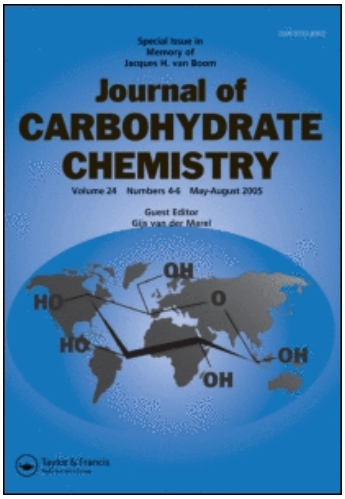

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CHEMOENZYMATIC SYNTHESIS OF NeuAcα-(2→3)-Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAcα1- *O*-(*Z*)-Serine (*N*-PROTECTED MUC II OLIGOSACCHARIDE-SERINE)

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CHEMOENZYMATIC SYNTHESIS OF NeuAcα-(2→3)-Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAcα1-*O*-(*Z*)-Serine (*N*-PROTECTED MUC II OLIGOSACCHARIDE–SERINE)

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ABSTRACT

An efficient synthesis of NeuAcα-(2→3)-Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAcα1-*O*-(*Z*)-Serine (*N*-protected MUC II oligosaccharide–serine, **14**) by a chemoenzymatic strategy is described. The enzymatic reaction of GalNAcα1-*O*-(*Z*)-Ser-*O*All **7** with *p*NP-β-Gal in the presence of recombinant β1,3-galactosidase from *Bacillus circulans* gave Galβ-(1→3)-GalNAcα1-*O*-(*Z*)-Ser-*O*All **3** in 68%. The introduction of two sialic acids into **3** was accomplished by a stepwise method. The branched Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAcα1-*O*-(*Z*)-Ser-*O*All **11** was constructed by a chemical method. Sialylation at the C-3 position of the terminal Gal residue on Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAcα1-*O*-(*Z*)-Serine **2** using α2,3-(*O*)-sialyltransferase from rat liver gave a target compound **14** in a practical yield.

Keywords: Mucin II oligosaccharide; Glycopeptide; Chemoenzymatic synthesis

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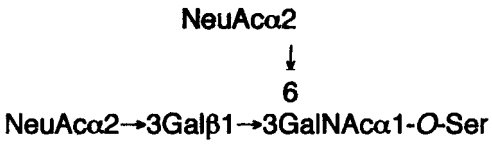


Mucin II tetrasaccharide-serine 1

Figure 1.

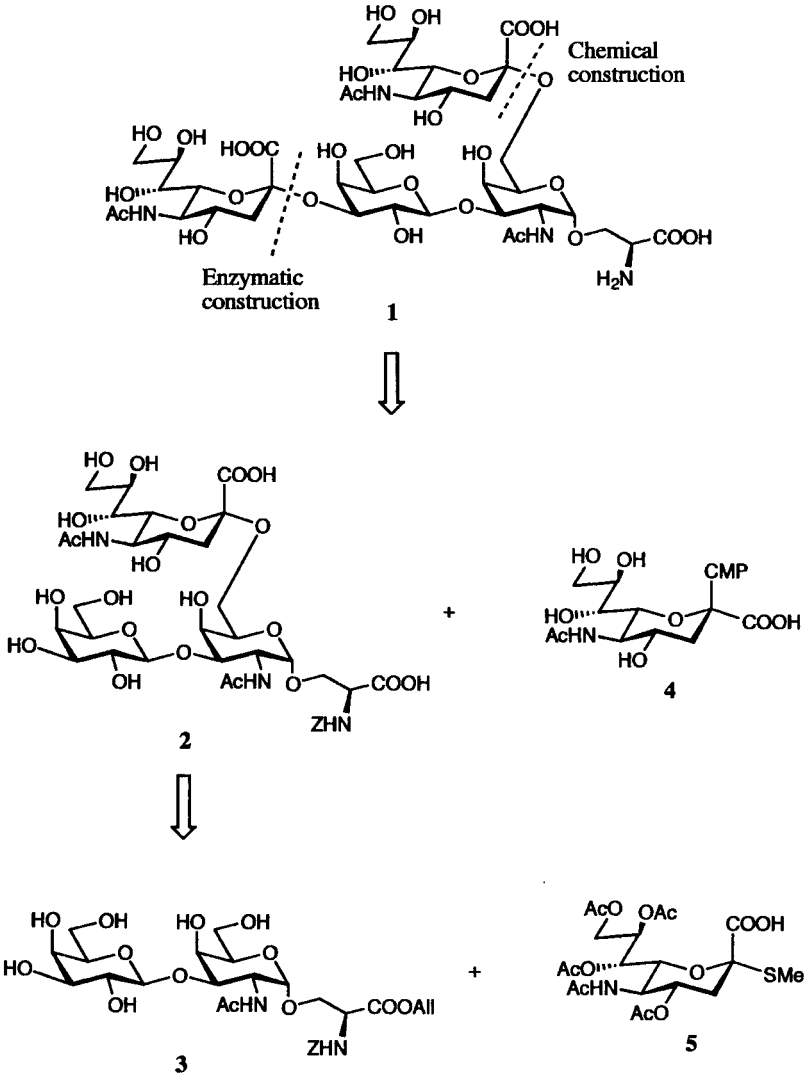
INTRODUCTION

Natural type oligosaccharides are necessary for understanding various biological phenomena originated by sugar chains in glycoconjugates. To use oligosaccharides as tools for biological research, many chemical synthetic reactions of carbohydrates have been reported,[1a,1b] but large-scale synthesis of oligosaccharides for glycoconjugates requires many steps due to the protection of hydroxyl groups for unambiguous glycosylations resulting in low yields. On the other hand, enzymatic synthesis using glycosyltransferases[2a–2j] or glycosidases,[3a–3c] which catalyzes glycosylation without protection of hydroxyl groups, can be an efficient method. For example, sialyl-T antigen was synthesized using a one-pot multi-enzyme system.[4a–4c] Recently, various glycosidases have become readily available, and various oligosaccharide units have been synthesized by two types of reactions, reverse hydrolysis and transglycosylation.[5a–5h] We have discovered a useful enzyme, a recombinant β-galactosidase, from *Bacillus circulans*[6,7] for the synthesis of the Galβ-(1 → 3)-GalNAc linkage, which is a partial structure of the mucin-type glycopeptide. Additionally, we have demonstrated the synthesis of the Galβ-(1→3)-GalNAcα1-*O*-Ser derivative in excellent yield.[8] The appropriately protected Galβ-(1→3)-GalNAcα1-*O*-Ser derivative was expected to be a key compound for the synthesis of various mucin type oligosaccharides.

MUC II **1** is disialylated tetrasaccharide-L-serine isolated from the urine of a patient suffering from mucolipidosis I.[9a,9b] The MUC II oligosaccharide, linking two sialic acids on Galβ-(1→3)-GalNAcα1-*O*-Serine, has already been synthesized by a pure chemical approach.[10] In the present report, we would like to show an efficient synthesis of MUC II as *N*-protected form by the combination of chemical and enzymatic methods (Figure 1).

RESULTS AND DISCUSSION

Our synthetic plan for obtaining **1** is based on a stepwise introduction of two sialic acids into Galβ-(1→3)-GalNAcα1-*O*-Ser derivative (Scheme 1). The route in Scheme 1, including individual glycosylation at the C-3 position of Gal and the C-6 position of GalNAc, seems to be widely applicable for the synthesis of various mucin type oligosaccharides which have Galβ-(1→3)-GalNAcα1-*O*-Ser/Thr at the reducing end as a core structure. Sialylation at the terminal Gal residue on trisaccharide **2** might be carried out using α2,3-(*O*)-sialyltransferase.[11] However, the enzymatic construction



Scheme 1.

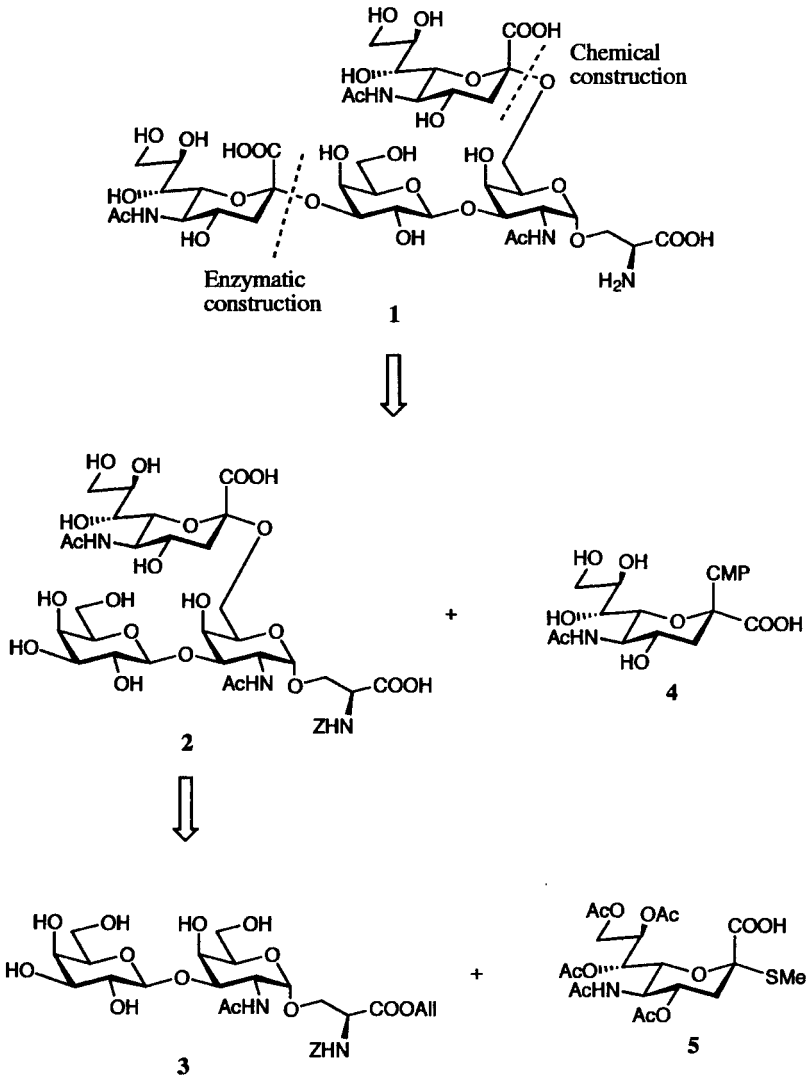
of a branched structure at the GalNAc residue of Galβ-(1 → 3)-[NeuAcα-(2 → 6)]-GalNAc]-Ser/Thr derivative is still challenging even at present. In this study, we undertook construction of a branched NeuAcα-(2 → 6)-GalNAc structure by a chemical method.

We first began synthesis of a Galβ-(1→3)-GalNAcα1-*O*-Ser derivative, which was essential for the total synthesis of mucin-type oligosaccharides of various structures. GalNAcα1-*O*-Ser **6** was synthesized by a reverse hydrolysis reaction using GalNAc and serine in the presence of *N*-acetyl-α-D-galactosaminidase from *Aspergillus niger* [EC 3.2.1.49].[12a,12b] The transglycosylation of the Gal residue of *p*NP-β-D-galactoside to GalNAcα1-*O*-Ser **6** was performed by using recombinant β1,3-galacto-

sidase from *B. circulans* [EC 3.2.0.23]. The mixture of GalNAcα1-*O*-Ser **6** and *p*NP-β-Gal in 20% DMF−80% potassium phosphate buffer (KPB, pH 6.0, 0.1 M) was incubated at 37 °C in the presence of the β-galactosidase. The progress of the reaction was monitored by a HPLC fitted with a UV detector (215 nm) and a NH_2-column (CAPCELL PAK). Two small peaks appeared after 3 hours but thereafter the reaction proceeded no further. The yield of the transglycosylation product was very low. We considered that the low reactivity and regioselectivity of this transglycosylation reaction might be due to the carboxyl and/or amino group of the serine residue interacting with the enzyme.

Therefore, we used a serine derivative as an acceptor whose amino and carboxyl groups were protected. Treatment of GalNAcα1-*O*-Ser **6** with benzyl chloroformate in aqueous NaOH afforded GalNAcα1-*O*-(*Z*)-Ser. Reaction of GalNAcα1-*O*-(*Z*)-Ser with allyl bromide in the presence of triethylamine gave GalNAcα1-*O*-(*Z*)-Ser-*O*All **7** in 77% yield. Transglycosylation using GalNAcα1-*O*-(*Z*)-Ser-*O*All **7** and *p*NP-β-Gal in the presence of β1,3-galactosidase from *B. circulans* gave Galβ-(1→3)-GalNAcα1-*O*-(*Z*)-Ser-*O*All **3** as a single product in 68% yield. The structure of the product was supported by ^{1}H and ^{13}C NMR spectroscopy and the coupling constant $J_{1',2'} = 7.8$ Hz proved the newly constructed β-structure. The Galβ-(1→3)-linkage was also confirmed by an NOE interaction between the H-1′ and H-3 protons.[8]

For the chemical construction of the branched Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAc-structure, protection of hydroxyl groups on **3** was performed. Isopropylidenation of disaccharide-serine **3** by use of 2,2-dimethoxypropane in the presence of (±)-camphorsulfonic acid gave a di-*O*-isopropylidene derivative **8** in good yield. After the toluoylation of remaining hydroxyl groups, the 4,6-*O*-isopropylidene group on the GalNAc residue was cleaved selectively to afford a diol derivative **10**. Sialylation of **10** using the *S*-methyl donor **5**[13] promoted by NIS-TfOH[14] at −40 °C gave monosialoside **11** as an anomeric mixture at the NeuAc residue (α:β = 74:26). The α-(2→6)-linkage of **11** was determined by conversion into the acetylated derivative **12**, which showed a signal for H-4 at δ 5.44 as a doublet with $J_{3,4} = 2.6$ Hz in the ^{1}H NMR spectrum. The anomeric configuration of the NeuAc residue at C-2 of **11** was determined as α by NMR (NeuAc H-3e δ 2.57, NeuAc H-3a δ 1.89, NeuAc C-3 δ 37.33).[15] The 3,4-*O*-Isopropylidene group of **11** was easily removed by 70% aq HOAc to give a triol **13**. Deallylation of **13** was cleanly achieved by use of $Pd(PPh_3)_4$ in EtOH−H_2O,[16] and the product was deacylated to give OH-free trisaccharide **2**. Two methods (chemical and enzymatic) were considered to introduce a second NeuAc residue into the triol **13**, but we chose to use α2,3-(*O*)-sialyltransferase [EC 2.4.99.4] from rat liver, which was known to be highly regio- and stereo-selective for the synthesis of NeuAcα-(2→3)-Galβ-(1→3)-GalNAc derivatives. A mixture of trisaccharide **2** and CMP-NeuAc **4** was incubated for 12 hours at 37 °C in the presence of α2,3-(*O*)-sialyltransferase in 0.1 M cacodylate buffer (0.2 mL, pH 6.0) containing bovine serum albumin. The reaction was monitored with HPLC using an ODS column (Mightysil) connected to a UV monitor (215 nm). The target tetrasaccharide **14** was produced quantitatively. In the ^{1}H NMR spectrum of **14**, the H-3a and H-3e signals of α-(2→6)-GalNAc-linked sialic acid appeared at δ 1.59 and δ 2.59, respectively, and those of α-(2→3)-Gal-linked sialic acid appeared at δ 1.69 and δ 2.64, respectively. From the empirical rule of the chemical shifts in sialic acid linkage,[17] the structure of **14** was confirmed to be NeuAcα-(2→3)-Galβ-(1→3)-[NeuAcα-(2→6)]-GalNAc (Scheme 2).



Scheme 2. a) 1. Benzyl chloroformate, 1M NaOH 2. Allyl bromide, Et_3N, DMF; b) *p*NP-β-galactoside, β(1,3)-galactosidase, DMF, 0.1M KPB; c) 2,2-Dimethoxypropane, (±)-Camphorsulfonic acid, acetone; d) Toluoyl chloride, pyridine; e) Me_3SiCl, ethylene glycol, MeOH; f) **5**, NIS, TfOH, MS3A, MeCN, −40 °C; g) Ac_2O, Pyridine; h) 70% HOAc; i) 1. $Pd(PPh_3)_4$, EtOH, H_2O 2. 1 M NaOH, MeOH; j) **4**, α(2,3)-*O*-Sialyltransferase, BSA, cacodylate buffer (0.1 M, pH 6.0).

In conclusion, the mucin type oligosaccharide NeuAcα-(2 → 3)-Galβ-(1 → 3)-[NeuAcα-(2 → 6)]-GalNAcα1-*O*-Ser was synthesized by the stepwise introduction of two sialic acids into a Galβ-(1→3)-GalNAcα-1-*O*-(*Z*)-Ser-*O*All. The branched Galβ-(1 → 3)-NeuAcα-(2 → 6)-GalNAc derivative was constructed by use of a chemical method. Sialylation on the terminal Gal residue was accomplished by use of sialyltransferase from rat liver. The present method, including individual glycosylation at the C-3 position of Gal and the C-6 position of a GalNAc of Galβ-(1 → 3)-GalNAcα1-*O*-Ser/

Thr derivative by a chemoenzymatic strategy, was shown to be effective for the synthesis of various mucin type oligosaccharides.

EXPERIMENTAL

General Methods. Optical rotations were measured at 25 °C with a HORIBA polarimeter SEPA-300. NMR spectra were measured on a UNITY INOVA 500 spectrometer (500 MHz) or JEOL JNM-ECP 400 spectrometer (400 MHz). ^{1}H NMR were recorded in $CDCl_3$ or D_2O using Me_4Si (δ 0.00) or DOH (δ 4.65) as the internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O using $CDCl_3$ (δ 77.0) or 1,4-dioxane (δ 67.6) as the internal standard. Coupling constants were measured in Hz. TOF–MS spectra were recorded on Finnigan mat LASERMAT 2000 with 2,5-dihydroxybenzoic acid as the matrix. High resolution mass spectra were obtained on JMS-HX110 (Jeol) spectrometer with diethanolamine as the matrix. Silica gel column chromatography was performed using Wakogel C-300 (Wako Pure Chemical Industries). Analytical TLC was performed on aluminium plates coated with silica gel 60 F254 (Merck). Preparative TLC was performed on glass plates coated with silica gel 60 F254 (Merck). Molecular sieves (3 Å) were purchased from Aldrich Chemical Company, Inc., and activated at 180 °C under vacuum immediately prior to use. α2,3-(*O*)-Sialyltransferase (EC 2.4.99.4) was purchased from Carbiochem–Novabiochem Corporation.

***N*-(Benzyloxycarbonyl)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (7).** To a 1 M NaOH solution (50 mL) of GalNAcα1-*O*-Ser **6** (4.0 g, 13.0 mmol) was added slowly benzyl chloroformate (6.6 g, 39.0 mmol), and the mixture was stirred for 10 hours at 0 °C. After the reaction mixture was washed with $CHCl_3$, an aqueous layer was neutralized with ion-exchange resin (Dowex 50W) and concentrated in vacuo. To the DMF solution (130 mL) of the residue containing Et_3N (3.95 g, 39.0 mmol) was added allyl bromide (4.72 g, 39.0 mmol) at 0 °C. The mixture was stirred for 20 hours at room temperature and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc ~ EtOAc:MeOH = 7:3) to afford GalNAcα1-*O*-(*Z*)-Ser *O*All **7** (4.83 g, 77%) as crystals: mp 185–187 °C (EtOH); $[\alpha]_D$ + 115.3° (*c* 2.08, MeOH); ^{1}H NMR (500 MHz, D_2O) δ 7.36–7.29 (m, 5H, Phenyl), 5.95–5.87 (m, 1H, CH_2–C*H*=CH_2), 5.33 (d, 1H, CH_2–CH=C*H*$_2$, *J* = 16.9 Hz), 5.23 (d, 1H, CH_2–CH=C*H*$_2$, *J* = 10.3 Hz), 5.13 (d, 1H, benzyl, *J* = 12.1 Hz), 5.09 (d, 1H, benzyl), 4.78 (d, 1H, H-1, *J* = 3.3 Hz), 4.62 (m, 2H, C*H*$_2$–CH=CH_2), 4.49 (br s, 1H, Ser-α), 4.22 (dd, 1H, H-2, *J* = 11.0 Hz), 3.94 (dd, 1H, Ser-β, *J* = 3.7, 11.0 Hz), 3.89 (dd, 1H, Ser-β, *J* = 4.4 Hz), 3.86 (d, 1H, H-4, *J* = 2.2 Hz), 3.79–3.65 (m, 4H, H-3,5,6,6′), 1.95 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.76, 171.53, 158.53, 138.01, 133.08, 129.49, 129.10, 128.96, 119.06, 99.96, 72.83, 70.20, 69.48, 68.90, 67.87, 67.07, 62.73, 56.00, 51.34, 22.90.

Anal. Calcd for $C_{22}H_{30}N_2O_{10}$: C, 54.77; H, 6.27; N, 5.81. Found: C, 54.56; H, 6.31; N, 5.82.

***N*-(Benzyloxycarbonyl)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (3).** To the β1,3-galactosi-

dase solution (recombinant, from *B. circulans*, 0.1 mL, 130 mU)[6,7] was added 20% DMF–80% KPB (pH 6.0, 0.1 M) buffer (0.6 mL) including *p*NP-β-Gal (10.7 mg, 35.3×10^{-3} mmol) and GalNAcα1-*O*-(*Z*)-Ser-*O*All **7** (51.1 mg, 106×10^{-3} mmol) and the mixture was incubated for 4.5 hours at 37 °C. The reaction was stopped by heating at 100 °C for 5 min, then filtered and purified by HPLC [ODS (Waters AccQ TAG), eluent: 30% aq MeOH–50% aq MeOH] to afford Galβ1-3GalNAcα1-*O*-(*Z*)-Ser-*O*All **3** (15.5 mg, 68%) as crystals: mp 196–198 °C (EtOH); $[\alpha]_D+70.0°$ (*c* 1.10, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 7.42–7.34 (m, 5H, Phenyl), 5.88–5.85 (m, 2H, $CH_2-CH=CH_2$), 5.29 (d, 1H, $CH_2-CH=CH_2$, *J* = 17.1 Hz), 5.23 (d, 1H, $CH_2-CH=CH_2$, *J* = 10.5 Hz), 5.14 (d, 1H, benzyl, *J* = 12.5 Hz), 5.09 (d, 1H, benzyl), 4.81 (d, 1H, H-1, *J* = 3.2 Hz), 4.51 (br s, 1H, Ser-α), 4.40 (d, 1H, H-1′, *J* = 7.8 Hz), 4.23 (dd, 1H, H-2, *J* = 11.0 Hz), 4.17 (d, 1H, H-4, *J* = 2.9 Hz), 3.94 (dd, 1H, H-3, *J* = 3.2 Hz), 3.85 (d, 1H, H-4′, *J* = 2.9 Hz), 3.59 (m, 1H, H-5′), 3.56 (dd, 1H, H-3′, *J* = 10.0 Hz), 3.46 (dd, 1H, H-2′), 1.93 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 103.57 (C-1′), 97.03 (C-1), 75.87 (C-3), 73.96 (C-5′), 71.49 (C-3′), 69.88 (C-5), 69.58 (C-2′), 67.55, 67.52 (C-4,4′), 60.06, 59.94 (C-6,6′), 47.51 (C-2).

Anal. Calcd for $C_{28}H_{40}N_2O_{15}$: C, 52.17; H, 6.25; N, 4.35. Found: C, 51.83; H, 6.27; N, 4.37.

***N*-(Benzyloxycarbonyl)-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (8).** To an acetone solution (46 mL) of Galβ1-3GalNAcα1-*O*-(*Z*)-Ser-*O*All **3** (1.16 g, 1.8 mmol) was added 2,2-dimethoxypropane (2.2 g, 21.6 mmol) containing (±)-camphorsulfonic acid (230 mg, 0.99 mmol) and the mixture was stirred for 24 hours at room temperature. After the reaction was stopped by addition of Et_3N (0.165 mL, 1.19 mmol), the solution was concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc:MeOH = 98:2) to afford diacetonide **8** (910 mg, 70%): $[\alpha]_D+77.0°$ (*c* 0.96, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.34 (m, 5H, Phenyl), 5.92–5.82 (m, 2H, $CH_2-CH=CH_2$ and N*H*), 5.70 (br d, 1H, N*H*, *J* = 5.7 Hz), 5.34 (d, 1H, $CH_2-CH=CH_2$, *J* = 17.3 Hz), 5.30 (d, 1H, $CH_2-CH=CH_2$, *J* = 10.5 Hz), 5.14 (s, 2H, benzyl), 4.87 (d, 1H, H-1, *J* = 3.7 Hz), 4.67 (d, 2H, $CH_2-CH=CH_2$, *J* = 5.1 Hz), 4.61 (ddd, 1H, H-2, *J* = 9.3, 10.7 Hz), 4.58 (br s, 1H, Ser-α), 4.30 (d, 1H, H-4, *J* = 2.9 Hz), 4.26 (d, 1H, H-1′, *J* = 8.1 Hz), 4.15 (dd, 1H, H-4′, *J* = 1.7, 5.5 Hz), 4.03 (dd, 1H, H-3′, *J* = 7.7 Hz), 4.01–3.65 (m, 9H, H-3, 5, 6a, 6b, 5′, 6′a, 6′b, Ser-β), 3.59 (dd, 1H, H-2′), 1.98 (s, 3H, Ac), 1.52, 1.49, 1.46, 1.34 (each s, 12H, acetonide); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 170.2, 155.9, 135.9, 130.9, 128.6, 128.4, 128.3, 119.6, 110.4, 104.4, 99.1, 78.6, 77.2, 73.8, 73.4, 72.2, 69.0, 68.7, 67.3, 66.5, 63.3, 62.5, 54.5, 47.8, 29.3, 28.0, 26.3, 23.3, 18.4.

Anal. Calcd for $C_{34}H_{48}N_2O_{15}$: C, 56.35; H, 6.68; N, 3.87. Found: C, 56.00; H, 6.74; N, 3.68.

***N*-(Benzyloxycarbonyl)-*O*-(3,4-*O*-isopropylidene-2,6-di-*O*-toluoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (9).** To a pyridine solution (5 mL) of diacetonide **8** (300 mg, 0.414 mmol) was added toluoyl chloride (320 mg, 2.07 mmol) and the mixture was stirred for 2 h at room temperature. After the reaction was stopped by addition of MeOH (5 mL), the solution was concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc = 1:1 ∼ 1:2 ∼ EtOAc) to afford ditoluoyl

derivative **9** (328 mg, 82%): $[\alpha]_D + 69.7°$ (*c* 1.01, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.94–7.91 (m, 4H, Phenyl), 7.36–7.20 (m, 13H, Phenyl), 5.89–5.78 (m, 2H, CH_2–$CH{=}CH_2$ and N*H*), 5.59 (br d, 1H, N*H*, *J* = 6.9 Hz), 5.30 (d, 1H, CH_2–$CH{=}CH_2$, *J* = 16.9 Hz), 5.24 (d, 1H, CH_2–$CH{=}CH_2$, *J* = 10.1 Hz), 5.13–5.10 (m, 3H, H-2′, benzyl, H-1), 4.88 (d, 1H, H-1′, *J* = 6.4 Hz), 4.41 (ddd, 1H, H-2, *J* = 11.1 Hz), 4.18 (m, 1H, H-5′), 3.92 (dd, 1H, Ser-β, *J* = 3.2, 11.3 Hz), 3.85 (dd, 1H, Ser-β, *J* = 2.7 Hz), 3.75 (dd, 1H, H-3, *J* = 2.5 Hz), 2.41, 2.40 (each s, 6H, toluoyl), 1.62 (s, 3H, Ac), 1.47, 1.41, 1.36, 1.31 (12H, each s, acetonide); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.21, 169.83, 166.29, 165.45, 155.90, 144.36, 144.22, 135.99, 131.19, 129.92, 129.55, 129.22, 128.55, 128.29, 128.21, 126.62, 119.15, 110.93, 102.67, 99.30, 98.91, 76.22, 73.18, 72.94, 71.17, 69.28, 68.03, 67.18, 66.22, 63.78, 63.08, 62.54, 54.60, 48.27.

Anal. Calcd for $C_{50}H_{60}N_2O_{17}$: C, 62.49; H, 6.29; N, 2.91. Found: C, 62.08; H, 6.48; N, 2.88.

***N*-(Benzyloxycarbonyl)-*O*-(3,4-*O*-isopropylidene-2,6-di-*O*-toluoyl-β-D-galacto-pyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (10).** To a MeOH solution (10 mL) of ditoluoyl derivative **9** (338 mg, 0.352 mmol) containing ethylene glycol (39×10^{-3} mL, 0.704 mmol) was added Me_3SiCl (22×10^{-3} mL, 0.176 mmol) at 0 °C. After being stirred for 30 min, the reaction was stopped by addition of Et_3N (0.102 mL, 0.704 mmol) and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc = 1:2 ~EtOAc) to afford monoacetonide **10** (243 mg, 75%): $[\alpha]_D + 203.7°$ (*c* 0.35, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.96–7.90 (m, 4H, Phenyl), 7.35–7.26 (m, 13H, Phenyl), 5.84–5.77 (m, 1H, CH_2–$CH{=}CH_2$), 5.73 (d, 1H, N*H*, *J* = 8.4 Hz), 5.29 (d, 1H, N*H*, *J* = 7.6 Hz), 5.26 (d, 1H, CH_2–$CH{=}CH_2$, *J* = 15.5 Hz), 5.21 (d, 1H, CH_2–$CH{=}CH_2$, *J* = 10.6 Hz), 5.17 (dd, 1H, H-2′, *J* = 6.6 Hz), 5.11 (d, 1H, benzyl, *J* = 12.2 Hz), 5.07 (d, 1H, benzyl), 4.87 (d, 1H, H-1, *J* = 3.7 Hz), 4.74 (d, 1H, H-1′, *J* = 6.9 Hz), 3.89 (dd, 1H, Ser-β, *J* = 2.2, 10.8 Hz), 3.83 (dd, 1H, Ser-β, *J* = 3.2 Hz), 2.42, 2.40 (each s, 6H, toluoyl), 1.63 (s, 3H, Ac), 1.45, 1.36 (each s, 6H, acetonide); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.79, 169.68, 166.16, 164.89, 155.90, 144.14, 144.10, 135.90, 130.87, 129.66, 129.48, 129.05, 128.33, 128.08, 127.98, 126.52, 126.44, 119.01, 110.89, 100.60, 98.67, 78.83, 75.86, 72.79, 72.56, 70.88, 69.57, 68.85, 68.25, 66.99, 63.43, 62.31, 54.26.

Anal. Calcd for $C_{47}H_{56}N_2O_{17}$: C, 61.30; H, 6.13; N, 3.04. Found: C, 60.97; H, 6.19; N, 3.08.

***N*-(Benzyloxycarbonyl)-*O*-(3,4-*O*-isopropylidene-2,6-di-*O*-toluoyl-β-D-galacto-pyranosyl)-(1→3)-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl)onate-(2→6)]-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (11).** The mixture of acceptor **10** (261 mg, 0.284 mmol), donor **5** (296 mg, 0.568 mmol) and molecular sieves 3 Å (600 mg) in MeCN (0.5 mL) was stirred for 1 h at room temperature under an argon atmosphere. To this mixture was added the MeCN solution (0.5 mL) of NIS (383 mg, 1.7 mmol) containing TfOH (50×10^{-3} mL, 0.568 mmol) at −40 °C. After being stirred for 14 hours, the solution was diluted with CH_2Cl_2, filtered and washed with saturated $NaHSO_3$ and brine, respectively. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by gel filtration (Sephadex LH-20, MeOH) to afford sialosides

as an anomeric mixture (α:β = 76:24). These isomers were separated by preparative TLC (CH_2Cl_2:MeOH = 19:1, 2 times) to give α isomer **11** (166 mg, 42%) and β isomer (50 mg, 13%) respectively. Compound **11**: $[\alpha]_D + 34.5°$ (*c* 0.35, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94–7.90 (m, 4H, Phenyl), 7.37–7.23 (m, 13H, Phenyl), 4.87 (d, 1H, H-1′, *J* = 6.1 Hz), 4.78 (d, 1H, H-1, *J* = 3.7 Hz), 3.77 (s, 3H, OMe), 2.57 (dd, 1H, NeuAc H-3e, *J* = 4.7, 12.3 Hz), 2.42, 2.40 (each s, 6H, toluoyl), 2.11, 2.02, 1.98, 1.87, 1.63, 1.53, 1.36 (each s, 24H, Ac and acetonide), 1.89 (dd, 1H, NeuAc H-3a, *J* = 12.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.63, 170.50, 169.97, 169.93, 167.75, 165.97, 164.99, 144.05, 143.86, 131.01, 129.71, 129.53, 129.05, 128.38, 128.11, 128.02, 126.81, 126.57, 119.16, 110.74, 101.04, 98.78, 98.70, 78.68, 75.40, 72.68, 72.49, 72.44, 70.22, 68.99, 68.92, 68.44, 67.50, 67.03, 66.00, 63.17, 63.04, 62.30, 52.59, 49.31, 47.77, 37.33, 29.48, 26.77, 25.55, 22.97, 22.40, 21.45, 20.79, 20.56, 20.51.

Anal. Calcd for $C_{67}H_{83}N_3O_{29}$: C, 57.71; H, 6.00; N, 3.01. Found: C, 57.27; H, 5.52; N, 3.05.

***N*-(Benzyloxycarbonyl)-*O*-(3,4-*O*-isopropylidene-2,6-di-*O*-toluoyl-β-D-galacto-pyranosyl)-(1→3)-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl)onate-(2→6)]-*O*-(2-acetamido-4-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (12).** To a pyridine solution (1 mL) of **11** (35 mg, 25.1×10^{-3} mmol) was added Ac_2O (0.5 mL) and the mixture was stirred for 30 min at 70 °C. The reaction was stopped by addition of MeOH (5 mL), and the solution was concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 15:1) to afford acetate **12** (30 mg, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–7.90 (m, 4H, Phenyl), 7.36–7.16 (m, 13H, Phenyl), 5.82 (m, 1H, CH_2–CH=C*H*$_2$), 5.76 (d, 1H, N*H*, *J* = 8.1 Hz), 5.66 (d, 1H, N*H*, *J* = 7.7 Hz), 5.44 (d, 1H, H-4, *J* = 2.6 Hz), 5.15 (dd, 1H, H-2′, *J* = 6.1, 6.4 Hz), 4.92 (d, 1H, H-1, *J* = 3.3 Hz), 4.85 (m, 1H, NeuAc H-4), 4.73 (d, 1H, H-1′, *J* = 6.4 Hz), 4.39 (dd, 1H, H-3′, *J* = 6.1 Hz), 3.73 (s, 3H, OMe), 2.54 (dd, 1H, NeuAc H-3e, *J* = 4.8, 12.8 Hz), 2.41, 2.38 (each s, 6H, toluoyl), 2.11, 2.09, 2.07, 2.02, 1.87, 1.64, 1.57, 1.33 (each s, 27H, Ac and acetonide); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.87, 170.64, 170.17, 170.12, 169.93, 169.68, 167.73, 166.29, 165.38, 155.83, 144.28, 143.91, 136.06, 131.21, 129.86, 129.69, 129.21, 129.14, 128.54, 128.28, 128.22, 119.28, 110.69, 100.62, 98.53, 98.49, 77.21, 76.21, 73.18, 72.81, 72.57, 71.00, 96.03, 68.44, 68.21, 67.30, 67.12, 66.17, 63.76, 63.01, 62.36, 54.48, 52.79, 49.28, 49.10, 37.57, 27.20, 25.92, 23.16, 21.64, 20.93, 20.81, 20.78, 20.70.

***N*-(Benzyloxycarbonyl)-*O*-(2,6-di-*O*-toluoyl-β-D-galactopyranosyl)-(1→3)-*O*-[methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl)onate-(2→6)]-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine Allyl Ester (13).** The aq HOAc solution (70%, 5 mL) of sialoside **11** (129 mg, 93×10^{-3} mmol) was stirred for 48 hours at room temperature. The solution was concentrated in vacuo to give a triol **13** (95 mg, 76%): $[\alpha]_D + 38.0°$ (*c* 0.90, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92–7.88 (m, 4H, Phenyl), 7.37–7.18 (m, 13H, Phenyl), 6.00 (d, 1H, N*H*, *J* = 9.9 Hz), 5.71 (d, 1H, N*H*, *J* = 8.1 Hz), 5.56 (d, 1H, N*H*, *J* = 9.2 Hz), 5.27 (d, 1H, CH_2–CH=C*H*$_2$, *J* = 17.2 Hz), 5.21 (d, 1H, CH_2–CH=C*H*$_2$, *J* = 10.3 Hz), 4.78 (d, 1H, H-1′, *J* = 7.3 Hz), 4.61 (d, 1H, H-1, *J* = 3.3 Hz), 3.84 (s, 3H, OMe), 2.56 (dd,

1H, NeuAc H-3e, $J = 4.8, 12.8$ Hz), 2.40, 2.36 (each s, 6H, toluoyl), 2.14, 2.12, 2.03, 1.97, 1.85, 1.19 (each s, 18H, Ac and acetonide).

Anal. Calcd for $C_{64}H_{79}N_3O_{29}$: C, 56.76; H, 5.88; N, 3.10. Found: C, 56.42; H, 5.83; N, 3.16.

***N*-(Benzyloxycarbonyl)-*O*-(β-D-galactopyranosyl)-(1→3)-O-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)]-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine (2).** To an EtOH–H_2O solution (2 mL, 1:1) of triol **13** (14.7 mg, 10.9×10^{-3} mmol) was added $Pd(PPh_3)_4$ (12.5 mg, 10.9×10^{-3} mmol), and the mixture was stirred for 18 hours at room temperature. The mixture was filtered through a celite pad and the filtrate was concentrated in vacuo. The residue was purified by preparative TLC (EtOAc:MeOH = 7:3) to afford an acid. To the MeOH solution (1 mL) of the acid was added 1 M NaOH (1 mL), and the mixture was stirred for 10 hours at room temperature. The solution was neutralized with ion-exchange resin (Dowex 50Wx8, H^+ form) and concentrated in vacuo. The residue was purified by gel filtration (Sephadex G-15, H_2O) to give compound **2** (7.8 mg, 80%): $[\alpha]_D + 27.8°$ (*c* 0.44, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 7.35–7.29 (m, 5H, Phenyl), 5.06 (s, 2H, benzyl), 4.33 (d, 1H, H-1′, $J = 7.9$ Hz), 4.18 (dd, 1H, $J = 3.7, 11.0$ Hz), 4.06 (d, 1H, H-1, $J = 3.0$ Hz), 4.08 (m, 1H, Ser-α), 3.38 (dd, 1H, H-2′, $J = 9.8$ Hz), 2.61 (dd, 1H, NeuAc H-3e, $J = 4.4, 12.3$ Hz), 1.92, 1.87 (each s, 6H, Ac), 1.55 (dd, 1H, NeuAc H-3a, $J = 12.3$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 104.87, 100.54, 98.15. TOFMS *m/z*: 918.6 $[M+Na]^+$. HRMS (FAB^-) Calcd for $C_{36}H_{52}O_{23}N_3$ $[M-H]^-$: 894.2992; Found: 849.2957.

***N*-(Benzyloxycarbonyl)-*O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)]-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine (14).** A mixture of compound **2** (13.2 mg, 14.7×10^{-3} mmol), CMP-NeuAc **4** (19.4 mg, 29.4×10^{-3} mmol), α2,3-(*O*)-sialyltransferase (EC 2.4.99.4) from rat liver (100 mU in 50 mM MES, pH 6.0, 250 mM NaCl, 0.5 mM β-mercaptoethanol, 50% glycerol) in 0.1 M cacodylate buffer (0.7 mL, pH 6.0) containing bovine serum albumin (2 mg) was incubated for 12 hours at 37 °C. The solution was purified by HPLC (ODS, 10% MeCN containing 0.1% TFA) and gel filtration (Sephadex G-15, H_2O) to afford tetrasaccharide-serine **14** (16 mg, 83%): $[\alpha]_D + 19.3°$ (*c* 0.29, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 7.38–7.28 (m, 5H, Phenyl), 5.07 (s, 2H, benzyl), 4.40 (d, 1H, H-1′, $J = 7.8$ Hz), 4.15 (dd, 1H, $J = 3.7, 10.8$ Hz), 4.12 (br s, 1H, H-1), 3.41 (dd, 1H, H-2′, $J = 9.8$ Hz), 2.64 (dd, 1H, NeuAc H-3e, $J = 4.4, 12.3$ Hz), 2.59 (dd, 1H, NeuAc H-3e, $J = 4.7, 12.5$ Hz), 1.92, 1.86 (each s, 6H, Ac), 1.69 (dd, 1H, NeuAc H-3a, $J = 12.3$ Hz), 1.59 (dd, 1H, NeuAc H-3a, $J = 12.3$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 104.87, 100.54, 98.15. TOFMS *m/z*: 1211.9 $[M+Na]^+$. HRMS (FAB^-) Calcd for $C_{47}H_{69}O_{31}N_4$ $[M-H]^-$: 1185.3946; Found: 1185.3993.

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REFERENCES

1. (a) Schmidt, R.R. New methods for the synthesis of glycosides and oligosaccharides — are there alternatives to the Koenigs–Knorr method? Angew. Chem. Int. Ed. Engl. **1986**, *25*, 212–235. (b) Kunz, H. Synthesis of glycopeptides, partial structures of biological recognition components. Angew. Chem. Int. Ed. Engl. **1987**, *26*, 294–308.
2. (a) Thiem, J.; Treder, W. Synthesis of the trisaccharide neu-5-Ac-α(2 → 6)Gal-β(1 → 4)GlcNAc by the use of immobilized enzymes. Angew. Chem. Int. Ed. Engl. **1986**, *25*, 1096–1097. (b) Augé, C.; Gautheron, C.; Pora, H. Enzymic synthesis of the sialylglycopeptide, α-D-Neu*p*Ac-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 4N)-L-Asn. Carbohydr. Res. **1989**, *193*, 288–293. (c) Toone, E.J.; Simon, E.S.; Bednarski, M.D.; Whitesides, G.M. Enzyme-catalyzed synthesis of carbohydrates. Tetrahedron **1989**, *45* (17), 5365–5422. (d) Thiem, J.; Wiemann, T. Galactosyltransferase-catalyzed synthesis of 2′-deoxy-*N*-acetyllactosamine. Angew. Chem. Int. Ed. Engl. **1991**, *30*, 1163–1164. (e) David, S.; Augé, C.; Gautheron, C. Enzymatic methods in preparative carbohydrate chemistry. Adv. Carbohydr. Chem. Biochem. **1991**, *49*, 175–237. (f) Wong, C.-H.; Dumas, D.P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S.J.; Weston, B.W.; Lowe, J.B. Specificity, inhibition, and synthetic utility of a recombinant human α-1,3-fucosyltransferase. J. Am. Chem. Soc. **1992**, *114* (18), 7321–7322. (g) Ichikawa, Y.; Look, G.-C.; Wong, C.-H. Enzyme-catalyzed oligosaccharide synthesis. Anal. Biochem. **1992**, *202*, 215–238. (h) Ichikawa, Y.; Lin, Y.-C.; Dumas, D.P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M.A.; Bayer, R.; Ketcham, C.; Walker, L.E.; Paulson, J.C.; Wong, C.-H. Chemical-enzymatic synthesis and conformational analysis of sialyl Lewis x and derivatives. J. Am. Chem. Soc. **1992**, *114* (24), 9283–9298. (i) Ito, Y.; Paulson, J.C. Combined use of trans-sialidase and sialyltransferase for enzymatic synthesis of αNeuAc2 → 3βGal-OR. J. Am. Chem. Soc. **1993**, *115* (17), 7862–7863. (j) Wong, C.-H.; Whitesides, G.M. *Enzymes in Synthetic Organic Chemistry*; Tetrahedron Org. Chem. Ser.; Baldwin, J.E., Magnus, P.D., Eds.; Pergamon: Oxford, 1994; Vol. 12.
3. (a) Ajisaka, K.; Matsuo, I.; Isomura, M.; Fujimoto, H.; Shirakabe, M.; Okawa, M. Enzymatic synthesis of mannobioses and mannotrioses by reverse hydrolysis using α-mannosidase from Aspergillus niger. Carbohydr. Res. **1995**, *270*, 123–130. (b) Yoon, J.-H.; Ajisaka, K. The synthesis of galactopyranosyl derivatives with β-galactosidases of different origins. Carbohydr. Res. **1996**, *292*, 153–163. (c) Křen, V.; Thiem, J. Glycosylation employing bio-systems: from enzymes to whole cells. Chem. Soc. Rev. **1997**, 26, 463–473.
4. (a) Křen, V.; Thiem, J. A multienzyme system for a one-pot synthesis of sialyl T-antigen. Angew. Chem. Int. Ed. Engl. **1995**, *34*, 893–895. (b) Gambert, U.; Thiem, J. Multienzyme system for synthesis of the sialylated Thomsen–Friedenreich antigen determinant. Eur. J. Org. Chem. **1999**, 107–110. (c) Gonzales Lio, R.; Thiem, J. Chemoenzymatic synthesis of spacer-linked oligosaccharides for the preparation of neoglycoproteins. Carbohydr. Res. **1999**, *317*, 180–190.
5. (a) Hedbys, L.; Larsson, P.-O.; Mosbach, K.; Svensson, S. Synthesis of the disaccharide 6-*O*-β-D-galactopyranosyl-2-acetamido-2-deoxy-D-galactose using immobilized β-galactosidase. Biochem. Biophys. Res. Commun. **1984**, *123* (1), 8–15. (b) Johansson, E.; Hedbys, L.; Larsson, P.-O. Synthesis of mannose oligosaccharides

via reversal of the α-mannosidase reaction. Biotechnol. Lett. **1986**, *8*, 421–424. (c) Ajisaka, K.; Nishida, H.; Fujimoto, H. Use of an activated carbon column for the synthesis of disaccharides by use of a reversed hydrolysis activity of β-galactosidase. Biotechnol. Lett. **1987**, *9* (6), 387–392. (d) Ajisaka, K.; Nishida, H.; Fujimoto, H. The synthesis of oligosaccharides by the reversed hydrolysis reaction of β-glucosidase at high substrate concentration and at high temperature. Biotechnol. Lett. **1987**, *9* (4), 243–248. (e) Nilsson, K.G.I. A Comparison of the enzyme catalyzed formation of peptides and oligosaccharides in various hydroorganic solutions using the nonequilibrium approach. Biocat. Org. Med. **1987**, 369–374. (f) Fujimoto, H.; Nishida, H.; Ajisaka, K. Enzymatic synthesis of glucobioses by a condensation reaction with α-glucosidase, β-glucosidase and glucoamylase. Agric. Biol. Chem. **1988**, *52* (6), 1345–1351. (g) Sauerbrei, B.; Thiem, J. Galactosylation and glucosylation by use of β-galactosidase. Tetrahedron Lett. **1992**, *33* (2), 201–204. (h) Fukase, K.; Yasukochi, T.; Suda, Y.; Yoshida, M.; Kusumoto, S. Chemoenzymatic synthesis of Gal(β1-3)Gal(β1-4)Xyl(β)-L-Ser and Gal(β1-3)Gal(β1-4)Xyl(β)-MU by the use of β-D-galactosidase. Tetrahedron Lett. **1996**, *37* (37), 6763–6766.

6. Ito, Y.; Sasaki, T. Cloning and characterization of the gene encoding a novel β-galactosidase from *Bacillus circulans*. Biosci. Biochem. Biotechnol. **1997**, *61* (8), 1270–1276.
7. Fujimoto, H. Regioselective synthesis of β-D-Gal-(1→3)-D-GlcNAc using β-galactosidase from *Xanthomonas manihotis*. J. Carbohydr. Chem. **1997**, *16* (6), 967–970.
8. Suzuki, K.; Fujimoto, H.; Ito, Y.; Sasaki, T.; Ajisaka, K. An efficient synthesis of a Galβ1-3GalNAc-serine derivative using β-galactosidase. Tetrahedron Lett. **1997**, *38* (7), 1211–1214.
9. (a) Parkkinen, J.; Finne, J. Isolation and structural characterization of five major sialyloligosaccharides and a sialylglycopeptide from normal human urine. Eur. J. Biochem. **1983**, *136*, 355–361. (b) Lecat, J.D.; Lemonnier, M.; Derappe, C.; Lhermitte, M.; Van Halbeek, H.; Dorland, L.; Vliegenthart, J.F.G. The structure of sialyl-glycopeptides of the *O*-glycosidic type, isolated from sialidosis (mucolipidosis I) urine. Eur. J. Biochem. **1984**, *140*, 415–420.
10. Iijima, H.; Ogawa, T. Synthesis of a fully protected derivative of *O*-(*N*-acetyl-α-D-neuraminyl)-(2→3)-*O*-β-D-galactopyranosyl-(1→3)-*O*-[(*N*-acetyl-α-D-neuraminyl)-(2→6)]-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-L-serine. Carbohydr. Res. **1989**, *186*, 107–118.
11. Williams, M.A.; Kitagawa, H.; Datta, A.K.; Paulson, J.C.; Jamieson, J. Large-scale expression of recombinant sialyltransferases and comparison of their kinetic properties with native enzymes. Glycoconjugate J. **1995**, *12*, 755–761.
12. (a) Johansson, E.; Hedbys, L.; Larsson, P.-O. Enzymatic synthesis of monosaccharide-amino acid conjugates. Enzyme Microb. Technol. **1991**, *13*, 781–787. (b) Naundorf, A; Ajisaka, K. Purification of α-*N*-acetylgalactosaminidase from *Aspergillus niger* and its use in the synthesis of GalNAc-α-(1→*O*)-serine. Enzyme Microb. Technol. **1999**, *25*, 483–488.
13. Kanie, O.; Kiso, M.; Hasegawa, A. Glycosylation using methylthioglycosides of *N*-acetylneuraminic acid and dimethyl(methylthio)sulfonium triflate. J. Carbohydr. Chem. **1986**, *7*, 501–506.
14. (a) Konradsson, P.; Mootoo, D.R.; McDevitt, R.E.; Fraser-Reid, B. Iodonium ion generated in situ from *N*-iodosuccinimide and trifluoromethanesulphonic acid pro-

motes direct linkage of 'Disarmed' pent-4-enyl glycosides. J. Chem. Soc., Chem. Commun. **1990**, 270–272. (b) Veeneman, G.H.; Van Leeuwen, S.H.; Van Boom, J.H. Iodonium ion promoted reactions at the anomeric center. II. An efficient thioglycoside mediated approach toward the formation of 1,2-trans linked glycosides and glycosidic esters. Tetrahedron Lett. **1990**, *31* (9), 1331–1334. (c) Konradsson, P.; Udodong, U.E.; Fraser-Reid, B. Iodonium promoted reactions of disarmed thioglycosides. Tetrahedron Lett. **1990**, *31* (30), 4313–4316.

15. Paulsen, H.; Tietz, H. Synthese eines Trisaccharides aus *N*-acetylneuraminsäure und *N*-acetyllactosamin. Carbohydr. Res. **1984**, *125*, 47–64.
16. Kunz, H.; Waldmann, H. Synthesis of the glycopeptide partial sequence A^{80}–A^{84} of human fibroblast interferon. Helv. Chim. Acta. **1985**, *68*, 618–622.
17. Vliegenthart, J.F.G.; Dorland, L.; Halbeek, H. High-resolution, ^{1}H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. Adv. Carbohydr. Chem. Biochem. **1983**, *41*, 209–374.

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