Synthesis of a Bis(sialic acid) 8,9-Lactam

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Methyl [2-(trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate was sialylated with methyl (ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-thio-3-(phenylthio)-2,3,5-trideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate to give the corresponding bis(sialic acid) derivative in 23% yield. Removal of protecting groups, reduction of the azido group to an amino group, and removal of the auxiliary thiophenyl group gave the desired bis(sialic acid) 8,9-lactam. Comparison of the ^{1}H NMR spectra and energy-minimized (MM3) structures of the bis(sialic acid) lactam with those of the corresponding bis(sialic acid) lactone showed the conformations of the two compounds to be very similar (RMS = 0.027 Å).

Introduction

Glycosphingolipids that contain sialic acid moieties (gangliosides) are present on the surface of mammalian cells.[1] Tumor cells often carry abnormal amounts of some gangliosides, which has led to the concept of gangliosides as tumor-associated antigens.[2] It is well known that gangliosides form δ-lactones under acidic conditions,[3] and furthermore, the high density of gangliosidic sialic *acid* on many tumor cells may induce the formation of lactones *in vivo*. The δ-lactones were suggested to be the true immunogens in the preparation of antiganglioside antibodies, but the hydrolytic lability of the lactones would make them poor immunogens due to the difficulty of maintaining a high *in vivo* concentration during immunization.[2b,4]

We have prepared ganglioside lactams[5] (G_{M1-4}) and used them as immunogens in order to raise antibodies that cross-react with the corresponding lactones on murine melanoma cells. This work demonstrated that G_{M3}-lactone is indeed present on the cell surface.[6] The lactams have proved to be hydrolytically stable and structurally similar to their lactone counterparts.[5]

Ganglioside G_{D3}, which carries two sialic acid moieties, is abnormally frequent on human malignant melanoma cells as compared to healthy cells.[7] G_{D3} is capable of forming several lactones, of which the 8,9-lactone is formed most readily (Figure 1).[8] We now describe the synthesis of a bis(sialic acid) lactam (**1**), suitable for further elaboration into G_{D3}-lactam and other ganglioside lactams containing a bis(sialic acid) moiety. The synthesis is based on our novel sialyl donor[9] **12** and acceptor **6**.

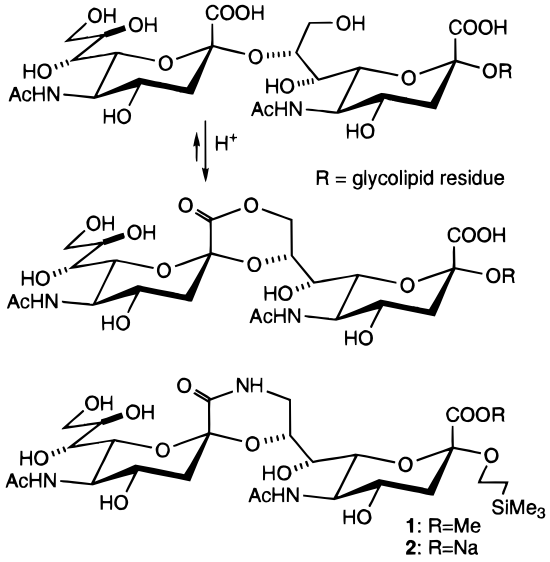

Figure 1. Bis(sialic acid) lactone and the synthetic lactam analogs **1** and **2**.

Results and Discussion

Our synthetic strategy was based on stereoselective 8-*O*-sialylation of the acceptor **6**, rather than introduction of a nitrogen functionality in the 9-position of commercially available bis(sialic acid) (for numbering, see Figure 2). The latter forms a quite stable 8,9-lactone,[10]

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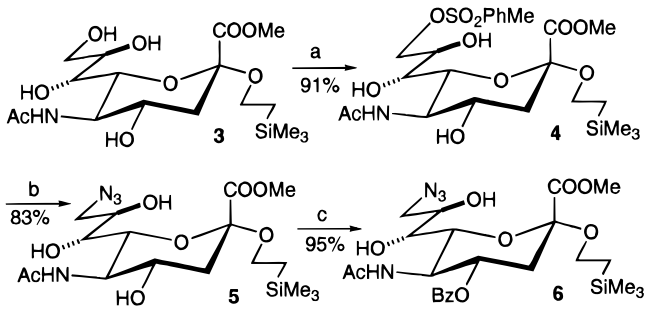
Scheme 1[a]



[a] Key: (a) *p*-$MePhSO_2Cl$, CH_2Cl_2/pyridine, −80 °C, 48 h; (b) NaN_3, 18-crown-6, DMF, 60 °C, 20 h; (c) BzCl, Et_3N, −30 °C, 18 h.

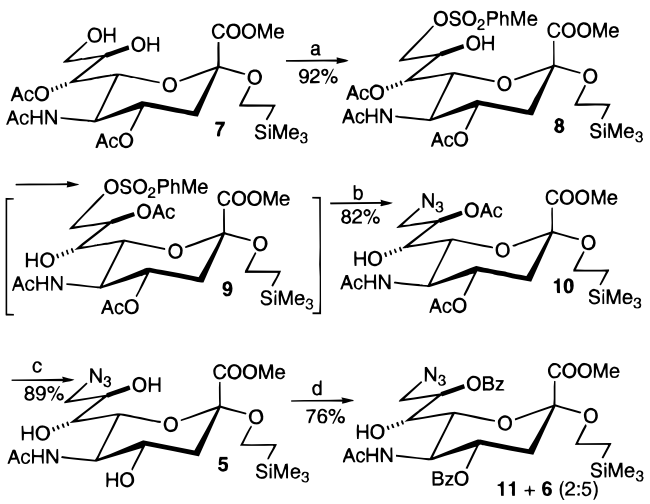
and it is by no means trivial to exploit this for regioselective introduction of nitrogen at C-9.

The sialic ester glycoside **3**[11] was treated with 2 equiv of *p*-toluenesulfonyl chloride at −80 °C, which gave the 9-*O*-tosylate **4** (91%) after chromatography (Scheme 1). Regioselective 9-*O*-tosylations of sialic ester glycosides have been conducted earlier.[12] Treatment of the tosylate **4** with sodium azide in *N*,*N*-dimethylformamide in the presence of 18-crown-6 ether as catalyst gave the 9-azido compound **5** (83%).

At this point, the reactivity order was not settled for the three secondary hydroxyl groups in **5**. Treatment of **5** with benzoyl chloride in dichloromethane/pyridine at −80 °C (Scheme 2) gave the desired monobenzoate **6** (55%) and the dibenzoate **11** (21%). In order to improve the yield of **6**, alternative benzoylation procedures were investigated. Regioselective *O*-benzoylation has been performed using benzoyl cyanide/triethylamine or 1-(benzoyloxy)benzotriazole/triethylamine.[13] Since benzoyl cyanide is reported to be unreactive in the absense of triethylamine in aprotic solvents,[13a] we thought that benzoyltriethylammonium cyanide was the reactive species needed for regioselective benzoylation to occur. Hence, we expected that the new combination benzoyl chloride/triethylamine (Scheme 1) would constitute a viable alternative. Treatment of **5** with the latter reagent combination in dichloromethane at −30 °C gave the desired sialyl acceptor **6** (95%) as the only product formed. The order of reactivity of the four hydroxyl groups in **3** was thus established to be HO-9 >> HO-4 > HO-8 >> HO-7.

Prior to the successful preparation of **6** (Scheme 1), we investigated the regioselective introduction of a 9-azido group in the known diol **7**[14] (Scheme 2). Treatment of **7** with a large excess of *p*-toluenesulfonyl chloride gave the monotosylate **8** (92%). However, treatment of **8** with lithium azide in tetrahydrofuran, using 15-crown-5 ether as catalyst, caused the 7-*O*-acetyl group to migrate to the 8-position, and **10** (82%) was the only compound isolated. Monitoring the reaction by TLC revealed the intermediate formation of a strongly UV-absorbing compound,

Scheme 2[a]



[a] Key: (a) *p*-$MePhSO_2Cl$, pyridine, 0 → 20 °C, 22 h; (b) LiN_3, 15-crown-5, THF, 45 °C, 24 h; (c) MeONa, MeOH, 20 °C, 2 h; (d) BzCl, CH_2Cl_2/pyridine, −80 °C, 96 h.

which had disappeared when the reaction was complete. It is thus expected that the transformation of **8** into **10** proceeds *via* the tosylate **9** (Scheme 2). ^{1}H-NMR analysis of the reaction mixture supported this assumption. Tetrabutylammonium azide in acetonitrile gave rapidly the desired product according to TLC analysis. However, rearrangement into **10** occurred during workup of the reaction mixture. De-*O*-acetylation of **10** gave **5** (89%), identical with the product obtained by azide treatment of **4** (Scheme 1). As a consequence of these studies, acyl protection of HO-7 in sialic acids should be avoided and sialylations in the 8-position be performed with acceptors such as **6** that have both HO-7 and HO-8 unprotected, as shown in Scheme 3.

The joining of two sialic acid units *via* an α2→8 glycosidic bond in high yield and α*β* selectivity is difficult, and consequently, many synthetic approaches have been published.[15] In a recent paper, we reported the synthesis of the novel sialyl donor **12**, which was found to be superior to conventional donors. Sialylation yields were higher, even with sterically hindered and unreactive sialyl acceptors, and the α*β*-selectivity was virtually complete.[9] Compound **12**, which carries an auxiliary phenylthio substituent[16] in the 3-position, was synthesized from *N*-acetylneuraminic acid in six steps and 47% overall yield.[9]

The 9-azido diol **6** was sialylated by the donor **12** at −40 °C in acetonitrile, using methyl sulfenyl bromide[17] /silver trifluoromethane sulfonate as promotor, to give the α2→8 bis(sialoside) **13** in 28% yield (Scheme 3). When the concentration of the reactants was halved, the yield of **13** was reduced to 23%. However, the main part (63%) of acceptor **6** was thus recovered, and the yield, based on consumed **6**, was raised from 47% to 64% when the more diluted reaction mixture was employed. According to TLC and NMR analysis, additional bis(sialosides) were formed, but these were extremely labile and could not be isolated; their identity remains un-

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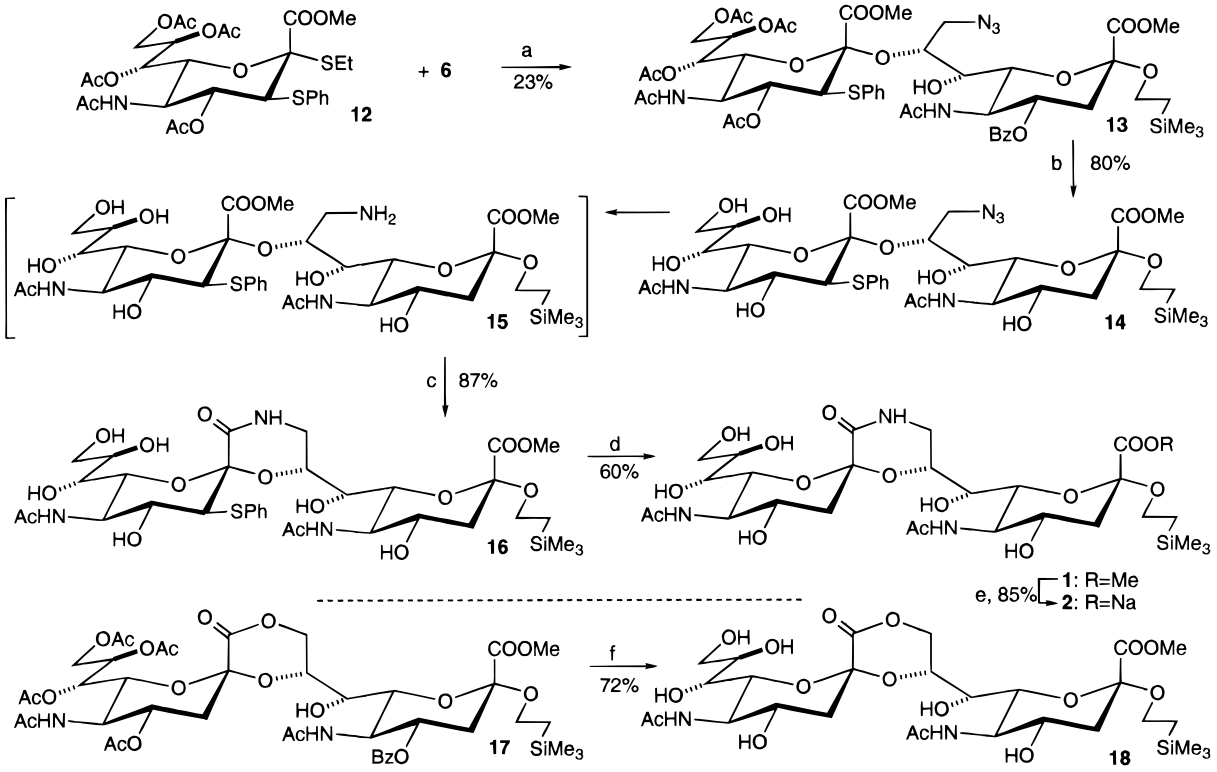
Scheme 3[a]



[a] Key: (a) MeSBr, CF_3SO_3Ag, 3 Å MS, MeCN, −40 °C, 2 h; (b) MeONa, MeOH/toluene, 20 °C, 16 h; (c) Ph_3P, THF/H_2O, 40 °C, 20 h; (d) Ra-Ni, EtOH, 20 °C, 3 h, then Ph_3P, 1 h; (e) NaOH, MeOH/H_2O, 20 °C, 24 h, Sephadex G10; (f) MeONa, MeOH, 20 °C, 24 h, then HOAc, 20 °C, 22 h, freeze-drying.

known. All of the donor **12** was destroyed in the reaction. Since **12** gave high yields (71−77%) with galactoside and lactoside diol acceptors,[9] the low yields obtained with sialic acid diol acceptors indicate that the latter are highly hindered. More stable, yet highly reactive, sialyl donors are thus required in order to obtain high yields in sialylations of sialic acid-derived acceptors in the 8-position. Such donors remain to be developed.

In our previous syntheses of ganglioside lactams, we found that the conditions used for reduction of the azide functionality caused the intermediate amino ester to lactamize.[5] However, reduction of the 9-azido group of **13**, using hydrogen sulfide as reducing agent, gave almost no lactamization. De-*O*-acylation of **13** (→ **14**, 80%), followed by reduction of **14** with triphenylphosphine, gave the desired 8,9-lactam **16** (presumably via the amine **15**) in 87% yield.

Reductive removal of the auxiliary phenylthio substituent of **16** was performed with Raney nickel in ethanol. Initial attempts gave the desired lactam **1** in <20% yield, due to absorption of **1** to the surface of the Raney nickel particles. However, addition of triphenylphosphine to the reaction mixture prior to filtration caused desorption of **1** and an acceptable yield of 60% was obtained. The yield might well be improved by optimizing the type and amount of the desorption agent. It deserves to be mentioned that the phenylthio group could not be removed by traditional tin hydride reagents;[16,18] either several products were formed or no reaction occurred.

Hydrolysis of the methyl ester function of **1** gave the sodium salt **2** in 85% yield. The successful synthesis of **1** has provided useful information for our planned synthesis of the tetrasaccharidic G_{D3}-ganglioside lactam. Furthermore, compound **1** is well suited as starting material for the preparation of neoglycoprotein antigens, similar in scope to G_{M3}-lactam-BSA used for raising monoclonal antibodies that crossreacted with G_{M3}-lactone on cell surfaces.[6]

The bis(sialic acid) lactone **17**[9b] was de-*O*-acylated, and the crude product was freeze-dried from an acetic acid solution to give lactone **18** (72%) for comparison with **1** concerning the solution conformations of the two compounds. The lactone ring of **18** was partially opened by methanol to give an equilibrium mixture (∼9:1) of **18** and the corresponding bis(methyl ester), as indicated in Figure 2C. FAB mass spectroscopy of the mixture gave peaks at *m/z* 697.3 (**18**) and *m/z* 729.3 (bis(methyl ester)), thus confirming the presence of the bis(methyl ester).

Comparison of the ^{1}H-NMR spectra (Figure 2) of **1** and **18** showed that coupling constants diagnostic of the sialic acid- and lactam/lactone-ring conformations are very similar (Figure 2 and Table 1). The conformational similarity was corroborated by a molecular mechanics calculation [MM3(92)][19] of **1** and **18** (Figure 2). Superimposition and RMS-fitting of the low energy conformations of **1** and **18** (using all ring atoms) showed them to have very similar overall shapes (rms = 0.027 Å), as depicted in Figure 2.

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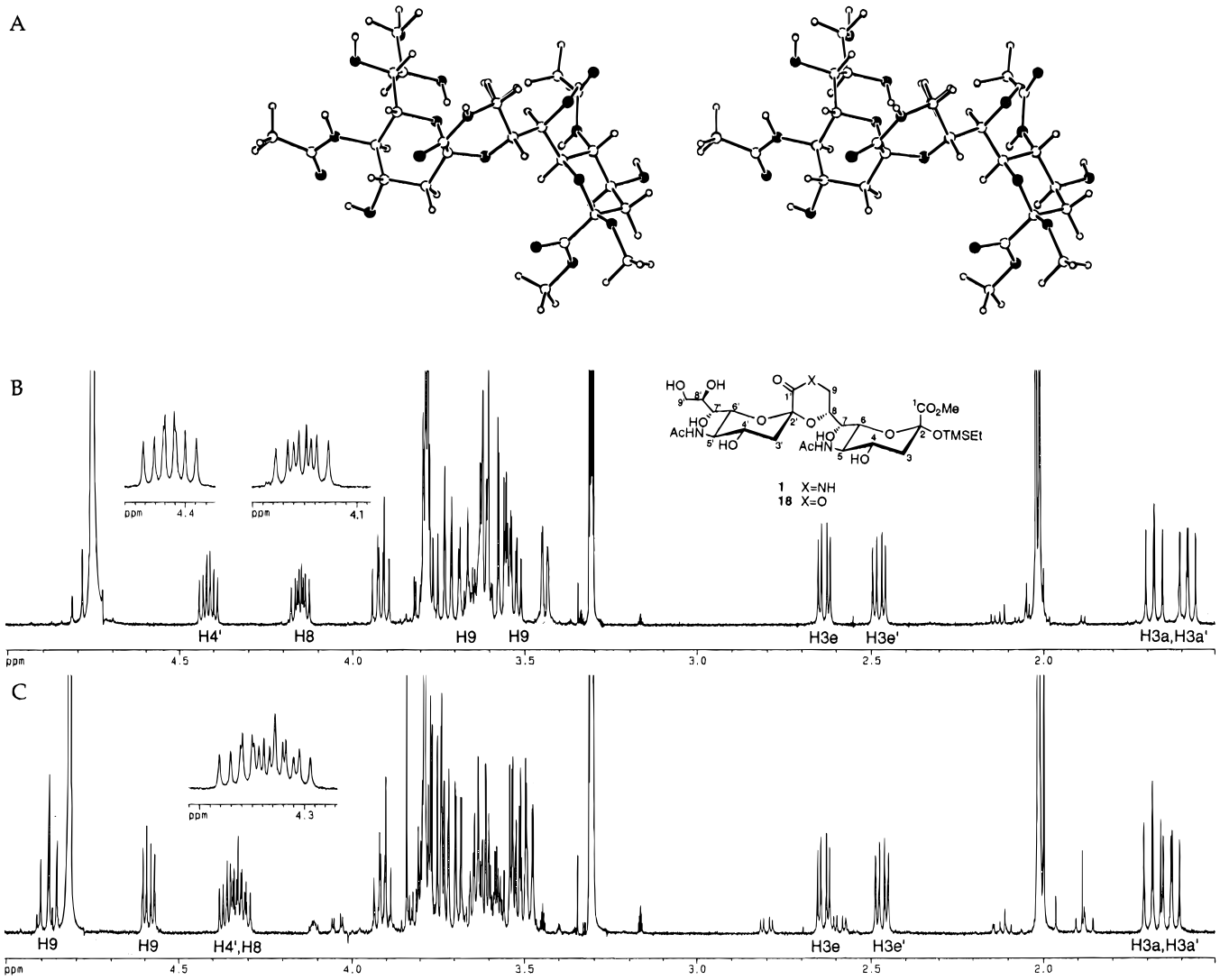

Figure 2. (A) Stereoview of the superimposed low energy [MM3(92)][19] conformers of the methyl glycosides that correspond to **1** and **18**. (B) ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **1**. (C) ^{1}H-NMR (500 MHz, CD_3OD) spectrum of **18** (contaminated by ~10% of the corresponding bis(methyl ester), obtained by reaction of **18** with the solvent).

Table 1. Selected ^{1}H NMR coupling constants for 1 and 18

	coupling constant (Hz)	
coupling[a]	**1**	**18**
$H_{3a}-H_{3e}$	12.6	12.7
$H_{3a}-H_4$	11.9	12.3
$H_{3e}-H_4$	4.6	4.6
H_7-H_8	8.6	7.7
$H_{9A}-H_{9B}$	13.1	11.8
H_8-H_{9A}	11.0	11.6
H_8-H_{9B}	5.9	5.2
$H_{3'a}-H_{3'e}$	12.7	13.1
$H_{3'a}-H_{4'}$	10.9	11.2
$H_{3'e}-H_{4'}$	5.4	5.4

[a] Assignments were made by COSY and HETCOR procedures.

The ^{1}H NMR spectra of **1** and **18** require some additional comments. In both compounds, the ring hydrogen H-4′ is observed at an unusually large chemical shift (~4.4 ppm). This is probably due to close proximity between H-4′ and the carbonyl oxygen of the lactam and lactone rings. The H-4′−O-distance is ~2.6 Å in the energy-minimized structures obtained by the MM3-calculations. Such oxygen-induced chemical shifts have been observed in other saccharides.[20]

As with the mono(sialic acid)-containing lactams reported earlier,[5] bis(sialic acid)-containing lactams also seem to be good substitutes for the natural sialic acid lactones, thus making the lactams potentially useful as hydrolytically stable immunogens for the preparation of, e.g., anti-lactone antibodies.

Experimental Section

General. NMR spectra were recorded at 500 and 300 MHz. Assignment of ^{1}H NMR spectra was achieved using 2D-methods (COSY, HETCOR). Optical rotations were measured at +20 °C. Chemical shifts are expressed in ppm using residual $CHCl_3$, CHD_2OD as reference. Reactions were monitored by TLC using alumina plates coated with silica gel 60 F_{254} (Merck) and visualized using either UV light or by charring with H_3PO_4 (aqueous 10% spray solution). Preparative chromatography was performed with Merck silica gel (35−70 μm, 60 Å). CH_2Cl_2, toluene, and THF were distilled under N_2 over CaH_2 and sodium benzophenone ketyl, respectively. MeCN and NEt_3 were stored over 3 Å molecular sieves and filtered through a column of Al_2O_3 (activity I, Merck) im-

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mediately before use. Methanol was dried over 3 Å molecular sieves >3 days before use. Compounds obtained as white powders were precipitated with *n*-hexane from a chloroform/diethyl ether solution.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (1). Compound **16** (8.7 mg, 0.0108 mmol) was stirred vigorously with Raney-Ni (1 mL, 50% slurry in water) in ethanol (4 mL) at room temperature for 3 h. Triphenylphosphine (0.35 g) was added, and the stirring was continued for 1 h. The reaction mixture was filtered through glass fiber paper (Whatman) on a Celite pad (MeOH). The eluate was concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH/H_2O 40:10:1) to give **1** (4.5 mg, 60%) as a white solid: $[\alpha]_D$ −36° (*c* 0.34, CH_3OH); IR (neat) 1738, 1680, 1635 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 4.42 (ddd, 1 H, J = 10.9, 5.4, 10.0 Hz), 4.15 (ddd, 1 H, J = 5.9, 8.6, 11.0 Hz), 3.92 (m, 1 H), 3.79 (s, 3 H), 3.73 (t, 1 H, J = 10.3 Hz), 3.69 (dd, 1 H, J = 13.1 Hz), 3.62 (m, 1 H), 3.56 (dd, 1 H, J = 1.5, 10.5 Hz), 3.53 (dd, 1 H), 3.44 (m, 1 H), 2.64 (dd, 1 H, J = 4.6, 12.6 Hz), 2.48 (dd, 1 H, J = 12.7 Hz), 2.02, 2.01, (2 s, 6 H), 1.68 (dd, 1 H, J = 11.9 Hz), 1.58 (dd, 1 H), 0.88 (m, 2 H), 0.01 (s, 9 H); ^{13}C NMR (CD_3OD) δ 175.8, 175.4, 171.1, 170.7, 100.7, 99.0, 74.5, 74.1, 73.5, 72.3, 72.0, 69.1, 69.0, 65.4, 62.9, 54.4, 53.8, 42.8, 42.0, 41.9, 23.0, 22.7, 19.2. HR FAB-MS calcd for $C_{28}H_{50}N_3O_{15}Si$ (M + H) 696.3011, found 696.3019.

Sodium {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (2). To a stirred solution of **1** (4.3 mg, 0.0062 mmol) in methanol (0.3 mL) were added water (0.2 mL) and aqueous sodium hydroxide (0.050 mL, 0.2654 M, 0.0133 mmol). After 24 h at room temperature, the mixture was chromatographed (Sephadex G10, H_2O). The eluate was freeze-dried to give **2** (3.7 mg, 85%) as a white foam: $[\alpha]_D$ +6.2° (*c* 0.32, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.33 (ddd, 1 H, J = 5.4, 10.0, 11.0 Hz), 4.23 (ddd, 1 H, J = 4.7, 6.4, 11.1 Hz), 4.07 (dd, 1 H, J = 1.0, 10.6 Hz), 3.91 (t, 1 H, J = 10.1 Hz), 3.86 (t, 1 H, J = 10.2 Hz), 3.83 (m, 1 H), 3.83 (dd, 1 H, J = 2.4, 11.8 Hz), 3.76 (dd, 1 H, J = 1.2, 10.5 Hz), 3.70 (dd, 1 H, J = 1.2, 6.6 Hz), 3.63 (m, 1 H), 3.54 (m, 1 H), 3.54 (dd, 1 H, J = 1.1, 9.3 Hz), 2.66 (dd, 1 H, J = 4.4, 12.2 Hz), 2.58 (dd, 1 H, J = 13.2 Hz), 2.06, 2.05 (2 s, 6 H), 1.78 (dd, 1 H, J = 11.4 Hz), 1.59 (t, 1 H, J = 12.2 Hz), 0.94 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{27}H_{47}N_3O_{15}SiNa$ (M + H): 704.2674, found 704.2675.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-9-*O*-(*p*-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (4). A stirred solution of **3**[11] (244.5 mg, 0.577 mmol) in dry dichloromethane/pyridine 2:1 (4.5 mL) was cooled to −70 °C under an argon atmosphere, and *p*-TsCl (226 mg, 1.18 mmol) was added. The reaction was left at −80 °C without stirring for 2 d, after which water (0.1 mL) was added. Stirring for 10 min at −70 °C and 30 min at rt followed by concentration to a brown syrup which upon chromatography (dichloromethane/methanol 25:1) gave **4** (303 mg, 91%) as a white foam: $[\alpha]_D$ −8.6° (*c* 0.93, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$/CD_3OD ~97:3) δ 7.76 (d, 2 H, J = 8.3 Hz), 7.32 (d, 2 H), 4.30 (dd, 1 H, J = 2.2, 10.0 Hz), 4.12 (d, 1 H, J = 5.7 Hz), 4.00 (ddd, 1 H, J = 9.2 Hz), 3.79 (m, 1 H), 3.78 (s, 3 H), 3.66 (t, 1 H, J = 10.1 Hz), 3.47 (m, 1 H), 3.42 (dd, 1 H, J = 1.7 Hz), 3.36−3.26 (m, 2 H), 2.69 (dd, 1 H, J = 13.0, 4.7 Hz), 2.41 (s, 3 H), 2.00 (s, 3 H), 1.79 (t, 1 H, J = 11.4 Hz), 0.82 (m, 2 H), 0.00 (s, 9 H); HR FAB-MS calcd for $C_{24}H_{40}NO_{11}SSi$ (M + H^+) 578.2091, found 578.2096.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (5). (a) A solution of **4** (256.0 mg, 0.443 mmol), 18-crown-6 ether (44.4 mg, 0.168 mmol), and sodium azide (148 mg, 2.27 mmol) in dry *N*,*N*-dimethylformamide (1.5 mL) was stirred vigorously at 60 °C for 20 h after which the reaction mixture was filtered, coconcentrated with toluene, and chromatographed (CH_2Cl_2 → CH_2Cl_2/EtOH 12:1, gradient) to give **5** (165 mg, 83%) as a white powder: $[\alpha]_D$ +1.1° (*c* 0.95, $CHCl_3$); IR (neat) 2090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/CD_3OD ~97:3) δ 3.97 (ddd, 1 H, J = 9.0, 2.6, 6.3 Hz), 3.82 (m, 1 H), 3.78 (s, 3 H), 3.66 (t, 1 H, J = 10.1 Hz), 3.57 (dd, 1 H, J = 12.7 Hz), 3.45 (m, 1 H), 3.40 (dd, 1 H, J = 1.9 Hz), 3.37−3.27 (m, 3 H), 2.69 (dd, 1 H, J = 13.1, 4.7 Hz), 1.99 (s, 3 H), 1.79 (t, 1 H, J = 12.5 Hz), 0.82 (m, 2 H), −0.05 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.6, 169.9, 98.6, 73.7, 70.6, 69.5, 68.3, 62.0, 53.6, 53.2, 53.0, 40.8, 29.7, 23.2, 18.0. HR FAB-MS calcd for $C_{17}H_{33}N_4O_8Si$ (M + H) 449.2067, found 449.2069.

(b) To a stirred solution of **10** (233 mg, 0.437 mmol) in dry methanol (3 mL) was added a solution of NaOMe in methanol (0.050 mL, ~2 M) under N_2. After 2 h at rt, Amberlite IR-120 was added and the reaction mixture was filtered, washed with methanol, concentrated, and chromatographed (toluene/EtOH 15:1 → 10:1, gradient) to give **5** (175 mg, 89%) as a white powder.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (6) and Methyl [2-(Trimethylsilyl)-ethyl 5-acetamido-9-azido-4,8-di-*O*-benzoyl-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (11). (a) A stirred solution of **5** (132 mg, 0.295 mmol) in dry dichloromethane/pyridine 2:1 (1.5 mL) was cooled to −70 °C under Ar, and benzoyl chloride (0.034 mL, 0.29 mmol) was added. The mixture was stirred vigorously for 20 min and then kept at −80 °C for 3 d, after which additional benzoyl chloride (0.007 mL, 60 μmol) was added. After 24 h at −80 °C, water (0.050 mL) was added, and the mixture was concentrated and chromatographed (toluene/EtOH 80:1 → 50:1 → 10:1, gradient) to give **11** (41 mg, 21%) and **6** (90 mg, 55%) as white powders and recovered **7** (20 mg, 15%) as a syrup. Compound **6**: $[\alpha]_D$ −31° (*c* 1.00, $CHCl_3$); IR (neat) 2090, 1720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$/CD_3OD ~97:3) δ 7.99, 7.60, 7.45 (5 H), 5.12 (ddd, 1 H, J = 12.2, 4.9, 10.5 Hz), 4.14−4.04 (m, 2 H), 3.90 (m, 1 H), 3.86 (s, 3 H), 3.63 (dd, 1 H, J = 2.5, 12.8 Hz), 3.52−3.45 (m, 2 H), 3.41 (dd, 1 H, J' = 6.3 Hz), 3.39 (m, 1 H), 2.82 (dd, 1 H, J = 12.9 Hz), 2.10 (t, 1 H), 1.91 (s, 3 H), 0.86 (m, 2 H), −0.01 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 173.0, 169.5, 167.5, 133.9, 129.8, 128.7, 128.6, 98.4, 73.9, 70.5, 69.6, 69.4, 62.1, 53.6, 53.3, 51.7, 37.5, 23.0, 17.9; HR FAB-MS calcd for $C_{24}H_{37}N_4O_9Si$ (M + H) 553.2329, found 533.2330.

Compound **11**: $[\alpha]_D$ +8.2° (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.06−7.40 (m, 10 H), 6.21 (d, 1 H, J = 10.1 Hz), 5.58 (ddd, 1 H, J = 8.4, 2.9, 4.2 Hz), 5.18 (ddd, 1 H, J = 12.1, 4.7, 10.5 Hz), 4.13 (q, 1 H), 3.95 (m, 1 H), 3.87−3.80 (m, 3 H), 3.75 (dd, 1 H, J = 10.5, 1.8 Hz), 3.37 (m, 1 H), 3.31 (s, 3 H), 2.70 (dd, 1 H, J = 12.7 Hz), 2.10 (t, 1 H), 1.95 (s, 3 H), 0.91 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{31}H_{41}N_4O_{10}Si$ (M + H) 657.2592, found 657.2583.

(b) To a stirred solution of **5** (200.4 mg, 0.447 mmol) in dichloromethane (3 mL) was added dry triethylamine (0.090 mL, 0.65 mmol) at rt under Ar. After cooling to −50 °C and addition of benzoyl chloride (0.055 mL, 0.48 mmol), the mixture was left at −30 °C for 18 h. Methanol (0.010 mL) was added, the mixture was concentrated, and the residue was chromatographed (toluene/EtOH 80:1 → 40:1, gradient) to give **6** (234 mg, 95%) as a white powder.

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4,7-di-*O*-acetyl-3,5-dideoxy-9-*O*-(*p*-toluenesulfonyl)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (8). To an ice-cooled, stirred solution of **7**[14] (235.8 mg, 0.465 mmol) in dry pyridine (5 mL) was added *p*-toluenesulfonyl chloride (0.30 g, 1.6 mmol) under N_2. After 5 h at 0 °C, the mixture was left at room temperature for 17 h. Methanol was added, the mixture was concentrated, and the residue was chromatographed (toluene/EtOH 30:1) to give **8** (284 mg, 92%) as a white powder: $[\alpha]_D$ −3.3° (*c* 1.01, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80, 7.33 (2 d, 4 H), 5.24 (d, 1 H, J = 10.1 Hz), 4.98 (dd, 1 H, J = 2.2, 8.7 Hz), 4.81 (ddd, 1 H, J = 12.1, 4.8, 10.3 Hz), 4.17 (m, 1 H), 4.11 (q, 1 H, J = 10.3 Hz), 4.08 (dd, 1 H, J = 2.8, 10.8 Hz), 3.95 (dd, 1 H, J' = 6.7 Hz), 3.85 (s, 3 H), 3.89−3.78 (m, 2 H), 3.40 (m, 1 H), 2.67 (dd, 1 H, J = 13.0 Hz), 2.44 (s, 3 H), 2.08, 2.03 (2 s, 6 H), 1.99 (t, 1 H), 1.85 (s, 3 H), 0.89 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{28}H_{44}NO_{13}SSi$ (M + H) 662.2302, found 662.2299.

A sample of **8** was treated with acetic anhydride/pyridine to give the fully acetylated derivative: ^1H NMR (300 MHz,

$CDCl_3$) δ 5.35−5.27 (m, 2 H), 4.35 (dd, 1 H, J = 2.4, 11.5 Hz), 4.01 (dd, 1 H, J = 7.1 Hz), 2.07, 2.06, 2.02 (3 s, 9 H), 1.88 (s, 3 H).

Methyl [2-(Trimethylsilyl)ethyl 5-acetamido-4,8-di-*O*-acetyl-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (10). To a stirred solution of **8** (20.0 mg, 0.0302 mmol) in dry tetrahydrofuran (0.6 mL) was added lithium azide (22 mg, 0.45 mmol) and 15-crown-5 ether (0.010 mL). After 24 h at 45 °C, the mixture was filtered and concentrated and the residue was chromatographed (toluene/EtOH 50:1) to give **10** (13.2 mg, 82%) as a colorless syrup: $[\alpha]_D$ −26° (*c* 0.37, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (d, 1 H, J = 7.5 Hz), 5.28 (ddd, 1 H, J = 9.1, 2.9, 3.8 Hz), 4.90 (ddd, 1 H, J = 12.2, 4.6, 10.2 Hz), 4.86 (d, 1 H, J = 4.7 Hz), 3.95 (m, 1 H), 3.89 (m, 1 H), 3.78 (s, 3 H), 3.77−3.59 (m, 4 H), 3.24 (dt, 1 H, J = 7.3, 9.2 Hz), 2.59 (dd, 1 H, J = 12.8 Hz), 2.16, 2.10, 2.01 (3 s, 9 H), 1.95 (t, 1 H), 0.86 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{21}H_{37}N_4O_{10}Si$ (M + H) 533.2279, found 533.2264.

A sample of **10** was treated with acetic anhydride/pyridine to give the fully acetylated derivative: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.34−5.28 (m, 2 H), 3.66−3.62 (m, 2 H), 2.17, 2.16, 2.03 (3 s, 9 H), 1.88 (s, 3 H).

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-4-*O*-benzoyl-3,5,9-trideoxy-8-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3-(phenylthio)-3,5-dideoxy-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (13). A solution of **6** (84.7 mg, 0.153 mmol) and **12** (160.1 mg, 0.249 mmol) in acetonitrile (1.6 mL) was stirred for 40 min at rt with 3 Å molecular sieves (0.2 g). Silver trifluoromethanesulfonate (72.8 mg, 0.283 mmol) in acetonitrile (0.3 mL) was added under Ar, and the temperature was lowered to −40 °C, after which a solution of methyl sulfenyl bromide[17] in 1,2-dichloromethane (0.103 mL, 2.8 M, 0.28 mmol) was added dropwise over a period of 10 min. After 2 h, diisopropylamine (0.1 mL) was added, the stirring was continued for 5 min at −40 °C, and the mixture was allowed to attain room temperature. The mixture was filtered through a short column of silica gel (toluene/acetone 1:1), the eluate was concentrated, and the residue was chromatographed (toluene/acetone 8:1 → 6:1 → 4:1 → 3:1 → 2:1, gradient) to give recovered **6** (53.6 mg, 63%) as a syrup and **13** (40.5 mg, 23%) as a white powder: $[\alpha]_D$ +14.5° (*c* 1.00, $CHCl_3$); IR (neat) 2095, 1735, 1660 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03−7.23 (m, 10 H), 6.05 (d, 1 H, J = 8.8 Hz), 5.47 (dd, 1 H, J = 10.6, 9.9 Hz), 5.38 (ddd, 1 H, J = 2.8, 6.4, 8.9 Hz), 5.33 (d, 1 H, J = 9.5 Hz), 5.27 (m, 1 H), 5.26 (dd, 1 H, J = 1.6 Hz), 4.75 (dt, 1 H, J = 2.4, 7.3 Hz), 4.37 (dd, 1 H, J = 12.3 Hz), 4.20 (q, 2 H), 4.15 (dd, 1 H, J = 10.9 Hz), 4.12 (dd, 1 H), 3.99 (broad d, 1 H), 3.94 (dd, 1 H, J = 13.3 Hz), 3.88 (m, 1 H), 3.84, 3.82 (2 s, 6 H), 3.78 (dd, 1 H, J = 10.5, 1.4 Hz), 3.50 (d, 1 H), 3.48 (m, 1 H), 3.40 (dd, 1 H), 2.77 (dd, 1 H, J = 12.8, 4.9 Hz), 2.19, 2.10, 2.07, 2.03 (4 s, 12 H), 2.09 (1 H), 1.92, 1.89 (2 s, 6 H), 0.89 (m, 2 H), 0.02 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 172.2, 171.1, 171.0, 170.6, 170.1, 169.7, 168.3, 168.2, 166.8, 135.4, 133.5, 132.0, 129.8, 129.3, 128.9, 128.5, 127.5, 100.7, 98.6, 75.4, 75.3, 73.0, 72.1, 69.5, 69.4, 68.5, 67.2, 62.7, 62.2, 58.1, 52.5, 52.2, 51.1, 50.0, 37.8, 23.2, 23.0, 20.9, 20.8, 20.7, 20.6, 18.1; HR FAB-MS calcd for $C_{50}H_{68}N_5O_{21}SSi$ (M + H) 1134.3897, found 1134.3956.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-azido-3,5,9-trideoxy-8-*O*-[methyl (5-acetamido-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosid)onate]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate (14). To a stirred solution of **13** (62.7 mg, 0.0553 mmol) in toluene (0.8 mL) was added methanol (1.6 mL) and sodium methoxide in methanol (0.020 mL, 0.04 mmol, ∼2 M) under Ar at room temperature. After 16 h, the mixture was neutralized with acetic acid and concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH 25:1 → CH_2Cl_2/MeOH/H_2O 60:10:1, gradient) to give **14** (38 mg, 80%) as a white solid: $[\alpha]_D$ −36° (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.67−7.19 (m, 5 H), 4.63 (dt, 1 H, J = 6.7 Hz), 4.12 (t, 1 H, J = 10.1 Hz), 4.04 (dd, 1 H, J = 10.6 Hz), 4.00 (dd, 1 H, J = 1.4, 1.9 Hz), 3.86, 3.79 (2 s, 6 H), 3.77 (dd, 1 H, J = 1.5, 7.3 Hz), 3.76 (dd, 1 H), 3.58 (dd, 1 H, J = 2.8, 13.1 Hz), 3.47 (dd, 1 H, J = 9.2 Hz), 3.41 (m, 1 H), 3.33 (d, 1 H), 2.65 (dd, 1 H, J = 4.6, 12.8 Hz), 2.02, 2.02 (2 s, 6 H), 1.66 (dd, 1 H, J = 11.6 Hz), 0.84 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{35}H_{56}N_5O_{16}SSi$ (M + H) 862.3212, found 862.3224.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-9-amino-3,5,9-trideoxy-8-*O*-[5-acetamido-3,5-dideoxy-3-(phenylthio)-D-*erythro*-α-L-*gluco*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactam (16). To a stirred solution of **14** (17.9 mg, 0.0208 mmol) in tetrahydrofuran/water 8:1 (0.23 mL) was added triphenylphosphine (22.1 mg, 0.0843 mmol), and the mixture was kept at 40 °C for 20 h. Chloroform/methanol (∼1:1) was added, the mixture was concentrated, and the residue was chromatographed (CH_2Cl_2/MeOH/H_2O 50:10:1) to give **16** (14.5 mg, 87%) as a white solid: $[\alpha]_D$ −44° (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 7.63−7.16 (m, 5 H,), 4.39 (t, 1 H, J = 10.1 Hz), 4.22 (dt, 1 H, J = 5.4, 9.9 Hz), 3.90 (t, 1 H, J = 10.3 Hz), 3.90 (m, 1 H), 3.68 (s, 3 H), 3.58 (ddd, 1 H, J = 11.8, 4.6, 9.9 Hz), 3.44 (m, 1 H), 3.00 (d, 1 H, J = 10.4 Hz), 2.58 (dd, 1 H, J = 12.7 Hz), 2.01, 2.00 (2 s, 6 H), 1.69 (dd, 1 H), 0.86 (m, 2 H), 0.01 (s, 9 H); HR FAB-MS calcd for $C_{34}H_{54}N_3O_{15}SSi$ (M + H) 804.3045, found 804.3024.

Methyl {2-(Trimethylsilyl)ethyl 5-acetamido-3,5-dideoxy-8-*O*-[5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl]-D-*glycero*-α-D-*galacto*-2-nonulopyranosid}onate 1′→9-Lactone (18). To a solution of **17**[9b] (14.0 mg, 0.0145 mmol) in methanol (0.7 mL) was added sodium methoxide in methanol (0.003 mL, ∼2 M, ∼0.006 mmol), and the mixture was left at room temperature for 24 h and then neutralized with acetic acid, concentrated, and dried *in vacuo* for 3 h. The crude product was dissolved in acetic acid (0.5 mL), and the mixture was left at room temperature for 22 h. The mixture was freeze-dried and the residue was chromatographed (SiO_2, CH_2Cl_2/MeOH/H_2O 50:10:1 → 40:10:1, gradient) to give **18** as a syrup (7.2 mg, 72%): $[\alpha]_D$ −64° (*c* 0.14, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 4.88 (t, 1 H, J = 11.7 Hz), 4.59 (dd, 1 H, J = 5.2, 11.8 Hz), 4.36 (ddd, 1 H, J = 5.4, 11.2, 10.3 Hz), 4.32 (ddd, 1 H, J = 7.7, 11.6 Hz), 3.92 (m, 1 H), 3.79 (s, 3 H), 3.75 (t, 1 H, J = 10.3 Hz), 3.73 (dd, 1 H, J = 10.4, 1.2 Hz), 3.69 (dd, 1 H, J = 8.0 Hz), 3.60 (m, 1 H), 3.53 (m, 1 H), 3.52 (dd, 1 H, J = 1.7, 10.6 Hz), 3.49 (dd, 1 H, J = 9.3 Hz), 2.64 (dd, 1 H, J = 4.6, 12.7 Hz), 2.47 (dd, 1 H, J = 5.4, 13.1 Hz), 2.02, 2.01 (2 s, 6 H), 1.69 (t, 1 H, J = 12.3 Hz), 1.63 (dd, 1 H, J = 11.2 Hz), 0.88 (m, 2 H), 0.02 (s, 9 H); HR FAB-MS calcd for $C_{28}H_{49}N_2O_{16}Si$ (M + H) 697.2851, found 697.2865.

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Supporting Information Available: ^{1}H NMR spectra and ^{1}H NMR data with assigned signals for all title compounds described in the Experimental Section (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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