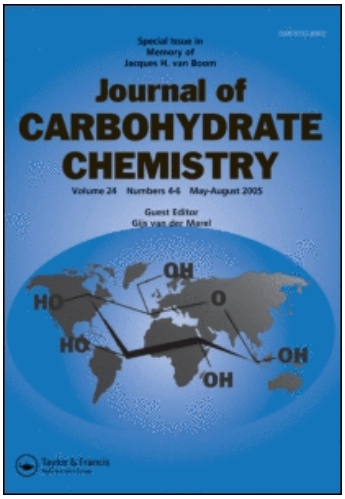

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Glycosidase-Catalyzed Synthesis of Fucosyl Di- and Trisaccharide Derivatives Using α-l-Fucosidase from *Alcaligenes sp*

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ABSTRACT

p-Nitrophenyl glycosides of di-and trisaccharides containing a (1→2)-, (1→3)-, (1→4)-, or (1→6)-linked α-L-fucosyl group were synthesized using the transglycosylation reaction mediated by α-L-fucosidase from *Alcaligenes sp*. with *p*-nitrophenyl glycosides of *N*-acetyllactosamine, lactose, D-GlcNAc, and D-Glc as acceptors. The enzymatic process for preparing these compounds is simple, and the yield is sufficiently high to make the method practical.

Key Words: Transglycosylation; Regioselectivity; α-L-Fucosidase; Fucosyl oligosaccharide.

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INTRODUCTION

The L-sugar fucose is an important recognition component of cell-surface glycans. A number of L-fucose-containing glycoconjugates have been reported to be involved in a variety of biological functions, such as growth regulation, receptor function, cell-cell interaction, and antigenicity.[1] To elucidate the biological functions of L-fucose-containing glycoconjugates, great attention have been paid to the chemical and enzymatic synthesis of fucosyl oligosaccharides. Chemical procedures have been extensively developed,[2–4] but they are frequently complicated due to multiple steps of protection and deprotection. In contrast, protection and deprotection are not required for enzymatic procedures. The enzymatic approach has been done in part with glycosyltransferases and glycosidases.[5] From a practical viewpoint, the use of glycosidases is attractive for oligosaccharides synthesis because glycosidases are inexpensive, stable, and readily available. In the case of α-L-fucosidase, various sources of α-L-fucosidase have been investigated for the ability to transfer a fucose residue to D-Gal, D-Glc, and D-GlcNAc.[6–11] In our previous works, we synthesized α-L-Fuc-(1→3)-β-D-Gal-(1→4)-D-GlcNAc, α-L-Fuc-(1→3)-β-D-Gal-(1→4)-D-Glc, and their analogues through the transglycosylation reaction mediated by α-L-fucosidase from *Alcaligenes sp*.[8] Here, we describe the transglycosylation mediated by α-L-fucosidase from *Alcaligenes sp*. for the synthesis of *p*-nitrophenyl glycosides of fucosyl di- and trisaccharides that could be useful as starting substances for glycopolymers[12,13] or as intermediates for further sugar elongation.

RESULTS AND DISCUSSIONS

It has been reported that α-L-fucosidase from *Alcaligenes sp*. mediates the regioselective transglycosylation from α-L-Fuc-$OC_6H_4NO_2$-*p* **1** to the 3-position of the Gal moiety of β-D-Gal-(1→4)-β-D-GlcNAc (acetyllactosamine), lactose, β-D-Gal-(1→4)-β-D-GlcNAc-OMe, β-D-Gal-(1→4)-β-D-Glc-OMe, and β-D-Gal-(1→3)-β-D-Glc-OMe as the acceptors. The enzyme, however, shows hydrolytic activity to α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-Glc (2′-fucosyllactose), and the relative hydrolysis rate of 2′-fucosyllactose is 77.5 as compared to that of α-L-Fuc-(1→3)-β-D-Gal-(1→4)-D-Glc whose hydrolytic rate is set arbitrarily at 100.[8] Thus, it should form α(1→2)-linked transfer product rather than the α(1→3)-linked product by transglycosylation. Therefore, we chose the *p*-nitrophenyl derivatives of *N*-acetyllactosamine, lactose, D-Glc, and D-GlcNAc as acceptors in order to investigate the regioselectivity of the enzyme. The rational choice of *p*-nitrophenyl derivative as an acceptor was advantageous for detecting and separating transfer products by chromatography due to the presence of the *p*-nitrophenyl group.

At first, **1** and β-D-Gal-(1→4)-β-D-GlcNAc-$OC_6H_4NO_2$-*p* **2**, which was prepared as described previously,[14] were incubated with α-L-fucosidase from *Alcaligenes sp*. at 50°C. The extent of enzymatic conversion was monitored in the course of reaction by HPLC of aliquots (10 μL) taken from the incubation mixture. The chromatogram (Figure 1) showed that two transfer products (26.091 min and 30.809 min, respectively) were formed during the incubation. The reaction mixture was separated by ODS and Toyopearl HW-40S, giving two products in a total yield of 38% based on **1** added.

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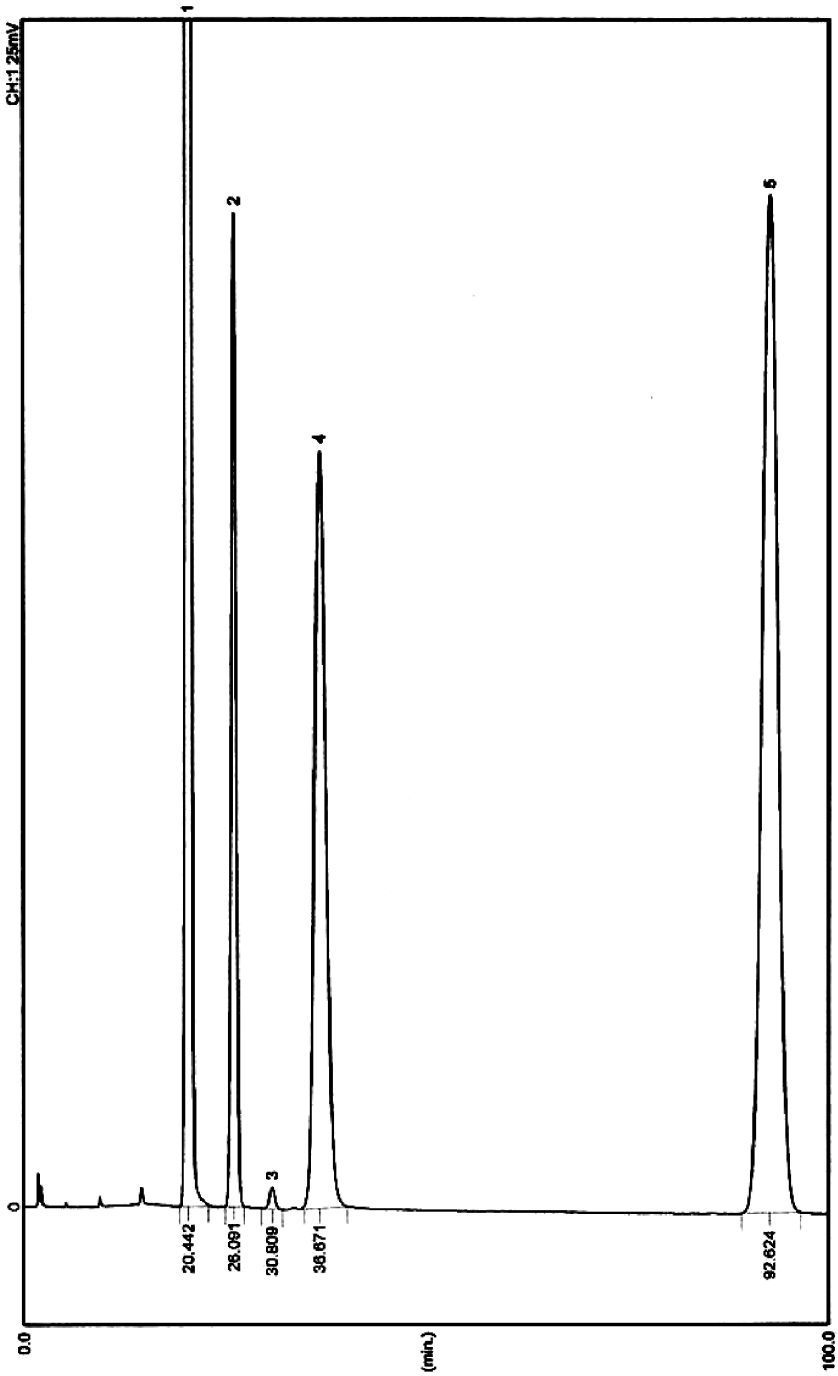

Figure 1. HPLC chromatogram of *Alcaligenes sp.* α-L-fucosidase-mediated transglycosylation reaction mixture with **1** and **2** as substrates. 1: compound **2**, 2: compound **4**, 3: compound **3**, 4: *p*-nitrophenol, 5: compound **1**.

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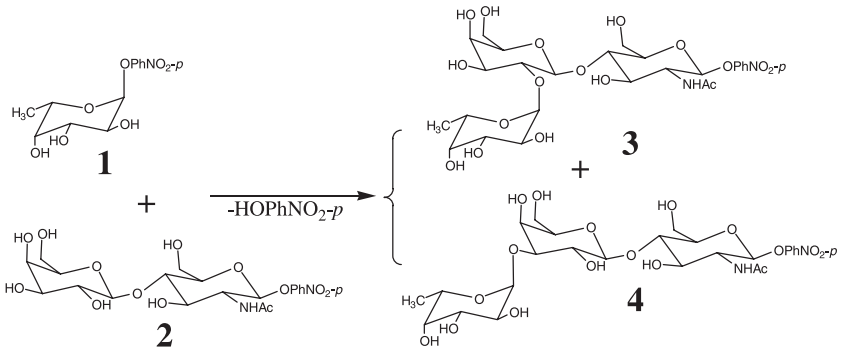

Scheme 1. Formation of fucosyl trisaccharides **3** and **4** by *Alcaligenes sp.* α-L-fucosidase-mediated transglycosylation.

The α(1→3)-linked product **4** as a main transfer product along with α(1→2)-linked isomer **3** was formed in a ratio of 97:3 (Scheme 1, Table 1). By replacing β-D-Gal-(1→4)-β-D-Glc-$OC_6H_4NO_2$-*p* **5**[15] with **2** as acceptor, the α(1→2)-linked transfer product was also detected. However in this case, the α(1→6)-linked isomer was formed in addition to the α(1→2)-and α(1→3)-linked products. The three transfer products **6**–**8** were obtained in an overall yield of 38% and in a molar ratio of 8:88:4 (Scheme 2, Table 1). In both cases, the fucosylation favored the 3-position of the Gal moiety rather than the 2-position.

When β-D-Glc-$OC_6H_4NO_2$-*p* **9** was used as the acceptor instead of **2**, the α(1→3)-linked product **10** was the main product along with the α(1→4)-linked isomer **11**. The two products were obtained in 53% overall yield and in a molar ratio of 85:15. When β-D-GlcNAc-$OC_6H_4NO_2$-*p* **12** was used as acceptor, the α(1→3)-(**13**) and α(1→4)-linked (**14**) transfer products were obtained in a ratio of 91:9, and the yield (13% overall yield) was much lower than that obtained with **9** as the acceptor (Scheme 3, Table 1). It appears that the regioselectivity of fucosyl transfer to the D-GlcNAc moiety is not influenced by the presence of the acetyl group. The low yield may be due to the low solubility of **12** (<1%) in aqueous solution as compared to the higher aqueous

Table 1. Transfer products and yields mediated by α-L-fucosidase from *Alcaligenes sp.*

Acceptor		Product	Yield* (%)
2	**3**	α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-GlcNAc-$OC_6H_4NO_2$-*p*	1
	4	α-L-Fuc-(1→3)-β-D-Gal-(1→4)-D-GlcNAc-$OC_6H_4NO_2$-*p*	37
5	**6**	α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-Glc-$OC_6H_4NO_2$-*p*	3
	7	α-L-Fuc-(1→3)-β-D-Gal-(1→4)-D-Glc-$OC_6H_4NO_2$-*p*	34
	8	α-L-Fuc-(1→6)-β-D-Gal-(1→4)-D-Glc-$OC_6H_4NO_2$-*p*	1
9	**10**	α-L-Fuc-(1→3)-β-D-Glc-$OC_6H_4NO_2$-*p*	45
	11	α-L-Fuc-(1→4)-β-D-Glc-$OC_6H_4NO_2$-*p*	8
12	**13**	α-L-Fuc-(1→3)-β-D-GlcNAc-$OC_6H_4NO_2$-*p*	12
	14	α-L-Fuc-(1→4)-β-D-GlcNAc-$OC_6H_4NO_2$-*p*	1

*Isolated yield based on the donor substrate added.

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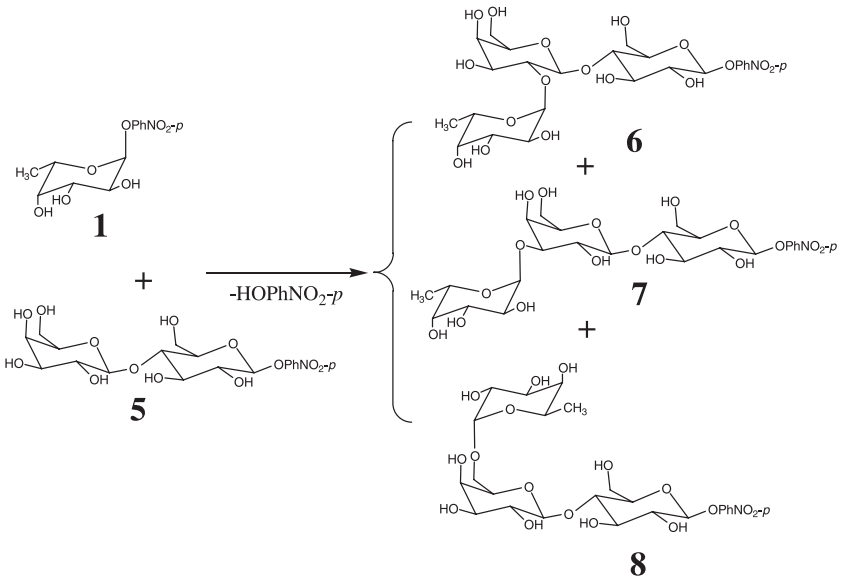
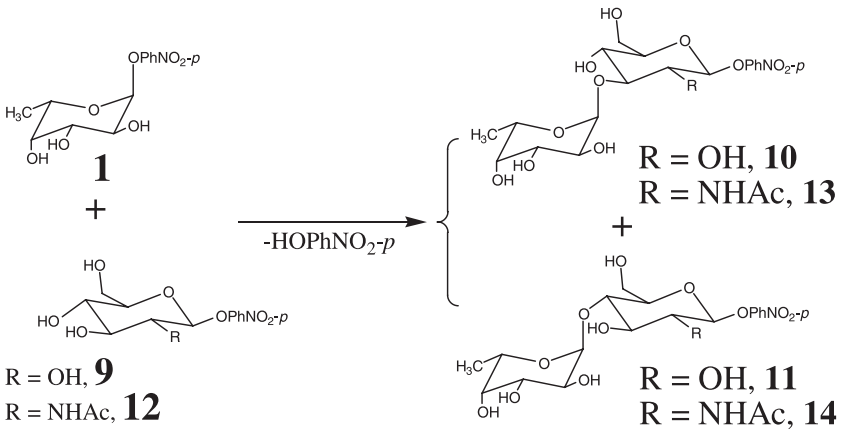


Scheme 2. Formation of fucosyl trisaccharides **6**–**8** by *Alcaligenes sp.* α-L-fucosidase-mediated transglycosylation.

solubility of **9** (>10%), because it is known that the efficiency of the transglycosylation process is dependent on an excess of substrate.

With *p*-nitrophenyl glycoside as acceptor, α-L-fucosidase from *Alcaligenes sp.* catalyzed the transfer of the α-L-fucosyl group to the 2-, 3-, 6-positions of the D-Gal residue, and to the 3- and 4-positions of the D-Glc or D-GlcNAc residues. The transfer products were detected and separated easily by chromatography due to the presence of



Scheme 3. Formation of fucosyl disaccharides **10**, **11**, **13**, **14** by *Alcaligenes sp.* α-L-fucosidase-mediated transglycosylation.

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the *p*-nitrophenyl group. The enzymatic process for preparation of fucosyl di- and trisaccharide derivatives is simple and the yield is sufficiently high to make the method practical. These synthesized compounds will be useful as starting substances for glycopolymers that are valuable for investigation of their biological recognition phenomena by lectins or as intermediates for further sugar elongation.

EXPERIMENTAL SECTION

Analytical methods. HPLC was done with a Jasco Gulliver series liquid chromatography with a column of Mightsyl RP-18 GP (5 × 150 mm, Kanto Chemical Co., Inc., Tokyo, Japan) eluted with 10% MeOH at a flow rate of 1.0 mL/min. ^{1}H and ^{13}C NMR spectra were recorded with a Jeol JNM-EX 270 Fourier transform NMR spectrometer at 30°C. Chemical shifts are expressed in δ units relative to sodium 4,4-dimethyl-4-silapentanoate (TPS) as the external standard in D_2O. Specific rotation was determined with a Digital Automatic Polarimeter DIP-1000 apparatus (Jasco, Japan).

Enzyme assay. The activity of α-L-fucosidase from *Alcaligenes sp.* (kindly supplied by Kumiai Chemical Industry Co., Ltd., Shizuoka, Japan) was assayed as follows. A mixture containing 0.4 mM **1** in 0.9 mL of 50 mM potassium phosphate buffer (pH 7.0) and an appropriate amount of enzyme in a total volume of 1.0 mL was incubated for 10 min at 50°C. Adding 0.5 mL of 1.0 M Na_2CO_3 solution stopped the reaction, and the liberated *p*-nitrophenol was determined spectrophotometrically at 410 nm. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzes 1 μmol of **1** per min.

α-L-Fuc-(1→2)-β-D-Gal-(1→4)-β-D-GlcNAc-$OC_6H_4NO_2$-*p*(3) and α-L-Fuc-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc-$OC_6H_4NO_2$-*p* (4). To a solution of **1** (50 mg) and **2** (200 mg) in 12 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.8 mL of dimethyl sulfoxide (DMSO) was added α-L-fucosidase from *Alcaligenes sp.* (0.47 U). The reaction mixture was incubated for 80 h at 50°C, and heating in a boiling water bath for 5 min terminated the reaction. The resulting insoluble material was filtered off. The solution was directly loaded onto a Chromatorex-ODS DM 1020T column (3 × 50 cm) equilibrated with 10% MeOH in aqueous solution. The column was eluted with the same solution. The fractions (tubes 41–75, 60 mL/tube) were collected, concentrated, and loaded onto a column of Toyopearl HW-40S (4.5 × 90 cm) equilibrated with 25% MeOH in water. The column was eluted with the same solution. F-1 (tubes 89–93, 20 mL/tube) and F-2 (tubes 95–101) were collected, concentrated, and lyophilized to afford compounds **3** (1.2 mg) and **4** (40.8 mg), respectively. NMR data for compound **3**: ^{1}H NMR δ 8.30 (d, 2H, *J* 9.2 Hz, *m*-Ph), 7.24 (d, 2H, *J* 9.2 Hz, *o*-Ph), 5.40 (d, 1H, *J* 4.3 Hz, H-1″), 4.63 (d, 1H, *J* 7.3 Hz, H-1), 2.09 (s, 3H, Me of Ac), and 1.31 (3H, Me of fucosyl residue). ^{13}C NMR δ 177.50 (C═O of Ac), 164.58 (Ph carbon attached to the phenolic oxygen), 145.69 (*p*-Ph carbon), 129.05 (*m*-Ph carbon), 119.53 (*o*-Ph carbon), 103.27 (C-1′), 102.33 (C-1), 101.54 (C-1″), 79.33 (C-4), 78.79 (C-2′), 78.63 (C-5′), 78.24 (C-5), 76.49 (C-3″), 74.63 (C-4″, C-3), 72.59 (C-3″), 72.06 (C-4″), 71.17 (C-2″), 69.92 (C-5″), 64.04 (C-6′), 62.93 (C-6), 57.74 (C-2), 25.03 (Me of Ac), and 18.29 (C-6″). Compound **4** had $[\alpha]_D^{25}$-75.6 (*c* 0.7, water). NMR data: ^{1}H NMR δ 8.24

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(d, 2H, *J* 9.2 Hz, *m*-Ph), 7.18 (d, 2H, *J* 9.2 Hz, *o*-Ph), 5.33 (d, 1H, *J* 8.4 Hz, H-1′), 5.17 (d, 1H, *J* 3.8 Hz, H-1″), 4.57 (d, 1H, H-1), 2.02 (s, 3H, Me of Ac), and 1.20 (3H, Me of fucosyl residue). ^{13}C NMR δ 177.79 (C═O of Ac), 164.54 (Ph carbon attached to the phenolic oxygen), 145.57 (*p*-Ph), 128.97 (*m*-Ph), 119.39 (*o*-Ph), 105.55 (C-1′), 103.79 (C-1), 101.38 (C-1″), 83.18 (C-3′), 80.86 (C-4), 78.17 (C-5′), 78.00 (C-5), 74.91 (C-3), 73.29 (C-2′), 72.27 (C-3″), 71.50 (C-4′), 71.28 (C-2″), 70.04 (C-5″), 63.83 (C-6′), 62.66 (C-6), 57.72 (C-2), 24.94 (Me of Ac), and 18.20 (C-6″).

α-L-Fuc-(1→2)-β-D-Gal-(1→4)-β-D-Glc-OC$_6$H$_4$NO$_2$-*p* (6), α-L-Fuc-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OC$_6$H$_4$NO$_2$-*p* (7), and α-L-Fuc-(1→6)-β-D-Gal-(1→4)-β-D-Glc-OC$_6$H$_4$NO$_2$-*p* (8). To a solution of **1** (100 mg) and **5** (500 mg) in 24 mL of 0.1 M potassium phosphate buffer (pH 7.0) containing 1.0 mL of DMSO was added α-L-fucosidase from *Alcaligenes sp*. (1.0 U). The mixture solution was incubated for 68 h at 50°C; heating in a boiling water bath for 5 min terminated the reaction. The reaction mixture was treated with Chromatorex-ODS DM 1020T and Toyopearl HW-40S columns as described as above to afford **6** (6.5 mg), **7** (69.6 mg), and **8** (2.7 mg). The NMR data of **6**–**8** were identical to those corresponding compounds reported previously.[16]

α-L-Fuc-(1→3)-β-D-Glc-OC$_6$H$_4$NO$_2$-*p* (10), α-L-Fuc-(1→4)-β-D-Glc-OC$_6$H$_4$NO$_2$-*p* (11), α-L-Fuc-(1→3)-β-D-GlcNAc-OC$_6$H$_4$NO$_2$-*p* (13), and α-L-Fuc-(1→4)-β-D-GlcNAc-OC$_6$H$_4$NO$_2$-*p* (14). In a similar manner, compounds **10**, **11**, **13**, and **14** were prepared from **9** and **12** as acceptors, respectively, by use of the transglycosylation of α-L-fucosidase from *Alcaligenes sp*. with **1** as the donor. The NMR data of these compounds were identical to those corresponding compounds reported previously.[16]

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