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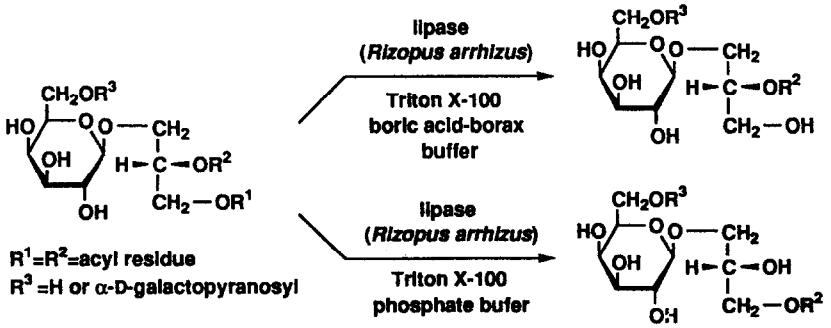
Enzymatic Transformation of Glyceroglycolipids into *sn*-1 and *sn*-2 Lysoglyceroglycolipids by Use of *Rhizopus arrhizus* Lipase[1]

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

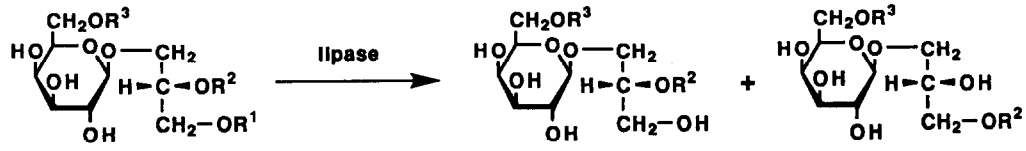
Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Abstract: Lipase from *Rhizopus arrhizus* catalyzed deacylation of two classes of glyceroglycolipids, monogalactosyl diacylglycerol(MGDG), and digalactosyl diacylglycerol(DGDG), proceeded regiospecifically to furnish *sn*-1 lysoglyceroglycolipids quantitatively. The lipase also catalyzed complete acyl migration of *sn*-1 lysoglycerogalactolipids leading to *sn*-2 lysoglycerogalactolipids.

The occurrence, biosynthesis, and metabolic functions of glyceroglycolipids, the major lipids constructing plant membrane, have been an area of intense interest and investigation over the past twenty years.[3] Furthermore, some glyceroglycolipids possessing pharmacological activity and/or unique structural features have been isolated and characterized from various organisms.[4] The physical and biological properties of such compounds are directly related to their chemical structure. In particular, the location and kinds of substituents esterified with a glycerol moiety influence not only the physical properties but also the biological function of glyceroglycolipids.[5] In the structural elucidation of glyceroglycolipids, location of fatty acid substituents linked to a glycerol moiety have been determined by enzymatic hydrolysis using lipase. However, regioselectivity and yield in the enzymatic reaction were ambiguous and resulting lysoglycolipids have never been isolated and characterized.[6]



Scheme 1



1a: R^1=linolenoyl, R^2=myristoyl, R^3=H

5a: R^1=linolenoyl, R^2=myristoyl, R^3=α-D-Gal
α-D-Gal=α-D-galactopyranosyl

2a: R^2=myristoyl, R^3=H

4: R^2=R^3=H

6a: R^2=myristoyl, R^3=α-D-Gal

3a: R^2=myristoyl R^3=H,

7a: R^2=myristoyl R^3=α-D-Gal

Scheme 2

On the other hand, lysoglycerophospholipids have been investigated intensively from various points of view because of a variety of bioactivities and important precursors for elucidating biogenetic pathway of glycerophospholipids.[7] The physical properties and biological activities of lysoglyceroglycolipids, however, have been little studied since their efficient and practical preparation procedure has never been established. This circumstance prompted us to examine regiospecific deacylation of glyceroglycolipid by enzymatic hydrolysis using lipase in the course of our investigation on structural elucidation of the biologically active monogalactosyl diacylglycerol (MGDG) from the cyanobacterium *Phormidium tenue*.[8] Here, we present the full detail of lipase catalyzed regiospecific deacylation of glyceroglycolipids and acyl migration of *sn*-1 lysoglyceroglycolipids into *sn*-2 lysocongeners [1] (Scheme 1).

First of all, we screened several readily available lipase for deacylation of the monogalactosyl diacylglycerol (**1a**), 1-*O*-linolenoyl-2-*O*-myristoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol, from *P. tenue* in phosphate buffer (pH=7.0). The results of enzymatic hydrolysis of **1a** using the lipase are summarized in Table 1. *Aspergillus niger* lipase (lipase A), *Mucor javanicus* lipase (lipase M), and porcine pancreas lipase (PPL) hydrolyzed **1a** to a mixture of two deacylated products (**2a** and **3a**) together with recovery of **1a**, while *Candida cylindracea* lipase (CCL) gave galactosylglycerol (**4**) as a sole hydrolyzed product. In the four lipase catalyzed hydrolysis, prolongation of reaction time and addition of lipase resulted in no obvious alternation,

Table 1. Lipase Catalyzed Hydrolysis of Monogalactosyl Diacylglycerol (**1a**)

lipase[a)]	conversion ratio[b)]	product distribution[b)] **2a : 3a : 4**
M	43 %	93 : 7 : 0
A	15 %	90 : 10 : 0
PPL	25 %	59 : 41 : 0
CCL	32 %	0 : 0 : 100
RAL	100 %	71 : 29 : 0

a) All reactions were carried out using 2.0 mg of substrate and 720 unit of lipase in 0.25 ml of phosphate buffer (pH 7.0).

b) In the lipase catalyzed hydrolysis of **1a**, no products other than fatty acids were observed. The conversion ratio and product distribution were determined by HPLC analysis after 2.5 hr.

Table 2. *Rhizopus arrhizus* Lipase (RAL) Catalyzed Deacylation of Galactolipids (**1a**, **5a**)

substrate [a]	buffer [b]	amounts of lipase	Triton X-100	reaction time [c]	**2a**:**3a** (**6a**:**7a**) [d]
1a	tris	1800 unit	no addition	2.5 h	76 : 24
(MGDG)	phosphate	1800 unit	no addition	2.5 h	71 : 29
	boric acid-borax	1800 unit	no addition	2 h	84 : 16
	tris	1800 unit	2.5 mg	1 h	79 : 21
	phosphate	1800 unit	2.5 mg	1.5 h	60 : 40
	boric acid-borax	1800 unit	2.5 mg	1 h	100 : 0
5a	tris	1800 unit	2.5 mg	6 h	63 : 37
(DGDG)	phosphate	1800 unit	2.5 mg	1.5 h	50 : 50
	boric acid-borax	1800 unit	2.5 mg	2.5 h	100 : 0

a)All reactions were performed using 5 mg of substrate in 0.6 ml of buffer solution.
b)All buffer solutions were adjusted to pH 7.7.
c)Time to consume substrate completely was shown.
d)A mixture of lysogalactolipids was yielded quantitatively and the product ratio was determined by HPLC analysis.

although more than half of substrates were remained. In contrast, *Rhizopus arrhizus* lipase (RAL) thoroughly converted **1a** into a mixture of two products (**2a** and **3a**) and linolenic acid.

Compound **2a** was obtained as a colorless oil and gave a quasimolecular ion peak at 487 $(M+Na)^+$ in its FAB-MS spectrum. In the ^1H NMR spectrum, the signal due to methylene protons on *sn*-1 carbon were observed in a higher field (δ 3.66-3.79) than those of **1a** (δ 4.43, 1H, dd, *J*=3.1, 12.0 Hz; δ 4.22, 1H, dd, *J*=6.8, 12.0 Hz). Furthermore, the *sn*-1 carbon signal (δc 61.7) was observed to shift upfield by 2.3 ppm and the *sn*-2 one (δc 74.7) downfield 2.9 ppm as compared the carbon chemical shifts of **2a** with those of **1a**. These alteration in the chemical shifts of two carbon signals was rationalized by acylation shift.[9] On the basis of the above physicochemical properties, the structure of **2a** was determined to be 2-*O*-myristoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol. Compound **3a**, a colorless oil, also exhibited a quasimolecular ion peak at 487 $(M+Na)^+$ in its FAB-MS spectrum. Detailed comparison of ^1H NMR spectra of **3a** with **1a** disclosed that the signal ascribable to the methine proton on *sn*-2 carbon appeared in a higher field (δ 3.98, 1H, m) than that of **1a** (δ 5.26, 1H, m). In the ^{13}C NMR spectrum of **3a**, each signal due to *sn*-1, *sn*-2, and *sn*-3 carbon was respectively shifted by +2.6, -2.1, and +3.2 ppm in comparison with that of **1a**. Based on these spectral data, the structure of **3a** was elucidated to be 1-*O*-myristoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol. The structures of **2a** and **3a** were also substantiated by the result that NaOMe-MeOH treatment gave methyl myristate and 3-*O*-β-D-galactopyranosyl-*sn*-glycerol[10] (**5a**) quantitatively (Scheme 2).

The configuration at *sn*-2 carbon of **3a** was determined as follows. H. Ohrui *et al.* reported that apparent difference was observed between (2*R*) and (2*S*) 1-*O*-acyl-3-*O*-β-D-galactosyl-*sn*-glycerol in the ^1H NMR spectra (CD_3OD) regardless of acyl substituents.[11] Comparison of ^1H NMR spectrum of **3a** with an authentic one showed that the stereochemistry at *sn*-2 carbon in **3a** proved to be *S*-configuration and be retained in the present enzymatic transformation.

Table 3. Enzymatic Transformation of Galactolipids (**1a**, **5a**) into *sn*-2 Lysogalactolipids (**3a**, **7a**)

substrate[a]	buffer[a]	amounts of lipase	Triton X-100	reaction time [b]	**2a**:**3a** (**6a**:**7a**)[a]
1a	tris	1800 unit	no addition	18 h	0 : 100
(MGDG)	phosphate	1800 unit	no addition	25 h	0 : 100
	boric acid -borax	1800 unit	no addition	6 h	84 : 16
	tris	700 unit	2.5 mg	18 h	0 : 100
	phosphate	700 unit	2.5 mg	23 h	0 : 100
	boric acid -borax	700 unit	2.5 mg	6 h	78 : 22
5a	tris	700 unit	2.5 mg	25 h	0 : 100
(DGDG)	phosphate	700 unit	2.5 mg	16 h	6 : 94
	boric acid -borax	700 unit	2.5 mg	30 h	11 : 89

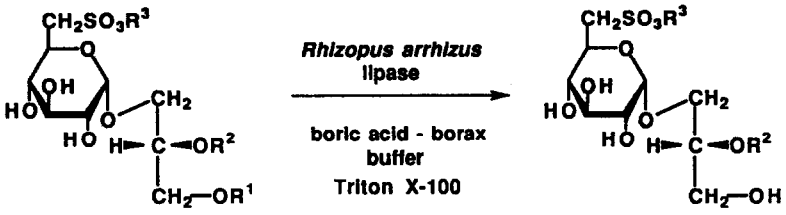
a) These conditions are the same as shown in Table 1.
b) Time to reach constant ratio of **2** and **3** (**5** and **6**) is shown, and the product ratio was determined by HPLC analysis.

In order to improve the selectivity of products, several reaction conditions were examined. Use of organic solvents such as MeOH and acetone brought about decrease of reaction rate and no apparent change in selectivity was observed. Modification of buffer also resulted in formation of **3a**. On the other hand, addition of Triton X-100 stimulated the lipase catalyzed deacylation of **1a** and the hydrolysis in boric acid-borax buffer gave only the *sn*-1 lysogalactolipid (**2a**) quantitatively at the point of consuming **1a** (Table 2).

The results of enzymatic hydrolysis using *Rhizopus arrhizus* lipase in phosphate and tris buffers indicate that the lipase deacylated **1a** regiospecifically at *sn*-1 position and *sn*-2 lysogalactolipid (**3a**) was formed subsequently by transacylation of *sn*-1 lysogalactolipid (**2a**) in the reaction medium. These findings indicate that *Rhizopus arrhizus* lipase transfers the acyl substituent from *sn*-2 position to *sn*-1 position. Further investigation of the lipase catalyzed hydrolysis of **1a** revealed that *sn*-2 lysoderivative increased along with prolonging reaction time as shown in Table 3. The *sn*-2 lysoderivative (**3a**) was yielded exclusively and quantitatively in tris and phosphate buffer without Triton X-100. Use of Triton X-100 enabled to reduce the amounts of lipase. In this transformation, acyl migration from **2a** to **3a** was considered to proceed enzymatically because treatment of **2a** with only Triton X-100 in phosphate or tris buffer gave little amount of **3a** and the conversion from **2a** to **3a** was achieved in the presence of the lipase. This finding suggests that RAL possessed acyltransferase activity toward *sn*-1 lysogalactolipids or contained acyltransferase as an impurity.

The selective enzymatic transformation was applicable to digalactosyl diacylglycerol (DGDG, **5a**) contained abundantly in a wide variety of plants. In the case of DGDG(**5a**), the reaction in boric acid-borax buffer and the one in tris buffer gave the *sn*-1 lysoderivative (**6a**) and *sn*-2 lysoderivative (**7a**), respectively (Table 2 and 3).

Finally, we applied the transformation to sulfoquinovosyl diacylglycerol (SQDG), which is also a major membrane lipid. The conversion from **8a**[13] to the corresponding *sn*-1 lysosulfolipd (**9a**) similarly proceeded

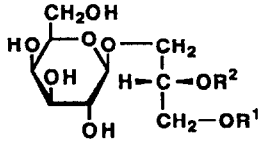


8a: R^1= linolenoyl, R^2=linoleoyl
R^3=Na, K, 1/2Ca, 1/2Mg, NH_4

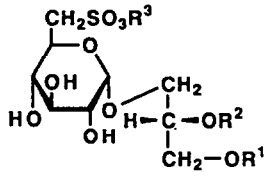
9a

Scheme 3

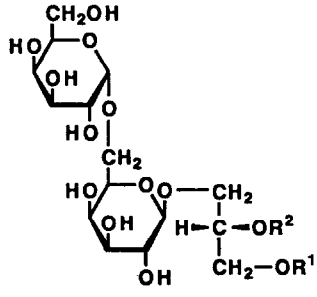
selectively and quantitatively under the same reaction conditions as the preparation of **2a** and **5a** (Scheme 3). However, the enzymatic transformation into the corresponding *sn*-2 lysosulfolipid was little observed in tris buffer. Examination in pH and buffer never resulted in apparent change. Treatment of *sn*-1 lysosulfolipid (**9a**) with the lipase afforded trace amount of *sn*-2 lysosulfolipid.



2b: R^1=H, R^2=palmiteraldoyl
2c: R^1=H, R^2=palmitoyl
2d: R^1=H, R^2=linoleoyl
3b: R^1=palmiteraldoyl, R^2=H
3c: R^1=palmitoyl, R^2=H
3d: R^1=linoleoyl, R^2=H



9b: R^1=H, R^2=isoheptadecanoyl
R^3=Na, K, NH_4, 1/2Ca, 1/2Mg
9c: R^1=H, R^2=palmitoyl
R^3=Na, K, NH_4, 1/2Ca, 1/2Mg



6b: R^1=H, R^2=linoleoyl
6c: R^1=H, R^2=palmitoyl
7b: R^1=linoleoyl, R^2=H
7c: R^1=palmitoyl, R^2=H

Fig. 1

By use of the present enzymatic transformation, we characterized eight MGDGs and nine DGDGs from the cyanobacterium *Phormidium tenue* and five SQDGs from the green alga *Chlorella vulgaris*.[12,13] Additionally, *sn*-1 lysogalactolipids (**2a-2d, 6a-6c**), *sn*-2 lysogalactolipids (**3a-3d, 7a-7c**), and *sn*-1 lysosulfolipids (**9a-9c**) were prepared (Fig. 1). While the appearance of selectivity in the reaction seems to be related with buffer solutions, the reason why acyl migrations of *sn*-1 lysogalactolipids in boric acid-borax buffer is depressed remains unknown. However, it should be noted that *sn*-1 and *sn*-2 lysogalactolipids, and *sn*-1 lysosulfolipids were prepared quantitatively and selectively in this conversion method.[14] Since the physical properties and biological activities of lysoglyceroglycolipids (lysoMGDG, lysoDGDG, lysoSQDG) have been little studied,[4c,15] our present conversion would be an efficient procedure for preparing sufficient amount of samples to examine them.

EXPERIMENTAL

General Method

IR spectra were recorded on a JASCO IRA-2 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL GSX-400 (400 MHz) spectrometer using tetramethylsilane as an internal standard. FAB-MS and high resolution FAB-MS were determined with a JMS SX-102 spectrometer. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. Gas-liquid chromatography (GLC) was carried out on a Shimadzu GC-13A. The conditions for identification of methyl esters of fatty acids were as follows: column, ULBON HR-SS-10 (0.25 mm i.d. x 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. Thin layer chromatography (TLC) was performed on Merck precoated Kieselgel $60F_{254}$, and spots were detected by spraying with 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.). *Rhizopus arrhizus* lipase (RAL) , porcine pancreas lipase (PPL), and *Candida cylindracea* lipase (CCL) were purchased from Sigma Co., Ltd. Lipase M (from *Mucor javanicus*) and lipase A (from *Aspergillus niger*) were supplied from Amano Pharmaceutical Co., Ltd.

General procedure for preparation of sn-1 lysoglyceroglycolipids

A solution of monogalactosyl diacylglycerol **1a** (5.0 mg) and *Rhizopus arrhizus* lipase (700 unit) in the presence of Triton X-100 (2.5 mg) in boric acid-borax buffer (0.63 ml, pH 7.7) was stirred at 38 °C for 1h. The reaction was quenched with acetic acid (0.1 ml), then EtOH (3.0 ml) was added to the reaction mixture. The solvent was removed under reduced pressure and the resulting residue was chromatographed on silica gel using $CHCl_3$:MeOH (7:1) as eluent to yield **2a** (3.2 mg) and linolenic acid (1.8 mg). In the case of lipase-catalyzed hydrolysis of digalactosyl diacylglycerol (**5a**) and sulfoquinovosyl diacylglycerol (**8a**), **6a** and **9a** were respectively purified by silica gel column chromatography with $CHCl_3$:MeOH:H_2O (7:3:1, lower layer) and $CHCl_3$:MeOH:H_2O (65:35:10, lower layer) after enzymatic hydrolysis followed by work up as described for preparation of **2a**.

2a: a colorless oil, $[\alpha]^{26}$ -7.1° (*c*=0.7, MeOH), IR (KBr, cm^{-1}): 3425, 1730, High resolution FAB-MS: Calcd for $C_{23}H_{44}O_9Na$ 487.2883, Found 487.2861, ^{1}H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=6.8 Hz), 2.35 (2H, t, *J*=7.5 Hz), 3.45 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.50 (1H, ddd-like, 5'-H), 3.51 (1H, dd, *J*=7.3, 9.7 Hz, 2'-H), 3.66-

3.79 (5H, m, 6'-H_2, *sn*-1-H_2, *sn*-3-H), 3.82 (1H, dd, *J*=0.9, 3.3 Hz, 4'-H), 3.96 (1H, dd, *J*=5.7, 11.0 Hz, *sn*-3-H), 4.23 (1H, d, *J*=7.3Hz, 1'-H), 5.04 (1H, m, *sn*-2-H), ^{13}C NMR (CD_3OD, δc): 175.3 (C=O), 105.3 (1'-C), 72.5 (2'-C), 74.9 (3'-C), 70.3 (4'-C), 76.8 (5'-C), 62.5 (6'-C), 61.7 (*sn*-1-C), 74.7 (*sn*-2-C), 68.8 (*sn*-3-C), FAB-MS (*m/z*): 487 $(M+Na)^+$.

2b: a colorless oil, $[\alpha]^{26}$ -6.8° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3410, 1725, High resolution FAB-MS: Calcd for $C_{25}H_{46}O_9Na$ 513.3039, Found 513.3052 , ^1H NMR (CD_3OD, δ): 0.89 (3H, t, *J*=7.0 Hz), 2.07 (4H, q-like), 2.37 (2H, t, *J*=7.5 Hz), 3.45 (1H, dd, *J*=3.1, 9.7 Hz, 3'-H), 3.49-3.52 (2H, m, 2'-H, 5'-H), 3.67-3.78 (5H, m, 6'-H_2, *sn*-1-H_2, *sn*-3-H), 3.82 (1H, dd-like, 4'-H), 3.97 (1H, dd, *J*=5.3, 10.8 Hz, *sn*-3-H) 4.22 (1H, d, *J*=7.3 Hz, 1'-H), 5.03 (1H, m, *sn*-2-H), 5.37 (2H, m), FAB-MS (*m/z*): 513 $(M+Na)^+$.

2c: a colorless oil, $[\alpha]^{26}$ -5.9° (*c*=0.3, MeOH), IR (KBr, cm^{-1}): 3390, 1725, High resolution FAB-MS: Calcd for $C_{25}H_{48}O_9Na$ 515.3196, Found 515.3160, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=7.0 Hz), 2.35 (2H, t, *J*=7.7 Hz), 3.45 (1H, dd, *J*=3.1, 9.7Hz, 3'-H), 3.49-3.56 (2H, m, 2'-H, 5'-H), 3.63-3.78 (5H, m, *sn*-1-H_2, *sn*-3-H, 6'-H_2), 3.82 (1H, dd, *J*=0.9, 3.1 Hz, 4'-H), 3.97 (1H, dd, *J*=5.3, 10.8 Hz, *sn*-3-H), 4.23 (1H, d, *J*=7.5 Hz, 1'-H), 5.04 (1H, m, *sn*-2-H), FAB-MS (*m/z*): 515 $(M+Na)^+$.

2d: a colorless oil, $[\alpha]^{26}$ -6.5° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3380, 1725, High resolution FAB-MS: Calcd for $C_{27}H_{48}O_9Na$ 539.6680, Found 539.6673, ^1H NMR (CD_3OD, δ): 0.97 (3H, t, *J*=7.5 Hz), 2.06 (4H, m), 2.35 (2H, t, *J*=7.3 Hz), 2.81 (2H, dd, *J*=6.2, 6.2 Hz), 3.45 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.50 (1H, ddd-like, 5'-H), 3.51 (1H, dd, *J*=7.5, 9.7 Hz, 2'-H), 3.67-3.77 (5H, m, 6'-H_2, *sn*-1-H_2, *sn*-3-H), 3.81 (1H, dd, *J*=1.1, 3.3 Hz, 4'-H), 3.96 (1H, dd, *J*=5.7, 10.8 Hz, *sn*-3-H), 4.23 (1H, d, *J*=7.5 Hz, 1'-H), 5.04 (1H, m, *sn*-2-H), 5.34 (4H, m), FAB-MS (*m/z*): 537 $(M+Na)^+$.

6a: a colorless oil, $[\alpha]^{26}$ +17.0° (*c*=0.4, MeOH). IR (KBr, cm^{-1}): 3400, 1740, High resolution FAB-MS: Calcd for $C_{29}H_{54}O_{14}Na$ 649.3411, Found 649.3452, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=7.0 Hz), 2.35 (2H, t, *J*=7.0 Hz), 3.49 (2H, m, 2'-H, 3'-H), 3.63-3.80 (10H, m), 3.84-3.95 (5H, m), 4.24 (1H, d, *J*=7.3 Hz, 1'-H), 5.04 (1H, m, *sn*-2-H), ^{13}C NMR (CD_3OD, δc): 175.3 (C=O), 105. 2 (1'-C), 72.5 (2'-C), 74.7* (3'-C), 70.2 (4'-C), 74.6* (5'-C), 67.9 (6'-C), 100.6 (1"-C), 70.2 (2"-C), 71.5 (3"-C), 71.1 (4"-C), 72.5 (5"-C), 62.8 (6"-C), 61.8 (*sn*-1-C), 74.6 (*sn*-2-C), 68.9 (*sn*-3-C), *Assignments may be interchangeable. FAB-MS (*m/z*): 649 $(M+Na)^+$.

6b: a colorless oil, $[\alpha]^{26}$ +13.1° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3405, 1730, High resolution FAB-MS: Calcd for $C_{33}H_{58}O_{14}Na$ 701.3724, Found 701.3701, ^1H NMR (CD_3OD, δ): 0.97 (3H, t, *J*=7.5 Hz), 2.08 (4H, m), 2.35 (2H, t, *J*=7.5 Hz), 2.81 (2H, dd, *J*=6.0, 6.0 Hz), 3.47 (1H, dd, *J*=3.1, 8.3 Hz, 3'-H), 3.51 (1H, dd, *J*=7.5, 8.3 Hz, 2'-H), 3.64-3.80 (10H, m), 3.84-3.95 (5H, m), 4.24 (1H, d, *J*=7.5 Hz, 1'-H), 5.03 (1H, m, *sn*-2-H), 5.35 (4H, m), FAB-MS (*m/z*): 701 $(M+Na)^+$.

6c: a colorless oil, $[\alpha]^{24}$ +20.1° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3410, 1730, High resolution FAB-MS: Calcd for $C_{31}H_{58}O_{14}Na$ 677.3724, Found 677.3761, ^1H NMR (CD_3OD, δ): 0.89 (3H, t, *J*=7.0 Hz), 2.35 (2H, t, *J*=7.7 Hz), 3.49 (2H, m, 2'-H, 3'-H), 3.63-3.80 (10H, m), 3.84-3.95 (5H, m), 4.23 (1H, d, *J*=7.5 Hz, 1'-H), 5.04 (1H, m, *sn*-2-H), FAB-MS (*m/z*): 677 $(M+Na)^+$.

General procedure for preparation of sn-2 lysoglyceroglycolipids

A solution of **1a** (5.0 mg) and *Rhizopus arrhizus* lipase (1800 unit) in the presence of Triton X-100 (2.5 mg) in tris buffer (0.63 ml, pH 7.7) was stirred at 38°C for 17h. Work up of the reaction mixture as described above gave a residue which was purified by silica gel column chromatography with $CHCl_3$-MeOH (7:1) to

furnish **3a** (3.2 mg) and linoleic acid (1.8 mg). In the case of lipase-catalyzed hydrolysis of **5a**, **7a** was isolated by silica gel column chromatography with $CHCl_3$-MeOH-H_2O (7:3:1, lower layer) after enzymatic hydrolysis in the similar manner.

3a: a colorless oil, $[\alpha]^{26}$-25.7° (*c*=0.6, MeOH), IR (KBr, cm^{-1}): 3390, 1740, High resolution FAB-MS Calcd for $C_{23}H_{44}O_9Na$ 487.2883, Found 487.2892, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=6.8 Hz), 2.34 (2H, t, *J*=7.3 Hz), 3.46 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.51 (1H, ddd, *J*=0.9, 6.1, 7.0 Hz, 5'-H), 3.53 (1H, dd, *J*=7.5, 9.7 Hz, 2'-H), 3.65 (1H, dd, *J*=4.4, 10.4, Hz *sn*-3-H), 3.69 (1H, dd, *J*=6.1, 11.4 Hz 6'-H), 3.76 (1H, dd, *J*=7.0, 11.4 Hz, 6'-H), 3.82 (1H, dd, *J*=0.9, 3.3 Hz, 4'-H), 3.91 (1H, dd, *J*=5.1, 10.4 Hz, *sn*-3-H), 3.99 (1H, m, *sn*-2-H), 4.13 (1H, dd, *J*=5.9, 11.2, Hz *sn*-1-H), 4.17 (1H, dd, *J*=4.8, 11.2 Hz, *sn*-1-H), 4.22 (1H, d, *J*=7.5 Hz, 1'-H), ^{13}C NMR (CD_3OD, δc): 175.5 (C=O), 105.4 (1'-C),72.6 (2'-C), 74.9 (3'-C), 70.3 (4'-C), 76.8 (5'-C), 62.5 (6'-C), 66.6 (*sn*-1-C), 69.7 (*sn*-2-C), 71.9 (*sn*-3-C), FAB-MS (*m/z*): 487 $(M+Na)^+$.

3b: a colorless oil, $[\alpha]^{26}$ -22.4° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3405, 1740, High resolution FAB-MS: Calcd for $C_{25}H_{46}O_9Na$ 513.3039, Found 513.3071, ^1H NMR (CD_3OD, δ): 0.89 (3H, t, *J*=7.0 Hz), 2.06 (2H, q-like), 2.37 (4H, m), 3.46 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.51 (1H, ddd, *J*=0.9, 5.7, 7.1 Hz, 5'-H), 3.54 (1H, dd, *J*=7.5, 9.7 Hz, 2'-H), 3.65 (1H, dd, *J*=4.4, 10.3 Hz, *sn*-3-H), 3.71 (1H, dd, *J*=5.7, 11.5 Hz, 6'-H), 3.76 (1H, dd, *J*=7.1, 11.5, 6'-H), 3.81 (1H, dd, *J*=0.9, 3.3, 4'-H), 3.91 (1H, dd, *J*=5.1, 10.3, *sn*-3-H), 3.98 (1H, m, *sn*-2-H), 4.13 (1H, dd, *J*=6.0, 11.4 Hz, *sn*-1-H), 4.17 (1H, dd, *J*=5.0, 11.4 Hz, *sn*-1-H), 4.22 (1H, d, *J*=7.5 Hz, 1'-H), 5.37 (2H, m), FAB-MS (*m/z*): 513 $(M+Na)^+$.

3c: a colorless oil, $[\alpha]^{26}$ -29.3° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3405, 1745, High resolution FAB-MS: Calcd for $C_{25}H_{48}O_9Na$ 515.3196, Found 515.3230, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=6.8 Hz), 2.33 (2H, t, *J*=7.2 Hz), 3.46 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.51 (1H, ddd, *J*=0.9, 6.1, 7.0 Hz, 5'-H), 3.53 (1H, dd, *J*=7.5, 9.7 Hz, 2'-H), 3.65 (1H, dd, *J*=4.4, 10.4 Hz, *sn*-3-H), 3.69 (1H, dd, *J*=6.1, 11.4 Hz, 6'-H), 3.76 (1H, dd, *J*=7.0, 11.4 Hz, 6'-H), 3.82 (1H, dd, *J*=0.9, 3.3 Hz, 4'-H), 3.91 (1H, dd, *J*=5.1, 10.4 Hz, *sn*-3-H), 3.99 (1H, m, *sn*-2-H), 4.13 (1H, dd, *J*=5.9, 11.2 Hz, *sn*-1-H), 4.17 (1H, dd, *J*=4.8, 11.2 Hz, *sn*-1-H), 4.22 (1H, d, *J*=7.5 Hz, 1'-H), FAB-MS (*m/z*): 515 $(M+Na)^+$.

3d: a colorless oil, $[\alpha]^{26}$ -24.6° (*c*=0.2, MeOH), IR (KBr, cm^{-1}): 3400, 1730, High resolution FAB-MS: $C_{27}H_{48}O_9Na$ 539.6680, Found 539.6661, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=7.0 Hz), 2.05 (4H, m, q-like), 2.36 (2H, t, *J*=7.3 Hz), 2.79 (2H, t, *J*=6.1 Hz), 3.46 (1H, dd, *J*=3.3, 9.7 Hz, 3'-H), 3.51 (1H, ddd, *J*=0.9, 5.9, 7.1 Hz, 5'-H), 3.53 (1H, dd, *J*=7.5, 9.7 Hz, 2'-H), 3.65 (1H, dd, *J*=4.3, 10.4 Hz, *sn*-3-H), 3.69 (1H, dd, *J*=6.1, 11.4 Hz, 6'-H), 3.76 (1H, dd, *J*=7.0, 11.3 Hz, 6'-H), 3.81 (1H, dd, *J*=0.9, 3.4 Hz, 4'-H), 3.91 (1H, dd, *J*=5.1, 10.4 Hz, *sn*-3-H), 3.99 (1H, m, *sn*-2-H), 4.13 (1H, dd, *J*=5.9, 11.2 Hz, *sn*-1-H), 4.17 (1H, dd, *J*=4.8, 11.2 Hz, *sn*-1-H), 4.22 (1H, d, *J*=7.5 Hz, 1'-H), 5.35 (4H, m), FAB-MS (*m/z*): 537 $(M+Na)^+$.

7a: a colorless oil, $[\alpha]^{26}$ +5.3° (*c*=0.4, MeOH), IR (KBr, cm^{-1}): 3400, 1735, High resolution FAB-MS: Calcd for $C_{29}H_{54}O_{14}Na$ 649.3411, Found 649.3421, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=7.0 Hz), 2.35 (2H, t, *J*=7.5 Hz), 3.49 (1H, dd, *J*=3.3, 9.3 Hz, 3'-H), 3.53 (1H, dd, *J*=7.3, 9.3 Hz, 2'-H), 3.61-3.80 (8H, m), 3.83-3.93 (5H, m), 3.98 (1H, m, *sn*-2-H), 4.12 (1H, dd, *J*=5.9, 11.6 Hz, *sn*-1-H), 4.16 (1H, dd, *J*=5.0, 11.6 Hz, *sn*-1-H), 4.24 (1H, d, *J*=7.3 Hz, 1'-H), ^{13}C NMR (CD_3OD, δc): 175.6 (C=O), 105.3 (1'-C), 72.6 (2'-C), 74.5* (3'-C), 70.2 (4'-C), 74.6* (5'-C), 67.8 (6'-C), 100.6 (1"-C), 70.3 (2"-C), 71.5 (3"-C), 71.1 (4"-C), 72.5 (5"-C), 62.8 (6"-C), 66.6 (*sn*-1-C), 69.7 (*sn*-2-C), 72.1 (*sn*-3-C), *Assignments may be interchangeable. FAB-MS (*m/z*): 649 $(M+Na)^+$.

7b: a colorless oil, $[\alpha]^{26}$ +3.9° (*c*=0.3, MeOH), IR (KBr, cm^{-1}): 3390, 1740, High resolution FAB-MS: Calcd

for $C_{33}H_{58}O_{14}Na$ 701.3724, Found 701.3701, ^1H NMR (CD_3OD, δ): 0.97 (3H, t, *J*=7.5), 2.07 (4H, m), 2.35 (2H, t, *J*=7.5 Hz), 2.81 (2H, dd, *J*=5.9, 5.9 Hz), 3.48 (1H, dd, *J*=3.4, 9.5 Hz, 3'-H), 3.54 (1H, dd, *J*=7.3, 9.5 Hz, 2'-H), 3.61-3.80 (8H, m), 3.83-3.94 (5H, m), 3.98 (1H, m, *sn*-2-H), 4.11 (1H, dd, *J*=5.8, 12.1 Hz, *sn*-1-H), 4.16 (1H, dd, *J*=5.7, 12.1 Hz, *sn*-1-H), 4.24 (1H, d, *J*=7.2 Hz, 1'-H), 5.34 (4H, m), FAB-MS (*m/z*): 701 $(M+Na)^+$.

7c: a colorless oil, $[\alpha]^{26}$+7.1° (*c*=0.1, MeOH), IR (KBr, cm^{-1}): 3380, 1730, High resolution FAB-MS: $C_{31}H_{58}O_{14}Na$ 677.3724, Found 677.3740, ^1H NMR (CD_3OD, δ): 0.90 (3H, t, *J*=6.9 Hz), 2.35 (2H, t, *J*=7.5 Hz), 3.49 (1H, dd, *J*=3.7, 9.7 Hz, 3'-H), 3.53 (1H, dd, *J*=7.3, 9.7 Hz, 2'-H), 3.62-3.80 (8H, m), 3.84-3.95 (5H, m), 3.98 (1H, m, *sn*-2-H), 4.12 (1H, dd, *J*=5.9, 11.2 Hz, *sn*-1-H), 4.16 (1H, dd, *J*=5.0, 11.2 Hz, *sn*-1-H), 4.24 (1H, d, *J*=7.3 Hz, 1'-H), FAB-MS (*m/z*): 677 $(M+Na)^+$.

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