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Published on Web 03/16/2002

Solid-Phase Synthesis of Oligosaccharides and On-Resin Quantitative Monitoring Using Gated Decoupling ^{13}C NMR†

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Received August 1, 2001

Abstract: A general strategy for solid-phase oligosaccharide synthesis capable of nondestructive quantitative monitoring has been developed. The synthesis was carried out on TentaGel using thioglycosides as glycosylating agents and dimethylthiomethylsulfonium triflate as the activator. An acylsulfonamide linker was introduced to cleave the oligosaccharide from the resin. The solid-phase reactions were monitored quantitatively by using the inverse gated decoupling technique of ^{13}C NMR, where two ^{13}C-enriched markers were used to monitor the reactions: one was ^{13}C-enriched glycine incorporated as a part of the linker and as an internal standard, and the other was a ^{13}C-enriched acetyl group used as a protecting group of the glycosylation reagent. A representative synthesis of sialyl Lewis X branched tetrasaccharide was demonstrated.

Introduction

Development of nondestructive quantitative methods to monitor the progress of a reaction is especially important for determination of the reaction conditions in solid-phase synthesis. Without such methods, it is very difficult and time-consuming to determine the reaction progress, which traditionally is done by taking aliquots of resins followed by thin-layer chromatography (TLC) analysis of the reaction products released from the support. Current efforts to solve this problem include the use of FT-IR and FT-Raman spectroscopy,[1] solid-state and gel-phase ^{13}C NMR spectroscopy, as well as ^{1}H and ^{13}C correlation NMR spectroscopy,[2] high-resolution ^{1}H MAS (magic angle spinning) and MAS−CH correlation NMR spectroscopy,[3] ^{19}F NMR spectroscopy,[4] matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS),[5] elemental analysis, titration of reactive groups (−NH_2, −COOH, ArOH, −SH), photometry (−NH_2 monitored by Fmoc determination), and gravimetric analysis. Among these techniques, the FT-IR- and NMR-based methods are often used to quantitatively monitor the progress in oligosaccharide synthesis.[1d,2g,3a−d,f,g,4c,6,7] In our effort to address this issue, we have developed a nondestructive quantitative monitoring method based on inverse gated decoupling ^{13}C NMR.[8] The gated decoupling technique

† This work was initiated as a Frontier Research Program of The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan, and was supported in part by the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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§ The Scripps Research Institute.

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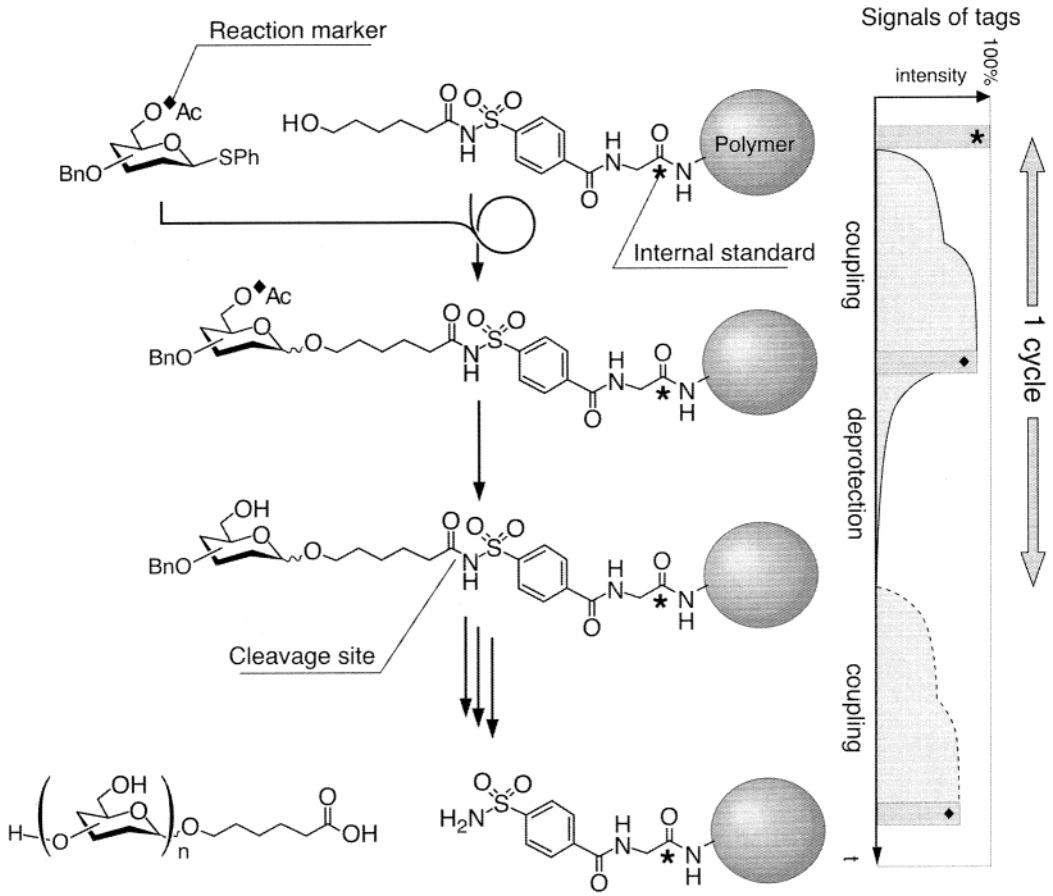

Figure 1. Schematic representation of the doubly tagged system that enables monitoring of the solid-phase oligosaccharide synthesis.

is not practical due to low signal intensities when a small amount of compound with no isotope enrichment is available for analysis. To improve the signal intensity, we used two ^{13}C-enriched tags for monitoring: one used as the internal integral marker and another as part of the glycosyl donor for determination of the coupling yield.[8b] Considering the fact that glycosylation reactions usually give rise to a mixture of α- and β-glycosides, the stereochemistry of glycosylation was not taken into consideration using this method (Figure 1). We incorporated a sulfonamide linker[9] to enable cleavage of the synthesized saccharide from the resin to make our method more practically useful, and report the application of the method to the solid-phase synthesis of an oligosaccharide, i.e., the cell adhesion ligand sialyl Lewis X (SLe^X) tetrasaccharide.[10]

Results and Discussion

Synthesis of the Spacer−Linker. The criteria to be fulfilled for a linker in solid-phase organic synthesis are (1) the attachment of the starting material is readily achieved in high yield, (2) the linker is stable to the chemistry used in the synthesis, and (3) the cleavage reaction is efficient under conditions that do not damage the final product. After several considerations, we chose an acylsulfonamide linker, which is completely stable under basic or strongly nucleophilic conditions, yet the sulfamyl group is activated by treatment with $(TMS)CHN_2$ or ICH_2CN and is then displaced with either an amine or a hydroxide to give the corresponding amide or the free acid.[9] We also decided to use a hydrophobic spacer that has been widely accepted due to the ease of product isolation and the possible route to various conjugates.[11]

Thus, the resin-bound spacer−linker **9** was synthesized as shown in Scheme 1. The spacer−linker **5** was obtained by hydrolysis of caprolactone, acetylation using ^{13}C-enriched acetic anhydride, and subsequent coupling of the ω-acetylated acid **1** with sulfonamide **2** (78.1%, three steps). Concurrently, Fmoc-glycine-*1*-^{13}C (**3**) was first incorporated into the amino-functionalized TentaGel **4**[12] as an internal integral marker (**6**).

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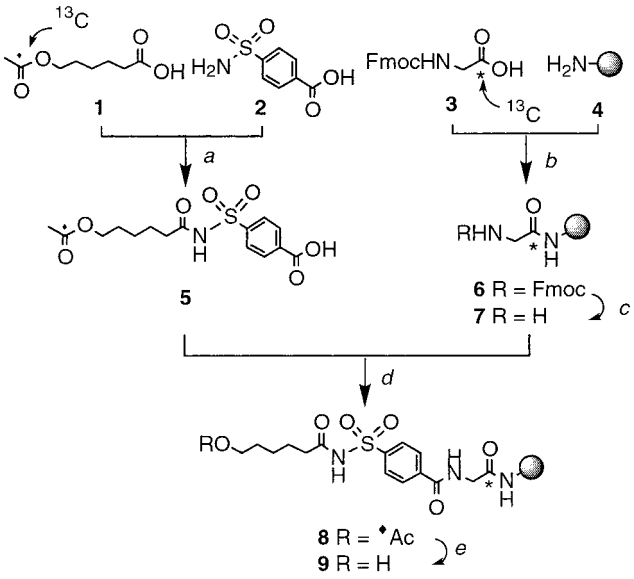
Scheme 1 [a]



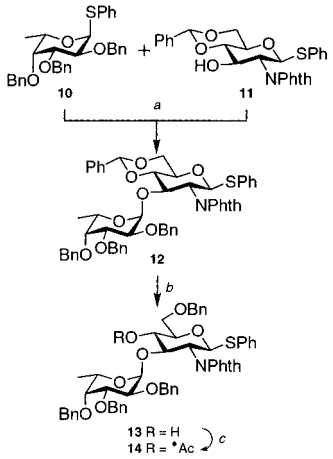
[a] Reagents and conditions: (a) (1) DCC, CH_2Cl_2; (2) 4-carboxybenzenesulfonamide, DMAP, DMF, 83%; (b) DIPC, HOBt, CH_2Cl_2, quantitative; (c) 20% piperidine/DMF, quantitative; (d) DIPC, HOBt, CH_2Cl_2, 97%; (e) 0.5 M NaOMe/MeOH−DMF, quantitative.

The Fmoc group was deprotected to afford **7**, which was then coupled with compound **5** in the presence of diisopropylcarbodiimide to give **8**. The coupling yield at this stage was estimated to be quantitative by inverse gated ^{13}C NMR. Subsequent deacetylation of the resin was achieved quantitatively to yield **9** having an internal integral marker, a cleavable linker, and a hydrophobic spacer.

Solid-Phase Synthesis of Sialyl Le^X. (a) Synthesis of Building Blocks. Our general strategy for the solid-phase oligosaccharide synthesis consists of the following principles: (1) The leaving group is a phenylthio group or an equivalent that can be activated under the same conditions. (2) The stereochemistry is not strictly controlled by neighboring group participation, but rather mildly controlled by a solvent effect. This strategy would reduce the number of required synthons (this consideration is important when a large number of compounds are targets). (3) Finally, the short-term protecting group is a (^{13}C-enriched) acetyl group. Our strategy is to simplify the reaction on the solid support. For this reason, the necessary building blocks have to be prepared prior to the solid-phase reactions. Also, to avoid complex orthogonal protecting group manipulations, we decided to use a presynthesized oligosaccharide as a building block to introduce a branching point.

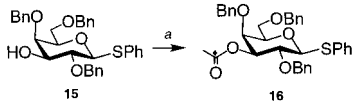
(1) αFuc-GlcN Disaccharide. A phenylthio group was chosen as a leaving group and protecting group at the anomeric center for both the glycosyl donor and acceptor (Scheme 2). This will also reduce the number of steps in the case of a combinatorial library synthesis. Thus, the disaccharide was synthesized on the basis of the armed and disarmed concept.[13] The more labile fucosyl phenylthio glycoside **10** was selectively activated in the presence of glucosamine derivative **11** using *N*-iodosuccinimide (NIS)−triflic acid (TfOH)[14] in CH_2Cl_2 at

Scheme 2 [a]



[a] Reagents and conditions: (a) NIS−TfOH, CH_2Cl_2, 3 Å MS, −20 °C, 30 min, 83.4%; (b) BH_3NMe_3–$AlCl_3$, THF, 3 Å MS, 0 °C, 4 h, then room temperature, 8 h, 80.6%; (c) $CH_3{}^{13}COOH$−DCC, DMAP, CH_2Cl_2, room temperature, 24 h, 89%.

Scheme 3 [a]



[a] Reagents and conditions: (a) $CH_3{}^{13}COOH$−DCC, DMAP, CH_2Cl_2, room temperature, 12 h, 88.9%.

−20 °C. After purification, α-linked disaccharide **12** was obtained in 83.4% yield. Ring opening of the 4,6-benzylidene group was achieved by using $BH_3{\cdot}NMe_3-AlCl_3$[15] in the presence of 3 Å molecular sieves (MS) to afford 6-benzylated compound **13** with 80.6% yield. Acetylation of the remaining hydroxyl group at the 4-position using ^{13}C-enriched acetic anhydride gave **14** (89%).

(2) Galactose Synthon. For the galactose residue, phenylthio glycoside **15** prepared according to the reported method[16] was acetylated (*1-*^{13}C) to give the glycosyl donor **16** (88.9%) (Scheme 3).

(3) Sialic Acid Synthon. The sialyl donor was synthesized according to a procedure described earlier:[17] methyl esterification of the carboxyl group with $^{13}CH_3I$ followed by acetylation (**18**, 65%) and glycosylation using methylthiotrimethylsilane [(TMS)SMe)] and trimethylsilyl triflate [(TMS)OTf)] (**19**, 62.8%) (Scheme 4).

(b) Building the Tetrasaccharide on Solid Supports. As shown in Scheme 5, the solid-bound spacer−linker **9** was glycosylated with disaccharide **14** (2 equiv) in the presence of dimethylthiomethylsulfonium triflate ($Me_2S^+SMe{\cdot}CF_3SO_3^-$, DMTST) (8 equiv)[18] in CH_2Cl_2 at 30 °C. The reaction was carried out twice to obtain an "acceptable" yield (85%), which is comparable to that obtained from solution chemistry. We did

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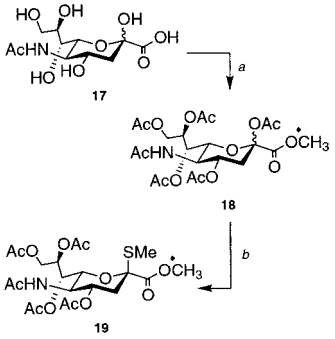
Scheme 4 [a]



[a] Reagents and conditions: (a) (1) $CsCO_3/H_2O$; (2) $^{13}CH_3I$/DMF, room temperature, 24 h; (3) $Ac_2O-Pyr-DMAP$, room temperature, 24 h, 65%; (b) TMSSMe−TMSOTf, $ClCH_2CH_2Cl$, 3 Å MS, 50 °C, 8 h, 62.8%.

not determine the anomeric configuration of the product (**20**), but we expected that the β-anomer would have been predominantly formed due to the neighboring phthalimide group. The remaining hydroxyl group was capped using *tert*-butyldimethylsilyl chloride [(TBDMS)Cl] to eliminate the truncated sequence. After deprotection of the acetyl group (**21**, quantitative), a second glycosylation reaction was performed using compound **16**. To use this solid-phase synthesis strategy to create diversity, a variety of synthetic units are required. In such a case, the structure of the glycosylating agent needs to be as simple as possible to avoid tedious preparative synthesis of individual synthetic units. On the basis of this consideration, the 2-*O*-Bn protecting group was chosen even though the desired anomer is a β-anomer. On the other hand, we expected to enhance β-selectivity by using CH_3CN as a cosolvent to enhance the β-stereoselectivity. The reaction conditions used were the same as used in the first coupling except for the temperature (0 °C) and the solvents. The glycosylation reaction yield thus obtained was 62% (**22**). After the capping and deacetylation protocols (**23**), the third coupling, the sialylation reaction, was carried out using compound **19** (**24**, 42%) in the presence of DMTST at −15 °C in CH_3CN.

(c) Cleavage and Deprotection Sequence. The resin-bound compound **24** was first treated with trimethylsilyldiazomethane and then with NaOH to give the tetrasaccharide **25** as a mixture of the combination of possible anomers (Scheme 6). The yield for the cleavage reaction was 80% on the basis of gravimetric analysis of the released products and the reaction yields estimated by the gated decoupling technique. This suggests that the accuracy of our monitoring is achieved with about 6% error in total and thus is very useful in determining the reaction conditions. The resin is glycosylated in various degrees of order since each glycosylation reaction was not quantitative, and individual compounds are given as a mixture of anomers. To determine the anomeric configurations in each glycosidic bond, we isolated all compounds formed during the solid-phase synthesis. First, tetrasaccharide **25** (17.7%) and trisaccharide **26** (25.9%) were isolated by gel permeation chromatography. Compound **25** was found to consist of four isomers based on the anomeric configurations of the Gal and Sia residues, which were isolated by preparative TLC on silica gel. It was revealed that the desired anomeric combination [β/α (Gal/Sia)] was a major component among the anomers: α/α (**25a**, 29%), α/β (**25b**, 12%), β/α (**25c**, 36%), and β/β (**25d**, 23%). In the same manner, **26** was also isolated to afford **26a** and **26b** at a ratio of 3.5:6.5 (α-Gal:β-Gal). It is, however, noted that the desired β/α linkages could be maximized if a neighboring group participation strategy was used in the synthesis. As we expected, no α-anomer for the GlcN residue was observed. The individual tetrasaccharides **25a−d** and trisaccharides **26a,b** were subjected further to dephthaloylation (ethylenediamine) followed by *N*-acetylation and hydrogenolysis to finally afford fully deprotected trisaccharides **32a,b** and tetrasaccharides **29a−d**. During this reacetylation reaction of the tetrasaccharides, we observed intramolecular dehydrative lactonization reactions using TLC and MALDI-TOF mass analyses. Although we did not fully characterize the structure of the products, these compounds were transformed back into compounds **28a−d** after saponification.

In summary, we have developed a nondestructive and quantitative monitoring method for solid-phase oligosaccharide synthesis by using two ^{13}C-enriched markers as an internal standard and protecting group of the growing sugar unit. Though the subsequent chromatographical purification has to be performed since the stereochemistry of each glycosidic linkage is not fully controlled, it should be realized that this process is necessary in any case because of the mechanism of the glycosylation reaction. Overall, there is only one protecting group manipulation (i.e., the acetyl group) in the iterative process, a strategy similar to that used in solid-phase synthesis of peptides and oligonucleotides, and if necessary, the acetyl group can be labeled for on-resin monitoring as illustrated. Compared to the solid-phase synthesis of peptides and nucleotides, however, a much greater number of carbohydrate building blocks would be necessary to create true molecular diversity. The problem of protecting group manipulation on resin is, however, inevitable in any stepwise solid-phase synthesis. To minimize the number of building blocks and completely eliminate protecting group manipulation during the iterative process, the programmable one-pot oligosaccharide synthesis strategy[19] and the orthogonal glycosylation method[2g] are alternatives.

Experimental Section

General Methods. TentaGel S-NH_2 resin was purchased from Fluka. Dried solvents were used for all reactions. Solutions were evaporated under reduced pressure at a bath temperature not exceeding 50 °C. Analytical TLC was performed on Merck Art. 5715, Kieselgel 60 F_{254}/0.25 mm thickness plates. Visualization was accomplished with UV light and phosphomolybdic acid and/or sulfuric acid solution followed by heating. Column chromatography was performed with silica gel FL-100D (Fuji Davison Co.). Optical rotations were measured on a Horiba SEPA-200 polarimeter with a sodium lamp (λ = 589 nm). ^1H NMR (270 MHz) and ^{13}C NMR (67.5 MHz) spectra were recorded with a JEOL EX-270 spectrometer in deuterated solvents using tetramethylsilane, $CHCl_3$, or DMSO as an internal standard. The MALDI-TOF mass spectra were recorded on a Perceptive Voyager mass spectrometer with 2,5-dihydroxybenzoic acid as a matrix. High-resolution mass spectra were recorded on a JEOL JMS-700 spectrometer under FAB conditions.

General Methods for the Monitoring of Resin-Bound Compounds with Inverse Gated Decoupling ^{13}C NMR Measurement. The dried

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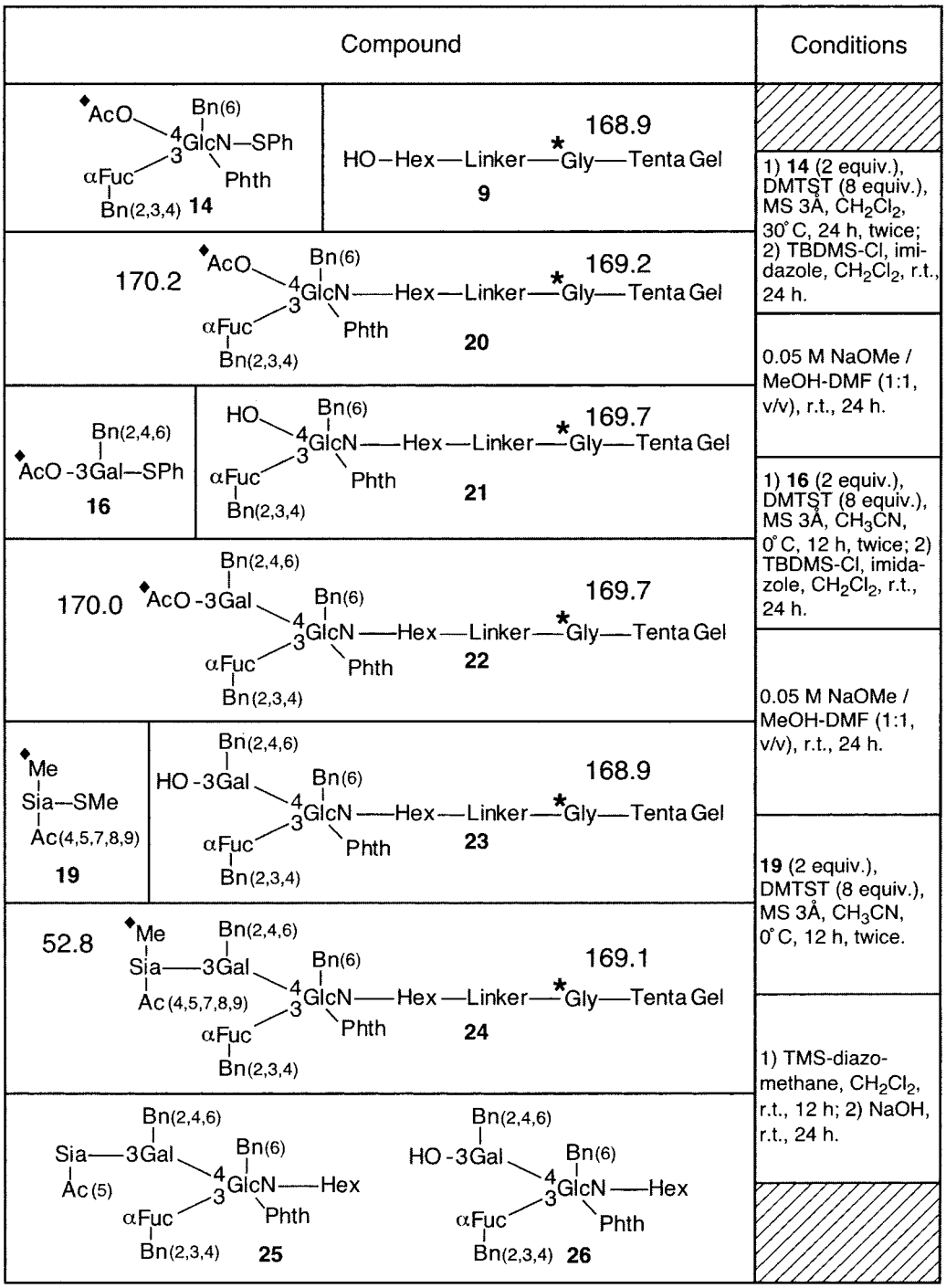
(19) Zhang,; Ollman, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734−753.

Scheme 5 [a]

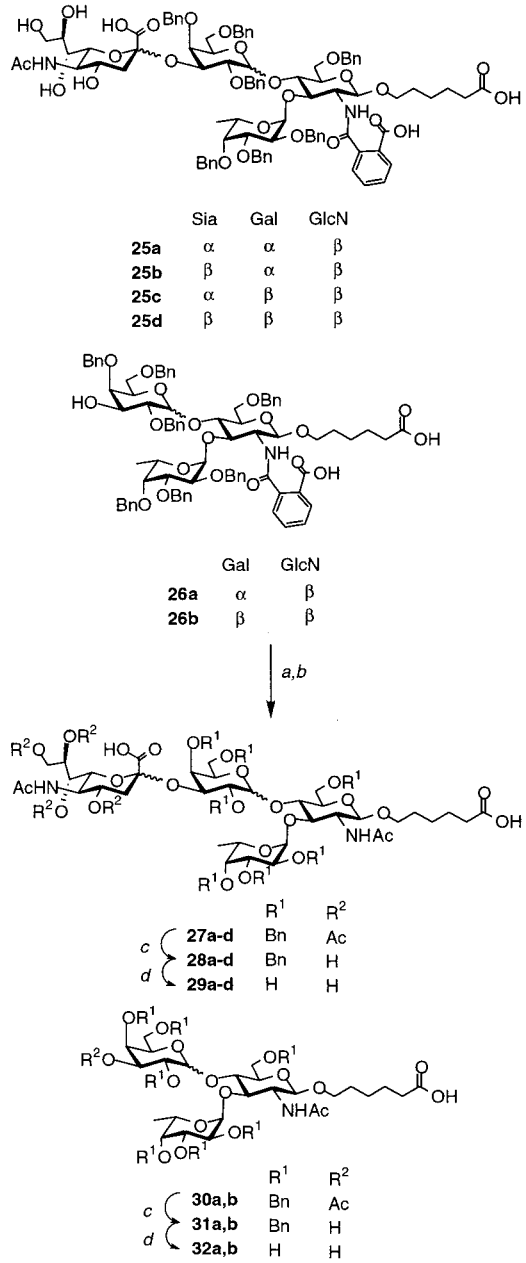


[a] The positions of each protecting group are shown in parentheses. The stereochemistry is indicated where it is fixed. The chemical shifts of ^{13}C-enriched atoms are shown. GlcN = glucosamine, Hex = 6-hydroxyhexanoic acid, Linker = sulfonamide linker, Fuc = fucose, Gal = galactose, and Sia = sialic acid.

resin (ca. 60 mg) was slurried in $CDCl_3$, and the sample was prepared to contain the relaxation agent chromium(III) 2,4-pentanedionate [Cr(AcAc)$_3$; 0.1 M] in ordinary 5 mm ∅ NMR tubes. ^{13}C NMR was measured using a JEOL EX-270 spectrometer at 67.5 MHz operated with a 9 s relaxation delay and gated decoupling without NOE (160 transients, 0.6 s acquisition time). The spectra were referred to the resonance for TMS. ^{13}C NMR spin−lattice relaxation times (T_1) were measured by using the inversion recovery method at 298 K (16 data points, 16 scans per point). T_1 values for methyl and carbonyl groups attached to the resin were first examined and were shorter than 1 s in the presence of Cr(AcAc)$_3$, whereas the T_1 values in the absence of the relaxation agent were 12 and 29 s, respectively.

Solution-Phase Chemistry. (a) 6-Hydroxyhexanoic Acid. A solution of ϵ-caprolactone (10.0 g, 69.4 mmol) in 0.5 N NaOH (200 mL) was stirred at room temperature for 12 h. The mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH/AcOH (20:1:0.5) as an eluent to provide 6-hydroxyhexanoic acid (11.3 g, 98%) as a colorless oil: R_f 0.45 (CH_2Cl_2/MeOH/AcOH, 10:1:0.5); ^{1}H NMR ($CDCl_3$) δ 4.24 (t, 2 H, J = 4.6 Hz), 2.67−2.62 (m, 2 H), 1.91−1.71 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 176.31 (C=O), 69.34, 34.56, 29.31, 28.93, 22.97; MALDI-TOF MS m/z calcd for $C_6H_{12}O_3$ 132, found 155 $(M + Na)^+$. Anal. Calcd for $C_6H_{12}O_3$: C, 54.53; H, 9.15. Found: C, 54.37; H, 9.11.

Scheme 6 [a]



[a] Reagents and conditions: (a) ethylenediamine−*n*-BuOH; (b) Ac_2O−Pyr; (c) NaOMe; (d) H_2 on $Pd(OH)_2$/MeOH−H_2O−AcOH.

(b) 6-(Acetyl-*1*-^{13}*C*)oxyhexanoic Acid (1). To a solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC; 178 mg, 0.86 mmol) in dry Et_2O (3.0 mL) was added acetic-*1*-^{13}*C* acid (100 μL, 1.72 mmol) at room temperature for 2 h. The mixture was filtered to remove urea. A solution containing 6-hydroxyhexanoic acid (230 mg, 1.74 mmol) and dimethylaminopyridine (DMAP; 21 mg, 0.17 mmol) in pyridine (2.0 mL) was added to the filtrate at room temperature, and the mixture was stirred for 3 h. The mixture was filtered, and the filtrate was diluted with EtOAc, washed with brine (3 times), dried with Na_2SO_4, filtered, and evaporated. The residue was purified on silica gel column chromatography using CH_2Cl_2/MeOH/AcOH (50:0.2:0.1) as an eluent to provide **1** (145 mg, 96%) as a colorless oil: R_f 0.65 (CH_2Cl_2/MeOH/AcOH, 20:0.5:0.25); ^{1}H NMR ($CDCl_3$) δ 4.10−4.04 (m, 2 H), 2.37 (t, 2 H, J = 7.3 Hz), 2.05 (d, 3H J = 6.9 Hz, Ac), 1.73−1.60 (m, 4 H), 1.47−1.36 (m, 2 H); HR-FAB MS *m*/*z* calcd for $C_7{}^{13}CH_{14}O_4$ (M) 175.0926, found 174.0868 (M − H)$^-$.

(c) [6-(Acetyl-*1*-^{13}*C*)oxyhexanoyl]amino-4-sulfonylbenzoic Acid (5). To a solution of DCC (1.46 g, 7.08 mmol) in dry CH_2Cl_2 (10 mL) was added compound **1** (2.46 g, 14.1 mmol) at room temperature. The mixture was stirred for 1 h and then filtered to remove urea. The filtrate was added to a solution containing 4-sulfamoylbenzoic acid (**2**; 1.40 g, 6.96 mmol; Tokyo Kasei, Japan) and DMAP (170 mg, 1.39 mmol) in DMF (15 mL) at room temperature. The mixture was then stirred for 4 h, diluted with EtOAc, washed with brine (3 times), dried with Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel column chromatograph using CH_2Cl_2/MeOH/AcOH (30:0.3:0.2) as an eluent to provide **5** (2.06 g, 83%) as a white solid: R_f 0.34 (CH_2Cl_2/MeOH/AcOH, 20:0.5:0.25); ^{1}H NMR ($CDCl_3$) δ 8.21−8.08 (m, 4 H, Ar), 3.98 (dt, J = 6.4, 3.0 Hz, 2 H), 2.25 (t, 2 H, J = 7.3 Hz), 1.99 (d, 3H J = 6.6 Hz, Ac), 1.58−1.49 (m, 4 H), 1.32−1.23 (m, 2 H); MALDI-TOF MS *m*/*z* calcd for $C_{14}{}^{13}CH_{19}NO_7S$ (M) 358, found 381 (M + Na)$^+$. Anal. Calcd for $C_{14}{}^{13}CH_{19}NO_7S$: C, 50.55; H, 5.34; N, 3.91. Found: C, 50.71; H, 5.41; N, 3.88.

(d) Phenyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-D-glucopyranoside (12). A mixture of phenyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (10, 4.14 g, 7.86 mmol), phenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-*β*-D-glucopyranoside (11, 2.40 g, 4.90 mmol), and 3 Å molecular sieves (3.5 g) in dry CH_2Cl_2 (30 mL) was stirred at room temperature for 7 h. To the stirred mixture were added *N*-iodosuccinimide (1.77 g, 7.87 mmol) and a solution of trifluoromethanesulfonic acid (400 mg 2.67 mmol) in CH_2Cl_2 (5 mL) at −60 °C. The mixture was stirred under a nitrogen atmosphere at −20 °C for 30 min. The reaction mixture was then filtered, and the filtrate was diluted with CH_2Cl_2, washed with 5% aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$, and water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel column using toluene/ethyl acetate (20:1) as an eluent to provide **12** (3.69 g, 83.4%): ^{1}H NMR ($CDCl_3$) δ 7.77−7.02 (m, 29 H, Ar), 5.75 (d, 1 H, $J_{1,2}$ = 10.7 Hz, H-1 of Glc), 5.55 (s, 1 H, CH of benzylidene), 4.81 (d, 1 H, $J_{1,2}$ = 2.3 Hz, H-1 of Fuc), 4.04 (q, 1 H, $J_{5,6}$ = 6.6 Hz, H-5 of Fuc), 0.87 (d, 3 H, H-6 of Fuc); ^{13}C NMR ($CDCl_3$) δ 168.4, 167.5, 138.8, 138.5, 138.2, 137.1, 133.9, 132.7, 131.9, 128.9, 128.7, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 127.6, 127.5, 127.3, 126.0, 123.2, 101.0, 99.5, 84.2, 81.8, 79.6, 77.9, 75.5, 74.7, 73.0, 72.7, 70.5, 68.5, 67.3, 54.6, 16.4; MALDI-TOF MS *m*/*z* calcd for $C_{54}H_{51}NO_{10}S$ (M) 905, found 928 (M + Na)$^+$. Anal. Calcd for $C_{54}H_{51}NO_{10}S$: C, 71.58; H, 5.67; N, 1.55. Found: C, 71.50; H, 5.61; N, 1.52.

(e) Phenyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-*β*-D-glucopyranoside (13). A mixture of compound **12** (3.60 g, 3.97 mmol) and 3 Å molecular sieves (2.0 g) in dry tetrahydrofuran (20 mL) was stirred at room temperature for 12 h. To the stirred mixture was added borane·trimethylamine (0.90 g, 12.3 mmol) at room temperature, and then aluminum chloride (1.65 g, 12.4 mmol) was added at 0 °C. The reaction temperature was allowed to reach room temperature over 8 h. The reaction mixture was filtered, and the filtrate was diluted with CH_2Cl_2, washed with brine, aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography with toluene/ethyl acetate (10:1) to provide **13** (2.90 g, 80.6%): ^{1}H NMR ($CDCl_3$) δ 7.86−6.93 (m, 29 H, Ar), 5.71 (d, 1 H, $J_{1,2}$ = 10.2 Hz, H-1 of Glc), 4.62 (d, 1 H, $J_{1,2}$ = 2.3 Hz, H-1 of Fuc), 4.34 (t, 1 H, $J_{2,3}$ = 10.2 Hz, H-2 of Glc), 4.19 (d, 1 H, H-3 of Glc), 4.05 (q, 1 H, $J_{5,6}$ = 6.3 Hz, H-5 of Fuc), 3.92 (d, 1 H, $J_{6,6'}$ = 9.9 Hz, H-6 of Glc), 3.81−3.66 (m, 4 H, H-5,6′ of Glc and H-2,3 of Fuc), 3.55 (t, 1 H, $J_{4,5}$ = 8.6 Hz, H-4 of Glc), 3.48 (s, 1 H, H-4 of Fuc), 1.05 (d, 3 H, H-6 of Fuc); ^{13}CNMR ($CDCl_3$) δ 168.6, 167.7, 100.8, 84.5, 83.2, 79.4, 78.9, 77.8, 74.7, 73.8, 73.4, 72.3, 71.0, 69.4, 68.5, 53.5, 16.4; HR-FAB MS *m*/*z* calcd for $C_{54}H_{53}NO_{10}S$ (M) 907.3390, found 1013.4253 [M + $(HOCH_2CH_2)_2$-NH + H]$^+$.

(f) Phenyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-4-*O*-(acetyl-1-^{13}C)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-*β*-D-glucopyranoside (14). To a solution of compound **13** (910 mg, 1.00 mmol)

were added DCC (425 mg, 2.06 mmol) and DMAP (20 mg, 0.16 mmol) in dry CH_2Cl_2 (10 mL), and acetic-*1*-^{13}C acid (100 mL, 1.72 mmol), at room temperature, and the reaction mixture was stirred for 24 h. The reaction mixture was filtered, and the filtrate was diluted with CH_2Cl_2, washed with brine (3 times), dried over Na_2SO_4, filtered, and concentrated. The residue was purified using a silica gel column eluted with toluene/ethyl acetate (20:1) to provide **14** (849 mg, 89.0%): ^{1}H NMR ($CDCl_3$) δ 7.83−6.99 (m, 29 H, Ar), 5.77 (d, 1 H, $J_{1,2}$ = 9.9 Hz, H-1 of Glc), 4.94 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 8.4 Hz, H-3 of Glc), 4.51 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H-1 of Fuc), 4.35 (t, 1 H, H-2 of Glc), 3.90−3.77 (m, 2 H, H-4 of Glc and H-5 of Fuc), 3.68 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 2.3 Hz, H-3 of Fuc), 3.62−3.45 (m, 4 H, H-5,6 of Glc and H-2 of Fuc), 3.41 (s, 1 H, H-4 of Fuc), 1.89 (d, 3H J = 6.9 Hz, Ac), 0.94 (d, 3 H, $J_{5,6}$ = 6.6 Hz, H-6 of Fuc); HR-FAB MS *m*/*z* calcd for $C_{55}{}^{13}CH_{55}NO_{11}S$ (M) 950.3529, found 1056.4390 [M + $(HOCH_2CH_2)_2NH$ + H]$^+$.

(g) Phenyl 3-*O*-(Acetyl-*1*-^{13}C)-2,4,6-tri-*O*-benzyl-1-thio-*β*-D-galactopyranoside (16). To a solution of phenyl 2,4,6-tri-*O*-benzyl-1-thio-*β*-D-galactopyranoside (**15**; 285 mg, 0.525 mmol) were added DCC (250 mg, 1.21 mmol) and DMAP (10 mg, 0.08 mmol) in dry CH_2Cl_2 (5 mL), and acetic-*1*-^{13}C acid (30 mL, 0.52 mmol), at room temperature, and the mixture was stirred for 12 h. The reaction mixture was filtered, and the filtrate was diluted with CH_2Cl_2, washed with brine (3 times), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography using toluene/ethyl acetate (20:1) to provide **16** (274 mg, 88.9%): ^{1}H NMR ($CDCl_3$) δ 7.58−7.21 (m, 20 H, Ar), 4.96 (dd, 1 H, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.6 Hz, H-3), 4.83 (d, 1 H, $J_{1,2}$ = 11.2 Hz, H-1), 4.71−4.40 (m, 6 H, 3 · Bn-CH_2), 4.03 (d, 1 H, $J_{5,6}$ = 2.6 Hz, H-5), 3.94 (t, 1 H, H-4), 3.77−3.63 (m, 3 H, H-2, 6), 1.88 (d, 3H J = 6.9 Hz, Ac); ^{13}C NMR ($CDCl_3$) δ 170.2 (^{13}C-enriched), 87.7, 75.4, 75.3, 74.9, 74.6, 73.5, 68.2, 20.9; HR-FAB MS *m*/*z* calcd for $C_{21}{}^{13}CH_{31}NO_{14}$ (M) 534.1778, found 557.1640 [M + Na]$^+$.

(h) Methyl-*1*-^{13}C 5-Acetoamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosylonate (18). Anhydrous cesium carbonate (632 mg, 1.94 mmol) was added to a solution of 5-acetoamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (**17**; 1.20 g, 3.88 mmol) in water (6 mL) at room temperature, and the solution was evaporated to dryness. The amorphous residue was suspended in DMF (6 mL), and iodomethane-^{13}C (559 mg, 3.91 mmol) was added. The mixture was stirred for 24 h at room temperature, and then pyridine (6 mL), acetic anhydride (2 mL), and DMAP (48 mg, 0.39 mmol) were added at 0 °C. The mixture was stirred for 24 h at room temperature, poured into 0.5 N HCl, and extracted with ethyl acetate. The combined extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (50:1) as an eluent to provide **18** (1.35 g, 65.0%): ^{1}H NMR ($CDCl_3$) δ 5.68 (br d, 1 H, NH), 5.40−5.39 (m, 1 H, H-7), 5.30−5.21 (m, 1 H, H-4), 5.09−5.04 (m, 1 H, H-8), 4.52 (dd, 1 H, $J_{8,9}$ = 2.5 Hz, $J_{9,9'}$ = 12.4 Hz, H-9), 4.17−4.07 (m, 3 H, H-5,6,9′), 3.52 (s, 3 H, Me), 2.55 (dd, 1 H, $J_{3_{ax},3_{eq}}$ = 13.2 Hz, $J_{3_{eq},4}$ = 5.0 Hz, H-3$_{eq}$), 2.15, 2.07, 2.04, 1.89 (each s, 18 H, 6 × Ac); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.6, 170.3 × 2, 168.3, 166.4, 97.5, 72.9, 71.5, 68.4, 67.8, 62.2, 53.2 (^{13}C-enriched), 49.2, 35.9, 23.1, 20.9 × 2, 20.8, 20.7; HR-FAB MS *m*/*z* calcd for $C_{54}H_{53}NO_{10}S$ (M) 907.3390, found 1013.4253 [M + $(HOCH_2CH_2)_2NH$ + H]$^+$.

(i) Methyl-*1*-^{13}C 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-methylthio-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosylonate (19). A mixture of compound **18** (600 mg, 1.12 mmol) and 3 Å molecular sieves (300 mg) in $ClCH_2CH_2Cl$ (10 mL) was stirred at room temperature for 4 h. Methylthiotrimethylsilane (400 mg, 3.33 mmol) and trimethylsilyl trifluoromethanesulfonate (200 mg, 0.90 mmol) were added to the stirred mixture at room temperature, and the mixture was stirred at 50 °C for 8 h. The reaction mixture was filtered, and the filtrate was diluted with $CHCl_3$, washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (30:1) as an eluent to provide **19** (360 mg, 62.8%): ^{1}H NMR ($CDCl_3$) δ 5.45 (t, 1 H, $J_{7,8}$ = 2.8 Hz, H-7), 5.27 (td, 1 H, $J_{3_{eq},4}$ = 5.0 Hz, $J_{4,5}$ = 10.9 Hz, H-4), 5.16 (dt, 1 H, $J_{8,9'}$ = 8.6 Hz, H-8), 4.81 (dd, 1 H, $J_{8,9}$ = 2.5 Hz, $J_{9,9'}$ = 12.4 Hz, H-9), 4.31 (dd, 1 H, $J_{5,6}$ = 2.3 Hz, H-5), 4.17 (dd, 1 H, H-9′), 4.11−4.07 (m, 1 H, H-6), 3.54 (s, 3 H, Me), 2.54 (dd, 1 H, $J_{3_{ax},3_{eq}}$ = 13.9 Hz, H-3$_{eq}$), 2.14, 2.09, 2.04, 2.03, 1.89 (each s, 21 H, 6 × Ac and SMe); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.9, 170.6, 170.3, 170.2, 167.9, 84.7, 72.5, 72.1, 69.3, 68.5, 62.4, 52.9 (^{13}C-enriched), 49.5, 36.9, 23.2, 21.0, 20.9, 20.8, 11.3; HR-FAB MS *m*/*z* calcd for $C_{20}{}^{13}CH_{31}NO_{12}S$ (M) 522.1601, found 545.1512 (M + Na)$^+$.

Solid-Phase Synthesis. (a) Resin 6. To a suspension of TentaGel amine resin (**4**; loading 0.26 mmol/g, 1.58 g; NovaSyn TG amino resin, Switzerland) and Fmoc-glycine-*1*-^{13}C (**3**; prepared by the reaction of glycine-*1*-^{13}C and 9-fluorenylmethyl succinimidyl carbonate in the presence of $NaHCO_3$ in aqueous 1,2-dimethoxyethane; 224 mg, 0.75 mmol) in dry CH_2Cl_2 (12 mL) were added *N*,*N*′-diisopropylcarbodiimide (DIPC; 160 mg, 1.27 mmol) and 1-hydroxybenzotriazole (HOBt; 110 mg, 0.81 mmol) at room temperature. After the mixture was stirred for 4 h, the resins were washed with CH_2Cl_2, MeOH, H_2O, DMF, and CH_2Cl_2 and then dried in vacuo to obtain glycine-attached resin **6** (1.69 g, quantitative). Data for compound **3**: R_f 0.58 (CH_2Cl_2/MeOH/AcOH, 10:1:0.5); ^{1}H NMR ($CDCl_3$) δ 7.76−7.27 (Fmoc aromatic protons, 8H), 5.25 (NH), 4.44 (d, 2H), 4.22 (t, 1H), 4.06 (t, 1H). The coupling yield was estimated to be quantitative according to the weight gain of the resin: ^{13}C NMR ($CDCl_3$) δ 169.0.

(b) Resin 7. Resin **6** (1.69 g) was treated with 20% piperidine in DMF at room temperature for 4 h, washed with DMF, H_2O, MeOH, and CH_2Cl_2, and then dried in vacuo to obtain resin **7** (1.60 g, quant.). The absence of a ^{13}C signal at δ 169.0 (Fmoc-Gly-resin) indicated reaction completion: ^{13}C NMR ($CDCl_3$) δ 172.7.

(c) Resin 8. To a suspension of resin **7** (1.60 g) and compound **5** (427 mg, 1.19 mmol) in dry DMF (12 mL) were added DIPC (155 mg, 1.23 mmol) and HOBt (165 mg, 1.22 mmol) at room temperature. After the mixture was stirred for 4 h, the resins were washed with DMF, H_2O, MeOH, and $CHCl_3$ and then dried in vacuo to obtain sulfonamide-linker-attached resin **8** (1.73 g, 97%). The yield was estimated by the integral obtained from inverse gated decoupling relative to the internal ^{13}C marker at 168.8 ppm: ^{13}C NMR ($CDCl_3$) δ 170.5, 168.8.

Deacetylation of Solid-Phase Compounds. The ^{13}C-enriched acetyl group protected resin was treated with 0.025 M NaOMe in MeOH−DMF (1:1, v/v) at room temperature for 8 h, washed with DMF, H_2O, DMF−AcOH, MeOH, and CH_2Cl_2, and then dried in vacuo to obtain the acetyl group deprotected resin. The deprotection was confirmed by disappearance of the carbonyl signal of the acetyl group in the ^{13}C NMR spectra.

Glycosylation Reaction on the Solid Phase. A mixture of resin acceptor, glycosyl donor (2 equiv), and 3 Å molecular sieves [0.5 g/(g of resin)] in dry CH_2Cl_2 or CH_3CN [20 mL/(g of resin)] was stirred at room temperature for 12 h. To the stirred mixture was added DMTST (8 equiv). The mixture was stirred under a nitrogen atmosphere at the designated temperature for 24 h. The resins were washed with CH_2Cl_2, MeOH, H_2O, DMF, and CH_2Cl_2, and this procedure was repeated twice. The resin was then suspended in CH_2Cl_2, *t*-$BuMe_2SiCl$ and imidazole were added at room temperature, and the mixture was stirred for 24 h. The resin was washed with CH_2Cl_2, MeOH, water, DMF, and CH_2Cl_2. The reaction yield was calculated by the integral of the signals obtained by ^{13}C NMR. The chemical shifts of individual carbons are shown in Scheme 5.

Cleavage of Glycosyl Compounds from the Solid Phase. Trimethylsilyldiazomethane (2.0 M solution in hexane, 0.6 mL) was added to a suspension of the resin **24** (510 mg) in dry THF (8 mL). The mixture was stirred under a nitrogen atmosphere for 12 h. The resin was washed with THF, DMF, and THF and suspended in THF (8 mL). Aqueous sodium hydroxide solution (1 N, 0.8 mL) was added to the suspension and the mixture stirred at room temperature for 12 h. The

resin was washed with THF, MeOH, and DMF. The filtrate was neutralized by Amberlite IR-120 (H^+), filtered, and concentrated. The residue was passed through an S-X1 gel permeation column with toluene/ethyl acetate (3:1) as an eluent to separate the trisaccharide (36 mg) and tetrasaccharide (30 mg) fractions. The trisaccharide fraction was then purified by silica gel column chromatography using CH_2Cl_2/MeOH/AcOH (40:1:0.5) as an eluent to provide **26a** (12.6 mg) and **26b** (23.4 mg). The tetrasaccharide fraction was also purified by silica gel column chromatography using CH_2Cl_2/MeOH/H_2O (15:5:0.8) as an eluent to provide **25a** (8.8 mg), **25b** (3.6 mg), **25c** (10.9 mg), and **25d** (6.9 mg). Data for **25a**: R_f 0.31 (CH_2Cl_2/MeOH/H_2O, 15:5:0.8); $[\alpha]_D$ −18.9° (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD) δ 7.97−7.09 (m, 39 H), 5.49 (d, 1 H, J = 3.0 Hz), 5.05 (d, 1 H, J = 3.3 Hz), 4.99−4.24 (m, 22 H), 4.07−3.29 (m, 18 H), 2.72 (dd, 1 H, J = 13.5, 3.6 Hz), 2.20 (t, 2 H, J = 7.4 Hz), 1.95 (s, 3 H), 1.85 (t, 1 H, J = 13.5 Hz), 1.63−1.52 (m, 4 H), 1.42−1.31 (m, 2 H), 1.02 (d, 3 H, J = 6.3); MALDI-TOF MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ 1670, found 1693 (M + Na)$^+$. Data for **25b**: R_f 0.38 (CH_2Cl_2/MeOH/H_2O, 15:5:0.8); $[\alpha]_D$ −10.5° (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD) δ 7.97−7.12 (m, 39 H), 5.49 (d, 1 H, J = 3.0 Hz), 5.01 (d, 1 H, J = 2.9 Hz), 4.88−4.25 (m, 22 H), 4.10−3.30 (m, 18 H), 2.90 (dd, 1 H, J = 14.0, 3.0 Hz), 2.23 (t, 2 H, J = 7.8 Hz), 1.96 (s, 3 H), 1.80 (t, 1 H, J = 14.0 Hz), 1.65−1.50 (m, 4 H), 1.44−1.31 (m, 2 H), 0.93 (d, 3 H, J = 5.9); MALDI-TOF MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ 1670, found 1693 (M + Na)$^+$; FAB MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ (M) 1670.7, found 1693.7 (M + Na)$^+$. Data for **25c**: R_f 0.30 (CH_2Cl_2/MeOH/H_2O, 15:5:0.8); $[\alpha]_D$ −52.8° (*c* 0.4, MeOH); ^{1}H NMR (CD_3OD) δ 7.83−7.00 (m, 39 H), 5.57 (d, 1 H, J = 3.3 Hz), 5.18−4.29 (m, 23 H), 4.04−3.27 (m, 18 H), 2.90 (dd, 1 H, J = 13.2, 5.0 Hz), 2.20 (t, 2 H, J = 7.3 Hz), 2.02 (s, 3 H), 1.85 (t, 1 H, J = 13.2 Hz), 1.59−1.52 (m, 4 H), 1.37−1.29 (m, 2 H), 0.99 (d, 3 H, J = 6.3); MALDI-TOF MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ 1670, found 1693 (M + Na)$^+$. Data for **25d**: R_f 0.34 (CH_2Cl_2/MeOH/H_2O, 15:5:0.8); $[\alpha]_D$ −38.9° (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD) δ 7.84−7.00 (m, 39 H), 5.57 (d, 1 H, J = 2.9 Hz), 5.08−4.23 (m, 23 H), 4.09−3.27 (m, 18 H), 2.78 (dd, 1 H, J = 13.2, 3.4 Hz), 2.19 (t, 2 H, J = 7.3 Hz), 1.93 (s, 3 H), 1.83 (t, 1 H, J = 13.2 Hz), 1.56−1.52 (m, 4 H), 1.40−1.28 (m, 2 H), 1.05 (d, 3 H, J = 5.9); MALDI-TOF MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ 1670, found 1693 (M + Na)$^+$; HR-FAB MS *m*/*z* calcd for $C_{92}H_{106}N_2O_{27}$ (M) 1670.6983, found 1708.6593 (M + K − H)$^+$. Data for **26a**: R_f 0.15 (CH_2Cl_2/MeOH/AcOH, 40:1:0.5); $[\alpha]_D$ −20.1° (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD) δ 7.79−7.13 (m, 39 H), 5.39 (d, 1 H, J = 3.0 Hz), 5.17 (d, 1 H, J = 3.3 Hz), 4.85−4.14 (m, 17 H), 4.04−3.29 (m, 16 H), 2.15 (t, 2 H, J = 7.4 Hz), 1.51 (m, 4 H), 1.30 (m, 2 H), 1.05 (d, 3 H, J = 6.3); ^{13}C NMR (CD_3OD) δ 177.5, 171.8, 170.1, 140.2, 140.1, 140.0, 139.7, 139.6, 139.5, 139.4, 139.1, 132.0, 129.8, 129.7, 129.6, 129.5, 129.5, 129.4, 129.4, 129.3, 129.3, 129.2, 129.1, 129.0, 129.0, 128.9, 128.9, 128.7, 128.7, 128.6, 128.6, 128.5, 128.2, 102.2, 98.3, 97.9, 79.6, 79.3, 79.1, 78.2, 77.1, 77.0, 76.7, 76.3, 76.2, 74.8, 74.3, 74.1, 74.0, 73.9, 73.4, 72.0, 71.4, 70.1, 70.0, 68.6, 54.0, 34.9, 30.3, 26.7, 25.8, 17.2; MALDI-TOF MS *m*/*z* calcd for $C_{81}H_{89}NO_{19}$ 1379, found 1402 (M + Na)$^+$; HR-FAB MS *m*/*z* calcd for $C_{81}H_{89}NO_{19}$ (M) 1379.6029, found 1402.5927 (M + Na)$^+$.

Data for **26b**: R_f 0.25 (CH_2Cl_2/MeOH/AcOH, 40:1:0.5); $[\alpha]_D$ −68.9° (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.77−6.91 (m, 39 H), 5.51 (d, 1 H, J = 3.3 Hz), 4.88−4.22 (m, 18 H), 4.02−3.14 (m, 16 H), 2.10 (t, 2 H, J = 7.4 Hz), 1.48−1.40 (m, 4 H), 1.32−1.23 (m, 2 H), 0.98 (d, 3 H, J = 6.3); ^{13}C NMR (CD_3OD) δ 177.6, 172.2, 170.0, 140.7, 140.4, 140.3, 140.2, 139.7, 139.6, 138.8, 132.5, 132.4, 131.2, 131.1, 130.0, 129.6, 129.5, 129.4, 129.4, 129.3, 129.3, 129.1, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 103.7, 101.3, 97.2, 81.8, 80.2, 78.7, 77.2, 76.6, 76.3, 76.2, 75.7, 75.4, 75.3, 74.3, 74.0, 73.5, 73.1, 70.5, 69.4, 67.1, 60.3, 34.9, 30.6, 26.8, 25.9, 17.2; MALDI-TOF MS *m*/*z* calcd for $C_{81}H_{89}NO_{19}$ 1379, found 1402 (M + Na)$^+$; LR-FAB MS *m*/*z* calcd for $C_{81}H_{89}NO_{19}$ (M) 1379.6, found 1423.3 (M + 2Na − H)$^+$.

Typical Procedure for the *N*-Acetylation Reaction. A solution of compound **26b** (30 mg, 0.022 mmol) in 1-butanol−ethylenediamine monohydrate (4:1, v/v; 5 mL) was stirred at 100 °C for 16 h. The reaction mixture was concentrated in vacuo. The residue was treated with pyridine (3 mL) and Ac_2O (1.5 mL) at room temperature for 24 h. The reaction mixture was concentrated in vacuo. The residue was then treated with 0.05 M NaOMe in MeOH (2 mL) at room temperature for 3 h, neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/AcOH (40:1:0.5) as an eluent to provide **31b** (20.0 mg 73%). Data for **28a**: R_f 0.22 (CH_2Cl_2/MeOH/H_2O, 8:2:0.3); ^{1}H NMR (CD_3OD) δ 7.51−7.13 (m, 35 H), 5.20 (d, 1 H, J = 3.1 Hz), 5.12 (d, 1 H, J = 3.0 Hz), 5.01−4.28 (m, 22 H), 3.98−3.24 (m, 18 H), 2.83 (dd, 1 H, J = 13.3, 4.9 Hz), 2.13 (t, 2 H, J = 7.0 Hz), 2.10 (s, 3 H), 1.98 (s, 3 H), 1.81 (t, 1 H, J = 13.3 Hz), 1.57−1.52 (m, 4 H), 1.34−1.25 (m, 2 H), 1.02 (d, 3 H, J = 6.0); MALDI-TOF MS *m*/*z* calcd for $C_{86}H_{104}N_2O_{25}$ (M) 1564, found 1587 (M + Na)$^+$. Data for **28b**: R_f 0.24 (CH_2Cl_2/MeOH/H_2O, 8:2:0.3); ^{1}H NMR (CD_3OD) δ 7.58−7.11 (m, 35 H), 5.18 (d, 1 H, J = 3.1 Hz), 5.00 (d, 1 H, J = 3.0 Hz), 4.95−4.20 (m, 22 H), 3.98−3.28 (m, 18 H), 2.75 (dd, 1 H, J = 12.7, 4.8 Hz), 2.11 (t, 2 H, J = 7.3 Hz), 2.10 (s, 3 H), 1.96 (s, 3 H), 1.79 (t, 1 H, J = 12.7 Hz), 1.57−1.51 (m, 4 H), 1.38−1.26 (m, 2 H), 0.96 (d, 3 H, J = 6.6); MALDI-TOF MS *m*/*z* calcd for $C_{88}H_{104}N_2O_{25}$ 1564, found 1587 (M + Na)$^+$. Data for **28c**: R_f 0.21 (CH_2Cl_2/MeOH/H_2O, 8:2:0.3); ^{1}H NMR (CD_3OD) δ 7.53−7.11 (m, 35 H), 5.17 (d, 1 H, J = 3.3 Hz), 5.14−4.28 (m, 23 H), 4.03−3.24 (m, 18 H), 2.91 (dd, 1 H, J = 13.0, 4.8 Hz), 2.14 (t, 2 H, J = 7.3 Hz), 2.02 (s, 3 H), 1.93 (s, 3 H), 1.85 (t, 1 H, J = 13.0 Hz), 1.58−1.56 (m, 4 H), 1.34−1.28 (m, 2 H), 0.98 (d, 3 H, J = 6.3); MALDI-TOF MS *m*/*z* calcd for $C_{86}H_{104}N_2O_{25}$ (M) 1564, found 1587 (M + Na)$^+$; FAB MS *m*/*z* calcd for $C_{86}H_{104}N_2O_{25}$ (M) 1564.7, found 1585.6 (M + Na − 2H)$^-$. Data for **28d**: R_f 0.22 (CH_2Cl_2/MeOH/H_2O, 8:2:0.3); ^{1}H NMR (CD_3OD) δ 7.51−7.13 (m, 35 H), 5.02 (d, 1 H, J = 3.0 Hz), 5.00−4.28 (m, 23 H), 4.00−3.24 (m, 18 H), 2.85 (dd, 1 H, J = 13.3, 5.2 Hz), 2.13 (t, 2 H, J = 7.3 Hz), 2.03 (s, 3 H), 1.91 (s, 3 H), 1.82 (t, 1 H, J = 13.3 Hz), 1.58−1.53 (m, 4 H), 1.34−1.25 (m, 2 H), 0.97 (d, 3 H, J = 6.3); MALDI-TOF MS *m*/*z* calcd for $C_{86}H_{104}N_2O_{25}$ (M) 1564, found 1587 (M + Na)$^+$. Data for **31a**: R_f 0.43 (CH_2Cl_2/MeOH/AcOH, 30:1:0.5); $[\alpha]_D$ +12.0° (*c* 1.0, MeOH); ^{1}H NMR (CD_3OD) δ 7.38−7.22 (m, 35 H), 5.25 (d, 1 H, J = 3.3 Hz), 5.20 (d, 1 H, J = 3.6 Hz), 4.93−4.00 (m, 18 H), 3.89−3.29 (m, 16 H), 2.18 (t, 2 H, J = 7.4 Hz), 1.68 (s, 3 H), 1.51 (m, 4 H), 1.28 (m, 2 H), 1.04 (d, 3 H, J = 6.6); ^{13}C NMR (CD_3OD) δ 180.3, 173.4, 141.0, 140.9, 140.8, 140.8, 140.6, 140.4, 140.3, 130.4, 130.3, 130.2, 130.2, 130.1, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 102.5, 99.6, 98.4, 80.5, 80.2, 79.8, 79.0, 78.4, 77.5, 77.2, 77.1, 76.9, 76.0, 75.2, 75.2, 74.8, 74.5, 74.5, 72.5, 72.4, 70.9, 69.5, 53.6, 37.2, 31.1, 27.6, 27.2, 23.7, 17.8; HR-FAB MS *m*/*z* calcd for $C_{75}H_{87}NO_{17}$ (M) 1273.5974, found 1296.5929 (M + Na)$^+$. Data for **31b**: R_f 0.47 (CH_2Cl_2/MeOH/AcOH, 30:1:0.5); $[\alpha]_D$ −72.8° (*c* 1.5, MeOH); ^{1}H NMR (CD_3OD) δ 7.32−7.06 (m, 35 H), 5.10 (d, 1 H, J = 3.3 Hz), 4.86−4.19 (m, 18 H), 3.88−3.16 (m, 16 H), 2.11 (t, 2 H, J = 7.4 Hz), 1.83 (s, 3 H), 1.51−1.45 (m, 4 H), 1.27−1.14 (m, 2 H), 0.98 (d, 3 H, J = 6.3); ^{13}C NMR (CD_3OD) δ 177.7, 173.2, 140.6, 140.4, 140.3, 140.3, 139.7, 139.6, 130.1, 130.0, 129.9, 129.8, 129.5, 129.5, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 103.8, 102.9, 97.9, 81.8, 80.1, 79.9, 78.6, 77.1, 76.8, 76.4, 76.4, 76.3, 75.8, 75.3, 74.2, 74.0, 73.5, 73.1, 70.4, 69.4, 69.3, 67.5, 57.9, 35.1, 30.3, 26.6, 25.8, 23.5, 17.2; HR-FAB MS *m*/*z* calcd for $C_{75}H_{87}NO_{17}$ (M) 1273.5974, found 1296.5891 (M + Na)$^+$.

Typical Procedure for the Debenzylation Reaction. A mixture of compound **31b** (36 mg, 0.028 mmol) and $Pd(OH)_2$ (30 mg) in MeOH−H_2O (4:1, v/v; 5 mL) and AcOH (1 drop) was stirred under a H_2 atmosphere for 48 h at room temperature. The reaction mixture was filtered through a pad of Celite and the filtrate concentrated. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/H_2O (5:4:1) as an eluent to provide **32b** (17.0 mg 94%). Data for **29a**: R_f 0.47 (CH_2Cl_2/MeOH/H_2O, 5:4:1); MALDI-TOF MS *m*/*z* calcd for $C_{37}H_{62}N_2O_{25}$ 934, found 957 (M + Na)$^+$. Data for **29b**: R_f 0.43 (CH_2-

Cl_2/MeOH/H_2O, 5:4:1); MALDI-TOF MS *m*/*z* calcd for $C_{37}H_{62}N_2O_{25}$ 934, found 957 $(M + Na)^+$. Data for **29c**: R_f 0.50 (CH_2Cl_2/MeOH/H_2O, 5:4:1); ^1H NMR (D_2O) δ 5.01 (d, 1 H, $J = 4.0$ Hz), 4.44 (d, 1 H, $J = 7.6$ Hz), 4.43 (d, 1 H, $J = 7.9$ Hz), 4.11−3.48 (m, H), 2.67 (dd, 1 H, $J = 12.3$, 4.8 Hz), 2.08 (t, 2 H, $J = 7.3$ Hz), 1.94 (s, 3 H), 1.93 (s, 3 H), 1.73 (t, 1 H, $J = 12.3$ Hz), 1.54−1.43 (m, 4 H), 1.24−1.15 (m, 2 H), 1.07 (d, 3 H, $J = 6.6$); HR-FAB MS *m*/*z* calcd for $C_{37}H_{62}N_2O_{25}$ (M) 934.3642, found 957.3475 $(M + Na)^+$. Data for **29d**: R_f 0.45 (CH_2Cl_2/MeOH/H_2O, 5:4:1); FAB MS *m*/*z* calcd for $C_{37}H_{62}N_2O_{25}$ (M) 934.4, found 957.7 $(M + Na)^+$. Data for **32a**: R_f 0.49 (CH_2Cl_2/MeOH/H_2O, 5:4:1); $[\alpha]_D$ −12.0° (*c* 0.8, MeOH); ^1H NMR (D_2O) δ 5.24 (br s, 1 H), 5.11 (br s, 1 H), 4.59 (d, 1 H, $J = 6.3$ Hz), 4.11−3.48 (m, 16 H), 2.29 (t, 2 H, $J = 7.4$ Hz), 1.93 (s, 3 H), 1.59−1.48 (m, 4 H), 1.34−1.25 (m, 2 H), 1.14 (d, 3 H, $J = 6.6$); ^{13}C NMR (D_2O) δ 187.4, 175.1, 102.4, 99.4, 98.5, 78.7, 77.0, 73.3, 71.7, 71.2, 70.9, 70.8, 69.6, 69.5, 69.1, 63.3, 62.6, 54.7, 50.5, 36.1, 29.9, 26.3, 25.8, 23.7, 16.9; HR-FAB MS *m*/*z* calcd for $C_{26}H_{45}NO_{17}$ (M) 643.2688, found 666.2563 $(M + Na)^+$. Data for **32b**: R_f 0.34 (CH_2Cl_2/MeOH/H_2O, 5:4:1); $[\alpha]_D$ −6.3° (*c* 0.8, MeOH); ^1H NMR (D_2O) δ 5.03 (d, 1 H, $J = 4.0$ Hz), 4.46 (d, 1 H, $J = 7.6$ Hz), 4.38 (d, 1 H, $J = 7.9$ Hz), 3.94−3.39 (m, 16 H), 2.14 (t, 2 H, $J = 7.4$ Hz), 1.95 (s, 3 H), 1.55−1.44 (m, 4 H), 1.29−1.14 (m, 2 H), 1.10 (d, 3 H, $J = 6.3$); ^{13}C NMR (D_2O) δ 184.2, 175.5, 103.1, 102.2, 99.9, 76.6, 76.2, 76.2, 74.7, 73.7, 73.2, 72.3, 71.6, 70.5, 69.6, 69.0, 68.0, 62.7, 61.1, 57.1, 38.1, 29.6, 26.5, 26.2, 23.6, 16.6; HR-FAB MS *m*/*z* calcd for $C_{26}H_{45}NO_{17}$ (M) 643.26875, found 666.2559 $(M + Na)^+$.

Acknowledgment. We thank Ms. J. Hada (Kyoritsu College of Pharmacy) for the high-resolution mass spectrometry measurements. This research was supported in part by the Science and Technology Agency of the Japanese Government through the Special Postdoctoral Researchers Program (T.K.) and the FRP.

JA0167396