ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 325 (2000) 120–131

CARBOHYDRATE RESEARCH

Regioselective synthesis of *p*-nitrophenyl glycosides of β-D-galactopyranosyl-disaccharides by transglycosylation with β-D-galactosidases

Xiaoxiong Zeng [a], Rika Yoshino [a], Takeomi Murata [a], Katsumi Ajisaka [b], Taichi Usui [a,*]

[a] *Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka 422-8529, Japan*

[b] *Meiji Institute of Health Science, Meiji Milk Products Co., Ltd., 540 Naruda, Odawara 250-0862, Japan*

Received 1 November 1999; accepted 5 November 1999

Abstract

The β-D-galactosidase from porcine liver induced regiospecific transglycosylation of β-D-galactose from β-D-Gal-$OC_6H_4NO_2$-*o* to OH-6 of, respectively, *p*-nitrophenyl glycoside acceptors of Gal, GlcNAc and GalNAc to afford β-Gal-(1→6)-α-Gal-$OC_6H_4NO_2$-*p*, β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p*, β-Gal-(1→6)-α-GalNAc-$OC_6H_4NO_2$-*p*, β-Gal-(1→6)-β-GalNAc-$OC_6H_4NO_2$-*p*, β-Gal-(1→6)-α-GlcNAc-$OC_6H_4NO_2$-*p*, and β-Gal-(1→6)-β-GlcNAc-$OC_6H_4NO_2$-*p*. The enzyme showed much higher transglycosylation activity for the α-glycoside acceptors than the corresponding β-glycoside acceptors. The regioselectivity of the β-D-galactosidase from *Bacillus circulans* ATCC 31382 greatly depended on the nature of the acceptor. When α-D-GalNAc-$OC_6H_4NO_2$-*p* and α-D-GlcNAc-$OC_6H_4NO_2$-*p* were used as acceptors, the enzyme showed high potency for regioselective synthesis of β-Gal-(1→3)-α-GalNAc-$OC_6H_4NO_2$-*p* and β-Gal-(1→3)-α-GlcNAc-$OC_6H_4NO_2$-*p* in high respective yields of 75.9 and 79.3% based on the acceptors added. However, replacement of β-D-Gal-$OC_6H_4NO_2$-*p* by β-D-GalNAc-$OC_6H_4NO_2$-*p* did change the direction of galactosylation. The enzyme formed regioselectively β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p* with (β-Gal-1→(6-β-Gal-1→)$_n$6-β-Gal-$OC_6H_4NO_2$-*p*, $n = 1–4$). No β-(1→3)-linked product was detected during the reaction. Use of the two readily available β-D-galactosidases facilitates the preparation of (1→3)- and (1→6)-linked disaccharide glycosides of β-D-Gal-GalNAc and β-D-Gal-GlcNAc.

Keywords: β-D-Galactosidase; *p*-Nitrophenyl glycoside of β-D-galactosyl-disaccharides; *p*-Nitrophenyl β-glycoside of (1→6)-galactosyl-oligosaccharides; Regioselectivity; Transglycosylation

1. Introduction

D-Galactose is an important constituent of the carbohydrate chains of glycoconjugates involved in a variety of biological recognition events. The D-galactose residue is generally found at terminal positions in the sugar moieties of asialoglycoproteins, and the usual linkages are (1→4) to GlcNAc, (1→3) to GlcNAc, and (1→3) to GalNAc in sugar-chain components of glycoproteins [1–4]. There is consequently much interest in the chemical or enzymatic synthesis of oligosaccharides containing these linkages. From a practical viewpoint, the use of glycosidases is attractive for oligosaccharide synthesis [5–12], because glycosidases do have some regioselec-

* Corresponding author. Tel./fax: +81-54-238-4873.
E-mail address: actusui@agr.shizuoka.ac.jp (T. Usui)

0008-6215/00/$ - see front matter
PII: S0008-6215(99)00303-1

tivity for the hydroxyl linkage to the acceptor, and their selectivity may vary with different enzymes [13]. For example, the β-D-galactosidase from *Bacillus circulans* mediated the transfer of β-D-galactosyl from lactose to the secondary hydroxyl group (OH-4) over the primary hydroxyl group of GlcNAc, whereas the β-D-galactosidase from *Kluyveromyces lactis* affords β-D-Gal-(1→6)-D-GlcNAc as the main transglycosylation product from lactose and GlcNAc [14]. Our interest is aimed at β-D-galactosidase-mediated synthesis of *p*-nitrophenyl glycosides of galactosyl-disaccharides with different linkages as the units mimicking the carbohydrate chains of glycoconjugates [15–17]. In our recent work, α-L-fucosidase and β-D-galactosidase from porcine liver have been separated from each other. The former catalyzes the formation of an α-L-(1→2)-linked product with its isomers fucosylated at OH-3 or OH-6 of the galactosyl residue of LacNAc or *p*-nitrophenyl β-D-lactoside [18,19]. In the present report, we describe the transglycosylation by β-D-galactosidases from porcine liver and *B. circulans* ATCC 31382 for the synthesis of *p*-nitrophenyl glycosides of galactosyl-disaccharides and the regioselectivity of the two enzymes toward various of *p*-nitrophenyl glycoside acceptors.

2. Results and discussion

Transglycosylation of β-D-galactosidase from porcine liver.—The fresh extract from porcine liver was first dialyzed against 50 mM NaOAc buffer (pH 5.2) overnight. The supernatant from the dialyzate was loaded directly onto an ion-exchange column pre-equilibrated with 50 mM NaOAc buffer (pH 5.2). The fractions containing β-D-galactosidase activity were pooled, concentrated to low volume, and loaded onto the same column treated as already described, but the pH of the buffer was changed to 4.5. The partially purified β-D-galactosidase, completely devoid of *N*-acetylhexosaminidases [which degrades the acceptor substrates α- and β-HexNAc-$OC_6H_4NO_2$-*p* (see later)], was obtained in a specific activity of 1.9 U/mg protein with a 51-fold purification. The enzyme was used for the regioselective synthesis of *p*-nitrophenyl galactosyl-disaccharides without further purification. The galactosidase from porcine liver had an optimum pH of 5.5 with β-D-Gal-$OC_6H_4NO_2$-*o* as substrate. The enzyme showed an optimum temperature of 50 °C, while the enzyme was stable only below 40 °C. The enzyme hydrolyzed β-D-Gal-$OC_6H_4NO_2$-*o* and β-D-Gal-$OC_6H_4NO_2$-*p* as good substrates, but not lactose and *N*-acetyllactosamine (data not shown).

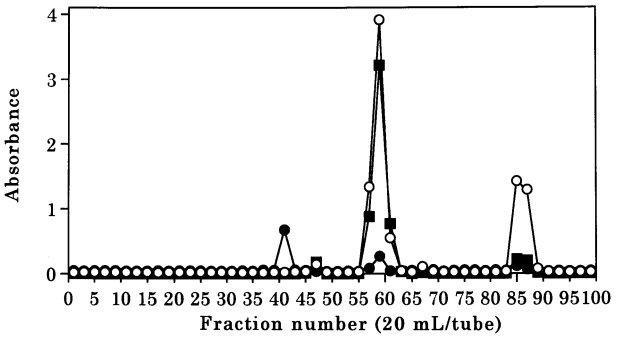

Fig. 1. Toyopearl HW-40S chromatographic separation of transglycosylation products with β-D-Gal-$OC_6H_4NO_2$-*o* and α-D-GalNAc-$OC_6H_4NO_2$-*p* as substrates by use of β-D-galactosidase from porcine liver. (○) Absorbance at 210 nm, (■) absorbance at 300 nm, and (●) absorbance at 485 nm.

When α-D-GalNAc-$OC_6H_4NO_2$-*p* and β-D-Gal-$OC_6H_4NO_2$-*o* were incubated with β-D-galactosidase from porcine liver, the transfer product was monitored by high-performance liquid chromatography (HPLC). HPLC showed that one transfer product was formed during the incubation. The mixture was readily separated on a column of Toyopearl HW-40S to afford compound **1** in a yield of 78.7% based on the acceptor added (Fig. 1). Similarly with β-D-GalNAc-$OC_6H_4NO_2$-*p*, α-D-GlcNAc-$OC_6H_4NO_2$-*p*, β-D-GlcNAc-$OC_6H_4NO_2$-*p*, β-D-Gal-$OC_6H_4NO_2$-*p*, and α-D-Gal-$OC_6H_4NO_2$-*p* as acceptors, the β-(1→6)-linked transfer products were obtained in yields of 28.1, 83.8, 25.7, 21.2, and 63.9%, respectively (Table 1). No β-(1→4)- or β-(1→3)-linked product was detected during the reaction. The use of α-glycoside acceptors gave much higher yields (63–84%) than those of their corresponding β-glycoside acceptors (21–28%). The enzyme transferred the Gal

Table 1
The yields of transfer products mediated by β-D-galactosidases from porcine liver and *Bacillus circulans* ATCC 31382

Sources of β-D-galactosidase	Acceptor	Product	Yield [a] (%)
Porcine liver	GalNAcα-*p*NP	Galβ1→6GalNAcα-*p*NP (**1**)	78.7
	GalNAcβ-*p*NP	Galβ1→6GalNAcβ-*p*NP (**3**)	28.1
	GlcNAcα-*p*NP	Galβ1→6GlcNAcα-*p*NP (**7**)	83.8
	GlcNAcβ-*p*NP	Galβ1→6GlcNAcβ-*p*NP (**5**)	25.7
	Galα-*p*NP	Galβ1→6Galα-*p*NP (**14**)	63.9
	Galβ-*p*NP	Galβ1→6Galβ-*p*NP (**9**)	21.2
Bacillus circulans ATCC 31382	GalNAcα-*p*NP	Galβ1→3GalNAcα-*p*NP (**2**)	75.9
		Galβ1→6GalNAcα-*p*NP (**1**)	3.2
	GalNAcβ-*p*NP	Galβ1→3GalNAcβ-*p*NP (**4**)	20.9
		Galβ1→6GalNAcβ-*p*NP (**3**)	18.6
	GlcNAcα-*p*NP	Galβ1→3GlcNAcα-*p*NP (**8**)	79.3
		Galβ1→6GlcNAcα-*p*NP (**7**)	0.3
	GlcNAcβ-*p*NP	Galβ1→3GlcNAcβ-*p*NP (**6**)	29.5
		Galβ1→6GlcNAcβ-*p*NP (**5**)	6.0
	Galα-*p*NP	Galβ1→3Galα-*p*NP (**15**)	9.3
		Galβ1→6Galα-*p*NP (**14**)	22.8
		Galβ1→6Galβ1→6Galα-*p*NP (**16**)	4.3
		Galβ1→(6Galβ1→)$_2$6Galα-*p*NP (**17**)	2.6
	Galβ-*p*NP	Galβ1→6Galβ-*p*NP (**9**)	11.7
		Galβ1→6Galβ1→6Galβ-*p*NP (**10**)	6.8
		Galβ1→(6Galβ1→)$_2$6Galβ-*p*NP (**11**)	4.3
		Galβ1→(6Galβ1→)$_3$6Galβ-*p*NP (**12**)	1.7
		Galβ1→(6Galβ1→)$_4$6Galβ-*p*NP (**13**)	0.5

[a] The yield is calculated based on the acceptor added.

group much more extensively to OH-6 of the α-glycoside acceptor than that of the corresponding β-glycoside. This shows that the orientation of the hydrophobic *p*-nitrophenyl group in the acceptor greatly influences the efficiency of transglycosylation. The efficiency of the transglycosylation was strongly dependent on the molar ratio of the donor to acceptor substrates. The reaction employing the molar ratio of 3.0 was suitable for the effective syntheses of the corresponding β-(1→6)-linked disaccharide glycosides (Fig. 2). Once formation of compound **7** reached its maximum, no decomposition of product could be observed. This was also the same trend for other acceptors (data not shown).

In a separate experiment, the relative hydrolytic rates of *p*-nitrophenyl galactosyl-*N*-acetylglycosaminides toward porcine liver β-D-galactosidase were measured and are shown in Table 2. The initial rate of reaction was (1→6) > (1→3). The enzyme did not hydrolyze the (1→4) linkage. In general, the order of hydrolytic activity toward those disaccharides closely corresponds to that of transglycosylation activity. In this case, however, the enzyme produced only (1→6)-linked transfer product, and no (1→3) transfer product was detected during the reaction.

Transglycosylation of β-D-galactosidase from B. circulans *ATCC 31382.*—β-D-Galac-

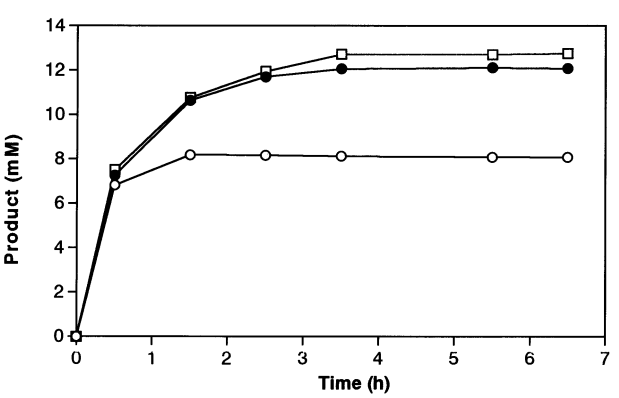

Fig. 2. Effects of the molar ratio of donor/acceptor on the formation of β-Gal-(1→6)-α-GlcNAc-$OC_6H_4NO_2$-*p* mediated by β-D-galactosidase from porcine liver. (○), (●), and (■) represent molar ratios of 1.5, 3.0, and 4.0 for donor/acceptor, respectively.

Table 2
The hydrolytic rates of β-D-galactosidase acting on *p*-nitrophenyl galactosyl-*N*-acetylglucosaminides

Compounds	Enzyme	
	β-D-Galactosidase from porcine liver	β-D-Galactosidase from *B. cirulans* ATCC 31382
β-Gal-(1→3)-β-GlcNAc-$OC_6H_4NO_2$-*p*	18	100 [a]
β-Gal-(1→4)-β-GlcNAc-$OC_6H_4NO_2$-*p*	0	5
β-Gal-(1→6)-β-GlcNAc-$OC_6H_4NO_2$-*p*	100 [b]	14

[a] The hydrolytic rate on β-Gal-(1→3)-β-GlcNAc-$OC_6H_4NO_2$-*p* by β-D-galactosidasc from *B. cirulans* ATCC 31382 was arbitrarily set at 100.

[b] The hydrolytic rate on β-Gal-(1→6)-β-GlcNAc-$OC_6H_4NO_2$-*p* by β-D-galactosidase from porcine liver was arbitrarily set at 100.

tosidase from *B. circulans* ATCC 31382 predominated the formation of the (1→3)-linked compound **2** over the (1→6)-linked isomer (**1**) through Gal transfer from β-D-Gal-$OC_6H_4NO_2$-*o* to α-D-GalNAc-$OC_6H_4NO_2$-*p*. The transfer products **2** and **1** were obtained in 79.1% overall yield based on the acceptor added and in a ratio of 96:4. This was also the case for the formation of compound **8** with α-D-GlcNAc-$OC_6H_4NO_2$-*p* as acceptor (Table 1). These transfer products were readily separated on Chromatorex-ODS and Toyopearl HW-40S columns. The unreacted acceptors could be recovered by straightforward chromatography and reutilized. The enzymatic process for obtaining the disaccharides **2** and **8** was simple and the yield is sufficiently high to make the method practical. The enzyme formed regioselectively the β-(1→6)-linked disaccharide **9** along with a series of β-(1→6)-linked glycosides of galactopyranosyl-oligosaccharides (**10**–**13**). No other linkage in the transfer products was detected during the reaction. In the case of the α-D-Gal-$OC_6H_4NO_2$-*p* acceptor, the β-(1→6)-linked disaccharide (**14**) was formed along with the β-(1→3)-linked one (**15**) in a molar ratio of 19:6, and together with the β-(1→6)-linked galactosyl-trisaccharide (**16**) and galactosyl-tetrasaccharide (**17**). In both reactions, β-(1→6)-linked galactosyl-oligosaccharides larger than the dimer were formed as transfer products. It is apparent that these oligomers are produced by successive regioselective galactosylation from the disaccharides **9** and **14** formed initially. The results indicate that the enzyme can be used to synthesize β-glycosides of (1→6)-β-D-galactopyranosyl-oligosaccharides (degree of polymerization 2–5) in one step. Much work has been done on the chemical synthesis of *O*-β-(1→6)-D-galactopyranosyl-oligosaccharides that act as ligands of anti-(1→6)-β-D-galactopyranan antibodies [20–24]. For example, Ekborg et al. have reported the synthesis of compounds **9** and **10** from β-D-Gal-$OC_6H_4NO_2$-*p* using a chemical method [20]. The present process for obtaining β-glycosides of (1→6)-β-D-galactosyl-oligosaccharides is simple and the yield is sufficiently high to make the method practical. Recently, Fransen et al. have reported the enzymatic synthesis of [(β-D-Gal*p*-(1→4))$_n$-D-Glc*p*] and [(β-D-Gal*p*-(1→6))$_n$-β-D-Gal*p*-(1→4)-D-Glc*p*] ($n = 0$–5) from lactose using β-D-galactosidase [25].

Regioselectivity of formation of p-*nitrophenyl glycosides of β-D-galactopyranosyl-disaccharides by β-D-galactosidase from* B. circulans *ATCC 31382.*—The positions of galactosylation mediated by β-D-galactosidase from *B. circulans* ATCC 31382 with α-D-GalNAc-$OC_6H_4NO_2$-*p*, β-D-GalNAc-$OC_6H_4NO_2$-*p*, α-D-GlcNAc-$OC_6H_4NO_2$-*p*, β-D-GlcNAc-$OC_6H_4NO_2$-*p*, α-D-Gal-$OC_6H_4NO_2$-*p*, and β-D-Gal-$OC_6H_4NO_2$-*p* acceptors are depicted by arrows in Fig. 3. It may be seen that the regioselectivity of the β-D-galactosidase from *B. circulans* ATCC 31382 is markedly influenced by the presence of the 2-acetamido group and the anomeric configuration of the aglycon in the acceptor. In the case of α-D-GalNAc-$OC_6H_4NO_2$-*p* and α-D-GlcNAc-$OC_6H_4NO_2$-*p* acceptors, galactosylation occurs

overwhelmingly at OH-3 and is accompanied by a high efficiency of transglycosylation. This can be tentatively explained by comparing the stereochemical environment between acceptor and the active site of the enzyme with regard to reaction sites. Thus, two hydrophobic groups, the 2-acetamido and *p*-nitrophenyl groups in the α-glycoside acceptor, may bind to the enzyme and position the substrate molecular properly on the active site so that they are located in relation to the hydrophobic site of the enzyme, as shown in Fig. 4(A). This shows that the regioselectivity of β-D-galactosyl transfer to the acceptor catalyzed by the enzyme is strongly determined by the 1,2-cis configuration of 2-acetamido and 1-*p*-nitrophenyl groups, which adopt an orientation favorable to the enzyme. Replacement of β-glycosides of GalNAc and GlcNAc by their corresponding α-glycosides diminishes not

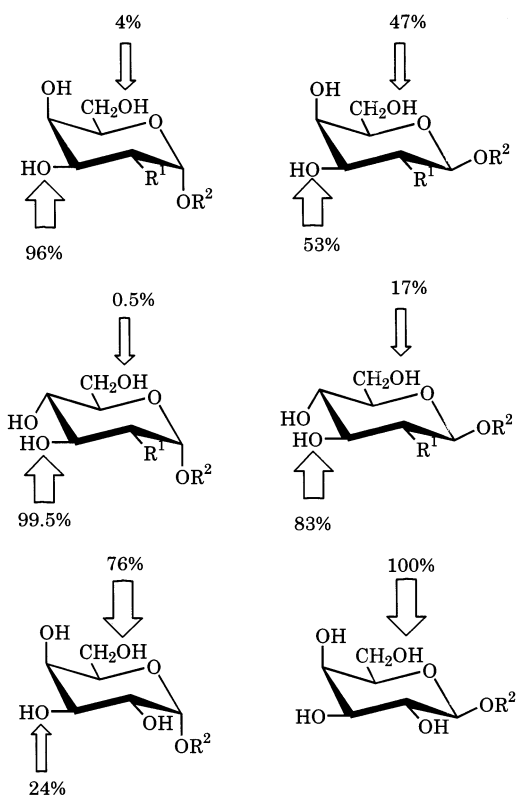

Fig. 3. Structures of acceptors for β-D-galactosidase-mediated galactosylation (β-D-galactosidase from *B. circulans* ATCC 31382). Arrows indicate the positions of galactosylation. Percentages above and below the arrows are of the formation of a given transglycosylation as compared with the total. The percentages of galactosylation are based on the time at which the (1→3)-linked disaccharide derivative production reaches its maximum. R^1: *N*-acetyl group, and R^2: *p*-nitrophenyl group.

only the regioselectivity at OH-3, but also the efficiency of transglycosylation. The decrease in activity may be attributed to the unsuitable configuration of the *p*-nitrophenyl group to the hydrophobic binding locus in the active site. On the other hand, if this concept is applied to the β-D-Gal-$OC_6H_4NO_2$-*p* acceptor, it would not adopt a favorable orientation along C-1 to C-2, because of an unsuitable configuration of the 1-*p*-nitrophenyl group and a repulsion of the hydrophilic 2-hydroxyl group to the hydrophobic binding locus in the active site, as shown in Fig. 4(B-a). In an alternative plan (shown in Fig. 4(B-b)), when the structural form of β-D-Gal-$OC_6H_4NO_2$-*p* to the binding locus is reversed along C-1 to C-4, the acceptor will be properly located in relation to the hydrophobic site so that it adopts a favorable orientation along C-1 to the ring oxygen atoms. This enables us to assume that only OH-6 of the β-D-Gal-$OC_6H_4NO_2$-*p* acceptor is galactosylated to form the β-(1→6)-galactosyl-disaccharide glycoside. It has already been reported that the regioselectivity of glycosidase-catalyzed formation of disaccharides is greatly influenced by the nature of the acceptor, such as its anomeric configuration, presence of a 2-acetamido group, and the substitution mode at the anomeric carbon atoms [15,26–28].

Table 2 shows the relative hydrolytic rates of *p*-nitrophenyl galactosyl-*N*-acetylglucosaminide toward β-D-galactosidase from *B. circulans* ATCC 31382. The relative rates of hydrolysis for compound **5** and β-Gal-(1→4)-β-GlcNAc-$OC_6H_4NO_2$-*p* as compared with compound **6** (set at 100) were 5 and 14, respectively, namely, 7–14-fold differences. Compound **6** should be a much better substrate than compound **5**. Thus, the order of the hydrolytic rates of compounds **6** and **5** corresponds to that of their transglycosylation.

In conclusion, the regiospecific synthesis of β-(1→6)-galactosyl-disaccharide glycosides was achieved by using transglycosylation by the readily available β-D-galactosidase from porcine liver. We also developed a synthetic method for compound **2**, which is a mimic unit of mucin type I and its analogs, by transglycosylation mediated by the β-D-galactosi-

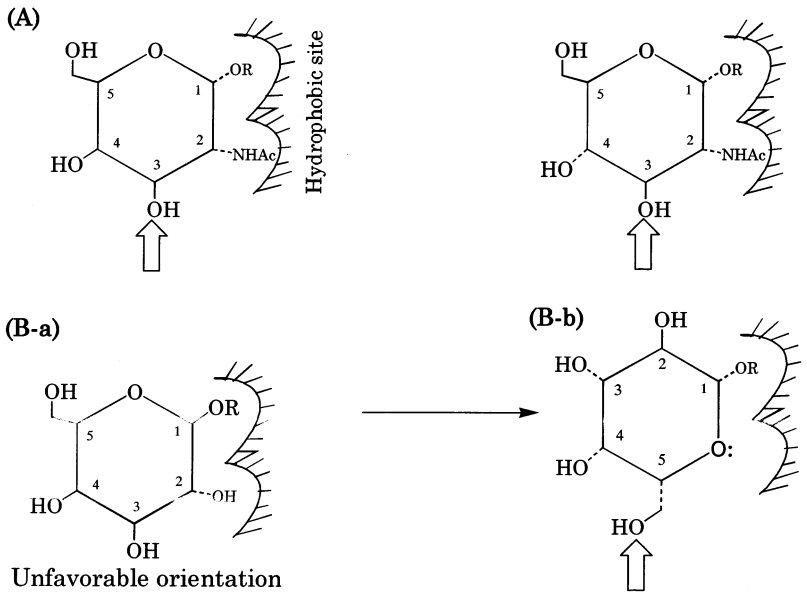

Fig. 4. (A) The postulated hydrophobic–acceptor binding-site of β-D-galactosidase, which positions the acceptor properly on the active site. Arrows indicate the positions of galactosylation. (A) α-D-GalNAc-$OC_6H_4NO_2$-*p* and α-D-GlcNAc-$OC_6H_4NO_2$-*p*, (B) β-D-Gal-$OC_6H_4NO_2$-*p*.

dase from *B. circulans* ATCC 31382. The enzymatic process for obtaining the desired compounds **2** and **8** is simple and the yields (75–80%) are remarkably high. The synthetic oligosaccharide glycosides are useful as starting substances for glycopolymers, which are valuable for investigating biological recognition phenomena using lectins [29–31].

3. Experimental

Materials.—α - D - GalNAc - $OC_6H_4NO_2$ - *p* was purchased from Yaizu Suisan Kagaku Co., Ltd. (Yaizu, Japan). α-D-Gal-$OC_6H_4NO_2$-*p*, β-D-Gal-$OC_6H_4NO_2$-*p*, β-D-GalNAc-$OC_6H_4NO_2$-*p*, and α-D-GlcNAc-$OC_6H_4NO_2$-*p* were obtained from Sigma Chemical Co. (St Louis, MO, USA). CM-Sepharose Fast Flow was a product of Pharmacia. β-D-Galactosidase from *B. circulans* ATCC 31382 was kindly supplied from Meiji Institute of Health Science (Odawara, Japan).

Assay of β-D-galactosidase activity.—The activity was assayed as follows. A mixture containing 2.0 mM β-D-Gal-$OC_6H_4NO_2$-*o* in 0.9 mL NaOAc buffer (pH 5.5) and an appropriate amount of enzyme to a total volume of 1.0 mL was incubated for 10 min at 37 °C. The reaction was stopped by adding 0.5 mL of 1.0 M Na_2CO_3 soln, and the liberated *o*-nitrophenol was determined spectrophotometrically at 420 nm. One unit of enzyme activity was defined as the amount of enzyme hydrolyzing 1 μmol of β-D-Gal-$OC_6H_4NO_2$-*o* per minute.

Analytical methods.—HPLC was performed with a Hitachi 6000 liquid chromatograph with a column of Shodex Asahipak NH2P-50 4E (4.6 × 250 mm, Showa Denko Corp., Tokyo, Japan) developed with 80% of MeCN in aq soln at a flow rate of 0.8 mL/min, and a column of TSKgel Super ODS (4.6 × 100 mm, Tosoh Corp., Tokyo, Japan) eluted with 10% MeOH at a flow rate of 1.0 mL/min, both with an L-4000 UV detector. ^1H and ^{13}C NMR spectra were recorded with a Jeol JNM-EX 270 Fourier transform NMR spectrometer at 25 °C. Chemical shifts are expressed in δ relative to sodium 4,4-dimethy-4-silapentanoate (TPS) as the external standard in D_2O. The assignment of signals in the transfer products was obtained by ^{13}C–^1H COSY and also by analogy with the chemical shifts of related compounds based on earlier data [7,16,17,20]. FABMS was performed in the positive mode using a Jeol JMS DX-303 HF mass spectrometer coupled to a Jeol DA-800 mass data system. An accelerating voltage of 10 kV and a mass soln of 1000 were em-

ployed. Mass calibration was achieved using Ultramark. Specific rotation was determined with a Digital Automatic Polarimeter DIP-1000 apparatus (Jasco, Japan).

Preparation of β-D-galactosidase from porcine liver.—A crude enzyme soln from fresh porcine liver was prepared by the method reported [19]. The extract was dialyzed overnight against 50 mM NaOAc buffer (pH 5.2). After centrifugation (24,100*g*, 20 min), the supernatant (20 mL) was applied to a column (3.2 × 26 cm) of CM-Sepharose Fast Flow pre-equilibrated with 50 mM NaOAc buffer (pH 5.2). The column was washed with 400 mL of the same buffer. Next the column was eluted with a linear gradient of NaCl concentration from 0 (400 mL) to 1.0 M (400 mL). The fractions containing β-D-galactosidase activity (20 mL/tube, tubes 9–13) were combined, concentrated to a low volume with an Amicon Diaflo unit equipped with a PM10 membrane operating at 2 kg/cm^2, and loaded to the same column pre-equilibrated with 50 mM NaOAc buffer (pH 4.5). Then the column was treated as already described. The fractions containing β-D-galactosidase activity (tubes 31–35) were combined and concentrated to a low volume, giving 1.9 U/mg protein of specific activity.

Effects of pH and temperature on the activity of β-D-galactosidase from porcine liver.—For the determination of optimum pH, the activity of β-D-galactosidase from porcine liver was measured at 37 °C for 10 min at various pH values using sodium citrate buffer (pH 2.0–3.0), NaOAc buffer (pH 3.0–6.0), and sodium phosphate buffer (pH 6.0–9.0). To determine optimum temperature, the β-D-galactosidase was incubated for 10 min at various temperatures in 0.1 M NaOAc buffer (pH 5.5). The thermal stability of the enzyme was examined by incubating the enzyme at various temperatures for 30 min, and the activity was measured as already described.

Hydrolysis reaction by β-D-galactosidases on p-*nitrophenyl galactosyl*-N-*acetylglucosaminides.*—To a soln containing *p*-nitrophenyl galactosyl-*N*-acetylglucosaminide (250 μg) in 50 mM NaOAc buffer (pH 5.5, 1.0 mL) was added β-D-galactosidase from porcine liver (partially purified) or from *B. circulans* ATCC 31382 (0.4 U). The soln was incubated at 37 °C, samples (100 μL) were taken out at appropriate time intervals (0, 5, 10, 15, 20 min) during the incubation, and were analyzed by HPLC with a column of TSKgel G-Oligo-PW (4.6 × 100 mm, Tosoh Corp., Tokyo, Japan). The following *p*-nitrophenyl galactosyl-glycosides were used: β-Gal-(1 → 3)-β-GlcNAc-$OC_6H_4NO_2$-*p*, β-Gal-(1 → 4)-β-GlcNAc-$OC_6H_4NO_2$-*p*, and β-Gal-(1 → 6)-β-GlcNAc-$OC_6H_4NO_2$-*p*.

*Preparation of β-Gal-(1 → 6)-α-GalNAc-$OC_6H_4NO_2$-*p (**1**) *and β-Gal-(1 → 3)-α-GalNAc-$OC_6H_4NO_2$-*p (**2**)

(A) Using β-D-galactosidase from porcine liver. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (120 mg) and α-D-GalNAc-$OC_6H_4NO_2$-*p* (44 mg) in 8 mL of 40 mM NaOAc buffer (pH 5.5) was added partially purified β-D-galactosidase from porcine liver (0.8 U). The reaction was terminated by heating in a boiling-water bath for 5 min after 8.5 h of incubation at 40 °C. The supernatant from the centrifugation was extracted with diethyl ether to remove the *o*-nitrophenol liberated during the reaction, concentrated, and loaded onto a Toyopearl HW-40S column (4.5 × 90 cm) equilibrated with 25% MeOH in water. The column was eluted with the same soln. The elution was monitored by measuring the absorbances at 300 nm (the *p*-nitrophenyl group) and 485 nm (carbohydrate content, determined by the phenol–sulfuric acid method). The fractions (tubes 57–64) containing transfer product as shown in Fig. 1 were collected, concentrated, and lyophilized to afford compound **1** (51.0 mg). The physical and NMR data for compound **1** were identical to those of β-Gal-(1 → 6)-α-GalNAc-$OC_6H_4NO_2$-*p* reported previously [17].

(B) Using β-D-galactosidase from B. circulans *ATCC 31382.* To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (600 mg) and α-D-GalNAc-$OC_6H_4NO_2$-*p* (220 mg) in 40 mM NaOAc buffer (40 mL, pH 5.5) was added β-D-galactosidase from *B. circulans* ATCC 31382 (2.0 U). After 3.5 h of incubation at 50 °C, the reaction was terminated by heating in a boiling-water bath for 5 min. The supernatant from the centrifugation was applied to a column (3.6 × 52 cm) of Chromatorex-

ODS DM 1020T equilibrated with 10% MeOH in water. The column was eluted with the same soln. The fractions (60 mL/tube, tubes 58–101) were combined, concentrated, and loaded onto a Toyopearl HW-40S column treated as already described. The F-1 (30 mL/tube, tubes 59–63) and F-2 (tubes 67–75) fractions were combined, concentrated, and lyophilized to afford compounds **1** (10.5 mg) and **2** (245.7 mg), respectively. The physical and NMR data for compound **2** were identical to those of β-Gal-(1→3)-α-GalNAc-$OC_6H_4NO_2$-*p*, reported previously [17].

Preparation of β-Gal-(1→6)-β-GalNAc-$OC_6H_4NO_2$-p (**3**) *and β-Gal-(1→3)-α-GalNAc-$OC_6H_4NO_2$*-p (**4**)

*(A) Using β-*D*-galactosidase from porcine liver*. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (900 mg) and β-D-GalNAc-$OC_6H_4NO_2$-*p* (330 mg) in 40 mM NaOAc buffer (120 mL) was added partially purified β-D-galactosidase (5 U) from porcine liver. The reaction was terminated by heating in a boiling-water bath for 5 min after 8 h of incubation at 40 °C. The reaction mixture was treated as described for the preparation of compound **1** using β-D-galactosidase from porcine liver. The fractions (tubes 58–66) were combined, concentrated, and lyophilized to afford compound **3** (107.6 mg). The physical and NMR data for compound **3** were identical to those of β-Gal-(1→6)-β-GalNAc-$OC_6H_4NO_2$-*p*, reported previously [17].

*(B) Using β-*D*-galactosidase from* B. circulans *ATCC 31382*. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (900 mg) and β-D-GalNAc-$OC_6H_4NO_2$-*p* (330 mg) in 40 mM NaOAc buffer (120 mL) was added β-D-galactosidase (5.0 U) from *B. circulans* ATCC 31382. After 6 h of incubation at 50 °C, the reaction was terminated by heating in a boiling-water bath for 5 min. The supernatant from centrifugation was treated as described for the preparation of compounds **1** and **2** using β-D-galactosidase from *B. circulans* ATCC 31382. F-1 (20 mL/tube, tubes 84–97) and F-2 (tubes 104–117) fractions were concentrated and lyophilized to afford compounds **3** (90.1 mg) and **4** (101.5 mg), respectively. The physical and NMR data for compound **4** were identical to those of β-Gal-(1→3)-β-GalNAc-$OC_6H_4NO_2$-*p*, reported previously [17].

Preparation of β-Gal-(1→6)-β-GlcNAc-$OC_6H_4NO_2$-p (**5**) *and β-Gal-(1→3)-β-GlcNAc-$OC_6H_4NO_2$*-p (**6**)

*(A) Using β-*D*-galactosidase from porcine liver*. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (900 mg) and β-D-GlcNAc-$OC_6H_4NO_2$-*p* (330 mg) in 40 mM NaOAc buffer (120 mL) was added partially purified β-D-galactosidase (5 U) from porcine liver. The reaction was terminated by heating in a boiling-water bath for 5 min after 8 h of incubation at 40 °C. The supernatant from centrifugation was treated as described for the preparation of compound **1** using β-D-galactosidase from porcine liver. The fractions (tubes 60–71) were combined, concentrated, and lyophilized to afford compound **5** (98.4 mg). The physical and NMR data for compound **5** were identical to those of β-Gal-(1→6)-β-GlcNAc-$OC_6H_4NO_2$-*p*, reported previously [16].

*(B) Using β-*D*-galactosidase from* B. circulans *ATCC 31382*. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (900 mg) and β-D-GlcNAc-$OC_6H_4NO_2$-*p* (330 mg) in 40 mM NaOAc buffer (120 mL) was added β-D-galactosidase (5.0 U) from *B. circulans* ATCC 31382. After 9 h of incubation at 50 °C, the reaction mixture was terminated by heating in a boiling-water bath for 5 min. The supernatant from centrifugation was treated as described for the preparation of compounds **1** and **2** using β-D-galactosidase from *B. circulans* ATCC 31382. The F-1 (tubes 72–83) and F-2 (tubes 98–115) fractions were concentrated, and lyophilized to afford compounds **5** (29.2 mg) and **6** (143.9 mg), respectively. The physical and NMR data for compound **6** were identical to those of β-Gal-(1→3)-β-GlcNAc-$OC_6H_4NO_2$-*p*, reported previously [7].

Preparation of β-Gal-(1→6)-α-GlcNAc-$OC_6H_4NO_2$-p (**7**) *and β-Gal-(1→3)-α-GlcNAc-$OC_6H_4NO_2$*-p (**8**)

*(A) Using β-*D*-galactosidase from porcine liver*. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (200 mg) and α-D-GlcNAc-$OC_6H_4NO_2$-*p* (66 mg) in 40 mM NaOAc buffer (15 mL) was added partially purified β-D-galactosidase (1.8 U) from porcine liver. The reaction was terminated by heating in a boiling-water bath for 5 min after 6 h of incubation at 40 °C. The reaction mixture was treated as described for

the preparation of compound **1** using β-D-galactosidase from porcine liver. The fractions (tubes 53–61) were collected, concentrated, and lyophilized to afford compound **7** (81.5 mg).

Compound **7** had $[\alpha]_D^{25}$ + 181.9° (*c* 0.6, water) and *m*/*z* 505 $(M + H)^+$; ^{1}H NMR: δ 8.26 (d, 2 H, *J* 8.9 Hz, *m*-Ph), 7.30 (d, 2 H, *J* 8.9 Hz, *o*-Ph), 5.81 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.42 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), and 2.09 (s, 3 H, Me of Ac); ^{13}C NMR: δ 177.50 (C=O of Ac), 164.22 (Ph carbon attached to the phenolic oxygen), 145.35 (*p*-Ph), 128.97 (*m*-Ph carbon), 119.66 (*o*-Ph carbon), 106.25 (C-1′), 98.58 (C-1), 78.04 (C-5′), 75.67 (C-5), 75.09 (C-3′), 73.64 (C-2′, C-3), 72.31 (C-6), 71.57 (C-4), 71.03 (C-4′), 63.86 (C-6′), 56.14 (C-2), and 24.76 (Me of Ac).

(B) Using β-D-galactosidase from B. circulans *ATCC 31382.* To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (120 mg) and α-D-GlcNAc-$OC_6H_4NO_2$-*p* (44 mg) in 40 mM NaOAc buffer (8 mL) was added β-D-galactosidase (0.4 U) from *B. circulans* ATCC 31382. After 3.5 h of incubation at 50 °C, the reaction mixture was terminated by heating in a boiling-water bath for 5 min. The supernatant from centrifugation was treated as described for the preparation of compounds **1** and **2** using β-D-galactosidase from *B. circulans* ATCC 31382. The F-1 (tubes 54–58) and F-2 (tubes 67–77) fractions were concentrated, and lyophilized to afford compounds **7** (0.2 mg) and **8** (51.4 mg), respectively.

Compound **8** had $[\alpha]_D^{25}$ + 178.7° (*c* 0.6, water) and *m*/*z* 505 $(M + H)^+$; ^{1}H NMR: δ 8.26 (d, 2 H, *J* 8.9 Hz, *m*-Ph), 7.30 (d, 2 H, *J* 8.9 Hz, *o*-Ph), 5.81 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.42 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), and 2.09 (s, 3 H, Me of Ac); ^{13}C NMR: δ 177.41 (C=O of Ac), 163.92 (Ph carbon attached to the phenolic oxygen), 145.21 (*p*-Ph), 128.86 (*m*-Ph carbon), 119.35 (*o*-Ph carbon), 106.38 (C-1′), 98.49 (C-1), 82.48 (C-3), 78.13 (C-5′), 75.58 (C-5), 75.36 (C-3′), 73.51 (C-2′), 71.43 (C-4), 71.07 (C-4′), 63.89 (C-6′), 63.05 (C-6), 54.97 (C-2), and 24.74 (Me of Ac).

*Preparation of β-Gal-(1→6)-β-Gal-OC$_6$H$_4$NO$_2$-*p (**9**) *and β-Gal-1→(6-β-Gal-1→)$_n$-6-β-Gal-OC$_6$H$_4$NO$_2$-*p (**10**–**13**)

(A) Using β-D-galactosidase from porcine liver. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (600 mg) and β-D-Gal-$OC_6H_4NO_2$-*p* (200 mg) in 40 mM NaOAc buffer (50 mL) was added partially purified β-D-galactosidase (5.7 U) from porcine liver. The reaction was terminated by heating in a boiling-water bath for 5 min after 12 h of incubation at 40 °C. The reaction mixture was treated as described for the preparation of compound **1** using β-D-galactosidase from porcine liver. The fractions (tubes 63–72) were combined, concentrated, and lyophilized to afford compound **9** (65.3 mg). The NMR data for compound **9** were identical to those of β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p*, reported respectively [20].

(B) Using β-D-galactosidase from B. circulans *ATCC 31382.* To a soln of β-D-Gal-$OC_6H_4NO_2$-*p* (280 mg) in 40 mM NaOAc buffer (20 mL) was added β-D-galactosidase (4.0 U) from *B. circulans* ATCC 31382. After 20 h of incubation at 50 °C, the reaction mixture was treated as described for the preparation of compounds **1** and **2** using β-D-galactosidase from *B. circulans* ATCC 31382. For the separation on a Chromatorex-ODS DM 1020T column (Fig. 5(A)), F-2 (tubes 18–24) was concentrated and lyophilized to afford compound **9** (50.5 mg). The F-1 (tubes 9–17) fraction was concentrated and loaded onto a Toyopearl HW-40S column treated as already described (Fig. 5(B)). The F-1-1 (tubes 56–58), F-1-2 (tubes 60–64), F-1-3 (tubes 67–71), and F-1-4 (tubes 77–82) fractions were combined, concentrated, and lyophilized to afford compounds **13** (β-Gal-1→(6-β-Gal-1→)$_4$-6-β-Gal-$OC_6H_4NO_2$-*p*, 5.1 mg), **12** (β-Gal-1→(6-β-Gal-1→)$_3$6-β-Gal-$OC_6H_4NO_2$-*p*, 15.1 mg), **11** (β-Gal-1→(6-β-Gal-1→)$_2$6-β-Gal-$OC_6H_4NO_2$-*p*, 31.8 mg), and **10** (β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p*, 42.7 mg), respectively. The NMR data for compound **10** were identical to those of β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p*, reported respectively [20].

Compound **11** had $[\alpha]_D^{25}$ − 35.7° (*c* 0.5, water) and *m*/*z* 788 $(M + H)^+$; ^{1}H NMR δ 8.34 (d, 2 H, *J* 9.2 Hz, *m*-Ph), 7.38 (d, 2 H, *J* 9.2 Hz, *o*-Ph), 5.28 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.51 (d, 1 H, $J_{1''',2'''}$ 8.2 Hz, H-1‴), and 4.48 (2 H, *J* 7.9 Hz, H-1′, H-1″); ^{13}C NMR δ 164.83 (Ph

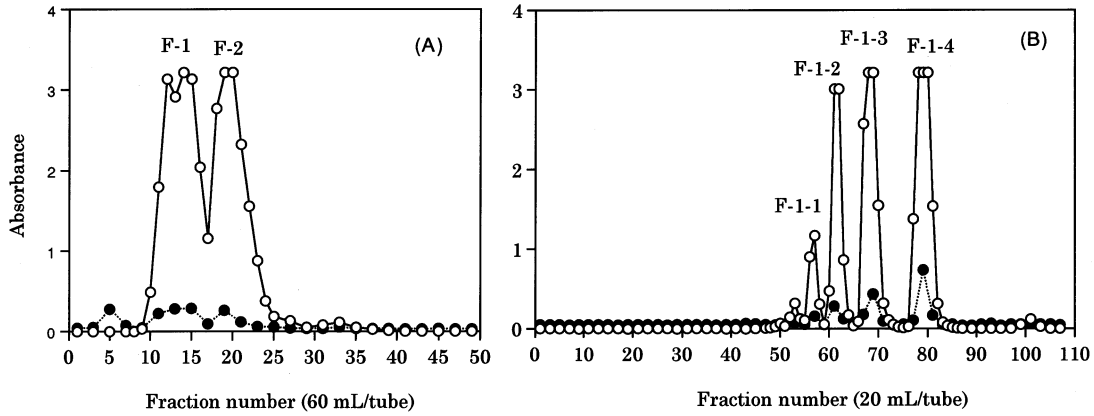

Fig. 5. Chromatorex-ODS DM 1020T (A) and Toyopearl HW-40S (B) chromatographic separation of transglycosylation product with β-D-Gal-$OC_6H_4NO_2$-*p* as substrate by use of β-D-galactosidase from *B. circulans* ATCC 31382. Absorbance at 300 nm (○) and absorbance at 485 nm (●).

carbon attached to the phenolic oxygen), 145.53 (*p*-Ph), 129.11 (*m*-Ph), 119.64 (*o*-Ph), 106.32 (C-1′, C-1″, C-1‴), 102.93 (C-1), 78.08, 77.38 and 76.66 (C-5, C-5′, C-5″, C-5‴), 75.69, 75.56, 75.51 and 75.24 (C-3, C-3′, C-3″, C-3‴), 73.67, 73.58 and 73.30 (C-2, C-2′, C-2″, C-2‴), 72.29 and 72.09 (C-6, C-6′, C-6″), 71.54 and 71.46 (C-4, C-4′, C-4″, C-4‴), and 63.93 (C-6‴).

Compound **12** had $[\alpha]_D^{25}$ −25.8° (*c* 0.5, water) and *m*/*z* 972 $(M + Na)^+$; ^1H NMR: δ 8.34 (d, 2 H, *J* 9.2 Hz, *m*-Ph), 7.38 (d, 2 H, *J* 9.2 Hz, *o*-Ph), 5.28 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.52 (d, 1 H, $J_{1'''',2''''}$ 6.8 Hz, H-1⁗), and 4.49 (3 H, *J* 6.5 Hz, H-1′, H-1″, H-1‴); ^{13}C NMR: δ 164.85 (Ph carbon attached to the phenolic oxygen), 145.55 (*p*-Ph), 129.11 (*m*-Ph), 119.66 (*o*-Ph), 106.32 (C-1′, C-1″, C-1‴, C-1⁗), 102.93 (C-1), 78.07, 77.39 and 76.64 (C-5, C-5′, C-5″, C-5‴, C-5⁗), 75.70, 75.54, 75.51 and 75.24 (C-3, C-3′, C-3″, C-3‴, C-3⁗), 73.69, 73.62 and 73.30 (C-2, C-2′, C-2″, C-2‴, C-2⁗), 72.31 and 72.13 (C-6, C-6′, C-6″, C-6‴), 71.55 (C-4, C-4′, C-4″, C-4‴,C-4⁗), and 63.93 (C-6⁗).

Compound **13** had *m*/*z* 1134 $(M + Na)^+$; ^1H NMR: δ 8.34 (d, 2 H, J 9.2 Hz, *m*-Ph), 7.38 (d, 2 H, J 9.2 Hz, *o*-Ph), 5.28 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.50 (d, 1 H, $J_{1'''',2''''}$ 7.6 Hz, H-1⁗), and 4.49 (4 H, *J* 7.6 Hz, H-1′, H-1″, H-1‴, H-1⁗); ^{13}C NMR: δ 164.85 (Ph carbon attached to the phenolic oxygen), 145.55 (*p*-Ph), 129.13 (*m*-Ph), 119.66 (*o*-Ph), 106.34 (C-1′, C-1″, C-1‴, C-1⁗, C-5⁗′), 102.95 (C-1), 78.07, 77.39 and 76.67 (C-5, C-5′, C-5″, C-5‴, C-5⁗, C-5⁗′), 75.70, 75.54, 75.51 and 75.24 (C-3, C-3′, C-3″, C-3‴, C-3⁗, C-3⁗′), 73.69, 73.64 and 73.30 (C-2, C-2′, C-2″, C-2‴, C-2⁗, C-2⁗′), 72.33 (C-6, C-6′, C-6″, C-6‴, C-6⁗), 71.57 (C-4, C-4′, C-4″, C-4‴, C-4⁗, C-4⁗′), and 63.95 (C-6⁗′).

Preparation of β-Gal-(1→6)-α-Gal-$OC_6H_4NO_2$-p (**14**), *β-Gal-(1→3)-α-Gal-$OC_6H_4NO_2$*-p (**15**), *β-Gal-(1→6)-β-Gal-(1→6)-α-Gal-$OC_6H_4NO_2$*-p (**16**), *β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-(1→6)-α-Gal-$OC_6H_4NO_2$*-p (**17**)

(A) Using β-D-galactosidase from porcine liver. To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (180 mg) and α-D-Gal-$OC_6H_4NO_2$-*p* (60 mg) in 40 mM NaOAc buffer (14 mL) was added partially purified β-D-galactosidase (2.0 U) from porcine liver. The reaction was terminated by heating in a boiling-water bath for 5 min after 12 h of incubation at 40 °C. The reaction mixture was treated as described for the preparation of compound **1** using β-D-galactosidase from porcine liver. The fractions (tubes 64–74) were combined, concentrated, and lyophilized to afford compound **14** (59 mg).

Compound **14** had *m*/*z* 464 $(M + H)^+$; ^1H NMR: δ 8.31 (d, 2 H, *J* 9.2 Hz, *m*-Ph), 7.34 (d, 2 H, *J* 9.2 Hz, *o*-Ph), 5.93 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.35 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′); ^{13}C NMR: δ 164.66 (Ph carbon attached to the phenolic oxygen), 145.35 (*p*-Ph), 129.00 (*m*-Ph), 119.98 (*o*-Ph), 105.89 (C-1′), 99.99 (C-1), 78.00 (C-5′), 75.65 (C-3′), 73.85 (C-2), 73.60

(C-2′), 72.09 (C-3), 72.00 (C-6), 71.52 (C-5), 71.46 (C-4′), 70.71 (C-4), and 63.91 (C-6′).

(B) Using β-D-galactosidase from B. circulans *ATCC 31382.* To a soln of β-D-Gal-$OC_6H_4NO_2$-*o* (240 mg) and α-D-Gal-$OC_6H_4NO_2$-*p* (80 mg) in 40 mM NaOAc buffer (16 mL) was added β-D-galactosidase (2.2 U) from *B. circulans* ATCC 31382. After 5.5 h of incubation at 50 °C, the reaction mixture was terminated by heating in a boiling-water bath for 5 min. The reaction mixture was treated as described for the preparation of compound **1** using β-D-galactosidase from porcine liver. The F-1 (tubes 68–71), F-2 (tubes 72–77), F-3 (tubes 78–82), F-4 (tubes 89–95), F-5 (tubes 98–103), and F-6 (tubes 121–127) fractions were combined, concentrated, and lyophilized to afford compounds **17** (5.5 mg), β-Gal-(1→6)-β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p* (18.7 mg, NMR data not shown), **16** (7.1 mg), β-Gal-(1→6)-β-Gal-$OC_6H_4NO_2$-*p* (84.7 mg, NMR data not shown), **14** (28.1 mg) and **15** (11.5 mg), respectively.

Compound **15** *m/z* 464 $(M+H)^+$; ^{1}H NMR: δ 8.30 (d, 2 H, *J* 9.0 Hz, *m*-Ph), 7.34 (d, 2 H, *J* 9.0 Hz, *o*-Ph), 5.93 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 4.35 (d, 1 H, $J_{1',2'}$ 8.9 Hz, H-1′); ^{13}C NMR: δ 164.38 (Ph carbon attached to the phenolic oxygen), 145.26 (*p*-Ph), 128.95 (*m*-Ph), 119.75 (*o*-Ph), 107.40 (C-1′), 99.71 (C-1), 82.01 (C-3), 78.04 (C-5′), 75.49 (C-3′), 74.81 (C-2), 74.05 (C-2′), 71.93 (C-5), 71.55 and 69.88 (C-4, C-4′), 63.93 and 63.82 (C-6, C-6′).

Compound **16** had *m/z* 626 $(M+H)^+$; ^{1}H NMR: δ 8.33 (d, 2 H, *J* 9.2 Hz, *m*-Ph), 7.36 (d, 2 H, *J* 9.2 Hz, *o*-Ph), 5.90 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.42 (d, 1 H, $J_{1'',2''}$ 8.9 Hz, H-1″), and 4.39 (d, 1 H, *J* 8.4 Hz, H-1′); ^{13}C NMR: δ 164.71 (Ph carbon attached to the phenolic oxygen), 145.39 (*p*-Ph), 129.02 (*m*-Ph), 120.09 (*o*-Ph), 106.29 and 106.07 (C-1′, C-1″), 100.11 (C-1), 78.00 and 76.62 (C-5′, C-5″), 75.69 and 75.49 (C-3′, C-3″), 73.93 (C-2), 73.66 and 73.53 (C-2′, C-2″), 72.06 (C-3), 72.00 (C-6, C-6′), 71.90 (C-5), 71.55 and 71.45 (C-4′, C-4″), 70.73 (C-4), and 63.92 (C-6″).

Compound **17** had *m/z* 788 $(M+H)^+$; ^{1}H NMR: δ 8.31 (d, 2 H, *J* 9.2 Hz, *m*-Ph), 7.36 (d, 2 H, *J* 9.2 Hz, *o*-Ph), 5.89 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.47 (d, 1 H, $J_{1''',2'''}$ 7.6 Hz, H-1‴), 4.45 (d, 1 H, $J_{1'',2''}$ 7.2 Hz, H-1″), and 4.40 (d, 1 H, *J* 6.8 Hz, H-1′); ^{13}C NMR: δ 164.71 (Ph carbon attached to the phenolic oxygen), 145.37 (*p*-Ph), 129.04 (*m*-Ph), 120.07 (*o*-Ph), 106.29 and 106.02 (C-1′, C-1″, C-1‴), 100.11 (C-1), 78.08, 76.67 and 76.59 (C-5′, C-5″, C-5‴), 75.70, 75.54 and 75.49 (C-3′, C-3″, C-3‴), 73.89 (C-2), 73.67 and 73.58 (C-2′, C-2″, C-2‴), 72.06 (C-3), 71.99 (C-6, C-6′, C-6″), 71.88 (C-5), 71.55 and 71.45 (C-4′, C-4″, C-4‴), 70.73 (C-4), and 63.93 (C-6‴).

Acknowledgements

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture, Japan, by a research grant for the Leading Research Utilizing Potential of Regional Science and Technology from the Science and Technology Agency, Japan, and by a research grant from the Ministry of Agriculture, Forestry, and Fisheries, Japan.

References

[1] M. Fukuda, *Biochim. Biophys. Acta*,, 780 (1985) 119–150.
[2] T. Feizi, H.C. Gooi, R.A. Childs, J.K. Pickard, K. Uemura, L.M. Loomes, S.J. Thorpe, E.F. Hounsell, *Biochem. Soc. Trans.*, 12 (1984) 591–596.
[3] R.A. Dwek, *Chem. Rev.*, 96 (1996) 683–720.
[4] H. Lis, N. Sharon, *Eur. J. Biochem.*, 218 (1993) 1–27.
[5] Y. Matahira, K. Ohno, M. Kawaguchi, H. Kawagishi, T. Usui, *J. Carbohydr. Chem.*, 14 (1995) 213–225.
[6] T. Usui, M. Suzuki, T. Sato, H. Kawagishi, K. Adachi, H. Sano, *Glycoconjugate J.*, 11 (1996) 105–110.
[7] T. Murata, S. Akimoto, M. Horimoto, T. Usui, *Biosci. Biotechnol. Biochem.*, 61 (1997) 1118–1120.
[8] D.H.G. Crout, G. Vic, *Curr. Opin. Chem. Biol.*, 2 (1998) 98–111.
[9] K.G.I. Nilsson, *Carbohydr. Res.*, 188 (1989) 9–17.
[10] A. Vetere, C. Galateo, S. Paoletti, *Biochem. Biophys. Res. Commun.*, 234 (1997) 358–361.
[11] S.C.T. Svensson, J. Thiem, *Carbohydr. Res.*, 200 (1990) 391–402.
[12] P. Finch, J.H. Yoon, *Carbohydr. Res.*, 303 (1997) 339–345.
[13] Y. Ichikawa, G.C. Look, C.-H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
[14] K. Sakai, R. Katsumi, H. Ohi, T. Usui, Y. Ishido, *J. Carbohydr. Chem.*, 11 (1992) 553–565.
[15] T. Usui, S. Morimoto, Y. Hayakawa, M. Kawaguchi, T. Murata, Y. Matahira, Y. Nishida, *Carbohydr. Res.*, 285 (1996) 29–39.

[16] T. Usui, S. Kubota, H. Ohi, *Carbohydr. Res.*, 244 (1993) 315–323.
[17] T. Murata, T. Itoh, Y. Hayakawa, T. Usui, *J. Biochem.*, 120 (1996) 851–855.
[18] T. Murata, S. Morimoto, X. Zeng, T. Watanabe, T. Usui, *Carbohydr. Res.*, 320 (1999) 192–199.
[19] X. Zeng, S. Morimoto, T. Murata, T. Usui, *J. Appl. Glycosci.*, 46 (1999) 241–247.
[20] G. Ekborg, B. Vranesic, A.K. Bhattacharjee, P. Kovac, C.P.J. Glaundemans, *Carbohydr. Res.*, 142 (1985) 203–211.
[21] J. Wang, P. Kovac, P. Sinaÿ, C.P.J. Glaudemans, *Carbohydr. Res.*, 308 (1998) 191–193.
[22] P. Kovac, *Carbohydr. Res.*, 153 (1986) 237–251.
[23] P. Kovac, H.C. Yeh, C.P.J. Glaudemans, *Carbohydr. Res.*, 140 (1985) 277–288.
[24] A.K. Bhattacharjee, E. Zissis, C.P.J. Glaudemans, *Carbohydr. Res.*, 89 (1981) 249–254.
[25] C.T.M. Fransen, K.M.J. Van Laere, A.A.C. van Wijk, L.P. Brüll, M. Dignum, J.E. Thomas-Oates, J. Haverkamp, H.A. Schols, A.G.J. Voragen, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 314 (1998) 101–114.
[26] R. Lopez, A. Feruaudez-Mayoralas, *J. Org. Chem.*, 59 (1994) 737–745.
[27] K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
[28] E. Montero, J. Alonso, F.J. Canada, A. Feruaudez-Mayoralas, M. Martin-Lomas, *Carbohydr. Res.*, 305 (1998) 383–391.
[29] A. Tsuchida, S. Akimoto, T. Usui, K. Kobayashi, *J. Biochem.*, 123 (1998) 715–721.
[30] X. Zeng, T. Murata, H. Kawagishi, T. Usui, K. Kobayashi, *Biosci. Biotechnol. Biochem.*, 62 (1998) 1171–1178.
[31] X. Zeng, T. Murata, H. Kawagishi, T. Usui, K. Kobayashi, *Carbohydr. Res.*, 312 (1998) 209–217.