Effective transgalactosylation catalysed by a lipid-coated β-D-galactosidase in organic solvents †

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A lipid-coated β-D-galactosidase was prepared, which was soluble in most organic solvents such as diisopropyl ether, 2,2,4-trimethylpentane and benzene, but insoluble in aqueous solution. It could act as an efficient transgalactosylation catalyst for various hydrophobic alcohols with *p*-nitrophenyl β-D-galactopyranoside in dry diisopropyl ether. When a native β-D-galactosidase was used in aqueous solution containing acetonitrile for the same reaction, the yield of the transgalactosylation was low due to the predominant process of hydrolysis. The enzyme activity for the galactosylation depended on coating lipid molecules, the origin of the enzyme used, the reaction's organic solvents and the chemical structures of the acceptor alcohols.

Introduction

Chemical syntheses of glycosides usually require skilful and selective protection and deprotection of the reactive hydroxy groups of carbohydrates. Enzymic synthesis can provide regio- and stereo-selective products without using protecting groups in a one-step reaction, in comparison with chemical synthesis. A glycosyl transferase catalyses the transfer of a glycosyl residue from a glycosyl donor activated as a sugar nucleotide to a glycosyl acceptor.[1-3] However, it is relatively difficult to obtain a pure glycosyl transferase in commercial form and to prepare the activated glycosyl donor by chemical syntheses. In recent years, many attempts have been made to use glycoside hydrolases as transglycosylation catalysts in aqueous solutions containing water-miscible organic solvents by utilizing a reverse hydrolysis reaction.[4-11] In these reactions, however, it has been difficult to obtain transglycosylation products in good yields, because the hydrolysis proceeded preferentially to the transglycosylation due to the presence of the aqueous solution.[12-21] If the reaction can be carried out in non-aqueous organic solvents without denaturation of enzymes, the transglycosylation products would be obtained in high yield.

We have recently reported a lipid-coated enzyme, in which hydrophilic head groups of lipids interact with the enzyme surface, and two long lipophilic alkyl chains extend away from its surface, to solubilize the enzyme in organic solvents (see illustrations in Fig. 1). The lipid-coated enzymes showed high catalytic activity in organic media.[22-26] For example, in dry 2,2,4-trimethylpentane ('isooctane') the lipid-coated lipase can catalyse triglyceride synthesis from 1-monoglyceride and aliphatic acids,[23] and can act as a catalyst for enantioselective esterification from racemic alcohols and aliphatic acids.[24]

In this paper, we apply our lipid-coating technique to transglycosylations by using a reverse hydrolysis of glycosidases. A lipid-coated β-D-galactosidase acts as an efficient transgalactosylation catalyst from *p*-nitrophenyl β-D-galactopyranoside (Gal-*p*NP) to respective alcohols in dry diisopropyl ether (see Fig. 1). β-D-Galactosidase was chosen due to it being a widely studied and easily obtained glycosidase enzyme.[5-9,13-17] Enzymic activity was studied under various conditions of coating lipid, enzyme origin, reaction organic solvent, and substrate structure. Catalyses by a lipid-coated enzyme in dry organic solvents are also compared with a conventional native enzyme catalysis in aqueous–organic solutions.

† Enzyme–Lipid complex 11. For part 10, see ref. 38.

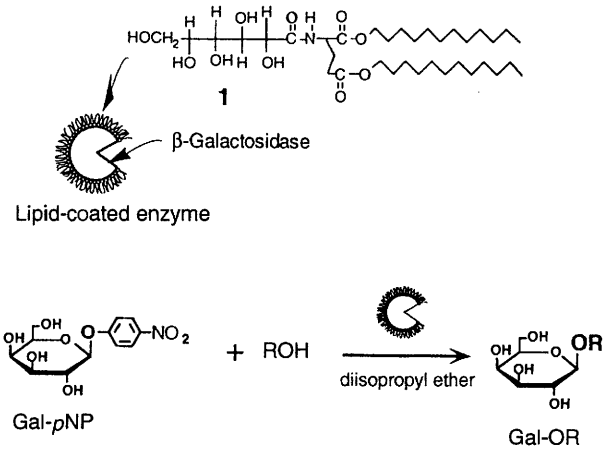


Fig. 1 Schematic illustrations of a lipid-coated β-D-galactosidase and transgalactosylation in dry organic solvents

Results and discussion

Fig. 2 shows typical time courses of transgalactosylation from *p*-nitrophenyl β-D-galactopyranoside (Gal-*p*NP) as a galactosyl donor to a 10-fold excess of 5-phenylpentan-1-ol (PhC_5-OH) as a galactosyl acceptor at 30 °C. Reactions were followed by liquid chromatography of the products from reduction of Gal-*p*NP and production of galactosides (Gal-OR) and *p*-nitrophenol (*p*NP).

When a native β-D-galactosidase was employed in acetonitrile–water (7:3) according to the conventional methods,[27] the yield of the transglycosylation product (Gal-OC_5Ph) was low (~ 30%) and ~ 70% of the galactosyl donor was hydrolysed to galactose (Gal) (see Fig. 2a). Similar results are reported in some aqueous buffer systems with catalysis by a native enzyme.[28-31] On the other hand, in the case of a β-D-galactosidase coated with the lipid **1** (didodecyl *N*-D-gluconoyl-L-glutamate) in dry diisopropyl ether, only the transglycosylated Gal-OC_5Ph was obtained, in 70% yield in 1 day, but the hydrolysis product galactose was not obtained even after 4 days (Fig. 2b). Thus, the galactose donor, Gal-*p*NP, was recovered in the remaining 30% yield. It was confirmed from ^1H and ^{13}C NMR analysis of the isolated products that the chemical structure of Gal-OC_5Ph kept the β-configuration of D-galactose (see Experimental section). When a native β-D-galactosidase was employed as a dispersion in diisopropyl

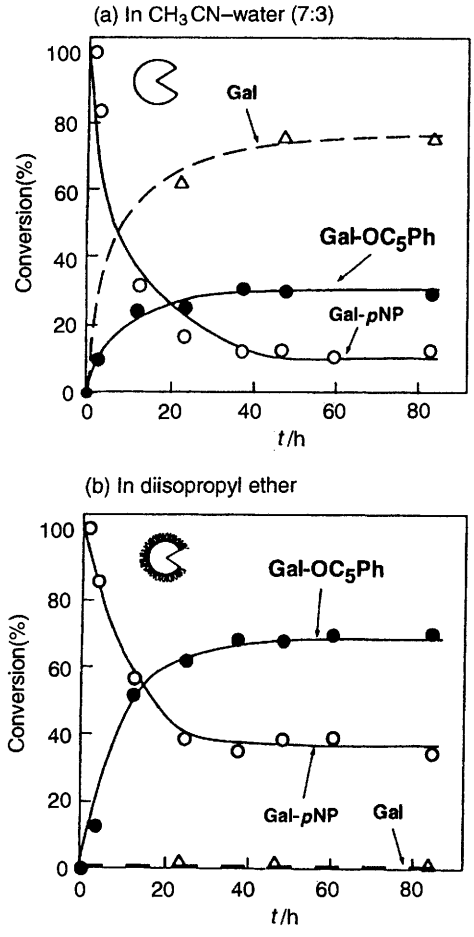


Fig. 2 Typical time courses of transgalactosylation from *p*-nitrophenyl β-D-galactopyranoside (Gal-*p*NP, 0.1 mM) and 5-phenylpentan-1-ol (PhC_5-OH, 1.0 mM) at 30 °C (a) in acetonitrile–water (7:3) catalysed by a native β-D-galactosidase from *E. coli*, and (b) in dry diisopropyl ether catalysed by a lipid (**1**)-coated β-D-galactosidase. [Enzyme] = 0.1 mg of protein in 2 ml.

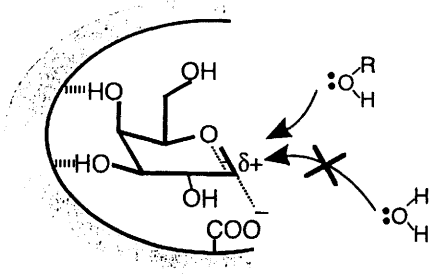


Fig. 3 A schematic illustration of transgalactosylation catalysed by a lipid-coated β-D-galactosidase in non-aqueous solvent

ether, the transgalactosylation or even hydrolysis hardly occurred, probably because of denaturation or low solubility of the enzyme in organic media.

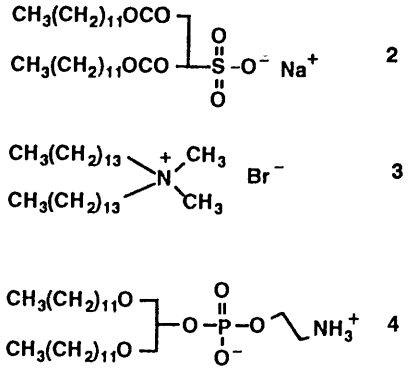
A schematic reaction mechanism is shown in Fig. 3. In the organic solution, the carbocation intermediate formed at the active side of the enzyme can be attacked by the alcohol as a galactosyl acceptor. In the conventional method using a native enzyme in aqueous–organic solutions, the competitive attack of water molecules and alcohols occurred, and the transglycosylation yield was lower.

The glycosylation catalysed by a lipid-coated enzyme in the organic solvent could be applied to a practical synthesis. The transgalactosylation product (Gal-OC_5Ph) could be isolated on a large scale (~1 g) in fair yield (50–60%) by recrystallization

Table 1 Effect of coating lipid structures on preparation of a lipid-coated β-D-galactosidase and its enzymic activity

Lipid	Preparation: Yield (mg)[a]	Preparation: Protein content (wt%)[b]	Enzymic activity conversion (%)[c,d]
Non-ionic lipid **1**	32.6	8.8	62
Anionic lipid **2**	40.5	7.0	21
Cationic lipid **3**	5.7	32	8
Zwitterionic lipid **4**	0		

[a] Both aqueous solutions of β-D-galactosidase (50 mg) and lipids (50 mg) were mixed and precipitates were lyophilized. [b] Obtained from UV absorption of aromatic amino acid residues in the protein, which was consistent with the protein content obtained from the C/N ratio of the elemental analyses. [c] Transgalactosylation from *p*-nitrophenyl β-D-galactopyranoside (Gal-*p*NP, 0.1 mM) and 5-phenylpentan-1-ol (PhC_5-OH, 1.0 mM) in dry diisopropyl ether (2 ml) at 30 °C catalysed by the respective lipid-coated enzyme ([β-D-galactosidase from *E. coli*] = 0.1 mg of protein). [d] The galactose donor (Gal-*p*NP) was recovered as the remaining yield of the conversion without hydrolysis.



from hexane under the following conditions: a lipid (**1**)-coated β-D-galactosidase (20 mg), Gal-*p*NP (2 g) and PhC_5-OH (10 g) all in 50 ml of dry diisopropyl ether at 30 °C.

It should be mentioned that the yield of the Gal-OC_5Ph product reached a limit at ~70% even after 4 days as shown in Fig. 2b. When the enzyme was added to the reaction mixture after the reaction had reached equilibrium, the yield did not increase. When the lipid-coated enzyme had been soaked in diisopropyl ether for 2 days in advance and then both substrates were added, the transgalactosylation proceeded in 65% yield in 2 days and reached equilibrium, similarly to the time courses shown in Fig 2(*b*). These results indicate that the lipid-coated enzyme was not consumed during the reaction in organic solvents. When the product, Gal-OC_5Ph, was added to the reaction mixture in advance, the yield of the transglycosylation product was decreased upon increasing the amount of added Gal-OC_5Ph. For example, when the equivalent amount of Gal-OC_5Ph to the substrate was added, the reaction was completely stopped. The saturation of the yield near 70% is explained by product inhibition, but not by the deactivation of the lipid-coated enzyme.

Effect of coating lipids

Lipid-coated enzymes were prepared from β-D-galactosidase (from *Escherichia coli*) and several kinds of dialkyl amphiphiles having nonionic, anionic, cationic and zwitterionic head groups. The results are summarized in Table 1. The lipid-coated β-D-galactosidase was obtained as a precipitate in fair yield when the nonionic **1** and anionic amphiphiles **2** were employed, but not when the cationic **3** and zwitterionic amphiphiles **4** were employed. Although the complexes were produced in fair yield by using the anionic amphiphile **2**, galactosyl activity was very low compared with those of complexes with the nonionic amphiphile **1**. This is probably because the strong electrostatic

Table 2 Effect of organic solvents on the galactosylations catalysed by a lipid (**1**)-coated β-D-galactosidase at 30 °C[a]

Solvents	Initial rate [10^{-4} μM s^{-1} (mg of protein)$^{-1}$]	Conversion after 2 days (%)[b]
Diisopropyl ether	7.1	62
'Isooctane'	6.1	39
Ethyl acetate	5.5	32
Benzene	2.6	24
Diethyl ether	2.8	23
1,4-Dioxane	2.4	12
Tetrahydrofuran	1.1	>5
Dimethylformamide	0.7	>5
Chloroform	0	
Acetone	0	
Acetonitrile	0	

[a] [Gal-*p*NP] = 0.1 mM, [PhC_5-OH] = 1.0 mM, [β-D-galactosidase from *E. coli*] = 0.1 mg of protein in 2 ml of dry organic solvent. [b] The galactose donor (Gal-*p*NP) was recovered as the remaining yield of the conversion without hydrolysis.

interaction between the anionic head groups of the amphiphiles and the hydrophilic surface of the β-D-galactosidase denatures and enzyme's structure. These results corresponded to those found with the lipid-coated lipase.[24] The nonionic dialkyl amphiphile **1** was chosen as the standard coating lipid for β-D-galactosidase in the following experiments.

Effect of reaction organic solvents

The transgalactosylation catalysed by lipid (**1**)-coated β-D-galactosidase (from *E. coli*) was carried out in various organic solvents. Initial rate and the conversion after 2 days are summarized in Table 2. The lipid-coated β-D-galactosidase was soluble in all the organic solvents listed in Table 2. The lipid-coated β-D-galactosidase showed fairly high initial rate and conversion in non-polar solvents such as diisopropyl ether. On the other hand, the enzyme activity decreased in polar solvents such as 1,4-dioxane and tetrahydrofuran (THF). In halogenated or water-miscible solvents such as chloroform, dimethylformamide (DMF), acetone and acetonitrile, the lipid-coated β-D-galactosidase showed hardly any catalytic activity although it is soluble. Even when the product yield was low, the galactose donor, Gal-*p*NP, was completely recovered from the remaining non-product material without hydrolysis (see footnote *b* in Table 2). Enzymes might be easily denatured in halogenated solvents, although if so the reason is not clear. In water-miscible solvents, enzymes may be denatured due to the removal of essential water from the enzyme. A similar tendency was observed in the case of lipid-coated lipase.[23,24] Diisopropyl ether was chosen as solvent for the following experiments.

Substrate selectivity

Table 3 shows the effect of the chemical structures of acceptor alcohols on the transgalactosylation from Gal-*p*NP catalysed by the lipid (**1**)-coated β-D-galactosidase in dry diisopropyl ether. The enzymic activity is shown as the initial rate and the equilibrium yield after 2 days. Structural data of the transgalactosylated products are shown in the Experimental section. Both the initial rate and the yield depended largely on the shape and the chain length of the alcohols. The unchanged galactose donor, Gal-*p*NP, was recovered as the remaining yield of the conversion without hydrolysis. When linear primary alcohols such as butan-1-ol and octan-1-ol were employed, the galactoside yields were nearly as high as in the case of 5-phenylpentan-1-ol (PhC_5-OH) shown in Fig. 2b. The reactivity decreased with increasing alkyl chain length, such as with decan-1-ol and dodecan-1-ol. When the primary (1°) alcohol was changed to a secondary (2°) or a tertiary (3 °) alcohol, the transglycosylation activity was reduced in the order 1° > 2° > 3° in butanols. It is interesting that transgalactosylation occurred with secondary alcohols having a large alkyl group, such as cholesterol or 1,3-didodecylglycerol, though the yields were generally low. It should be mentioned that the galactosylation to the primary alcohol of 1,2-didodecylglycerol having two long alkyl chains gave a fair yield of product (52%). This suggests that the lipid-coated enzyme system is suitable for preparations of glycolipids such as glycosyl ceramides in organic solvents.

Effect of origin of β-D-galactosidase

An advantage of the glycosylation using galactosidase is that many kinds of enzymes are easily obtained. Lipid-coated β-D-galactosidases from different origins were prepared with the nonionic amphiphile **1** and the yields of galactosylation to the primary, secondary, and tertiary butanols in dry diisopropyl ether are summarized in Table 4. Whenever the nonionic amphiphile **1** was used as coating lipid, the lipid-coated β-D-galactosidase was obtained in fair yield and in nearly constant protein content independent of the enzyme's origin (30 ± 5 mg in yield and 9 ± 3 wt% in protein content). However, the enzyme activity for the galactosylation largely depended on the origin of enzymes. In the case of β-D-galactosidase from bovine liver, only a small amount of the galactoside was formed. The lipid-coated β-D-galactosidase from *Aspergillus oryzae*, as well as that from *E. coli*, could catalyse the transglycosylation to the primary alcohol, but not to the secondary or tertiary alcohols. On the other hand, the enzyme from *Bacillus circulans* showed much more selectivity towards the secondary alcohol than to the primary alcohol. These results were consistent with the case of native enzymes in aqueous solutions.[32]

Since the lipid-coated β-D-galactosidase from *B. circulans* showed high transglycosylation activity towards the secondary alcohols, then enantioselective transglycosylation towards the racemic secondary alcohols is expected. As shown in Table 3, the transgalactosylation towards nonan-2-ol selectively occurred with the *R*-isomer as compared with the *S*-isomer. Similar enantioselectivity was observed for 1-phenylethanol, although the enantioselectivity for the *R*-isomer was small.

Conclusions

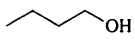
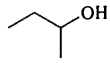
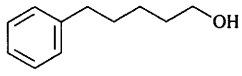
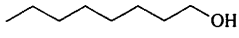
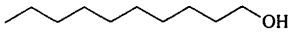
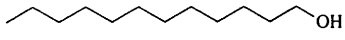
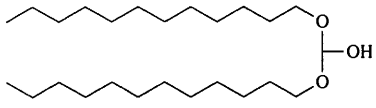
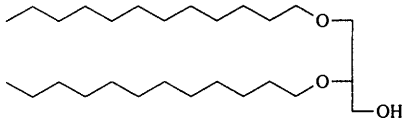
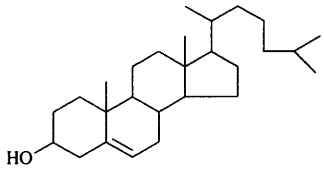
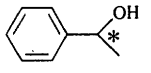
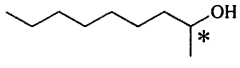
The lipid-coated β-D-galactosidase is easily prepared by mixing enzymes with lipids and is stable in hydrophobic organic solvents for several days. The lipid-coated β-D-galactosidase can catalyse efficiently the transgalactosylation without resulting in hydrolysis products in dry organic solvents. When the enzyme was coated with the nonionic lipid **1** and allowed to react in hydrophobic diisopropyl ether, a high reactivity was achieved. A variety of alcohols were found to be useful as galactosyl acceptors, depending on the origin of the enzyme used. The coating lipids act as lipophilic tails to be solubilized in organic media, but do not affect the enzyme selectivity. This method is obviously suitable for transglycosylation compared with the conventional method using a native enzyme is aqueous organic solvents. We believe that the lipid-coated enzyme system can be widely applicable to other glycoside hydrolyses as a transglycosylation catalyst in dry organic solvents.

Experimental

Materials

β-D-Galactosidases [EC 3.2.1.23] from *E. coli* (Toyobo Co.), *A. oryzae* (Sigma Chemicals Co.), bovine liver (Sigma Chemicals Co.) and *B. circulans* (Daiwa Kasei Co.) were used without further purification. Preparations of dialkyl amphiphiles as a coating lipid, didodecyl *N*-D-gluconoyl-L-glutamate **1**,[33] sodium 1,2-bis-(dodecyloxycarbonyl)ethanesulfonate **2**,[34] and 1,3-di-*O*-dodecyl-*rac*-glycero-2-phosphocholine **4**[35] were reported elsewhere. Dimethylbis(tetradecyl)ammonium bromide **3** was purchased as the finest grade from Sogo Pharmaceutical Co., Tokyo. Substrates 1,2-di-*O*-dodecylglycerol[36] (mp 40 °C) or 1,3-di-*O*-dodecylglycerol[37] (mp 36 °C) were prepared from

Table 3 Effect of chemical structures of acceptor alcohols on galactosylation catalysed by a lipid (**1**)-coated β-D-galactosidase in dry diisopropyl ether at 30 °C[a]

Acceptor ROH		Initial rate [10^{-4} μM s^{-1} (mg of protein)$^{-1}$]	Conversion after 2 days (%)[b]
		7.6	61
		4.8	42
		0.43	6.0
		7.1	62
		6.7	69
		2.1	13
		2.8	15
		0.72	9.0
		4.9	52
		0.59	7.2
	*R* *S*	4.1[c] 2.8[c]	41[c] 16[c]
	*R* *S*	3.1[c] 0.8[c]	36[c] >5[c]

[a] [Gal-*p*NP] = 0.1 mM, [acceptor alcohols] = 1.0 mM, [β-D-galactosidase from *E. coli*] = 0.1 mg of protein in 2 ml of dry diisopropyl ether. [b] The galactose donor (Gal-*p*NP) was recovered as the remaining yield of the conversion without hydrolysis. [c] β-D-Galactosidase from *B. circulans* was used as a lipid-coated enzyme, and other reaction conditions are the same as above.

dodecan-1-ol and 3-benzylglycerol or epichlorohydrin, respectively, according to the literature. (*R*)- and (*S*)-Nonan-2-ol and (*R*)- and (*S*)-1-phenylethanol were purchased from Chisso Co., Osaka. Other chemicals and organic solvents were purchased from Tokyo Kasei Co., Tokyo and Nacalai Tesque Co., Kyoto.

Preparation of a lipid-coated β-D-galactosidase

A lipid-coated β-D-galactosidase was prepared almost in accordance with our previous papers for other enzymes.[23–26] An aqueous buffer solution (50 ml; 0.05 M phosphate, pH 5.1) of the β-D-galactosidase (50 mg) was mixed with an aqueous dispersion (50 ml) of synthetic lipids (50 mg) at 4 °C and stirred for 1 day at 4 °C. Precipitates were gathered by centrifugation at 4 °C (5000 rpm; 15 min) and washed with buffer solution and distilled water, repeatedly, and then lyophilized. The resulting powder was soluble in most organic solvents such as acetonitrile, benzene and diisopropyl ether, but insoluble in aqueous buffer solution. The protein content of the lipid–enzyme complex was determined from both elemental analysis (C, H and N) and UV absorption from aromatic amino acid residues of proteins at 280 nm in chloroform solution.[23–26] UV Spectra were recorded on a Shimadzu UV-240 spectrophotometer.

Catalytic activity in organic solvents

A typical procedure is as follows. To a dry diisopropyl ether solution (2 ml) of a lipid-coated enzyme (1–2 mg, 0.1 mg of protein) was added Gal-*p*NP (0.06 mg; 0.1 mM) and an excess of alcohol substrate (R-OH, 0.14–0.85 mg; 1.0 mM), and the homogeneous solution was stirred at 30 °C. With the prescribed time interval, the reduction of Gal-*p*NP and the production of galactosides (Gal-OR) and *p*-nitrophenol (*p*NP) were followed by liquid chromatography: TSK-gel ODS-80Ts (4.6 mm × 25 cm) in a Tosoh CCPD-system liquid chromatography equipped with a UV (at 254 nm) and refractive index (RI) detector

Table 4 Effects of the origin of the β-D-galactosidase on transgalactosylations to primary, secondary, and tertiary butanols catalysed by the lipid (**1**)-coated enzyme

Origin of β-D-galactosidase	Conversion after 2 days (%)[a]		
	BuOH	Bu^{t}OH	Bu'OH
E. coli	61	42	6
A. oryzae	75	30	0
B. circulans	25	54	0
Bovine liver	8	0	0

[a] Transgalactosylation from Gal-*p*NP (0.1 mM) and 5-phenylpentan-1-ol (1.0 mM) in dry diisopropyl ether (2 ml) catalysed by a lipid-coated β-D-galactosidase ([enzyme] = 0.1 mg of protein) at 30 °C. The galactose donor (Gal-*p*NP) was recovered as the remaining yield of the conversion without hydrolysis.

(elution, acetonitrile–water 1 : 1; flow rate 1 ml/min^{-1}). Identification and quantification of substrates and products were made by comparison of the HPLC retention time and HPLC peak area with those of authentic samples. Production of D-galactose was followed with enzymic detection using D-galactose dehydrogenase [D-galactose: NAD^+ 1-oxidoreductase, EC 1.1.1.48] in the aqueous solution extracted from the reaction mixture.

Analyses of transgalactosylation products

The transgalactosylation products (β-D-Gal-OR) in Table 3 were isolated from the reaction mixture and their chemical structures were confirmed by ^1H (CD_3OD; 300 MHz) and ^{13}C NMR (CD_3OD; 75 MHz) spectra (Varian Gemini-30 instrument), and elemental analyses (C and H). Analysis results are summarized below. Coupling constants (*J*) are given in Hz.

Butyl β-D-galactopyranoside:[4] δ_H 0.8 (3 H, t), 1.2 (4 H, m), 3.4 (2 H, t), 3.5–4.2 (6 H, m) and 4.7 (1 H, d, *J* 7.0); δ_C 12.1 (C-4'), 23.0 (C-3'), 32.6 (C-2'), 62.9 (C-6), 70.1 (C-1'), 71.3 (C-4), 72.0 (C-2), 75.4 (C-3), 78.5 (C-5) and 104.3 (C-1, β-bond) (Found: C, 50.7; H, 8.6. Calc. for $C_{10}H_{20}O_6$: C, 50.84; H, 8.53%).

sec-Butyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.2 (2 H, m), 1.5 (3 H, m), 3.2 (1 H, m), 3.5–4.2 (6 H, m) and 4.6 (1 H, d, *J* 7.1); δ_C 10.4 (C-3'), 21.8 (C-1''), 33.6 (C-2'), 62.8 (C-6), 76.9 (C-1'), 71.4 (C-4), 72.0 (C-2), 75.3 (C-3), 78.1 (C-5) and 104.1 (C-1, β-bond) (Found: C, 50.8; H, 8.3%).

tert-Butyl β-D-galactopyranoside: δ_H 1.3 (9 H, s), 3.4–4.1 (6 H, m) and 4.7 (1 H, d, *J* 7.0); δ_C 37.1 (C-1'), 62.9 (C-6), 66.4 (C-2'), 71.1 (C-4), 72.9 (C-2), 75.4 (C-3), 78.4 (C-5) and 104.1 (C-1, β-bond) (Found: C, 50.7; H, 8.4%).

5-Phenylpentyl β-D-galactopyranoside: δ_H 1.2 (4 H, m), 1.6 (2 H, t), 2.1 (2 H, t), 3.3 (2 H, t), 3.4–4.1 (6 H, m), 4.7 (1 H, d, *J* 7.0) and 7.2–7.5 (5 H, m); δ_C 27.4 (C-3'), 31.5 (C-2'), 33.3 (C-5'), 48.8 (C-4'), 63.1 (C-6), 65.6 (C-1'), 71.2 (C-4), 72.0 (C-2), 75.5 (C-3), 78.5 (C-5), 104.6 (C-1, β-bond), 128.2 (Ph), 128.5 (Ph) and 129.6 (Ph) (Found: C, 62.7; H, 7.9. Calc. for $C_{17}H_{26}O_6$: C, 62.56; H, 8.03%).

Octyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.3 (10 H, m), 1.6 (2 H, t), 3.2 (2 H, t), 3.6–4.1 (6 H, m) and 4.7 (1 H, d, *J* 7.1); δ_C 12.6 (C-8'), 21.6 (C-7'), 26.3, 28.8, 29.0, 29.3 and 30.1 (C-2' to -6'), 60.9 (C-6), 69.1 (C-1'), 71.3 (C-4), 72.0 (C-2), 75.4 (C-3), 78.5 (C-5) and 104.1 (C1, β-bond) (Found: C, 57.7; H, 9.6. Calc. for $C_{14}H_{28}O_6$: C, 57.51; H, 9.65%).

Decyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.3 (14 H, m), 1.6 (2 H, t), 3.3 (2 H, t), 3.6–4.1 (6 H, m) and 4.6 (1 H, d, *J* 7.1); δ_C 10.9 (C-10'), 24.1 (C-9'), 25.9, 26.4, 29.1, 30.0 and 31.2 (C-2' to -8'), 62.1 (C-6), 68.4 (C-1'), 70.9 (C-4), 73.1 (C-2), 74.6 (C-3), 79.0 (C-5) and 104.1 (C-1, β-bond) (Found: C, 59.9; H, 10.15. Calc. for $C_{16}H_{32}O_6$: C, 59.97; H, 10.07%).

Dodecyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.3 (18 H, m), 1.6 (2 H, t), 3.2 (2 H, t), 3.5–4.1 (6 H, m) and 4.6 (1 H, d, *J* 7.1); δ_C 11.3 (C-11'), 23.7 (C-9'), 26.3, 27.2, 27.8, 28.6, 30.1 and 31.4 (C-2' to -11'), 62.1 (C-6), 67.4 (C-1'), 70.6 (C-4), 74.0 (C-2), 75.9 (C-3), 79.1 (C-5) and 103.8 (C-1, β-bond) (Found: C, 61.9; H, 10.2. Calc. for $C_{18}H_{36}O_6$: C, 62.04; H, 10.41%).

1,3-Di-*O*-dodecylglyceryl β-D-galactopyranoside: δ_H 0.9 (6 H, t), 1.3 (36 H, m), 1.6 (4 H, t), 3.4–3.6 (9 H, m), 3.6–4.1 (6 H, m) and 4.6 (1 H, d, *J* 6.9); δ_C 11.3 (C-12'), 23.7 (C-9'), 26.3, 27.2, 27.8, 28.6, 30.1 and 31.4 (C-2' to -11'), 60.4 (C-6), 64.4 (glycerol C-1), 67.4 (C-1'), 71.3 (C-4), 72.0 (C-2), 75.1 (C-3), 78.6 (C-5), 82.4 (glycerol C-2) and 103.9 (C-1, β-bond) (Found: C, 67.0; H, 11.1. Calc. for $C_{33}H_{66}O_8$: C, 67.08; H, 11.26%).

1,2-Di-*O*-dodecylglyceryl β-D-galactopyranoside: δ_H 0.9 (6 H, t), 1.3 (36 H, m), 1.6 (4 H, t), 3.4–3.6 (9 H, m), 3.6–4.2 (6 H, m) and 4.6 (1 H, d, *J* 6.9); δ_C 11.3 (C-12'), 23.7 (C-9'), 26.3, 27.2, 27.8, 28.6, 30.1 and 31.4 (C-2' to -11'), 59.8 (C-6), 67.4 (C-1'), 71.0 and 71.3 (C-4, glyceryl C-1), 73.2 (C-2), 74.1 and 75.4 (C-3, glyceryl C-2), 79.9 (C-5), 81.1 (glyceryl C-3) and 102.8 (C-1, β-bond) (Found: C, 67.2; H, 10.95%).

Cholesteryl β-D-galactopyranoside: δ_H 0.9–2.4 (44 H, t), 3.3 (1 H, m), 3.6–4.2 (6 H, m), 4.6 (1 H, d, *J* 6.9) and 5.4 (1 H, m); δ_C 105.8 (C-1, β-bond) (Found: C, 72.0; H, 10.5. Calc. for $C_{33}H_{56}O_6$: C, 72.22; H, 10.29%).

(*R*)-1-Phenylethyl β-D-galactopyranoside: δ_H 1.2 (3 H, m), 3.6–4.2 (6 H, m), 4.6 (1 H, d, *J* 6.9), 4.8 (1 H, m) and 7.2–7.5 (5 H, m); δ_C 21.4 (C-2'), 60.5 (C-6), 72.7 (C-4), 73.9 (C-2), 75.0 (C-3), 79.3 (C-5), 104.6 (C-1, β-bond), 121.3 (C-1'), 127.2 (Ph), 129.1 (Ph) and 129.6 (Ph) (Found: C, 59.5; H, 6.8. Calc. for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09%).

(*S*)-1-Phenylethyl β-D-galactopyranoside: δ_H 1.2 (3 H, m), 3.6–4.1 (6 H, m), 4.6 (1 H, d, *J* 7.0), 4.8 (1 H, m) and 7.2–7.5 (5 H, m); δ_C 20.3 (C-2'), 61.9 (C-6), 71.5 (C-4), 72.7 (C-2), 75.8 (C-3), 79.5 (C-5), 104.6 (C-1, β-bond), 120.2 (C-1'), 127.6 (Ph), 129.1 (Ph) and 129.6 (Ph) (Found: C, 59.4; H, 7.2%).

(*R*)-1-Methyloctyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.2–1.6 (16 H, m), 3.5–4.2 (6 H, m) and 4.6 (1 H, d, *J* 7.1); δ_C 12.3 (C-8'), 20.5 and 24.1 (C-7' and -1''), 26.7, 27.0, 29.6 and 31.1 (C-3' to -6'), 37.0 (C-2'), 60.0 (C-6), 72.3 (C-4), 73.8 (C-2), 75.1 (C-3), 78.8 (C-5), 84.1 (C-1') and 105.3 (C-1, β-bond) (Found: C, 58.45; H, 9.3. Calc. for $C_{15}H_{30}O_6$: C, 58.80; H, 9.87%).

(*S*)-1-Methyloctyl β-D-galactopyranoside: δ_H 0.9 (3 H, t), 1.2–1.6 (16 H, m), 3.5–4.2 (6 H, m) and 4.6 (1 H, d, *J* 7.1); δ_C 10.7 (C-8'), 21.0 and 23.1 (C-7' and -1''), 26.1, 27.4, 29.8 and 32.7 (C-3' to -6'), 35.1 (C-2'), 61.5 (C-6), 70.8 (C-4), 71.8 (C-2), 76.8 (C-3), 73.9 (C-5), 89.9 (C-1') and 103.8 (C-1, β-bond) (Found: C, 58.4; H, 9.9%).

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Paper 6/02640K
Received 16*th April* 1996
Accepted 13*th August* 1996