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CHEMOENZYMATIC SYNTHESIS OF 1-*O*-ACYL-3-*O*-(6'-*O*-ACYL-β-D-GALACTOPYRANOSYL)-*SN*-GLYCEROL[1]

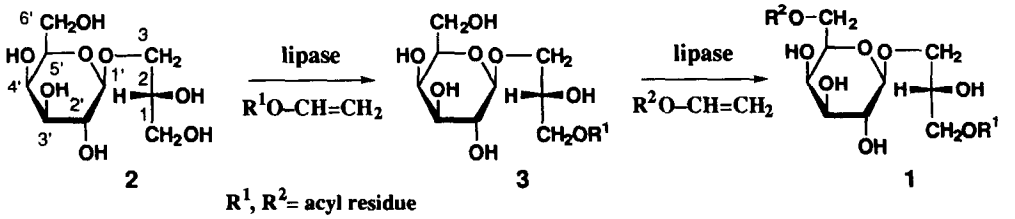
Takashi Morimoto, Akito Nagatsu, Nobutoshi Murakami[2] and Jinsaku Sakakibara*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Abstract : Convenient synthesis of 1-*O*-acyl-3-*O*-(6'-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol was studied. The lipase from *Achromobacter* sp. catalyzed acylation of 3-*O*-β-D-galactopyranosyl-*sn*-glycerol, which contains two primary hydroxyl functions, proceeded regioselectively to furnish 1-*O*-acyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol.

Glyceroglycolipids are major constituents of the chloroplast membrane in the plant kingdom. The biological function as well as occurrence and distribution of glyceroglycolipids has been an area of intense interest and investigation.[3] Some glycolipids with pharmacological activity have been isolated from various organisms other than higher plants, e.g. monogalactosyl diacylglycerols (MGDG) as anti-inflammatory substances[4] and sulfoquinovosyl diacylglycerols as anti-human immunodeficiency virus (HIV) active compounds[5]. MGDG and digalactosyl diacylglycerols (DGDG) inhibited tumor-promotion *in vitro*, and the strength of the activity was found to depend on their acyl pairs.[6] We reported that MGDG with oleoyl or myristoyl group at *sn*-1 position showed the stronger inhibition than those with other fatty acid residues.[7] We also reported that 1-*O*-acyl-3-*O*-(6'-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol (**1**) from the nitrogen-fixing cyanobacterium *Anabaena flos-aquae f. flos-aquae*[8] as a mixture possessing various acyl pairs. Since the galactolipid (**1**) was a minor component and it was difficult to separate the mixture into the compounds with a single acyl pairs, the biological activity has not been investigated. Recently, stereoselective or regioselective enzymatic acylation has been reported. In most case, the regioselectivity was depend on the difference of the reactivity between the secondary hydroxyl groups or between the primary and secondary hydroxyl groups of the substrates.[9] However, the regioselectivity between primary hydroxyl functions was scarcely investigated. This circumstance prompted us to investigate chemoenzymatic synthesis of **1** from 3-*O*-β-D-galactopyranosyl-

Scheme 1



sn-glycerol (**2**) which contains two primary hydroxyl functions (Scheme **1**). Here, we reported on convenient synthesis of the galactolipid (**1**) using lipase catalyzed regioselective acylation.

3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**) was prepared as follows. Dibenzyl-*sn*-glycerol[10] derived from D-mannitol was reacted with *tetra*-*O*-acetylgalactopyranosyl bromide[11] in the presence of HgO and $HgBr_2$ to furnish 1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*sn*-glycerol, which was deprotected with 5%-NaOMe/MeOH followed by hydrogenation to give 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**).

We screened several lipases for acylation of **2** with vinyl palmitate[12] as an acylating reagent. The acylation of **2** proceeded slightly in DMF or DMSO, and gave a trace amount of a diester in *t*-amyl alcohol or THF. On the other hand, use of pyridine as a reaction medium gave a mixture of monoester (**3a** and **4a**) in which the acyl residue was linked only to primary hydroxyl groups for all the lipases tested, *Achromobacter* sp. lipase (lipase AL), *Alcaligenes* sp. lipase (lipase PL), *Pseudomonas cepacia* lipase (lipase PS), *Mucor javanicus* lipase (lipase M), and *Rhizopus delemer* lipase (RDL) as shown in Table 1. Although lipase M, PS and RDL showed little selectivity, lipase AL and PL catalyzed the acylation selectively at *sn*-1 position of 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**), and lipase AL showed the best selectivity (**3a** : **4a**=95 : 5). The reaction was tested with other palmitate such as trichloroethyl ester, trifluoroethyl ester, and isopropenyl ester, and they exhibited lower reactivity and selectivity than vinyl palmitate. Furthermore, lipase AL catalyzed acylation of **2** with various vinyl esters in pyridine was carried out to obtain **3b**–**3g**. As shown in Table 2, variations of vinyl ester give no influence to the regioselectivity of the reaction. It is noteworthy that compound **3g** containing linoleic acid, which is liable to oxidation, was obtained in nearly the same yield as the others.

Then, we examined the optimal conditions to **1** from **3**. Acylation of **3f** with vinyl oleate using four lipases were tested in some organic solvents. Although lipase AL and PL catalyzed the acylation in CH_2Cl_2,

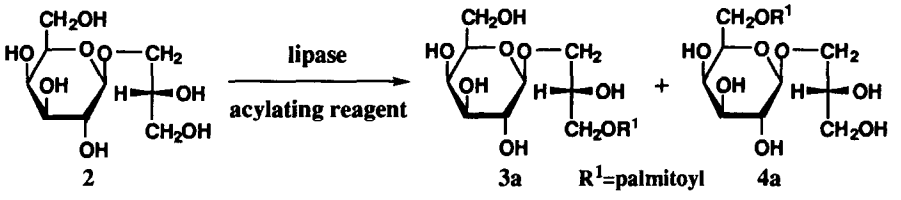


Table 1. Lipase Catalyzed Acylation of 3-*O*-β-D-Galactopyranosyl-*sn*-glycerol (**2**)[a]

lipase	acylating reagent	solvent	temp.	time (d)	conversion[b] (%)	**3a**:**7a**[c]
PS	vinyl palmitate	pyridine	40 °C	6	49	55 : 45
M	vinyl palmitate	pyridine	40 °C	7	11	46 : 54
RDL	vinyl palmitate	pyridine	40 °C	7	23	65 : 35
PL	vinyl palmitate	pyridine	40 °C	4	61	84 : 16
AL	vinyl palmitate	pyridine	40 °C	4	53	95 : 5
AL	vinyl palmitate	pyridine	room temp.	7	35	91 : 9
AL	trichloroethyl palmitate	pyridine	40 °C	7	42	85 : 15
AL	trifluoroethyl palmitate	pyridine	40 °C	7	45	83 : 17
AL	isopropenyl palmitate	pyridine	40 °C	7	37	91 : 9

a) All reaction were carried out using **2** (0.2 mM) and acylating reagent (0.6 mM) in the presence of lipase (150 mg) in pyridine (1.0 ml). b) The degree of conversion was determined by recovered substrate. c) The ratio of monoester was determined by HPLC analysis [J' sphere ODS-H80 (YMC), MeOH:H_2O=85:15].

Table 2. Lipase AL Catalyzed Acylation of **2** with Various Vinyl Esters of Fatty Acids

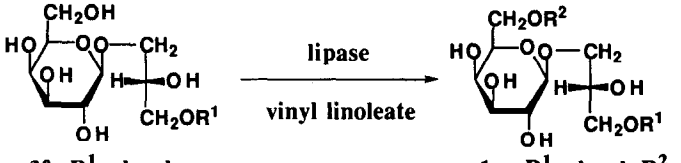
acylating reagent	time (d)	conversion[a] (%)	compound (isolated yield, %)	position of acylation C-1 : C-6'
vinyl laurate ($C_{12:0}$)	4	36	**3b** (32)	94 : 6
vinyl myristate ($C_{14:0}$)	4	42	**3c** (37)	92 : 8
vinyl palmitoleate ($C_{16:1}$)	3	47	**3d** (42)	93 : 7
vinyl stearate ($C_{18:0}$)	4	53	**3e** (48)	94 : 6
vinyl oleate ($C_{18:1}$)	3	53	**3f** (46)	94 : 6
vinyl linoleate ($C_{18:2}$)	4	51	**3g** (46)	93 : 7

a) The conversion rates were determined by recovered substrate.

they also catalyzed the hydrolysis to give **2** which was confirmed by HPLC analysis. The reaction with lipase M and lipase PS in CH_2Cl_2 exhibited good selectivity and gave **1a** without byproducts.

We applied the lipase-catalyzed acylation for preparation of 1-*O*-acyl-3-*O*-(6'-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerols possessing oleoyl or myristoyl group at the *sn*-1 position. As shown in Table 4, compound **3f** reacted with various vinyl esters in presence of lipase M in CH_2Cl_2 to give **1b**-**1d**. The acylation of **3c** also proceeded to give **1e**-**1g**.

In conclusion, we established the *sn*-1 regioselective enzymatic acylation of 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**) using *Achromobacter* sp. lipase (lipase AL) and vinyl esters of fatty acid in pyridine. Although many regioselective acylations with lipases have been demonstrated so far, to our best knowledge this is the first example discriminating between two primary hydroxyl groups in a polyhydroxyl molecule, which seems difficult to do with the usual organic reaction. We also found the regioselective acylation of 1-monoacylgalactosylglycerol (**3**) using *Mucor javanicus* lipase (lipase M) and vinyl esters of fatty acids in CH_2Cl_2, and the same time we established facile synthesis of 1-*O*-acyl-3-*O*-(6'-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol (**1**)[13] from 3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**) using the combination of two enzymatic acylation without any protecting groups.



3f : R^1=oleoyl **1a** : R^1=oleoyl, R^2=linoleoyl

Table 3. Lipase Catalyzed Acylation of 1-*O*-oleoyl-3-*O*-β-D-galactosyl-*sn*-glycerol (**3f**) with Vinyl linoleate[a]

lipase	solvent	temp.	time (d)	conversion[b] (%)	isolated yield (%)
AL	CH_2Cl_2	r. t.	4	85	73
PL	CH_2Cl_2	r. t.	4	95	79
M	CH_2Cl_2	r. t.	4	88	86
PS	CH_2Cl_2	r. t.	7	36	35

a) All reaction were carried out using **3f** (0.2 mM) and vinyl linoleate (0.6 mM) in the presence of lipase (75 mg) in CH_2Cl_2 (1.0 ml). b) The conversion rates were determined by recovered substrate.

Table 4. Lipase M Catalyzed Acylation of **3c** and **3f** with Vinyl Esters of Fatty Acids

product	R^1	R^2	time (d)	isolated yield (%)
1b	oleoyl (C18:1)	palmitoyl (C16:0)	4	89
1c	oleoyl	myristoyl (C14:0)	4	75
1d	oleoyl	oleoyl	4	90
1e	myristoyl	linoleoyl (C18:2)	4	85
1f	myristoyl	palmitoyl	4	78
1g	myristoyl	oleoyl	4	88

EXPERIMENTAL

General Method Lipase AL and PL were given from Meito Sangyo Co., Ltd. Lipase M and PS were given from Amano Pharmaceutical Co., Ltd. *Rhizopus delemer* lipase (RDL) was purchased from Biocatalyst Co., Ltd. ^1H NMR and ^{13}C NMR spectra were obtained with a JEOL GSX-400 (400 MHz) spectrometer using tetramethylsilane as an internal standard. FAB-MS were measured with JEOL DX-300 and JEOL DX-505 spectrometers. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. Gas-liquid chromatography (GLC) was carried out on a Shimadzu GC-8A. The conditions for identification of methyl esters of fatty acids were as follows: column, ULBON HR-SS-10 (0.25 mm i.d. × 50 m, Shinwa Kako Co., Ltd.); column temperature, 150-220 °C, 3 °C/min; injection temperature , 250 °C; carrier gas, N_2, 2.2 kg/cm^2. High performance liquid chromatography (HPLC) was performed using a JASCO 880-PU pump equipped with a Shodex RI SE-11 differential refractometer. Thin layer chromatography (TLC) was performed on Merck precoated Kieselgel $60F_{254}$, and spots were detected by illumination with an ultraviolet lamp, or by spraying 5% vanillin-70% $HClO_4$, 1% $Ce(SO_4)_2$-10% H_2SO_4 followed by heating. Column chromatography was performed on silica gel BW-200 or BW-300 (Fuji Davison Chemicals Co., Ltd.).

Preparation of 3-O-β-D-galactopyranosyl-sn-glycerol (2) 1,2-di-*O*-benzyl-*sn*-glycerol (0.68 g) and Drierite (2.5 g) were added in 1,2-dichloroethane (4.0 ml) and were stirred under atmosphere of nitrogen at room temperature for 0.5 h (flask I). 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.1 g, 3.0 eq) and Drierite (2.5 g) in 1,2-dichloroethane (4.0 ml) were stirred in a nitrogen atmosphere at room temperature for 0.5 h, and the mixture was added to flask I. Then, HgO (1.3 g, 2.4 eq) in 1,2-dichloroethane (4.0 ml) were stirred in a nitrogen atmosphere at room temperature for 0.5 h, and the mixture was added to flask I. Then, HgBr (0.081 g, 0.09 eq) was added to flask I, the whole was stirred in a nitrogen atmosphere at room temperature for 4.0 h. This reaction mixture was filtered through a plug of Celite, then the filtrate was washed with aqueous KBr (1M) and sat. NaCl. The dried ($MgSO_4$) organic layer was concentrated *in vacuo*. The residue was purified by SiO_2 column chromatography ($CHCl_3$:acetone=30:1) to furnish 1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-*sn*-glycerol (1.38 g). This compound (1.38 g) and palladium charcoal (10 %, 2.76 g) in EtOH (30 ml) was stirred under hydrogen (4.5 kg/m^2) for 48 h. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The residue was dissolved in dry MeOH (20 ml) and 5%-NaOMe/MeOH (20 ml) was added to the solution. After 10 min, the reaction mixture was neutralized by using ion-exchange resin (Dowex 50W × 8, 20-50 mesh), and the resin was removed by filtration. The filtrate was concentrated *in vacuo*. The resulting oil was purified by SiO_2 column chromatography ($CHCl_3$:MeOH:H_2O= 6:4:1) to yield **2** (0.55 g).

1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*sn*-glycerol: a colorless oil. $[\alpha]_D^{25}$ -10.5° (c =0.68, $CHCl_3$). IR (neat, cm^{-1}) : 1750, 1370, 1220, 1060, 750, 700. FAB-MS (m/z): 625(M+Na)$^+$. ^{1}H-NMR (270MHz, $CDCl_3$) δ: 1.95 (3H, s, -$COCH_3$), 1.98 (3H, s, -$COCH_3$), 2.04 (3H, s, -$COCH_3$), 2.15 (3H, s, -$COCH_3$), 3.53-3.79 (4H, m, *sn*-1-H_2, *sn*-3-H_2), 3.86 (1H, ddd, J=1.0, 6.6, 6.6 Hz, 5-H), 3.98 (1H, m, *sn*-2-H), 4.12 (1H, dd, *J*=6.6, 11.2 Hz, 6-H), 4.16(1H, dd, *J*=6.6, 11.2 Hz, 6-H), 4.50 (1H, d, *J*=7.9 Hz, 1-H), 4.53, 4.55 (each 1H, both d, *J*=12.0 Hz, -CH_2Ar), 4.65 (2H, s, -CH_2Ar), 4.99 (1H, dd, *J*=3.3, 10.6 Hz, 3-H), 5.20 (1H, dd, *J*=7.9, 10.6 Hz, 2-H), 5.38 (1H, dd, *J*=1.0, 3.3 Hz, 4-H), 7.26-7.40 (10H, m, Ar).

2: a colorless oil. $[\alpha]_D^{25}$ -8° (*c*=0.8, H_2O). IR (neat, cm^{-1}) : 3400. ^{1}H NMR (400MHz, C_5D_5N) δ : 4.08 (dd, *J*=5.3, 6.6 Hz, 5'-H), 4.13 (d, *J*=5.5 Hz, *sn*-1-H), 4.14 (d, *J*=4.9 Hz, *sn*-1-H), 4.17 (dd, *J*=3.3, 9.3 Hz, 3-H), 4.27(dd, *J*=3.8, 9.7 Hz, *sn*-3-H), 4.45 (3H, m, 6-H_2, *sn*-3-H, *sn*-2-H), 4.52 (dd, *J*=7.7, 9.3 Hz), 4.56 (d, *J*=3.3 Hz, 4-H), 4.91 (d, *J*=7.7 Hz, 1-H). ^{13}C NMR (100MHz, CD_3OD) δc: 105.3 (C-1'), 72.6 (C-2'), 74.9 (C-3'), 70.4 (C-4'), 76.8 (C-5'), 62.6 (C-6'), 64.1 (*sn*-1-C), 72.2 (*sn*-2-C), 72.1 (*sn*-3-C).

General procedure for preparation of 1-O-monoacyl-3-O-β-D-galactopyranosyl-sn-glycerol

A mixture of galactosyl glycerol (**2**,150 mg), vinyl palmitate (162 mg) and lipase AL(150 mg) in pyridine (1.0 ml) was stirred at 40°C. The reaction mixture was filtered and the solvent was removed *in vacuo*. The residue was separated by SiO_2 column chromatography ($CHCl_3$: MeOH:H_2O=10:3:1, lower layer) and HPLC (J' sphere ODS-H80, MeOH:H_2O=85:15) to give 1-*O*-palmitoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**3a**, 49.2 mg) and 3-*O*-(6'-*O*-palmitoyl-β-D-galactopyranosyl)-*sn*-glycerol (**7a**, 2.6 mg). Compounds (**3b**-**3g**) were obtained by similar method.

3a: a colorless oil, $[\alpha]_D^{25}$ -6.6° (*c*= 0.3 , MeOH). IR (film, cm^{-1}) : 3400, 1733. High resolution FAB-MS: Calcd for $C_{25}H_{48}O_9Na$ 515.3196, Found 515.3179, FAB-MS (m/z) : 515 (M+Na)$^+$ ^{1}H NMR (C_5D_5N, 400MHz) δ: 0.88 (3H, t, *J*=7.2 Hz), 2.33 (2H, t, *J*=7.2 Hz), 4.07 (1H, dt, *J*=2.5, 6.0 Hz, 5-H), 4.11 (1H, dd, *J*=5.1, 10.2 Hz, *sn*-3-H), 4.15 (1H, dd, *J*=3.5, 9.7 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.0 Hz, 6-H), 4.50 (1H, dd, *J*=7.7, 9.7 Hz, 2-H), 4.55 (1H, dd, *J*=3.5, 2.6 Hz, 4-H), 4.59 (1H, d, *J*=5.4 Hz, *sn*-1-H), 4.60 (1H, d, *J*=3.2 Hz, *sn*-1-H), 4.88 (1H, d, *J*=7.7 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.4 (C=O), 105.0 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.5 (*sn*-1-C), 62.4 (6-C).

7a: a colorless oil, $[\alpha]_D^{25}$ -10.1° (*c*=0.1, MeOH). IR (film, cm^{-1}) : 3400, 1733. High resolution FAB-MS: Calcd for $C_{25}H_{48}O_9Na$ 515.3196, Found 515.3188, FAB-MS (m/z) : 515 (M+Na)$^+$. ^{1}H NMR(500 MHz, C_5D_5N) δ: 4.91 (1H, d, *J*=7.9 Hz, 1-H), 4.88 (1H, dd, *J*=5.0, 10.9 Hz, 6-H), 4.80 (1H, dd, *J*=5.0, 10.9 Hz, 6-H), 4.52 (1H, dd, *J*=7.9, 9.8 Hz, 2-H), 4.50 (1H, dd, *J*=5.3, 9.7 Hz, *sn*-1-H), 4.45 (1H, m, *sn*-2-H), 4.40 (1H, d, *J*=3.4 Hz, 4-H), 4.29 (1H, dd, *J*=4.5, 9.7 Hz, *sn*-1-H), 4.20-4.15 (4H, m, 3-H, 5-H, *sn*-3-H_2), 2.39 (2H, t, *J*=7.3 Hz), 0.87 (1H, t, *J*=7.3 Hz). ^{13}C NMR (125MHz, C_5D_5N) δc: 173.7 (C=O), 105.6 (1-C), 74.8 (3-C), 73.7 (5-C), 72.7 (*sn*-2-C), 72.2 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 64.5 (6-C), 64.2 (*sn*-1-C).

3b: a colorless oil, $[\alpha]_D^{25}$ -8.0° (*c*= 0.3, MeOH). IR (film, cm^{-1}) : 3400, 1735. High resolution FAB-MS: Calcd for $C_{21}H_{40}O_9Na$ 459.2570, Found 459.2581, FAB-MS (m/z): 459 (M+Na)$^+$. ^{1}H NMR (C_5D_5N, 400MHz) δ: 0.88 (3H, t, *J*=7.4 Hz), 2.33 (2H, t, *J*=7.3 Hz), 4.06 (1H, dt, *J*=2.3, 6.0 Hz, 5-H), 4.12 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.14 (1H, dd, *J*=3.6, 9.7 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.1 Hz, 6-H), 4.49 (1H, dd, *J*=7.7, 9.7 Hz, 2-H), 4.55(1H, dd, *J*=3.6, 2.3 Hz, 4-H), 4.59 (1H, d, *J*=5.4 Hz, *sn*-1-H), 4.60 (1H, d, *J*=3.5 Hz, *sn*-1-H), 4.88 (1H, d, *J*=7.7 Hz, 1-H). ^{13}C

NMR(C_5D_5N, 100MHz) δc: 175.4 (C=O), 105.0 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.5 (*sn*-1-C), 62.4 (6-C).

3c: a colorless oil, $[\alpha]_D^{25}$ -7.0° (*c*= 0.3, MeOH). IR (film, cm^{-1}) : 3400, 1733. High resolution FAB-MS: Calcd for $C_{23}H_{44}O_9Na$ 487.2283, Found 487.2279, FAB-MS (m/z): 487 $(M+Na)^+$. ^1H NMR (C_5D_5N, 400MHz) δ: 0.89 (3H, t, *J*=7.0 Hz), 2.35 (2H, t, *J*=7.3 Hz), 4.06 (1H, dt, *J*=2.5, 6.1 Hz, 5-H), 4.11 (1H, dd, *J*=5.0, 10.1 Hz, *sn*-3-H), 4.14 (1H, dd, *J*=3.4, 9.8 Hz, 3-H), 4.43 (1H, dd, *J*=5.1, 10.2 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.0 Hz, 6-H), 4.50 (1H, dd, *J*=7.6, 9.8 Hz, 2-H), 4.56(1H, dd, *J*=3.4, 2.5 Hz, 4-H), 4.59 (2H, m, *sn*-1-H_2), 4.88 (1H, d, *J*=7.6 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.2 (C=O), 105.0 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.5 (*sn*-1-C), 62.4 (6-C).

3d: a colorless oil, $[\alpha]_D^{25}$ -6.8° (*c*= 0.2, MeOH). IR (film, cm^{-1}) : 3400, 1735. High resolution FAB-MS: Calcd for $C_{25}H_{46}O_9Na$ 513.3040, Found 513.3026, FAB-MS (m/z):513 $(M+Na)^+$. ^1H NMR (C_5D_5N, 400MHz) δ: 0.88 (3H, t, *J*=7.5 Hz), 2.35 (2H, t, *J*=7.3 Hz), 4.07 (1H, dt, *J*=2.3, 6.0 Hz, 5-H), 4.11 (1H, dd, *J*=5.1, 10.2 Hz, *sn*-3-H), 4.15 (1H, dd, *J*=3.5, 9.7 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.46 (1H, d, *J*=6.3 Hz, 6-H), 4.50 (1H, dd, *J*=7.7, 9.7 Hz, 2-H), 4.55(1H, dd, *J*=3.5, 2.3 Hz, 4-H), 4.59 (1H, d, *J*=5.9 Hz, *sn*-1-H), 4.61 (1H, d, *J*=3.7 Hz, *sn*-1-H), 4.89 (1H, d, *J*=7.7 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.4 (C=O), 105.0 (1-C), 76.6 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2(4-C), 69.6(*sn*-2-C), 66.5(*sn*-1-C), 62.4 (6-C).

3e: a colorless oil, $[\alpha]_D^{25}$ -6.6° (*c*= 0.3, MeOH). IR (film, cm^{-1}) : 3400, 1735. High resolution FAB-MS: Calcd for $C_{27}H_{52}O_9Na$ 543.3510, Found 543.3495, FAB-MS (m/z):543 $(M+Na)^+$. ^1H NMR (C_5D_5N, 400MHz) δ: 0.90 (3H, t, *J*=7.3 Hz), 2.34 (2H, t, *J*=7.5 Hz), 4.07 (1H, dt, *J*=2.5, 6.0 Hz, 5-H), 4.11 (1H, dd, *J*=5.3, 10.1 Hz, *sn*-3-H), 4.14 (1H, dd, *J*=3.6, 9.7 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.1 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.0 Hz, 6-H), 4.50 (1H, dd, *J*=7.6, 9.7 Hz, 2-H), 4.54(1H, dd, *J*=3.6, 2.6 Hz, 4-H), 4.59 (1H, d, *J*=5.4 Hz, *sn*-1-H), 4.60 (1H, d, *J*=3.2 Hz, *sn*-1-H), 4.88 (1H, d, *J*=7.6 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.4 (C=O), 105.0 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.5 (*sn*-1-C), 62.4 (6-C).

3f: a colorless oil, $[\alpha]_D^{25}$ -6.4° (*c*= 0.2, MeOH). IR (film, cm^{-1}) : 3400, 1735. High resolution FAB-MS: Calcd for $C_{27}H_{50}O_9Na$ 541.3353, Found 541.3369, FAB-MS (m/z):541 $(M+Na)^+$. ^1H NMR (C_5D_5N, 400MHz) δ: 0.89 (3H, t, *J*=7.3 Hz), 2.34 (2H, t, *J*=7.5 Hz), 4.07 (1H, dt, *J*=2.5, 6.0 Hz, 5-H), 4.11 (1H, dd, *J*=5.1, 10.2 Hz, *sn*-3-H), 4.15 (1H, dd, *J*=3.5, 9.7 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.0 Hz, 6-H), 4.50 (1H, dd, *J*=7.7, 9.7 Hz, 2-H), 4.55 (1H, dd, *J*=3.5, 2.6 Hz, 4-H), 4.59 (1H, d, *J*=5.4 Hz, *sn*-1-H), 4.60 (1H, d, *J*=3.2 Hz, *sn*-1-H), 4.88 (1H, d, *J*=7.7 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.5 (C=O), 105.1 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.5 (*sn*-1-C), 62.3 (6-C).

3g: A colorless oil, $[\alpha]_D^{25}$ -5.9° (*c*= 0.3, MeOH). IR (film, cm^{-1}) : 3400, 1735. High resolution FAB-MS: Calcd for $C_{27}H_{48}O_9Na$ 539.3196, Found 539.3198, FAB-MS (m/z):539 $(M+Na)^+$. ^1H NMR (C_5D_5N, 400MHz) δ: 0.94 (3H, t, *J*=7.5 Hz), 2.06 (4H, m) 2.35 (2H, t, *J*=7.3 Hz),4.07 (1H, dt, *J*=2.5, 6.0 Hz, 5-H), 4.11 (1H, dd, *J*=5.1, 10.2 Hz, *sn*-3-H), 4.15 (1H, dd, *J*=3.5, 9.8 Hz, 3-H), 4.43 (1H, dd, *J*=4.9, 10.2 Hz, *sn*-3-H), 4.45 (1H, d, *J*=6.0 Hz, 6-H), 4.50 (1H, dd, *J*=7.7, 9.8 Hz, 2-H), 4.54 (1H, dd, *J*=3.5, 2.6 Hz, 4-H), 4.59 (1H, d, *J*=5.4 Hz, *sn*-1-H), 4.60 (1H, d, *J*=3.2 Hz, *sn*-1-H), 4.89 (1H, d, *J*=7.7 Hz, 1-H). ^{13}C NMR(C_5D_5N, 100MHz) δc: 175.2 (C=O), 105.1 (1-C), 76.7 (5-C), 74.7 (3-C), 72.5 (2-C), 71.8 (*sn*-3-C), 70.2 (4-C), 69.6 (*sn*-2-C), 66.6 (*sn*-1-C), 62.4 (6-C).

General procedure for preparation of 1-O-acyl-3-O-(6'-O-acyl-β-D-galactopyranosyl)-sn-glycerol A mixture of 1-*O*-oleoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (25 mg), vinyl linoleate (44 mg) and lipase M (75 mg) in CH_2Cl_2(1.0 ml) was stirred at room temperature. The reaction mixture was filtered to remove the enzyme and the solvent was removed *in vacuo*. The residue was separated by SiO_2 column chromatography ($CHCl_3$:MeOH=15:1) to furnish 1-*O*-oleoyl-3-*O*-(6'-*O*-linoleoyl-β-D-galactopyranosyl)-*sn*-glycerol (**1a**, 30 mg). Compounds (**1b-1g**) were obtained by similar method.

1a: a colorless oil. $[\alpha]_D^{25}$ -2.2° (*c*=0.3, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. High resolution FAB-MS: Calcd for $C_{45}H_{80}O_{10}Na$ 803.5649, Found 803.5627. FAB- MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 5.40(6H, m, olefinic H), 4.90 (1H, dd, *J*=7.4, 11.0 Hz, 6-H), 4.89 (1H, d, *J*=7.7 Hz, 1-H), 4.80(1H, dd, *J*=5.5, 11.0 Hz, 6-H), 4.62 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.7, 9.5 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.3 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.4 Hz, 4-H), 4.18 (1H, dd, *J*=3.4, 9.5 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.4 Hz, 5-H), 4.14(1H, dd, *J*=5.1, 10.3 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.4 (C=O), 173.5 (C=O), 105.4 (1-C), 74.7 (3-C), 73.8 (5-C), 72.0 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

1b: a colorless oil. $[\alpha]_D^{25}$ -2.9° (*c*=0.2, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. FAB- MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 5.40 (2H, m, olefinic H), 4.90 (1H, dd, *J*=7.3, 10.8 Hz, 6-H), 4.89 (1H, d, *J*=7.6 Hz, 1-H), 4.80(1H, dd, *J*=5.3, 10.8 Hz, 6-H), 4.61 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.6, 9.4 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.4 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.3 Hz, 4-H), 4.18 (1H, dd, *J*=3.3, 9.4 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.3 Hz, 5-H), 4.14 (1H, dd, *J*=5.1, 10.4 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.4 (C=O), 173.5(C=O), 105.4 (1-C), 74.7 (3-C), 73.9 (5-C), 72.0 (2-C), 71.9(*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

1c: a colorless oil. $[\alpha]_D^{25}$ -3.3° (*c*=0.2, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. FAB- MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 5.40(2H, m, olefinic H), 4.90 (1H, dd, *J*=7.3, 10.8 Hz, 6-H), 4.89 (1H, d, *J*=7.6 Hz, 1-H), 4.80 (1H, dd, *J*=5.3, 10.8 Hz, 6-H), 4.62 (2H, m, *sn*-1-H_2), 4.51 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.6, 9.4 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.4 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.3 Hz, 4-H), 4.19 (1H, dd, *J*=3.3, 9.4 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.3 Hz, 5-H), 4.15 (1H, dd, *J*=5.1, 10.3 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.5 (C=O), 173.5(C=O), 105.4 (1-C), 74.7 (3-C), 73.8 (5-C), 72.0 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

1d: a colorless oil . $[\alpha]_D^{25}$ -3.1° (*c*=0.2, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720 FAB-MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 5.40 (4H, m, olefinic H), 4.89 (1H, dd, *J*=7.5, 11.0 Hz, 6-H), 4.88 (1H, d, *J*=7.7 Hz, 1-H), 4.80 (1H, dd, *J*=5.5, 11.0 Hz, 6-H), 4.62 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.49 (1H, dd, *J*=7.7, 9.5 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.3 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.4 Hz, 4-H), 4.18 (1H, dd, *J*=3.4, 9.5 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.5 Hz, 5-H), 4.14 (1H, dd, *J*=5.1, 10.3 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.5(C=O), 173.5 (C=O), 105.4 (1-C), 74.7 (3-C), 73.8 (5-C), 72.0 (2-C), 72.0 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

1e: a colorless oil. $[\alpha]_D^{25}$ -1.8° (*c*=0.3, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. FAB-MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 5.40 (4H, m, olefinic H), 4.91 (1H, dd, *J*=7.7, 10.9 Hz, 6-H), 4.89 (1H, d, *J*=7.6 Hz, 1-H), 4.80 (1H, dd, *J*=5.5, 10.9 Hz, 6-H), 4.62 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.7, 9.5 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.3 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.4 Hz, 4-H), 4.18 (1H, dd, *J*=3.4, 9.5 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.7 Hz, 5-H), 4.14 (1H, dd, *J*=5.1, 10.3 Hz,

sn-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.3 (C=O), 173.5 (C=O), 105.4 (1-C), 74.7 (3-C), 73.8 (5-C), 72.0 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.4 (*sn*-1-C).

1f: a colorless oil. $[\alpha]_D^{25}$ -2.0° (*c*=0.2, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. FAB-MS (m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ: 4.90 (1H, dd, *J*=7.5, 11.0 Hz, 6-H), 4.89 (1H, d, *J*=7.7 Hz, 1-H), 4.80 (1H, dd, *J*=5.5, 11.0 Hz, 6-H), 4.62 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.7 , 9.5 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.3 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.4 Hz, 4-H), 4.18 (1H, dd, *J*=3.4, 9.5 Hz, 3-H), 4.17 (1H, dd, *J*=5.3, 7.5 Hz, 5-H), 4.13 (1H, dd, *J*=5.0, 10.6 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.5 (C=O), 173.5(C=O), 105.4 (1-C), 74.7 (3-C), 73.8(5-C), 72.0 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

1g: a colorless oil. $[\alpha]_D^{25}$ -2.3°(*c*=0.3, $CHCl_3$). IR (film, cm^{-1}) : 3900, 1735, 1720. FAB- MS(m/z): 803 $(M+Na)^+$. ^1H NMR (C_5D_5N, 500 MHz) δ : 5.40 (2H, m, olefinic H), 4.91 (1H, dd, *J*=7.0, 10.8 Hz, 6-H), 4.89 (1H, d, *J*=7.7 Hz, 1-H), 4.79 (1H, dd, *J*=5.5, 10.8 Hz, 6-H), 4.60 (2H, m, *sn*-1-H_2), 4.52 (1H, m, *sn*-2-H), 4.50 (1H, dd, *J*=7.7, 9.5 Hz, 2-H), 4.45 (1H, dd, *J*=5.5, 10.3 Hz, *sn*-3-H), 4.41 (1H, d, *J*=3.4 Hz, 4-H), 4.18 (1H, dd, *J*=3.4, 9.5 Hz, 3-H), 4.16 (1H, dd, *J*=5.3, 7.4 Hz, 5-H), 4.14 (1H, dd, *J*=5.1, 10.3 Hz, *sn*-3-H). ^{13}C NMR (125 MHz, C_5D_5N) δc: 173.4 (C=O), 173.5 (C=O), 105.3 (1-C), 74.7 (3-C), 73.8 (5-C), 72.0 (2-C), 71.9 (*sn*-3-C), 69.9 (4-C), 68.8 (*sn*-2-C), 66.5 (*sn*-1-C).

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REFERENCES AND NOTES

1. Partly presented in our previous communication. Morimoto, T.; Murakami, N.; Nagatsu, A.; Sakakibara, J., *Chem. Pharm. Bull.*, **1994**, *42*, 751.
2. Present address: Kyoto Pharmaceutical University, 5 Misasagi, Yamashina-ku, Kyoto 607, Japan.
3. a) Ishizuka, I.; Yamakawa, T. *New Comprehensive Biochemistry*; Neuberger, A.; Van Deenen, L. L. M.; Wiegandt, H. Eds.; Elsevier: Amsterdam, Vol. 10, 1985; pp. 101-198. b) Van Hummel, H. C. *Fortsch. Org. Naturst.*, **1975**, *32*, 267. c) Kobayashi, M.; Hayashi, K.; Kawazoe, K.; Kitagawa, I. *Chem. Pharm. Bull.*, **1992**, *40*, 1404. d) Jiang, Z. D.; Gerwick, W. H. *Phytochemistry*, **1990**, *29*, 1433. e) *Idem*, *Lipids*, **1991**, *26*, 960. f) Sakata, K.; Ina, K. *Agric. Biol. Chem.*, **1990**, *47*, 2957.
4. T.; Nakanishi, H.; Kobayashi, M.; Kitagawa, I. *ibid.*, **1982**, *30*, 3544.
5. Gustafson, K. R.; Cardellia, J. H.; Fuller, R. W.; Weislow, O. S.; Kiser, R. H., *J. Natl. Cancer Inst.*, **1989**, *81*, 1254.
6. Shirahashi, H.; Murakami, N.; Watanabe, M.; Nagatsu, A.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A., *Chem. Pharm. Bull.*, **1993**, *41*, 1664.
7. Nagatsu, A.; Watanabe, M.; Ikemoto, K.; Hashimoto, M.; Murakami, N.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A.; Yazawa, K., *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 1619.
8. Murakami, N.; Shirahashi, H.; Nagatsu, A.; Sakakibara, J., *Chem. Pharm. Bull.*, **1993**, *41*, 1177.
9. a) Therisod, M.; Klibanov, A. M., *J. Am. Chem. Soc.*, **1986**, *108*, 5638; b) *idem*, *ibid.*, **1987**, *109*, 3977 ; c) Uemura, A.; Nozaki, K.; Yamashita, J.; Yasumoto, M., *Tetrahedron Lett.*, **1989**, *30*, 3817; d) Margolin, A. L.; Delinck, D. L.; Whalon, M. R., *J. Am. Chem. Soc.*, **1990**, *112*, 2849; e) Moris, F.; Gotor, V., *J. Org. Chem.*, **1992**, *57*, 2490.
10. van Boeckel, C. A. A.; Visser, G. M.; van Boom, J. H., *Tetrahedron*, **1985**, *41*, 4557.
11. Fletcher, Jr. H. G., *Methods Carbohydr. Chem.*; Whistler, R. L.; Wolform, M. L. ED., Academic Press; New York, 1963; Vol. II, pp. 226.
12. Swern, D.; Jordan, E. F., *Organic Syntheses*, Coll. Vol. IV, ed. by N. Rabjohn, John Wiley and Sons, Inc., New York, 1963, p. 977.
13. The investigation of the physicochemical properties and biological functions of **1** are now in progress.

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