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A convenient synthesis of β-D-galactosyl disaccharide derivatives using the β-D-galactosidase from *Bacillus circulans* *

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ABSTRACT

β-D-Gal-(1 → 4)-β-D-GlcNAc-$OC_6H_4NO_2$-*p* (*p*-nitrophenyl *N*-acetyl-β-lactosaminide) and β-D-Gal-(1 → 6)-β-D-GlcNAc-$OC_6H_4NO_2$-*p* (*p*-nitrophenyl *N*-acetyl-β-isolactosaminide) were regioselectively synthesized from lactose and *p*-nitrophenyl 2-acetamido-2-deoxy-glucopyranoside, employing transglycosylation by the β-D-galactosidase from *Bacillus circulans* and by controlling the concentration of organic solvent in the reaction system. The (1 → 4)-linked disaccharide was formed exclusively when the concentration of organic solvent was high, whereas the (1 → 6)-linked isomer was produced with a low concentration. Further utilization of the transglycosylation by the enzyme led to the regioselective formation of β-D-Gal-(1 → 4)-D-GalNAc and β-D-Gal-(1 → 4)-β-D-GalNAc-$OC_6H_4NO_2$-*p*. With the enzyme, β-D-galactosyl transfer occurred preferentially at the O-4 position of GlcNAc and GalNAc, regardless of the configuration of the hydroxyl group.

INTRODUCTION

There is high current interest in developing synthetic routes to oligosaccharides involved in glycoconjugates. From a practical viewpoint, transglycosylation for glycosidases is very attractive for oligosaccharide synthesis[1–5]. Our interest was directed to an enzymic approach to galactosyl-disaccharide units involved in glycoconjugates, because many D-galactose-containing glycoconjugates play an important role in biological recognition events. Generally, the D-galactose residues are found at nonreducing positions in the sugar moieties of asialoglycoproteins. The most abundant linkages are (1 → 4) to GlcNAc, forming the 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-acetyllactosamine) unit in sugar-chain components of glycoproteins[6,7]. We have recently reported that the β-D-galactosi-

* Abbreviations: *p*NP-GlcNAc, *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside; *p*NP-GalNAc, *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside.

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dase from *Bacillus circulans* regioselectively produces *N*-acetyllactosamine on a gram scale from lactose and GlcNAc through a transglycosylation reaction[8].

This report details transglycosylation by *B. circulans* β-D-galactosidase for the regioselective synthesis of β-(1 → 4)-linked galactosyl disaccharides from lactose.

EXPERIMENTAL

Materials.—Commercially available β-D-galactosidase preparations (Biolacta, Daiwa Kasei Co., Ltd., Osaka, Japan) prepared from the culture filtrates of *B. circulans* was used as an enzyme source. The charcoal–Celite column for the separation of transglycosylation products was prepared as follows: equal parts by weight of dry charcoal and Celite were slurried in water and packed into a glass column. All other chemicals were obtained from commercial sources.

Enzyme assay.—β-D-Galactosidase activity was assayed as follows. A mixture containing 2 mM *o*-nitrophenyl β-D-galactopyranoside in 0.9 mL of 50 mM sodium phosphate buffer (pH 7.0) and an appropriate amount of enzyme in a total volume of 0.1 mL was incubated for 10 min at 30°C. The reaction was stopped by adding 0.1 M Na_2CO_3 (2 mL), and then the liberated *o*-nitrophenol was determined spectrophotometrically at 420 nm. One unit of activity was defined as the amount of the enzyme releasing 1 μmol of *o*-nitrophenol per min.

Analytical methods.—HPLC was performed with a YMC-packed column Type A-014 (SIL) (6 mm i.d. × 30 cm) on a YMC-packed column Type AQ-312 (ODS) (6 mm i.d. × 15 cm) in a Shimadzu LC-6A liquid chromatograph equipped with an SPD-6A ultraviolet detector. Elution of the former column was effected with 1 : 4 H_2O–MeCN and the latter with 1 : 9 H_2O–MeOH. The flow rate was 1.0 mL/min at a pressure of 60 kg/cm^2. NMR spectra were determined with a Jeol GSX-400 spectrometer, operating for ^{13}C at 100.0 MHz in the pulsed Fourier-transform mode with computer proton decoupling, and for ^1H at 400 MHz. Chemical shifts are expressed in ppm relative to sodium 4,4-dimethyl-4-silapentanoate-d_4 as the internal standard. The FABMS spectra of the oligosaccharides were recorded with a Jeol DX-303 HF spectrometer, operating at the full accelerating potential (3 kV) and coupled to a Jeol DA-500 mass-data system. The sample in distilled water was added to the glycerol matrix. The molecular weight of the sample was estimated from the *m/z* value of the quasimolecular-ion $(M + H)^+$ peak. Specific rotations were determined with a digital automatic polarimeter PM-101 apparatus (Union Giken Corp, Ltd.). Elemental analyses were performed using a Perkin–Elmer 240C apparatus.

Partially purified β-D-galactosidase from B. circulans.—Crude β-D-galactosidase from *B. circulans* (20 U) was dissolved in 4 mL of 60 mM phosphate buffer (pH 6.0). The enzyme solution was directly loaded onto a GlcNAc-Sepharose CL 4B (tentatively named) column (1.3 × 4 cm) equilibrated with the same buffer. Most of the β-D-galactosidase activity emerged from the column and no β-*N*-acetylhexosaminidase (NAHase) activity was observed when the column was first washed with

the equilibrated buffer (84 mL). The eluates were combined, concentrated to low volume (2 mL) using an Amicon Diaflo unit equipped with a PM-10 membrane operating at 50 lb/in^{-2} pressure, lyophilized, and then stored over $CaSO_4$ at 4°C. This enzyme fraction not showing NAHase activity was used for the enzyme synthesis. When the column was changed to 0.1 M AcOH (36 mL) containing 1 M NaCl and 1% GlcNAc, most of the NAHase activity was eluted.

GlcNAc-Sepharose CL 4B was prepared by coupling *p*-aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside with formyl-Sepharose CL 4B according to our method[9].

Preparation of p-*nitrophenyl 2-acetamido-2-deoxy-4-*O-β-D-*galactopyranosyl-β-*D-*glucoside* (**1**), p-*nitrophenyl 2-acetamido-2-deoxy-6-*O-β-D-*galactopyranosyl-β-*D-*glucoside* (**2**), *and* p-*nitrophenyl 2-acetamido-2-deoxy-β-*D-*4-*O-*(4-*O-β-D-*galactopyranosyl-β-*D-*galactopyranosyl)-β-*D-*glucoside* (**3**).—To a solution of lactose (1.5 g) and *p*NP-GlcNAc (0.3 g) in 20 mM phosphate buffer (pH 7.0) containing 50% MeCN (10 mL) was added partially purified β-D-galactosidase from *B. circulans* (3 U). The molar ratio of the former and latter compounds was ~5:1 and the total substrate concentration was 18%. After the mixture was incubated for 8 h at 30°C, the solution was evaporated to a syrup under diminished pressure (0.1 Torr) at 40°C. The syrup was dissolved in 15 mL of water and then 1/5 vol of the solution was directly loaded onto a Toyopearl HW-40S column as in Fig. 1. The elution was monitored by measuring the absorbance at 300 nm (*p*-nitrophenyl group) and at 485 nm (carbohydrate content, determined by the phenol–H_2SO_4 method). The chromatogram showed one main peak (F-3, tube numbers 104–116) and two minor peaks (F-1: tube numbers 82–89 and F-2: tube numbers 92–98) as transglycosyla-

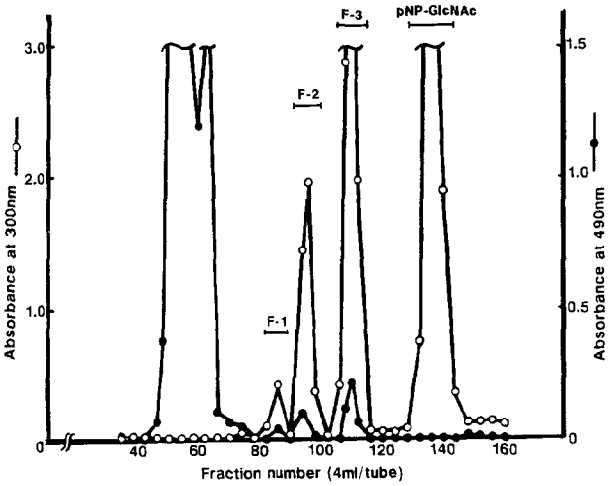

Fig. 1. Chromatographic separation of transglycosylation products by the action of β-D-galactosidase on lactose and *p*NP-GlcNAc, on a column (2.2×95 cm) of Toyopearl HW-40S eluted with 3:1 H_2O–MeOH at 40 mL/h.

tion products for which the absorbance at 300 nm coincides with that at 485 nm. The remaining aliquots were similarly treated. Fraction F-3, after concentration to dryness followed by crystallization from MeOH, gave compound **1** (63.1 mg). Other fractions, F-1 and F-2, were each combined, concentrated, and lyophilized to afford compounds **2** (2.5 mg) and **3** (28.4 mg), respectively. Peak F-4 (tube numbers 130–144) contained *p*NP-GlcNAc used as the acceptor.

Compound **1** had: $[\alpha]_D^{25}$ −18.4° (*c* 1, H_2O); mp 213°C (from MeOH); and *m/z* 505. NMR data (D_2O): ^1H, 8.242 (d, 2 H, *J* 8.1 Hz, *m*-Ph), 7.191 (d, 2H, *J* 9.1 Hz, *o*-Ph), 5.354 (d, 1 H, *J* 8.4 Hz, H-1), 4.531 (d, 1 H, *J* 7.6 Hz, H-1′), and 2.037 (s, 3 H, Ac); ^{13}C, 177.785 (C=O of Ac), 164.573 (Ph carbon attached to the phenolic oxygen), 145.515 (*p*-Ph carbon), 128.964 (*m*-Ph carbon × 2), 119.360 (*o*-Ph carbon × 2), 105.787 (C-1′), 101.379 (C-1), 80.887 (C-4), 78.260 (C-5′), 78.012 (C-5), 75.385 (C-3′), 74.888 (C-3), 73.852 (C-2′), 71.429 (C-4′), 63.927 (C-6′), 62.687 (C-6), 57.724 (C-2), and 24.973 (Me of Ac). Anal. Calcd for $C_{20}H_{28}N_2O_{13} \cdot H_2O$: C, 45.97; H, 5.78; N, 5.35. Found: C, 46.10; H, 5.64; N, 5.34.

Compound **2** had: $[\alpha]_D^{25}$ −12.49 (*c* 1, MeOH) and *m/z* 505. NMR data (D_2O): ^1H, 8.272 (d, 2 H, *J* 9.1 Hz, *m*-Ph), 7.245 (d, 2 H, *J* 9.1 Hz, *o*-Ph), 5.351 (d, 1 H, *J* 8.4 Hz, H-1), 4.440 (d, 1 H, *J* 7.2 Hz, H-1′), and 2.034 (s, 3 H, Ac); ^{13}C, 177.829 (C=O of Ac), 164.518 (Ph carbon attached to the phenolic oxygen), 145.588 (*p*-Ph carbon), 119.452 (*o*-Ph carbon × 2), 128.008 (*m*-Ph carbon × 2), 106.297 (C-1′), 101.452 (C-1), 78.260 (C-5′), 78.026 (C-5), 76.158 (C-3), 75.545 (C-3′), 73.619 (C-2′), 72.334 (C-4), 71.502 (C-4′), 71.254 (C-6), 63.840 (C-6′), 58.133 (C-2), and 24.958 (Me of Ac). Anal. Calcd for $C_{20}H_{28}N_2O_{13} \cdot H_2O$: C, 45.97; H, 5.78; N, 5.36. Found: C, 45.62; H, 5.49; N, 5.11.

Compound **3** had: $[\alpha]_D^{25}$ −4.8° (*c* 1, MeOH) and *m/z* 667. NMR data (D_2O): ^1H, 8.248 (d, 2 H, *J* 9.1 Hz, *m*-Ph), 7.194 (d, 2 H, *J* 9.1 Hz, *o*-Ph), 5.350 (d, 1 H, *J* 8.4 Hz, H-1), 4.623 (d, 1 H, *J* 7.6 Hz, H-1″), 4.560 (d, 1 H, *J* 8.0 Hz, H-1′), and 2.033 (s, 3 H, Ac); ^{13}C, 177.785 (C=O of Ac), 164.533 (Ph carbon attached to the phenolic oxygen), 145.515 (*p*-Ph carbon), 129.964 (*m*-Ph carbon × 2), 119.360 (*o*-Ph carbon × 2), 107.071 (C-1″), 105.801 (C-1′), 101.364 (C-1), 81.018 (C-4), 80.040 (C-4′), 77.997 (C-5′, C-5″), 77.399 (C-5), 76.076 (C-3′), 75.808 (C-3″), 74.334 (C-2″), 74.246 (C-2′), 74.859 (C-3), 71.473 (C-4″), 63.854 (C-6″), 63.606 (C-6′), 62.628 (C-6), 57.695 (C-2), and 24.973 (Me of Ac). Anal. Calcd for $C_{26}H_{38}N_2O_{18} \cdot H_2O$: C, 44.44; H, 5.98; N, 3.98. Found: C, 43.98; H, 5.64; N, 3.67.

Preparation of 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-D-galactose (**4**) *and 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-D-galactose* (**5**).—To a solution of lactose (0.62 g) and GalNAc (0.4 g) in 2.5 mL of 0.1 M phosphate buffer (pH 7.0) was added partially purified β-D-galactosidase from *B. circulans* (5 U). The mixture was incubated for 15 h at 40°C, and then the solution was directly applied to a charcoal–Celite column. The column was first eluted with water (20 mL) and then with a linear gradient of 0 (400 mL)–30% (400 mL) EtOH as in Fig. 2. The elution was monitored by measuring the absorbance at 210 nm (*N*-acetyl group) and at 485 nm (cabohydrate content, determined by the phenol–H_2SO_4 method).

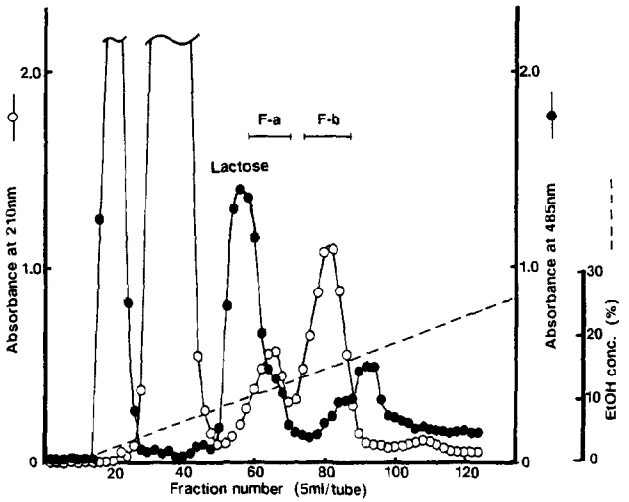

Fig. 2. Chromatography of transglycosylation products by the action of β-D-galactosidase on lactose and GalNAc, on a column (1.5×50 cm) of charcoal–Celite.

The chromatogram showed one minor peak (F-a: tubes 58–70) and one main peak (F-b: tubes 74–87) as transglycosylation products with absorbance at 210 nm. The former peak contained lactose, judging from overlapping of the absorption at 485 nm; it was collected, concentrated, lyophilized (24 mg), and further treated with the same enzyme in order to hydrolyze selectively the undesired lactose in the product mixture. F-a (24 mg) was dissolved in 5 mL of 20 mM phosphate buffer (pH 7.0) containing the *B. circulans* β-D-galactosidase (0.2 U) and the reaction was allowed to proceed at 40°C until lactose was no longer detected by HPLC; the time required for this reaction was 4 h. The reaction was terminated by heating for 15 min at 95°C after adjusting the pH to 3.5 with 0.3 M HCl. The mixture was again adjusted to pH 6.5 with 0.2 M NaOH and concentrated to low volume (2 mL), and then the solution was rechromatographed on the same column to afford a sharp peak (F-a′: tubes 59–68) eluted out with aq 10% EtOH. The elution corresponding to F-a′ was combined, concentrated, and lyophilized to afford compound **4** (14 mg). Fraction F-b was concentrated to low volume (4 mL), and then applied to a column of Bio-Gel P-2 (2.6 × 100 cm, 4 mL/tube). The F-b′ fraction, eluted as a sharp peak (tubes: 78–83), was combined, concentrated, and lyophilized to give compound **5**; yield 52 mg. The structural data for compound **4** was identical to those of β-D-Gal*p*-(1 → 6)-GalNAc already reported[10].

Compound **5** had: $[\alpha]_D^{25}$ +44.6° (*c* 1, H_2O) and *m/z* 383. NMR data (D_2O): ^1H, 5.241 (d, 1 H, *J* 3.3 Hz, H-1α), 4.694 (d, 1 H, *J* 6.7 Hz, H-1β), 4.593 (d, 1 H, *J* 7.0 Hz, H-1′), and 2.060 (s, 3 H, Ac); ^{13}C, 177.924 and 177.632 (C=O of Ac), 107.278 (C-1′), 98.097 (C-1β), 93.982 (C-1α), 80.294 (C-4α), 79.370 (C-4β), 77.962 (C-5′), 77.102 (C-5β), 75.566 (C-3′), 74.378 (C-3β), 74.213 (C-2′), 72.695 (C-5β), 71.433 (C-4′), 70.811 (C-3β), 63.844 (C-6′), 63.606 (C-6β), 63.405 (C-6α), 56.931 (C-2β),

53.639 (C-2α), 25.036 and 24.817 (Me of Ac). Anal. Calcd for $C_{14}H_{25}N_1O_{11} \cdot H_2O$: C, 41.90; H, 6.78; N, 3.49. Found: C, 41.84; H, 6.56; N, 3.25.

Preparation of p-*nitrophenyl 2-acetamido-2-deoxy-4-O-β-*D-*galactopyranosyl-β-*D-*galactoside* (**6**).—To a solution of lactose (1.3 g) and *p*-NP-GalNAc (0.1 g) in 50 mM phosphate buffer (pH 7.0) containing 50% MeCN (5.0 mL) was added partially purified β-D-galactosidase (3 U). The mixture was incubated for 20 h at 30°C, and then the solution was concentrated to a syrup. The syrup was dissolved in 15 mL of water and then 1/5 vol of the solution was directly loaded onto a column (2.2 × 95 cm) of Toyopearl HW-40S. The elution was monitored under the same conditions as in Fig. 1. The chromatogram showed a main peak (tubes 74–82) with two minor peaks as transglycosylation products. The remaining aliquots were similarly processed. The main fraction was combined, concentrated, and lyophilized to afford compound **6** (25.3 mg).

Compound **6** had $[\alpha]_D^{25}$ −4.8° (*c* 1, MeOH) and *m/z* 505. NMR data (D_2O): ^{1}H, 8.253 (d, 2 H, *J* 8.0 Hz, *m*-Ph), 7.211 (d, 2 H, *J* 7.3 Hz, *o*-Ph), 5.311 (d, 1 H, *J* 8.4 Hz, H-1), and 4.646 (d, 1 H, *J* 7.3 Hz, H-1′); ^{13}C, 178.135 (C=O of Ac), 164.562 (Ph carbon attached to the phenolic oxygen), 145.515 (*p*-Ph carbon), 128.949 (*m*-Ph carbon × 2), 119.419 (*o*-Ph carbon × 2), 107.231 (C-1′), 101.729 (C-1), 78.887 (C-4), 78.012 (C-5′), 77.647 (C-5), 75.604 (C-3′), 74.246 (C-3), 73.852 (C-2′), 71.473 (C-4′), 63.898 (C-6′), 63.154 (C-6), 55.477 (C-2), and 25.002 (Me of Ac). Anal. Calcd for $C_{20}H_{28}N_2O_{13} \cdot H_2O$: C, 45.97; H, 5.78; N, 5.35. Found: C, 45.53; H, 5.80; N, 5.09.

RESULTS AND DISCUSSION

Enzymic syntheses of **1**, **2**, *and* **3**.—Disaccharide glycoside **1** was effectively synthesized from lactose and *p*NP-GlcNac on mmolar scale by transglycosylation using the *B. circulans* β-D-galactosidase completely devoid of NAHase activity in an aqueous solution containing 50% acetonitrile. Compound **1** was obtained in a yield of 21.0% based on the *p*NP-GlcNAc added. Trisaccharide glycoside **3** was also obtained in significant amounts. This result suggests that **3** was also formed in significant quantities by successive galactosyl transfer to the 4-position at the nonreducing end galactosyl group of **1**. The sequence β-Gal-(1 → 4)-β-D-Gal-(1 → 4)-D-GlcNAc has been found in the sialooligosaccharide from embryos of *Oryzias latipes*[11]. There was only a small proportion of **2** under the present conditions.

The solvent effects at different concentrations (20, 30, 40, and 50%) in the presence of Me_2SO or acetonitrile on β-D-galactosidase-mediated transglycosylation were also investigated (Fig. 3). The production of **1** and **2** as a function of time were examined on the 0.5-mL scale (Experimental). Samples (10 μL) taken at intervals during incubation were inactivated by adding 20 μL of 1 M acetic acid, and then diluted with 4 vol of water for analysis by HPLC. Compound **1** predominanted over **2** in the initial stage of the reaction regardless of the concentration of organic solvent. However, after formation of **1** reached its maximum, the rate of

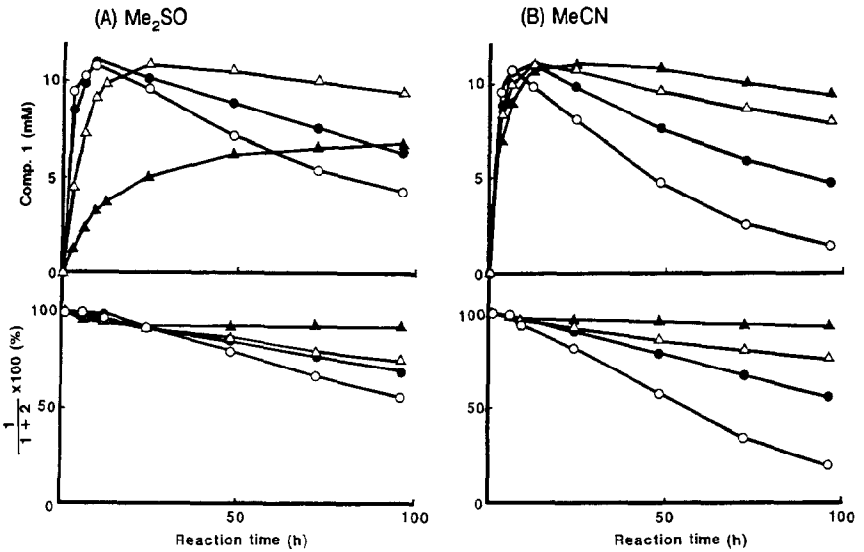

Fig. 3. Effects of Me_2SO and acetonitrile concentration on production of **1**. (A) The enzyme reaction was performed with lactose (75 mg), *p*NP-GlcNAc (15 mg), and β-D-galactosidase (0.2 U) at 30°C in 0.5 mL of 20 mM phosphate buffer (pH 7.0) containing different concentrations (20, ○; 30, ●; 40, △; 50%, ▲) of Me_2SO. (B) Substrates were dissolved in 0.5 mL of the same buffer containing different concentrations of acetonitrile. Other conditions were the same as in (A).

degradation was very dependent on the concentration of organic solvent. At 50% acetonitrile, the time at which maximal production of **1** was reached at 8 h and its concentration then varied little during the subsequent reaction. The use of 50% Me_2SO appears to considerably decrease the transferase activity, although it minimized the formation **2**. Thus, an aqueous-acetonitrile system at a high organic solvent concentration was better suited for production of **1** than that of aqueous Me_2SO. At 20% acetonitrile, formation of **2** was much slower during the entire course of reaction and the time for its maximal production was ~135 h. This result indicates that formation of the (1 → 6) linkage may be favored with a decreasing concentration of organic co-solvent (50–20%, acetonitrile). Based on this result, **2** was prepared from lactose and *p*NP-GlcNAc in a buffer system containing 20% acetonitrile for 135 h (Experimental), and compound **2** was obtained in a yield of 25.5 % based on the *p*NP-GlcNAc added. This was judged the best procedure, taking into account the efficiency of the transglycosylation in forming the desired compound. Organic chemical methods for obtaining **1** and **2** have been developed, but are characterized by elaborate protection and deprotection procedures[12].

Enzymic syntheses of **4**, **5**, *and* **6**.—When GalNAc was used as the acceptor, D-galactosyl transfer occurred preferentially at O-4 of the sugar moiety. Thus, the transglycosylation reaction led to the preferential production of **5** rather than its isomer **4** under these conditions. Compound **5** was obtained in a yield of 13.0%

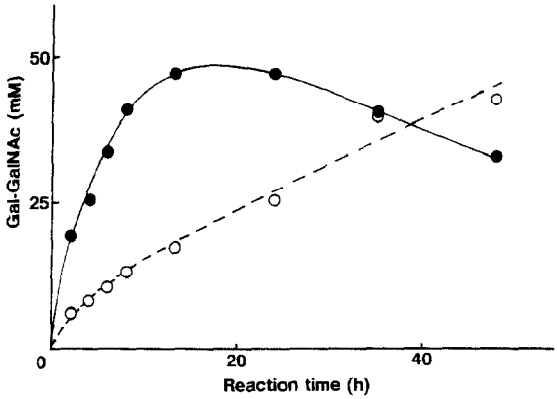

Fig. 4. Time course of β-D-galactosidase-mediated production of **4** and **5**. The amounts of **4** (○) and **5** (●) produced as a function of time were examined on the 0.5-mL scale, as described in the Experimental section, and samples were analyzed by HPLC during incubation.

based on the GalNAc added. Fig. 4 is a transglycosylation profile of the reaction of β-D-galactosidase with lactose and GalNAc. The time at which maximum production of **5** was reached was 15 h, at which time it preponderated 3-fold over **4**. However, once formation of **5** reached its maximum, the amount decreased markedly during the subsequent reaction. On the other hand, formation of **4** was much slower and the time for its maximumal production was ~50 h. The purification of compound **4** was a little more cumbersome than that for **5**, because **4** and the lactose contained in fraction F-a were not readily separable from each other on charcoal–Celite (Fig. 2). The unwanted lactose in the product mixture was therefore selectively removed by hydrolytic treatment with the same enzyme. When the fraction was incubated at a 0.5% concentration, the lactose were hydrolyzed completely after 4 h, whereas <5% of **4** was hydrolyzed during the entire course of the reaction. In a separate experiment, the relative rate of hydrolysis of **4** compared with lactose (100) was 4, a 25-fold difference. Lactose should thus be a much better substrate than **4** under hydrolytic conditions, enabling the selective removal of lactose from the mixture. When the hydrolysis was complete, it was necessary to lower the pH before heating because of the instability of **4** in solution at pH 7.0 at a higher temperature[8]. In the preparation of **6**, a molar excess of lactose was added to the expensive glycoside *p*NP-GalNAc used as an acceptor. The enzyme-catalyzed formation of **6** was also highly regioselective when the concentration of acetonitrile was high (data not shown).

An interesting result from these studies is the preponderant formation of the β-(1 → 4)-linked galactosyl-disaccharide by the *B. circulans* enzyme. In general, β-D-galactosyl transfer occurred preferentially at the primary alcohol group of D-glucose[13]. The β-D-galactosidase from *Kluyveromyces lactis* gave mainly β-D-Gal-(1 → 6)-β-D-GlcNAc (*N*-acetylisolactosamine) from lactose and GlcNAc[9], and *Escherichia coli* enzyme gave **4** from lactose and GalNAc in the transferase

reaction[10]. On the other hand, in a similar reaction, an enzyme from bovine testes induced β-D-galactosyl transfer to a secondary hydroxyl (OH-3) of GlcNAc or GalNAc rather than the primary hydroxyl group[1,2].

CONCLUSION

We have developed a practical route for the synthesis of **1** and its isomer **2**, by transglycosylation employing the β-D-galactosidase from *B. circulans* and controlling the concentration of organic solvent in the reaction system. Further transglycosylation by the enzyme led to the regioselective production of β-(1 → 4)-linked galactosyl-disaccharides containing the GalNAc group. β-D-Galactosyl transfer with the enzyme occurred preferentially at O-4 of *p*NP-GlcNAc (forming **1**), of GalNAc (forming **5**), and of *p*NP-GalNAc (forming **6**), regardless of the configuration of the hydroxyl group. This reaction was efficient enough to allow us to perform a one pot preparation of β-(1 → 4)-linked galactosyl-disaccharide from lactose.

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