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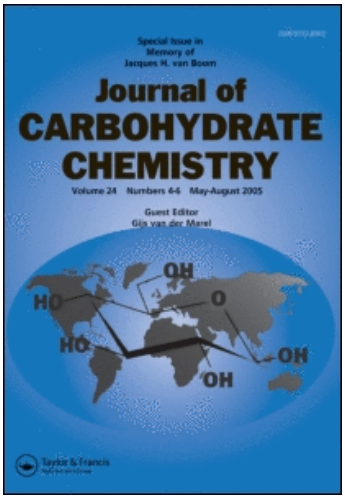


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J. CARBOHYDRATE CHEMISTRY, 18(2), 225-239 (1999)

# CHEMOENZYMATIC SYNTHESIS OF GANGLIOSIDE GM4 ANALOGS AS POTENTIAL IMMUNOSUPPRESSIVE AGENTS[1]

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## ABSTRACT

An efficient, chemoenzymatic synthesis of ganglioside GM4 analogs having a potent immunosuppressive activity is described. One-step and highly regioselective 6-*O*-acetylation of long-chain alkyl, 2-(trimethysilyl)ethyl and phenyl 1-thio β-D-galactopyranosides was performed by using vinyl acetate and lipase PS. The resulting 6-*O*-acetates (70-93%) were sialylated with methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate promoted by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH). The 2-(trimethylsilyl)ethyl glycoside derivative was converted to the imidate which was then coupled with dodecan-1-ol, hexadecan-1-ol, and 2-(tetradecyl)hexadecan-1-ol, respectively, to give the protected GM4 derivatives (90-96%). *O*-Deacylation and saponification of the methyl ester gave the target ganglioside GM4 analogs in high yields.

## INTRODUCTION

Gangliosides, sialic acid containing glycosphingolipids, have well documented implications in cell-cell recognitions, cell growth, differentiation, oncogenesis, infection and so on. However, gangliosides from natural sources are limited in amount and



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kinds. It has been proved that chemically synthesized gangliosides GM3 and GM4 had the same high degree of immunosuppressive activity as did natural GM3 and GM4 gangliosides.[2] GSC-53, a GM3 analog containing the 2-(tetradecyl)hexadecyl group in place of ceramide has been found to be a potent inhibitor of cellular immune response comparable to cyclosporin A.[3]

In order to elucidate the role of the ceramide and sialic acid components in the functions of GM3 and GM4, we have further synthesized their analogs containing a variety of lipophilic moieties in place of ceramide,[4,5] and found the length and branched structure of the fatty alkyl chain are critically important for immunosuppressive activity. We describe herein an efficient synthesis of ganglioside GM4 analogs containing long-chain alkyl residues consisting of 8, 12, 16, and 30 carbon atoms by use of chemoenzymatic method.

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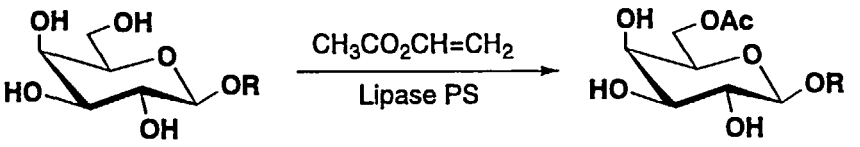
## RESULTS AND DISCUSSION

In the previous papers,[4,6] 2-(trimethysilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside has been employed for the synthesis of ganglioside GM4 and its analogs as the key glycosyl acceptor, on which a regio- and α-stereoselective sialylation was carried out by using methyl 2-thioglycoside of *N*-acetylneuraminic acid in the presence of dimethyl(methylthio)sulfonium triflate (DMTST) in acetonitrile.[7] However, five steps were required for the preparation of this glycosyl acceptor starting from acetobromogalactose including the regioselective 6-*O*-benzoylation. In order to shorten and simplify the reaction steps, we attempted one-step and highly regioselective 6-*O*-acetylation[8] of several long-chain alkyl (**1**-**4**), 2-(trimethylsily)ethyl (SE) (**5**) and phenyl-1-thio (SPh) (**6**) β-D-galactopyranosides by treatment with vinyl acetate and lipase PS (triacyglycerol lipase, EC. 3.1.1.3) from *Pseudomonas cepacia* (Scheme 1).

As shown in Table 1, all of the compounds examined gave the corresponding 6-*O*-acetates (**7**-**12**) regioselectively in 70-93% yields. In particular, 2-(tetradecyl)hexadecyl (B30) derivative (**10**) was obtained in 90% yield as a single product even at 45 °C, while the other long-chain alkyl derivatives were in relatively lower yields (70-75%), accompanied by two by-products. In contrast, compounds **5** and **6** afforded 93% and 80% of 6-*O*-acetates (**11** and **12**) under milder conditions, respectively. In fact, the synthesis of **11** was carried out in preparative scale. It is of




| | R | | R |
|---|---|---|---|
| 1 | $CH_2(CH_2)_6CH_3$ | 7 | $CH_2(CH_2)_6CH_3$ |
| 2 | $CH_2(CH_2)_{10}CH_3$ | 8 | $CH_2(CH_2)_{10}CH_3$ |
| 3 | $CH_2(CH_2)_{14}CH_3$ | 9 | $CH_2(CH_2)_{14}CH_3$ |
| 4 | B30 | 10 | B30 |
| 5 | SE | 11 | SE |
| 6 | SPh | 12 | SPh |

B30 = $CH_2CH[(CH_2)_{13}CH_3]_2$, SE = $CH_2CH_2Si(CH_3)_3$

**Scheme 1**

**Table 1.** Reaction conditions and yields for the regioselective 6-*O*-acetylation of compounds **1-6** catalyzed by lipase PS.[a]

| Starting compound | Reaction time (h) | Reaction temp. (°C) | Product | Yield (%) |
|---|---|---|---|---|
| **1** | 24 | 25 | **7** | 75 |
| **2** | 24 | 25 | **8** | 70 |
| **3** | 24 | 40 | **9** | 70 |
| **4** | 48 | 45 | **10** | 90 |
| **5** | 6 | 25 | **11** | 93 |
| **6** | 16 | 25 | **12** | 80 |

a. The reaction was conducted by treatment of **1-6** with the same amount of lipase PS in vinyl acetate.

worth to mention that the yields and the conditions required for the completion of the reactions are greatly influenced by the solubility of the starting materials in vinyl acetate.

Compounds **7-11** were each coupled with methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[9] **(13)**

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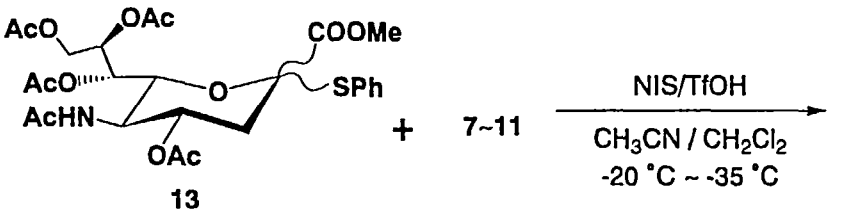


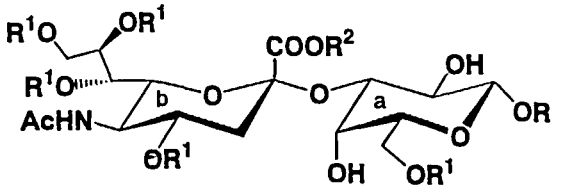


| | R | $R^1$ | $R^2$ |
|---|---|---|---|
| **14** | $CH_2(CH_2)_6CH_3$ | Ac | Me |
| **15** | $CH_2(CH_2)_{10}CH_3$ | Ac | Me |
| **16** | $CH_2(CH_2)_{14}CH_3$ | Ac | Me |
| **17** | SE | Ac | Me |
| **18** | $CH_2(CH_2)_6CH_3$ | H | H |
| **19** | $CH_2(CH_2)_{10}CH_3$ | H | H |
| **20** | $CH_2(CH_2)_{14}CH_3$ | H | H |

**Scheme 2**

promoted by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) at -20 °C in acetonitrile or 9:1-8:2 acetonitrile-dichloromethane to give the desired sialyl α(2→3) galactose derivatives **14** (55%), **15** (40%), **16** (10%) and **17** (66%) (Scheme 2). However no product was obtained from the B30 derivative **10**, due to the extremely low solubility in acetonitrile. We have also tried several procedures for the synthesis of GM4 analogs using phenyl 6-*O*-acetyl-1-thio-β-D-galactopyranoside (**12**) as a glycosyl acceptor to be coupled with methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate[7] or the phosphite derivatives[10] of *N*-acetylneuraminic acid. However, no hopeful result was

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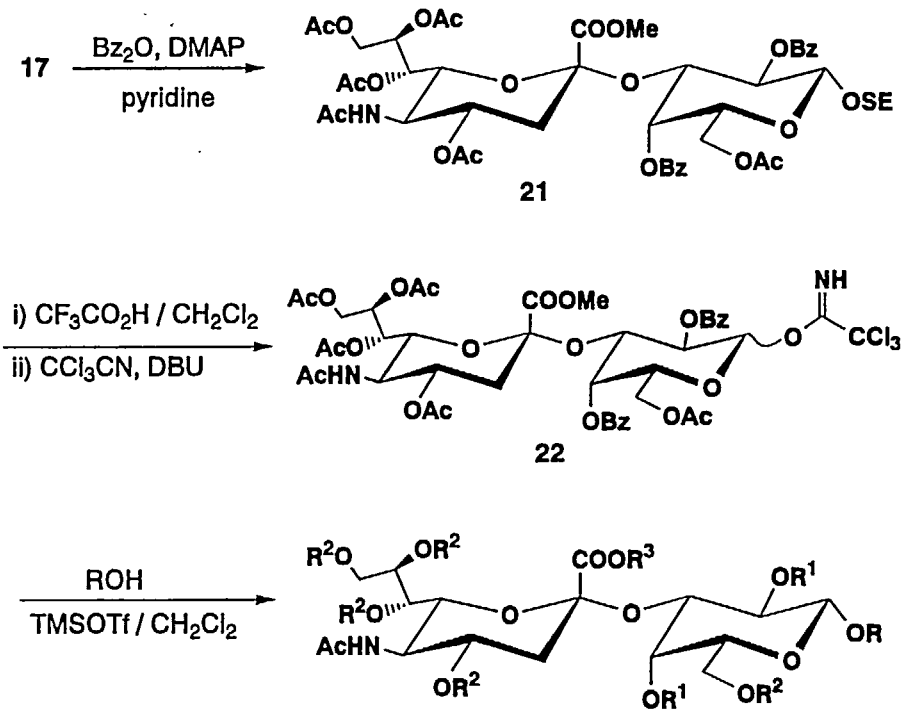


| | R | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| **23** | $CH_2(CH_2)_{10}CH_3$ | Bz | Ac | Me |
| **24** | $CH_3(CH_2)_{14}CH_2$ | Bz | Ac | Me |
| **25** | B30 | Bz | Ac | Me |
| **26** | B30 | H | H | H |

**Scheme 3**

obtained. Therefore, an alternative route (Scheme 3) was examined for the synthesis of GM4 analogs containing longer chain alkyl residues.

The SE glycoside derivative **17** was benzoylated and converted[11] to the imidate **22**, which was then coupled with long-chain alkyl alcohols such as dodecan-1-ol, hexadecan-1-ol, and 2-(tetradecyl)hexadecan-1-ol, respectively, to give a series of GM4 analogs **23-25** in 90-96% yields (Scheme 3). Treatment of **14-16** or **23-25** with

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methanolic sodium methoxide and saponification of the methyl ester afforded the target ganglioside GM4 analogs **18-20** and **26** in quantitative yields.

In conclusion, an efficient synthesis of ganglioside GM4 analogs has been achieved on a preparative scale by combining the enzymatic acetylation catalyzed by lipase PS with a chemical glycosylation method.

## EXPERIMENTAL

**General methods.** Melting points were determined with a Yanagimoto micro melting point apparatus and uncorrected. Optical rotations were calculated with a Union PM-201 polarimeter at 25 °C. $^1$H NMR spectra were recorded on Varian Unity Inova (400 MHz) spectrometer using deuterated solvents ($CDCl_3$, $CD_3OD$, $D_2O$) with TMS as the internal standard. All reactions were monitored by TLC (Merck silica gel aluminum plate 60F-254) and preparative column chromatography was performed on silica gel (Fuji Silysia Co. 300 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

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**Octyl β-D-Galactopyranoside (1).** To a solution of D-galactose pentaacetate (5 g, 12.8 mmol) in $CH_2Cl_2$ (50 mL) was added slowly 25% HBr/AcOH (20 mL) at 0 °C. The mixture was allowed to stand for 1.5 h at room temperature, washed with cold water and M $Na_2CO_3$, dried ($Na_2SO_4$), and concentrated to a residue, which crystallized from ether, to give 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (5.16 g, 98%). The bromide (5.16 g, 12.8 mmol) was coupled with octan-1-ol (4.1 mL, 25.6 mmol) by the Koenigs-Knorr reaction using MS-4A (3 g), $AgClO_4$ (2.92 g, 14.08 mmol), and $Ag_2CO_3$ (3.88 g, 14.08 mmol) in $CH_2Cl_2$ (30 mL), to afford octyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside. It was deacetylated with methanolic sodium methoxide, to give **1** (2.07 g, 80%) as an amorphous mass: mp 72-75 °C, $[\alpha]_D$ -14.1° (*c* 0.5, MeOH), $^1$H NMR (1:2 $CD_3OD$-$CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.26 and 1.64 (m, 12H, 6$CH_2$), 4.26 (d, 1H, $J_{1,2}$ = 7.69 Hz, H-1).

Anal. Calcd for $C_{14}H_{28}O_6$ (320.46): C, 52.47; H, 8.81. Found: C, 52.24; H, 8.63.

**Dodecyl β-D-Galactopyranoside (2).** Coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (7.4 g, 18.9 mmol) with dodecan-1-ol (4.2 mL, 18.9

mmol) and deprotection as described for **1**, gave crystalline **2** (5.35 g, 82%): mp 91-95 °C, $[\alpha]_D$ -15.8° (*c* 0.54, MeOH), $^1$H NMR (1:2 $CD_3OD$-$CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.35 and 1.65 (m, 20H, $10CH_2$), 4.24 (d, 1H, $J_{1,2}$ = 7.24 Hz, H-1).

Anal. Calcd for $C_{18}H_{36}O_6$ (348.48): C, 62.04; H, 10.41. Found: C, 62.03; H, 10.19.

**Hexadecyl β-D-Galactopyranoside (3).** Coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (7 g, 17.7 mmol) with hexadecan-1-ol (8.7 g, 35.4 mmol) as described for **1** gave crystalline **3** (5.4 g, 75%): mp 90-93 °C, $[\alpha]_D$ -5.9° (*c* 0.51, MeOH), $^1$H NMR (1:2 $CD_3OD$-$CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.26 and 1.63 (m, 28H, $14CH_2$), 4.24 (d, 1H, $J_{1,2}$ = 6.96 Hz, H-1).

Anal. Calcd for $C_{22}H_{44}O_6$ (404.59): C, 65.31; H, 10.96. Found: C, 65.21; H, 10.76.

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**Octyl 6-*O*-Acetyl-β-D-galactopyranoside (7).** To a suspension of compound **1** (1.15 g, 3.6 mmol) in vinyl acetate (65 mL) was added lipase PS (1.15 g) (Amano Pharmaseutical Co. Ltd., Japan). The mixture was stirred for one day at 25 °C and then filtered through filter paper. The filtrate was concentrated to a residue which was chromatographed (1:80 MeOH-$CHCl_3$) on a column of silica gel to give crystalline **7** (1 g, 75%): mp 90-92 °C; $[\alpha]_D$ -28.14° (*c* 0.4, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.27 and 1.62 (m, 12H, $6CH_2$), 2.08 (s, 3H, AcO), 3.66 (t, 1H, $J_{2,3}$ = 6.41 Hz, H-2), 4.22 (d, $J_{1,2}$ = 7.60 Hz, H-1), 4.29 and 4.36 (2dd, 2H, $J_{5,6} = J_{5,6'}$ = 6.18 Hz, $J_{6,6'}$ = 11.4, H-6,6').

Anal. Calcd for for $C_{16}H_{30}O_7$ (334.41): C, 57.47; H, 9.04. Found: C, 57.33; H, 8.87.

**Dodecyl 6-*O*-Acetyl-β-D-galactopyranoside (8).** A suspension of compound **2** (1.86 g, 5.3 mmol) in vinyl acetate (60 mL) was treated with lipase PS (1.86 g) as described for **7**, to give crystalline **8** (1.5 g, 70%): mp 95-98 °C; $[\alpha]_D$ -25.1° (*c* 0.39, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.27 and 1.61 (m, 20H, $10CH_2$), 2.08 (s, 3H, AcO), 4.20 (d, 1H, $J_{1,2}$ = 7.55 Hz, H-1), 4.29 and 4.36 (2dd, 2H, $J_{gem}$ = 11.44, $J_{5,6}$ = 6.18, $J_{5,6'}$ = 6.64 Hz, H-6,6').

Anal. Calcd for $C_{20}H_{38}O_7$ (390.52): C, 61.51; H, 9.81. Found: C, 61.29; H, 9.69.

**Hexadecyl 6-*O*-Acetyl-β-D-galactopyranoside (9).** A suspension of compound **3** (2.05 g, 5.1 mmol) in vinyl acetate (100 mL) was treated with lipase PS

(2.05 g) for two days at 40 °C and worked up as described for **7**, to give crystalline **9** (1.71 g, 70%): mp 98-102 °C, $[\alpha]_D$ -22.5° (*c* 0.37, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.27 and 1.53-1.68 (m, 28H, $14CH_2$), 2.10 (s, 3H, AcO), 2.43 (s, 1H, OH), 2.56 (s, 1H, OH), 2.67 (s, 1H, OH), 3.51 (m, 1H, J = 9.16, 6.96 Hz, $OCH_2CH_2$), 3.68 (dd, 1H, $J_{5,6}$ = 6.59, 6.23 Hz, H-5), 3.87-3.93 (m, 2H, H-4, $OCH_2CH_2$), 4.23 (d, 1H, $J_{1,2}$ = 7.32 Hz, H-1), 4.29, 4.40 (2dd, 2H, $J_{5,6}$ = 6.59 Hz, $J_{5,6'}$ = 6.23 Hz, $J_{6,6'}$ = 11.35 Hz, H-6,6').

Anal. Calcd for $C_{24}H_{46}O_7$ (446.43): C, 64.54; H, 10.38. Found: C, 64.47; H, 10.09.

**2-(Tetradecyl)hexadecyl 6-*O*-Acetyl-β-D-galactopyranoside (10).** A suspension of compound **4** (107 mg) in vinyl acetate (10 mL) was treated with lipase PS (107 mg) at 45 °C for two days, and worked up as just described for **7** to give **10** (103 mg, 90%): $[\alpha]_D$ -21.4° (*c* 0.44, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 6H, $2CH_3$), 1.26 and 1.55-1.65 (m, 53H, $26CH_2$ and CH), 2.09 (s, 3H, AcO), 2.46 (s, 1H, OH), 2.66 (s, 1H, OH), 2.82 (s, 1H, OH), 3.36 and 3.81 (2dd, 2H, $J_{gem}$ = 9.52, $J_{vic}$ = 6.23, 5.86 Hz, $OCH_2CH_2$), 3.67 (dt, 1H, $J_{5,6}$ = 6.59 Hz, $J_{5,6'}$ = 6.23 Hz, H-5), 3.90 (s, 1H, H-4), 4.19 (d, 1H, $J_{1,2}$ = 7.32 Hz, H-1), 4.29 and 4.38 (2dd, 2H, $J_{5,6}$ = 6.59 Hz, $J_{5,6'}$ = 6.23 Hz, $J_{6,6'}$ = 11.35 Hz, H-6,6')

Anal. Calcd for $C_{38}H_{74}O_7$ (643.00): C, 70.98; H, 11.60. Found: C, 70.87; H, 11.30.

**2-(Trimethylsilyl)ethyl 6-*O*-Acetyl-β-D-galactopyranoside (11).** To a suspension of **5** (150 mg, 0.537 mmol) in vinyl acetate (8 mL) was added lipase PS (150 mg). The mixture was stirred for 6 h at 25 °C and worked up as described for **7**. Column chromatography (20:1 $CHCl_3$-$CH_3OH$) of the product on silica gel, gave **11** (161 mg, 93%). When the reaction was conducted on a preparative scale, **5** (4 g, 12.4 mmol) was treated with vinyl acetate (100 mL) and lipase PS (4 g), to afford **11** (4.04 g, 80%): $[\alpha]_D$ -14.3° (*c* 1.1, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.02 (m, 2H, $Me_3SiCH_2CH_2$), 2.05 (s, 3H, AcO), 4.22 (d, 1H, $J_{1,2}$ = 7.69 Hz, H-1), 4.24 and 4.32 (2dd, 2H, $J_{5,6}$ = 6.96 Hz, $J_{5,6'}$ = 5.86 Hz, $J_{6,6'}$ = 11.35 Hz, H-6,6').

Anal. Calcd for $C_{13}H_{26}O_7Si$ (322.43): C, 48.43; H, 8.13. Found: C, 48.39; H, 8.02.

**Phenyl 6-*O*-Acetyl-1-thio-β-D-galactopyranoside (12).** To a solution of **6** (1 g, 3.66 mmol) in vinyl acetate (100 mL) was added lipase PS (300 mg), and the mixture was stirred for 14 h at 25 °C, and worked up as described for **7** to afford **12**

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(0.929 g, 80%): $[\alpha]_D$ = -13.0° (*c* 0.7, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 1.81 (s, 3H, AcO), 3.63 (dd, 1H, $J_{5,6}$ = 8.06, $J_{5,6'}$ = 4.03 Hz, H-5), 3.79 (dd, 1H, $J_{2,3}$ = 6.59 Hz, $J_{3,4}$ = 2.56, H-3), 3.97 (d, 1H, $J_{1,2}$ = 9.89 Hz, H-1), 4.03 (s, 1H, H-4), 4.15 and 4.30 (2dd, 2H, $J_{5,6}$ = 8.06 Hz, $J_{5,6'}$ = 4.03 Hz, $J_{6,6'}$ = 11.72 Hz, H- 6,6'), 4.43 (dd, 1H, H-2), 7.50 and 7.68 (m, 5H, SPh).

Anal. Calcd for $C_{14}H_{18}O_6S$ (314.36): C, 53.49; H, 5.77. Found: C 53.39; H, 5.70.

**Octyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-acetyl-β-D-galactopyranoside (14).** To a solution of **7** (473 mg, 1.49 mmol) and **13** (1.729 g, 3.98 mmol) in dry $CH_3CN/CH_2Cl_2$ (9:1, 5 mL) was added MS-3A (300 mg), and the mixture was stirred overnight at room temperature, and then it was cooled to -20 °C. *N*-iodosuccinimide (NIS, 2.507 g, 15.92 mmol) and trifluoromethanesulfonic acid (TfOH, 108.7 μL, 1.19 mmol) were added to the cooled mixture, and the mixture was stirred for 4 h at -20 °C, then neutralized with $Et_3N$, filtered through celite, washed with $Na_2CO_3$, $Na_2S_2O_3$ and water, dried ($Na_2SO_4$) and concentrated. Column chromatography (80:1 $CHCl_3$-MeOH) of the residue on silica gel (40 g) gave **14** (602 mg, 55.2%) as an amorphous mass: $[\alpha]_D$ -9.4° (*c* 0.44, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.2-1.4 and 1.70 (m, 12H, $6CH_2$), 1.90 (s, 3H, AcN), 2.04, 2.05, 2.08, 2.13, 2.16 (5s, 15H, 5AcO), 2.73 (dd, 1H, $J_{gem}$ = 13.0, $J_{3eq,4}$ = 4.6 Hz, H-3beq), 3.84 (s, 3H, MeO), 4.02 (q, 1H, $J_{4,5} = J_{5,6} = J_{5,NH}$ = 9.89 Hz, H-5b), 4.39 (d, 1H, $J_{1,2}$ = 8.06 Hz, H-1a), 4.98 (m, 1H, H-4b), 5.24 (d, 1H, $J_{5,NH}$ = 9.89 Hz, NH), 5.34 (dd, 1H, $J_{6,7}$ = 2.40, $J_{7,8}$ = 8.80 Hz, H-7b), 5.45 (m, 1H, H-8b),

Anal. Calcd for $C_{34}H_{57}NO_{19}$ (783.82): C, 52.10; H, 7.33; N, 1.79. Found: C, 51.80; H, 7.04; N, 1.69.

**Octyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic Acid)-(2→3)-β-D-galactopyranoside (18).** To a solution of **14** (602 mg, 0.75 mmol) in MeOH (10 mL) was added a catalytic amount of NaOMe and the mixture was stirred overnight at 40 °C. Water (0.5 mL) was added and the mixture stirred for an additional 8 h at 40 °C, then neutralized with Amberlite IR-120 ($H^+$) resin. The resin was filtered off and washed with 1:1 $CHCl_3$-MeOH. The filtrate and washings were combined and concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 gave **18** (480 mg, 95.6%) as an amorphous mass: $[\alpha]_D$ -2.9° (*c* 0.9, 1:1 $CHCl_3$-MeOH). $^1H$ NMR ($D_2O$) δ 0.74 (t, 3H,

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$CH_3$), 1.16 and 1.51 (m, 12H, $6CH_2$), 1.68 (t, 1H, $J_{gem} = J_{3ax,4} = 12.0$ Hz, H-3bax), 1.91 (s, 3H, AcN), 2.63 (dd, 1H, $J_{gem} = 12.0$ Hz, $J_{3eq,4} = 4.4$ Hz, $H_{3beq}$), 4.35 (d, $J_{1,2} = 7.69$ Hz, H-1a).

Anal. Calcd for $C_{23}H_{45}NO_{14}$ (559.61): C, 49.37; H, 8.11; N, 2.5. Found: C, 49.18; H, 8.02; N, 2.39.

**Dodecyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-acetyl-β-D-galactopyranoside (15).** Coupling of **8** (131 mg, 0.35 mmol) with **13** (407 mg, 0.7 mmol) as described for **14**, gave **15** (119 mg, 40%) as an amorphous mass: $[\alpha]_D$ +1.3° (*c* 0.54, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.25 and 1.65 (m, 20H, $10CH_2$), 1.90, 2.04, 2.05, 2.08, 2.13, 2.14 (6s, 18H, 5AcO, AcN), 2.73 (dd, 1H, $J_{gem} = 13.2$ Hz, $J_{3eq,4} = 4.4$ Hz, H-3beq), 3.83 (s, 3H, MeO), 4.02 (q, 1H, $J_{4,5} = J_{5,6} = J_{5,NH} = 9.89$ Hz, H-5b), 4.39 (d, 1H, $J_{1,2} = 7.69$ Hz, H-1a), 4.95 (m, 1H, H-4b), 5.23 (d, 1H, $J_{5,NH} = 9.89$ Hz, NH), 5.33 (dd, 1H, $J_{6,7} = 2.40$, $J_{7,8} = 8.79$ Hz, H-7b), 5.45 (m, 1H, H-8b).

Anal. Calcd for $C_{38}H_{65}NO_{19}$ (839.93): C, 54.34; H, 7.80; N, 1.67. Found: C, 54.20; H, 7.70; N, 1.56.

**Dodecyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic Acid)-(2→3)-β-D-galactopyranoside (19).** *O*-Deacylation and saponification of **15**, as described for **18**, yielded **19** as an amorphous mass: $[\alpha]_D$ -8.0° (*c* 0.16, 1:1 $CHCl_3$-MeOH), $^1H$ NMR ($CD_3OD$:$CDCl_3$ = 1:1) δ 0.87 (t, 3H, $CH_3$), 1.27 and 1.65 (m, 20H, $10CH_2$), 1.72 (t, 1H, $J_{gem} = J_{3ax,4} = 13.0$ Hz, H-3bax), 1.99 (s, 3H, AcN), 2.72 (dd, $J_{gem} = 13.2$ Hz, $J_{3eq,4} = 4.7$ Hz, H-3beq), 4.30 (d, $J_{1,2} = 8.02$ Hz, H-1a).

Anal. Calcd for $C_{27}H_{53}NO_{14}$ (615.71): C, 52.67; H, 8.68; N, 2.27. Found: C, 52.63; H, 8.49; N, 2.25.

**Hexadecyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate) - (2 → 3) -6-*O*-acetyl-β-D-galactopyranoside (16).** Coupling of **9** (186 mg, 0.43 mmol) with **13** (624 mg, 1.07 mmol) as described for **14**, gave **16** (39 mg, 10%) as an amorphous mass: $[\alpha]_D$ -0.4° (*c* 0.5, $CHCl_3$) $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.25 and 1.65 (m, 28H, $14CH_2$), 1.90, 2.02, 2.03, 2.10, 2.14, 2.15 (6s, 18H, 5AcO, AcN), 2.75 (dd, $J_{gem} = 13.2$ Hz, $J_{3eq,4} = 4.60$ Hz, H-3beq), 3.86 (s, 3H, MeO), 4.40 (d, $J_{1,2} = 7.69$ Hz, H-1a), 4.95 (m, 1H, H-4b), 5.25 (dd, 1H, H-7b), 5.43 (m, 1H, H-8b)

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Anal. Calcd for $C_{42}H_{73}NO_{19}$ (896.03): C, 56.30; H, 8.21; N, 1.56. Found: C, 56.04; H, 8.09; N, 1.46.

**Hexadecyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic Acid)-(2→3)-β-D-galactopyranoside (20).** Deacylation and saponification of **16**, as described for **18**, yielded **20** as an amorphous mass: $[\alpha]_D$ -1.23° (*c* 1.13, 1:1 $CHCl_3$-MeOH), $^1H$ NMR ($CD_3OD$: $CDCl_3$=1:1) δ 0.88 (t, 3H, $CH_3$), 1.25 and 1.65 (m, 28H, $14CH_2$), 1.78 (t, 1H, $J_{gem} = J_{3ax} = 13.0$Hz, H-3bax), 2.02 (s, 3H, AcN), 2.75 (dd, $J_{gem} = 13.2$ Hz, $J_{3eq,4} = 4.76$ Hz, 1H, H-3beq), 4.28 (1H, $J_{1,2} = 7.69$ Hz, H-1a).

Anal. Calcd for $C_{31}H_{61}NO_{14}$ (671.82): C, 55.42; H, 9.15; N, 2.08. Found: C, 55.21; H, 9.05; N,1.82.

**2-(Trimethylsilyl)ethyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α - D - *galacto*- 2 -nonulopyranosylonate)-(2→ 3)-6-*O*-acetyl-β-D-galatopyranoside (17).** To a solution of **11** (1.22 g, 2.09 mmol) and **13** (4.17 g, 3.13 mmol) in acetonitrile (10 mL) was added MS-3A (3g), and the mixture was stirred for 5 h at 25 °C, and cooled to -35 °C. NIS (2.41 g, 4.70 mmol) and TfOH (63 μL, 0.3 mmol) were added, and the mixture was stirred at -35 °C overnight, and then neutralized with $Et_3N$. The solids were filtered off through celite, and the filtrate was successively washed with $Na_2CO_3$, $Na_2S_2O_3$, and $H_2O$, dried ($Na_2SO_4$), and concentrated to a residue which was chromatograped (1:70 MeOH-$CHCl_3$) on a column of silica gel, to give **17** (2.28 g, 65.6%) as an amorphous mass. On a preparative scale, compound **11** (4.40 g, 15.9 mmol) and **13** (13.90 g, 23.9 mmol) were condensed as described above to afford **17** (8.42 g, 72%): $[\alpha]_D$ -6.0° (*c* 0.6, $CHCl_3$) $^1H$ NMR ($CDCl_3$) δ 1.01(m, 2H, $Me_3SiCH_2CH_2$), 1.89 (s, 3H, AcN), 2.02, 2.03, 2.05, 2.11, 2.13 (5s, 15H, 5AcO), 2.70 (dd, 1H, $J_{gem} = 13.0$, $J_{3eq,4} = 4.6$ Hz, H-3beq), 3.82 (s, 3H, MeO), 4.40 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1a), 4.94 (m, 1H, H-4b), 5.32 (dd, 1H, $J_{6,7} = 2.1$ Hz, $J_{7,8} = 8.9$ Hz, H-7b), 5.36 (d, 1H, $J_{5,NH} = 9.84$ Hz, NH), 5.43 (m, 1H, H-8b).

Anal. Calcd for $C_{33}H_{53}NO_{19}Si$ (795.86): C, 49.80; H, 6.71; N, 1.76. Found: C, 49.50; H, 6.52; N, 1.71.

**2-(Trimethylsilyl)ethyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl- 3,5 -dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate) - (2 → 3)-6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranoside (21).** To a solution of **17** (1.37 g, 1.72 mmol) in pyridine (12 mL) was added $Bz_2O$ (1.55 g, 6.90

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mmol) and 4-dimethylaminopyridine (DMAP, 147 mg, 1.20 mmol), and the mixture was stirred overnight at room temperature, and then treated with MeOH to decompose the excess reagent. Chloroform was added and the mixture was washed with 2M HCl and water, dried ($Na_2SO_4$), and concentrated. The residue was chromatographed (50:1 $CHCl_3$-MeOH) on a column of silica gel to give **21** (1.55 g, 90%) as an amorphous mass: $[\alpha]_D$ +54.4° (*c* 2.1, $CHCl_3$), $^1H$ NMR ($CDCl_3$); δ 0.97 (m, 2H, $Me_3SiCH_2CH_2$), 1.55 (s, 3H, AcN), 1.76 (t, 1H, $J_{gem} = J_{3ax,4} = 12.4$ Hz, H-3bax), 1.86, 2.02, 2.11, 2.18, 2.33 (5s, 15H, 5AcO), 2.55 (dd, 1H, $J_{3eq,4} = 4.30$ Hz, H-3beq), 3.98 (s, 3H, MeO), 4.91 (d, 1H, $J_{1,2} = 7.69$ Hz, H-1a), 4.92 (m, 1H, H-4b), 5.25 (d, 1H, $J_{5,NH} = 10.25$ Hz, NH), 5.30 (dd, 1H, $J_{6,7} = 2.93$ Hz, $J_{7,8} = 9.52$ Hz, H-7b), 5.33 (d, 1H, $J_{3,4} = 3.30$ Hz, H-4a), 5.48 (dd, 1H, $J_{1,2} = 8.06$ Hz, $J_{2,3} = 10.25$ Hz, H-2a), 5.72 (m, 1H, H-8b), 7.52-8.29 (m, 10H, 2Ph).

Anal. Calcd for $C_{47}H_{61}NO_{21}Si$ (1004.58): C, 56.22; H, 6.12; N, 1.40. Found: C, 55.99; H, 6.10; N, 1.39.

**(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-6-*O*-acetyl-2,4-di-*O*-benzoyl-D-galatopyranosyl trichloroacetimidate (22).** To a solution of **21** (1.23 g, 1.23 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (3.19 mL, 39.4 mmol) at 0 °C, and the mixture was stirred for 1.5 h at room temperature, and then concentrated at 30 °C with AcOEt. Column chromatography (1:100 MeOH-$CHCl_3$) of the residue on silica gel gave the 1-OH derivative. This compound (489 mg, 0.58 mmol) was dissolved in dichloromethane (8 mL) and treated with $CCl_3CN$ (1.76 mL, 17.4 mmol) and DBU (105 μL, 0.70 mmol) for 30 min at 0 °C. The mixture was concentrated at 30 °C and chromatographed (1:100 MeOH-$CHCl_3$) on a column of silica gel to give **22** (549 mg, 96%) as an amorphous mass: The anomeric ratio (α:β) was estimated as 1:8 from integration ratio of H-1α (δ 6.85) and H-1β (δ 6.23): For **22β**, $^1H$ NMR ($CDCl_3$) δ 1.46 (s, 3H, AcN), 1.70 (t, 1H, $J_{gem} = J_{3ax,4} = 12.6$ Hz, H-3bax), 1.78, 1.93, 2.04, 2.10, 2.25 (5s, 15H, 5AcO), 2.49 (dd, 1H, $J_{3eq,4} = 4.3$ Hz, H-3b eq), 3.92 (s, 3H, MeO), 3.96 (dd, $J_{gem} = 12.4$ Hz, $J_{8,9} = 8.0$ Hz, H-9b), 4.36 (dd, 1H, $J_{8,9'} = 2.5$ Hz, H-9b'), 4.83 (m, 1H, H-4b), 4.99 (dd, 1H, $J_{2,3} = 10.0$ Hz, $J_{3,4} = 3.30$ Hz, H-3a), 5.10 (d, 1H, $J_{5,NH} = 10.2$ Hz, NHAc), 5.22 (d, 1H, $J_{6,7} = 2.8$, $J_{7,8} = 9.3$ Hz, H-7b), 5.35 (d, 1H, $J_{3,4} = 3.3$ Hz, H-4a), 5.62 (m, 1H, H-8b), 5.69 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.0$ Hz, H-2a), 6.23 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 7.43-8.18 (m, 10H, 2Ph), 8.70 (s, 1H, C=NH).

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Anal. Calcd for $C_{44}H_{49}N_2O_{21}Cl_3$ (1048.23): C, 50.42; H, 4.71; N, 2.67. Found: C, 50.41; H, 4.48; N, 2.61.

**Dodecyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2 → 3)-6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranoside (23).** To a solution of the trichloroacetimidate **22** (329 mg, 0.31 mmol) and dodecanol (0.24 mL, 1.07 mmol) in dry $CH_2Cl_2$ (5 mL) were added molecular sieves 4A [MS-4A (AW-300), 2 g] and the mixture was stirred for 4 h at room temperature, then cooled to 0 °C. TMSOTf (34.4 μmol) was added and the mixture was stirred overnight at 0 °C and filtered through celite. The filtrate was washed with $Na_2CO_3$ and $H_2O$, dried ($Na_2SO_4$) and concentrated. Column chromatography (100:1 $CHCl_3$-MeOH) of the residue on silical gel (30 g), gave **23** (256 mg, 95%) as an amorphous mass: $[\alpha]_D$ +36.7° (*c* 0.55, $CHCl_3$), $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.0-1.3 and 1.5 (m, 20H, $10CH_2$), 1.46 (s, 3H, AcN), 1.67 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3bax), 1.77, 1.91, 2.03, 2.09, 2.24 (5s, 15H, 5AcO), 2.47 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.58$ Hz, H-3beq), 3.90 (s, 3H, MeO), 4.79 (d, 1H, $J_{1,2} = 8.24$ Hz, H-1a), 4.81 (m, 1H, H-4b), 5.14 (d, 1H, $J_{5,NH} = 10.1$ Hz, NH), 5.22 (dd, 1H, $J_{6,7} = 2.42$, $J_{7,8} = 8.30$ Hz, H-7b), 5.25 (d, 1H, $J_{3,4} = 3.4$ Hz, H-4a), 5.42 (dd, 1H, $J_{1,2} = 8.24$ Hz, $J_{2,3} = 10.0$ Hz, H-2a), 5.64 (m, 1H, H-8b), 7.4-8.2 (m, 10H, 2Ph).

Anal. Calcd for $C_{54}H_{74}NO_{21}$ (1073.17): C, 60.44; H, 6.95; N, 1.31. Found: C, 60.19; H, 6.92; N, 1.23.

**Hexadecyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate) - (2 → 3) -6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galactopyranoside (24).** Coupling of **22** (217 mg, 0.22 mmol) with hexadecanol (106 mg, 0.44 mmol), as described for **23**, gave **24** (141 mg, 90%) as an amorphous mass: $[\alpha]_D$ +47° (*c* 1.34, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 3H, $CH_3$), 1.0-1.3 and 1.5 (m, 28H, $14CH_2$), 1.45 (s, 3H, AcN), 1.78, 1.92, 2.03, 2.09, 2.24 (5s, 15H, 5AcO), 1.71 (t, 1H, $J_{gem} = J_{3ax,4} = 12.5$ Hz, H-3bax), 2.47 (dd, 1H, $J_{gem} = 12.8$ Hz, $J_{3eq,4} = 4.39$ Hz, H-3beq), 3.55 (s, 2H, $CH_2O$), 3.90 (s, 3H, MeO), 4.79 (d, 1H, $J_{1,2} = 8.06$ Hz, H-1a), 4.81 (m, 1H, H-4b), 5.06 (d, 1H, $J_{5,NH} = 9.89$ Hz, NH), 5.24 (dd, 1H, $J_{6,7} = 2.4$, $J_{7,8} = 8.3$ Hz, H-7b), 5.25 (d, 1H, $J_{3,4} = 3.3$ Hz, H-4a), 5.65 (m, 1H, H-8b), 7.35-8.25 (m, 10H, 2Ph).

Anal. Calcd for $C_{58}H_{82}NO_{21}$ (1129.28): C, 61.69; H, 7.32; N, 1.24. Found: C, 61.43; H, 7.24; N, 1.02.

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**2-(Tetradecyl)hexadecyl (Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-galatopyranoside (25).** Coupling of **22** (145 mg, 0.148 mmol) with 2-tetradecyhexadecanol (36 mg, 0.15 mmol), as described for **23**, gave **25** (195 mg, 95.6%) as an amorphous mass: $[\alpha]_D$ +4.1° (*c* 0.37, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 0.88 (t, 6H, $2CH_3$), 1.0-1.4 and 1.56 (m, 53H, $26CH_2$ and CH), 1.45 (s, 3H, AcN), 1.68 (t, 1H, $J_{gem} = J_{3ax,4} = 12.8$ Hz, H-3bax), 1.78, 1.92, 2.03, 2.09, 2.24 (5s, 15H, 5AcO), 2.47 (dd, 1H, $J_{gem} = 12.8$, $J_{3eq,4} = 4.76$ Hz, H-3beq), 3.33 (s, 1H, $OCH_2CH$), 3.62 (dd, 1H, $J_{5,6} = 10.6$ Hz, $J_{6,7} = 2.60$ Hz, H-6b), 3.90 (s, 3H, MeO), 3.98 (dd, 1H, $J_{gem} = 12.0$, $J_{5,6} = 5.90$ Hz, H-6a), 4.14 (d, 1H, $J_{5,6} = 5.50$ Hz, H-5a), 4.34 (dd, 1H, $J_{gem} = 12.0$ Hz, H-6a'), 4.75 (d, 1H, $J_{1,2} = 8.06$ Hz, H-1a), 4.79 (m, 1H, H-4b), 4.92 (d, 1H, $J_{5,NH} = 9.90$ Hz, NH), 5.22 (dd, 1H, H-7b), 5.24 (d, 1H, H-4a), 5.41 (dd, 1H, $J_{1,2} = 8.06$ Hz, $J_{2,3} = 9.90$ Hz, H-2a), 5.62 (m, 1H, H-8b), and 7.2-8.2 (m, 10H, 2Ph).

Anal. Calcd for $C_{72}H_{110}NO_{21}$ (1325.66): C, 65.23; H, 8.36; N,1.06. Found: C, 65.21; H, 8.10; N, 0.91.

**2-(Tetradecyl)hexadecyl (5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranoside (26).** *O*-Deacylation of **25** (195 mg) and saponification of the methyl ester as described for **14**, yielded **26**[4] (130 mg, 98%) as an amorphous mass: $[\alpha]_D$ -3.0° (*c* 1.12, 1:1 $CHCl_3$-MeOH); $^1H$ NMR (2:1 $CDCl_3$-$CD_3OD$) δ 0.89 (t, 6H, $2MeCH_2$), 1.28 and 1.62 (s, 53H, $26CH_2$ and CH), 1.76 (br t, 1H, H-3bax), 2.04 (s, 3H, AcN), 2.81 (dd, 1H, H-3beq), and 4.24 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1a).

Anal. Calcd for $C_{47}H_{89}NO_{14}$ (892.2): C, 63.27; H, 10.05; N, 1.57. Found: C, 63.05; H, 10.19; N,1.51.

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