″→4′)-β-glucopyranosyl-(1′↔1)-2-(hexadecanamido)-4-octadecene-1,3-diol (ganglioside GM3) (**6**).—To a stirred solution of β-**23** (72.2 mg, 42.7 μmol) in 3 mL of MeOH was added 0.20 mL of 1.3 M NaOMe in MeOH. After 3 h, 1 mL of water was added and the reaction mixture stirred for an additional 19 h. The reaction mixture was cooled to 0 °C (bt), and 0.11 g of Amberlite IRC-50 resin was added slowly over 1 min. The resin was removed by filtration through glass wool, washed with MeOH, and the filtrate concentrated. The residue was chromatographed (60:35:8 $CHCl_3$–MeOH–water) to give ganglioside GM3 **6** (49.0 mg, 42.5 μmol, 100 mol%) [18] as an amorphous white solid that was homogeneous by TLC (60:35:8 $CHCl_3$–MeOH–water, R_f 0.24) and by reversed-phase HPLC (17:3 MeOH–water): mp 180–190 °C (soften), >220 °C (decomp.); $[\alpha]_D^{22}$ $+0.8\pm0.8°$ (c 1.2, 1:1 $CHCl_3$–MeOH), lit. $[\alpha]_D^{22}$ $+1.5°$ (c 1.2, 1:1 $CHCl_3$–MeOH) [18]; ^1H NMR (Me_2SO-d_6): δ Glc, 4.17 (d, 1 H, $J_{1',2'}$ 7.7 Hz, 1′), 3.04 (dd, 1 H, $J_{2',3'}$ 8.0, 2′), 3.30–3.40 (m, 2 H, 3′, 4′), 3.28 (m, 1 H, 5′), 3.62 (m, 1 H, 6a′), 3.74 (m, 1 H, 6b′); Gal, 4.19 (d, 1 H, $J_{1'',2''}$ 7.6 Hz, 1″), 3.30–3.40 (m, 2 H, 2″, 5″), 3.94 (dd, 1 H, $J_{2'',3''}$ 9.8, $J_{3'',4''}$ 2.1 Hz, 3″), 3.72 (m, 1 H, 4″), 3.44 (dd, 1 H, $J_{5'',6a''}$ 6.6, $J_{6a'',6b''}$ 10.7 Hz, 6a″), 3.50 (dd, 1 H, $J_{5'',6b''}$ 4.9 Hz, 6b″); Neu, 1.38 (dd, 1 H, $J_{3ax''',3eq'''}$ 11.3, $J_{3ax''',4'''}$ 11.6 Hz, 3ax‴), 2.73 (dd, 1 H, $J_{3eq''',4'''}$ 3.8 Hz, 3eq‴), 3.55–3.65 (m, 1 H, 4‴), 3.30–3.40 (m, 3 H, 5‴, 6‴, 9a‴), 8.53 (br s, 1 H, 5‴-NH), 1.89 (s, 3 H, 5‴-NAc), 3.21 (br d, 1 H, $J_{7''',8'''}$ 8.9 Hz, 7‴), 3.55–3.65 (m, 1 H, 8‴), 3.55–3.65 (m, 1 H, 9b‴); Sph, 3.41 (dd, 1 H, $J_{1a,1b}$ 9.7, $J_{1a,2}$ 4 Hz, 1a), 4.00 (dd, 1 H, $J_{1b,2}$ 4.1 Hz, 1b), 3.78 (m, 1 H, 2), 7.52 (d, 1 H, $J_{2,2\text{-NH}}$ 9.0 Hz, 2-NH), 3.88 (dd, 1 H, $J_{2,3}$ 8.0, $J_{3,4}$ 7.1 Hz, 3), 5.34 (dd, 1 H, $J_{4,5}$ 15.2 Hz, 4), 5.52 (dt, 1 H, $J_{5,6}$ 6.7 Hz, 5), 1.92 (m, 2 H, 6), 1.32 (m, 2 H, 7), 1.15–1.35 (m, 20 H, 8–17), 0.85 (t, 3 H, $J_{17,18}$ 6.9 Hz, 18); FA, 2.02 (t, 2 H, $J_{2,3}$ 7.1 Hz, 2), 1.43 (m, 2 H, 3), 1.15–1.35 (m, 24 H, 4–15), 0.85 (t, 3 H, $J_{15,16}$ 6.9 Hz, 16); ^{13}C NMR (Me_2SO-d_6): δ Glc, 103.52 (1′), 73.23 (2′), 74.47 (3′), 80.47 (4′), 74.89 (5′), 60.32 (6′); Gal, 104.07 (1″), 68.55 (2″), 75.87 (2 C, 3″, 5″), 66.67 (4″), 60.36 (6″); Neu, 172.36 (1‴-C=O), 99.65 (2‴), 41.84 (3‴), 67.06 (4‴), 53.00 (5‴), 72.23 (6‴), 68.55 (7‴), 71.12 (8‴), 63.25 (9‴), 170.68 (5‴-NC=O), 22.43 (5‴-NAc); Sph, 69.16 (1), 53.00 (2), 70.67 (3), 131.45 (4), 131.29 (5), 31.75 (6), 28.71–29.08 (7–15), 31.29 (16), 22.07 (17), 13.88 (18); FA, 171.80 (NC=O), 35.60 (2), 25.38 (3), 28.71–29.08 (4–13), 31.29 (14), 22.07 (15), 13.88 (16); ESMS (pos, 20 V) m/z 1154 $[M+H]^+$; ESMS (neg, 60 V) m/z 1152 $[M-H]^-$.

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