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Efficient Synthesis of a Sialic Acid α(2→3)Galactose Building Block and Its Application to the Synthesis of Ganglioside GM3

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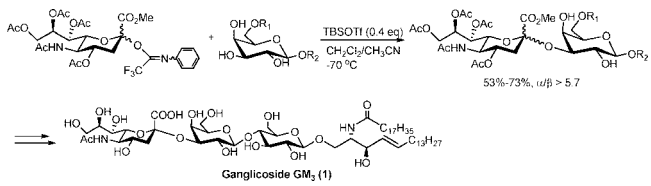

Glycosylation of various galactose derivatives with *O*-acetylated sialic acid *N*-phenyltrifluoroacetimidate as the donor was investigated. Efficient α(2,3)sialylation of galactose, with up to 73% yield and 8.4:1 stereoselectivity, was realized when 2,3,4-unprotected galactose derivatives and TBSOTf were used as acceptors and promoter, respectively. Sialylation of 2-(trimethylsilyl)ethyl 6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranoside (**3f**) gave the best result, and the resultant Neu5Ac α(2→3)Gal disaccharide was successfully used in the synthesis of ganglioside GM3.

N-Acetylneuraminic acid (Neu5Ac), the most abundant sialic acid congener in nature, is found at the termini of glycoproteins and glycolipids on mammalian cell surfaces.[1] In humans, Neu5Ac occurs frequently via an α(2→3)glycosidic linkage to galactose or α(2→6) linkages to galactose and *N*-acetylgalactosamine.[2] Since Neu5Ac on the cell surfaces plays diverse and important roles in cell−cell interaction processes, such as pathogen−host recognition, tumor metastasis, toxin−receptor interaction, malignant alteration, cell differentiation, and proliferation, much effort has been devoted to the efficient and stereoselective synthesis of α(2→3)- and α(2→6)Neu5Ac-Gal units in order to further investigate their biological functions.[3] However, sialylation to make the equatorial 2-α-ketosidic linkages is one of the most challenging tasks in glycosylation chemistry. The electron-withdrawing C-1 carboxylic function, disfavoring the oxocarbenium formation, restricts glycosidation both electronically and sterically. The lack of a substituent at C-3 precludes the suitable neighboring participation group leading to α-glycoside. In addition, the presence of the deoxy moiety in combination with the electron-withdrawing carboxylate group at the anomeric center make these derivatives prone to elimination to give 2,3-dehydro derivatives. These combined factors disfavor the desired α-glycoside formation, especially with secondary sugar hydroxyls. Ganglioside GM3 (NeuAcα-3Galβ4Glcβ1-Cer) containing a sialic acid α(2→3)galactose moiety is a ubiquitous metabolite with a variety of cellular activities.[4] The preparation of this ganglioside is important for further biophysical and biological studies. There are a number of the reports on the synthesis of ganglioside GM3.[5] Although the chemo-enzymatic approaches control the regio- and stereoselectivity of glycosylation, they have generally been demonstrated on smaller scales.[6] Chemical synthetic methods offer the flexibility to prepare analogues. However, the stereoselectivity is generally poor. Thus, highly regio- and stereoselective syntheses are desired.

Recently, Yu and co-workers have successfully enhanced the reactivity of sialic acid donors by utilizing *N*-phenyltrifluoroacetimidates as the leaving groups, and an α(2→3)-Neu5Ac-Gal synthesis has been realized in 81% yield, but the α-selectivity (α:β = 3:1) is not good enough for further chemical synthesis of complex sialylated oligosaccharides.[7] Fukase and co-workers[8] and, more recently, Seeberger's group[9] have evaluated different C-5-*N*-protecting groups of *N*-phenyltrifluoroacetimidate derivatives as the sialic acid donors and have shown good yields and selectivities in glycosylations. However, the modification of the C-5-*N*-protecting groups of the sialic acid donors required the additional chemical steps for introduction and removal of the C-5-*N*-protecting groups. Thus, finding a suitable galactose acceptor for improving the sialylation reaction becomes very important and practical in the synthesis of oligosaccharides containing a sialic acid α(2→3)galactose moiety. Here, we report the efficient synthesis of the sialic acid α(2→3)galactose building block with a *N*-phenyltrifluoroacetimidate donor by optimizing the galactose acceptor and its application to the synthesis of ganglioside GM3.

Coupled with our continuing interest in the *N*-phenyltrifluoroacetimidate method because of its accessibility, stability, and reactivity,[10] we first explored TMSOTf-promoted coupling of sialyl *N*-phenyltrifluoroacetimidate **2** (1.5 equiv) with the ethyl 2-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside **3a**, an ideal acceptor for the subsequent coupling with neighboring

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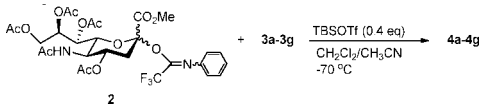
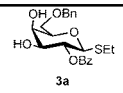
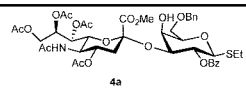
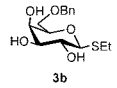
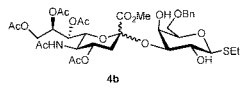
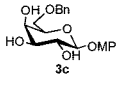
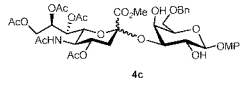
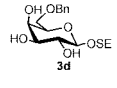
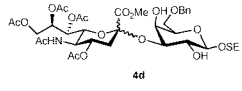
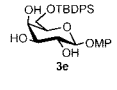
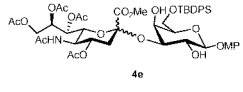
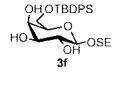
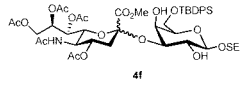
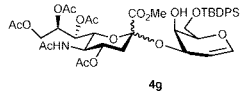
TABLE 1. Glycosylation of Galactose Nucleophiles 3a−g with Sialic Acid Building Block 2



Entry	Acceptor	Product	yield[a] (α:β)[b]
1	 3a	 4a	28% α only
2	 3b	 4b	53% (2.6:1)
3	 3c	 4c	53% (6.1:1)
4	 3d	 4d	64% (5.9:1)
5	 3e	 4e	62% (5.7:1)
6	 3f	 4f	73% (8.4:1)
7	 3g	 4g	70% (9.3:1)

[a] D/A = the ratio of donor and acceptor. [b] The α/β-ratio was determined by NMR analysis.

group participation, using the combination of $MeCN/CH_2Cl_2$ (1:1) as the solvent at −70 °C.[7] However, the reaction did not work well. The desired coupling product (**4a**) was provided in only 16% yield due to the decomposition of donor **2**. Then, we turned to survey the bulky promoter TBSOTf which was reported to be a milder activator than TMSOTf.[11] Examination of the reaction of **2** with **3a** under the influence of TBSOTf was carried out. Indeed, this coupling displayed a higher yield to give disaccharide **4a** in 28% yield, and the β-isomer was not isolated in sufficient quantity to identify (Table 1, entry 1). The α configuration of **4a** was implied by the NMR signals of H-3eq (δ = 2.55 ppm), H-4 (δ = 4.74−4.95 ppm), $J_{H\text{-}7,\ H\text{-}8}$ (9.1 Hz), and $\Delta\delta$ {H-9a-H-9b} (0.32−0.53 ppm), and the 2→3 linkage was determined by the correlation of HMBC between the signal of H-3 of the galactose unit and the signal of C-2 of the sialic acid moiety.[3]

However, besides the desired product **4a**, 2,3-dehydro compound **5** (Figure 1) as a major product was observed resulting from the competing elimination reaction of donor **2**. We assumed that the low sialylation yield was due to the poor nucleophilicity and steric hindrance of the C-3-hydroxyl group

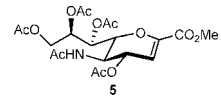


FIGURE 1. 2,3-Dehydro Compound **5**.

produced by the C-2-*O*-protection group of **3a**. In an attempt to enhance the reactivity of the acceptor alcohol and diminish the supposed steric hindrance, benzoyl protection on **3a** was removed with sodium methoxide in methanol to provide a new galactose acceptor **3b**.[12] As expected, TBSOTf-promoted coupling of **3b** with **2** at −70 °C displayed good chemo- and regioselectivity to give disaccharide **4b** in 53% yield and with a moderate α-selectivity (α:β = 2.6:1) (Table 1, entry 2).

Encouraged by the promising result, we further tested other triol acceptors **3c**−**f** and galactal acceptor **3g**. First, 4-methoxyphenyl and 2-(trimethylsilyl)ethyl (SE) protections were adopted to replace the anomeric thiol protection on **3b**, and the corresponding acceptors **3c** and **3d**[13] were designed. Second, the *tert*-butyldiphenylsilyl (TBDPS) group instead of benzyl group was introduced as the C-6-*O*-protection group to form another two acceptors **3e**[14] and **3f**. In addition, bearing in mind that galactal, with more nucleophilic C-3-hydroxyl group is an attractive acceptor in building α(2→3)-Neu5Ac-Gal unit;[9,15] here, galactal acceptor **3g**[15a] was also surveyed.

All glycosylation reactions were carried out at −70 °C in the presence of TBSOTf, and $MeCN/CH_2Cl_2$ (1:1) was used as the optimal solvent by taking advantage of "nitrile solvent effects". To our delight, when thiol protection on **3b** was switched to 4-methoxyphenyl protection on **3c**, the glycosylation proceeded with significantly increased α-selectivity (α:β = 6.1:1) and in good yield (53%, Table 1, entry 3). The reaction of acceptor **3d** with donor **2** (1.5 equiv) provided the corresponding disaccharide **4d** in an even better yield (64%) and good α-selectivity (α:β = 5.9:1, table 1, entry 4). More interestingly, when the TBDPS group was selected to protect the OH-6 of galactose acceptors, the sialylation yields of sialic acid *N*-phenyltrifluoroacetimidate **2** further increased. The glycosylation yield of acceptor **3e** with donor **2** increased up to 62% (Table 1, entry 3 vs 5), and the glycosylation of **3f** with donor **2** provided the desired **4f** in 73% yield and with very good α-selectivity (α:β = 8.4:1) (Table 1, entry 4 vs 6). Moreover, the latter result was retained on a gram-scale synthesis (70%, α:β = 8.3:1). All of the desired α-isomer products **4b**−**f** could be easily separated by column chromatography. However, under the same reaction conditions, the use of 1.5 equiv of donor **2** with 6-*O*-monoprotected galactal **3g** provided an inseparable mixture of α- and β-(2→3)-linked glycosylation products (α:β 9.3:1) in 70% yield (Table 1, entry 7). This observation is in accordance with that of Danishefsky and co-workers.[15a]

As can be seen from the results shown in Table 1, the triol galactose acceptor **3f** is optimal for constructing the sialic acid α(2→3)galactose building block by using *N*-phenyltrifluoroacetimidate as the leaving group. It is worthy of note that the sialylation yield of galactose acceptor markedly increased with

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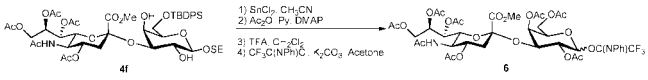
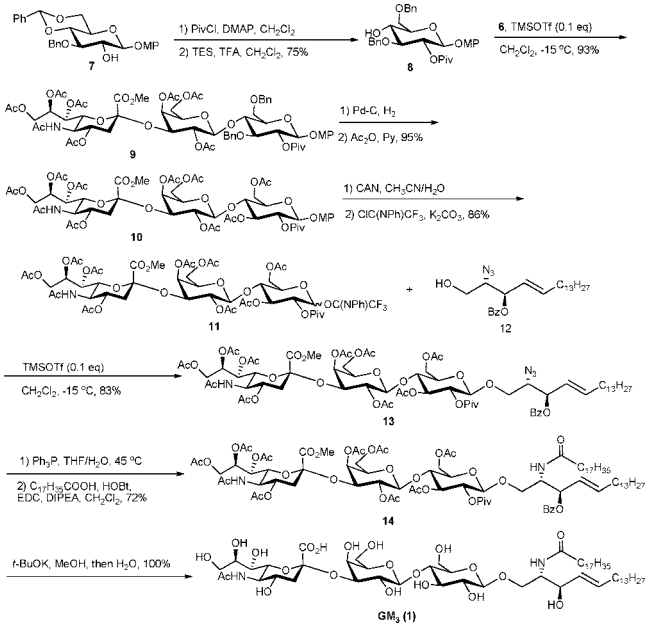
SCHEME 1. Transformation of 4f into Sialyl Galactose Building Block 6



SCHEME 2. Linear Synthesis of Ganglioside GM3



2-(trimethylsilyl)ethyl protection of OH-1 and *tert*-butyldiphenylsilyl (TBDPS) protection of OH-6 compared with 4-methoxyphenyl protection and benzyl protection. This might be attributed to that 2-(trimethylsilyl)ethyl and *tert*-butyldiphenylsilyl (TBDPS) protection, which enhanced the nucleophilicity of galactose acceptors. Thus, the reactivity of the acceptor nucleophiles strongly affects the stereoselectivity.[5f,16]

The disaccharide **4f** was equipped with a suitable anomeric leaving group (Scheme 1). Here *N*- phenyltrifluoroacetimidate was still adopted to prepare donor **6** easily from disaccharide **4f** in excellent yield (70% for four steps), involving the following procedures: (1) removal of the TBDPS protection by treatment with $SnCl_2$,[14] (2) peracetylation, (3) cleavage of the anomeric 2-(trimethylsilyl)ethyl protection with TFA,[15] and (4) introduction of the *N*-phenyltrifluoroacetimidate leaving group.

With the disaccharide donor **6** in hand, the synthesis of GM3 was then carried out (Scheme 2). A glucose acceptor **8** equipped with a neighboring participating group was synthesized using known procedures from **7**[19] and glycosylated with **6** in the presence of TMSOTf. The desired **9** was obtained as a single β-anomer in excellent yield. Debenzylation of the trisaccharide by hydrogenolysis with 10% Pd−C and peracetylation gave the desired product **10**. Deprotection of the 4-methoxyphenyl group using CAN in CH_3CN/H_2O,[20] followed by introduction of the anomeric *N*-phenyltrifluoroacetimidate, furnished trisaccharide donor **11** in 86% yield, and its condensation with (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-eicosene-1,3-diol **12**[21] afforded the β-linked glycosyl sphingosine derivative **13** in 83% yield. Reduction of the azido group with triphenylphosphine and subsequent coupling with octadecanoic acid in the presence of DIPEA, EDC, and HOBt provided amide **14** in good yield.[22] Finally, one-pot deprotection of **14** with potassium *tert*-butoxide in methanol and saponification of the methyl ester gave the ganglioside GM3 in quantitative yield.[20] The ^{1}H NMR data of the synthetic **1** were consistent with those reported for this natural product.[24]

In conclusion, an optimal triol galactose acceptor 2-(trimethylsilyl)ethyl 6-*O*-*tert*-butyldiphenylsilyl-β-D-galactopyranoside **3f** has been found to construct a sialic acid $\alpha(2\rightarrow3)$galactose building block in good yield (73%) and α-selectivity (8.4:1), in which *N*-phenyltrifluoroacetimidate was used as the leaving group and TBSOTf as the promotor. This practical procedure was applied successfully to the synthesis of ganglioside GM3. The results of the present investigation should be of value to synthesize complex sialylated oligosaccharides.

Experimental Section

Typical Procedure for Sialylation with TrifluoroacetimidateDonors 2. A mixture of the donor **2** (1.5 equiv), acceptors **3a**−**g** (1.0 equiv), and 4 Å molecular sieves in dry CH_2Cl_2/CH_3CN (1:1, 5 mL) was stirred at room temperature under argon for 30 min and then cooled to −70 °C. TBSOTf (0.4 equiv) was added. After being stirred at −70 °C for 1.5 h, the mixture was warmed to room temperature and quenched with a few drops of triethylamine. The resulting mixture was filtered and concentrated. The residue was chromatographed on a silica gel column to afford the desired coupling products **4a**−**g**.

Ethyl [Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate]-(2→3′)-6′-*O*-benzyl-1′-thio-β-D-galactopyranoside (4a). Following the abovementioned general procedure, coupling of **2** (118 mg, 0.23 mmol) with **3a** (50 mg, 0.12 mmol) afforded **4a** (30 mg, 28%) as a white solid. α Anomer: mp 104−106 °C (EtOAc/hexanes); $[\alpha]^{20}_D$ +4.67 (*c* 1.45, CH_2Cl_2); R_f 0.18 (3:1 toluene/CH_3CN); ^{1}H NMR($CDCl_3$) δ 7.09−8.14 (m, 10 H, ArH), 5.49−5.51 (m, 1 H, H-8), 5.42 (t, 1 H, J = 9.5 Hz, H-2′), 5.21 (dd, 2 H, J = 9.1, 2.2 Hz, H-7), 5.01 (d, 1 H, J = 9.5 Hz, NH), 4.74−4.76 (m, 1 H, H-4), 4.68 (d, 1 H, J = 9.9 Hz, H-1′), 4.59 (d, 2 H, J = 3.7 Hz, Ph-CH_2), 4.45 (dd, 1 H, J = 9.5, 3.3 Hz, H-3′), 4.27 (dd, 1 H, J = 12.1, 2.2 Hz, H-9a), 3.74−3.95 (m, 7 H, H-5, H-6, H-9b, H-4′, H-5′, H-6a′, H-6b′), 3.73 (s, 3 H, $COOCH_3$), 2.66−2.78 (m, 2 H, CH_2), 2.55 (dd, 1 H, J = 12.8, 4.4 Hz, H-3eq), 2.14, 2.06, 1.98, 1.81, 1.57 (5s, 15 H, 4 OAc and NHAc), 1.90−1.91 (m, 1 H, H-3ax), 1.23 (t, 3 H, J = 7.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.8, 170.6, 170.3, 170.2, 170.0, 168.4, 165.3, 138.0, 133.0, 130.1, 128.3, 127.6, 127.5, 97.0, 83.5, 76.5, 74.8, 73.4, 72.1, 68.9, 68.8, 67.7, 67.6, 66.7, 62.3, 53.0, 48.9, 37.4, 29.6, 23.8, 23.0, 21.2, 20.7, 20.2, 14.9; ESIHRMS calcd for $C_{42}H_{53}NO_{18}SNa$ $[M + Na]^+$ 914.2899, found 914.2876.

Methyl 4,7,8,9-Tetra-*O*-acetyl-3,5-dideoxy-5-acetamidyl-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonate-(2→3)-2,4,6-tri-*O*-acetyl-D-galactopyranosyl *N*-Phenyltrifluoroacetimidate (6). To the solution of **4f** (300 mg, 0.3 mmol) in acetonitrile (5 mL) was added $SnCl_2$ until the pH was 2. After the mixture was stirred for 10 h at room temperature, pyridine (10 mL) and acetic anhydride (5 mL) were added. The reaction mixture was stirred at room temperature for 8 h. Removal of the solvent yielded a crude mixture, which was then dissolved in EtOAc and washed with 1 M HCl, saturated

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$NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The resulting residue was dissolved in CH_2Cl_2 (4 mL) and TFA (2 mL). After the reaction mixture was stirred for 2 h and concentrated, the crude mixture was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was dissolved in acetone (10 mL) and K_2CO_3 solution (124 mg, 0.9 mmol), and $CF_3C(NPh)Cl$ (370 mg, 1.8 mmol) was added. After being stirred for 2 h at room temperature, the reaction mixture was filtered through Celite and concentrated to dryness. Purification with flash column silica gel chromatography (60:1 CH_2Cl_2/CH_3OH) gave *N*-phenyl trifluoroacetimidate **6** (200 mg, 70% for four steps): $[\alpha]^{20}D$ +27.6 (*c* 0.4, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.29−7.31 (m, 2 H, ArH), 7.10−7.12 (m, 1 H, ArH), 6.80−6.88 (m, 2 H, ArH), 5.54−5.58 (m, 1 H), 5.48−5.51 (m, 1 H), 5.26−5.41 (m, 3 H), 5.15−5.17 (m, 1 H), 4.88−5.05 (m, 2 H), 4.42−4.44 (m, 1 H), 3.95−4.25 (m, 6 H), 3.85 (s, 3 H, OCH_3), 3.72−3.75 (m, 1 H), 3.66−3.68 (m, 1 H), 2.60−2.64 (m, 1 H, H-3eq), 2.16 (s, 3 H), 2.13 (s, 3 H), 2.10 (s, 3 H), 2.18 (s, 3 H), 2.07 (s, 3 H), 2.04−2.06 (m, 1 H, H-3ax), 2.03 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.7, 170.6, 170.5, 170.4, 170.3, 169.9, 168.0, 167.9, 129.4, 128.8, 128.7, 124.4, 120.5, 119.3, 96.9, 96.6, 72.3, 71.6, 71.1, 70.7, 69.5, 69.2, 68.7, 68.2, 67.4, 67.3, 66.9, 62.6, 61.8, 53.2, 49.3, 49.0, 38.0, 37.5, 31.9, 29.7, 29.2, 20.6−23.2; ESIHRMS calcd for $C_{40}H_{49}N_2O_{21}F_3Na$ $[M + Na]^+$ 973.2679, found 973.2672.

4-Methoxyphenyl (Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-acetamidyl-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2-pivaloyl-3,6-di-*O*-benzyl-*β*-D-glucopyranoside (9). To a solution of sialyl galactose donor **6** (66 mg, 0.07 mmol) and glucose acceptor **8** (25 mg, 0.05 mmol) in CH_2Cl_2 (5 mL) was added powdered molecular sieves (4 Å, 100 mg). The reaction mixture was stirred for 30 min at room temperature, and then TMSOTf (1.9 uL, 0.007 mmol) was added at −20 °C. The stirring continued at −20 °C until TLC revealed full conversion of acceptor (about 30 min). The reaction was quenched with Et_3N, and the solid was then filtered off. The filtrate was concentrated under vacuum to yield a syrupy residue, which was purified by column chromatography on silica gel (80:1 CH_2Cl_2/CH_3OH) to give trisaccharide **9** (32 mg, 93%): $[\alpha]^{20}_D$ −5.6 (*c* 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.21−7.35 (m, 11 H, ArH), 6.95−6.96 (m, 2 H, ArH), 6.74−6.76 (m, 1 H, ArH), 5.60−5.62 (m, 1 H, H-8), 5.35−5.37 (m, 1 H, H-7), 5.28 (t, 1 H, J = 8.4 Hz, H-2″), 5.09 (d, 1 H, J = 10.2 Hz, N−H), 5.04 (t, 1 H, J = 8.4 Hz, H-2′), 5.00 (d, 1 H, J = 11.0 Hz, PhCH2), 4.94 (d, 1 H, J = 7.7 Hz, H-1′), 4.89 (dd, 1 H, J = 11.3, 4.4 Hz, H-4), 4.86 (d, 1 H, J = 3.3 Hz, H-4′), 4.84 (d, 1 H, J = 8.0 Hz, H-1″), 4.57−4.66 (m, 4 H, H-3′, $PhCH_2$), 3.35−4.36 (m, 1 H, H-9a), 4.04−4.09 (m, 2 H, H-5, H-5′), 3.89−3.96 (m, 3 H, H-4″, H-6a″, H-9b), 3.84 (s, 3 H, $-OCH_3$), 3.75−3.79 (m, 2 H, H-3″, H-6a′), 3.75 (s, 3 H, $-OCH_3$), 3.62−3.69 (m, 4 H, H-5″, H-6b′, H-6b″, H-6), 2.58 (dd, 1 H, J = 12.8, 4.3 Hz, H-3eq), 2.20 (s, 3 H, Ac), 2.15 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.01 (s, 3 H, Ac), 1.98 (s, 3 H, Ac), 1.87 (s, 3 H, Ac), 1.86 (s, 3 H, Ac), 1.72 (t, 1 H, J = 12.5 Hz, H-3ax), 1.16 (brs, 9 H); ^{13}C NMR ($CDCl_3$) δ 176.8, 170.9, 170.6, 170.4, 170.3, 170.2, 170.1, 170.0, 167.9, 155.3, 151.5, 138.5, 138.4, 128.3, 128.1, 127.4, 127.3, 127.2, 127.0, 118.3, 114.5, 100.6, 96.9, 81.3, 75.4, 74.2, 73.3, 72.5, 72.1, 71.5, 70.8, 70.6, 69.3, 68.7, 67.7, 67.4, 67.2, 62.4, 61.6, 55.6, 53.2, 49.1, 38.8, 37.5, 31.9, 29.7, 29.4, 29.2, 27.1, 23.2, 22.7, 21.4, 21.0, 20.8, 20.7, 20.6, 20.5; TOF MS ES+ calcd for $C_{64}H_{81}NO_{28}Na$ $[M + Na]^+$ 1334.4857, found 1334.4837.

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Supporting Information Available: Experimental procedures and copies of NMR spectra of **4a**−**c**,**e**−**g**, **6**, **8**, **9**−**11**, **13**, **14**, and GM3 **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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