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Synthesis of glycolipids containing disaccharides and two longer alkyl chains and their applications as enzyme modifiers

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Abstract

The aminolysis between *p*-(aminomethyl)benzoic acid and lactobiono-1,5-lactone was carried out in Me_2SO in quantitative yield. The amide formed thus was used directly for the final reaction without isolation of the intermediate from the reaction mixture. This simple one-pot procedure finished a convenient and useful synthesis of the target *N*-[*p*-(dialkyl-L-glutamatecarbonyl)-benzyl]lactobionamides. The phase-transition temperature of glycolipids was shown to greatly depend on the structure between the hydrophilic moiety and the hydrophobic segment of the glycolipids. The yield of proteins of lipases coated with the glycolipids containing disaccharides remarkably increased with alkyl-chain length, which was higher than that with glycolipids containing monosaccharides. The yields were also closely correlated to the origin of the lipases. The enzymatic reactivity of lipid-coated lipase PS was seldom affected by the hydrophobic segment of lipids, but its enantioselectivity was mainly affected by the hydrophilic moiety of lipids. © 1996 Elsevier Science Ltd.

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1. Introduction

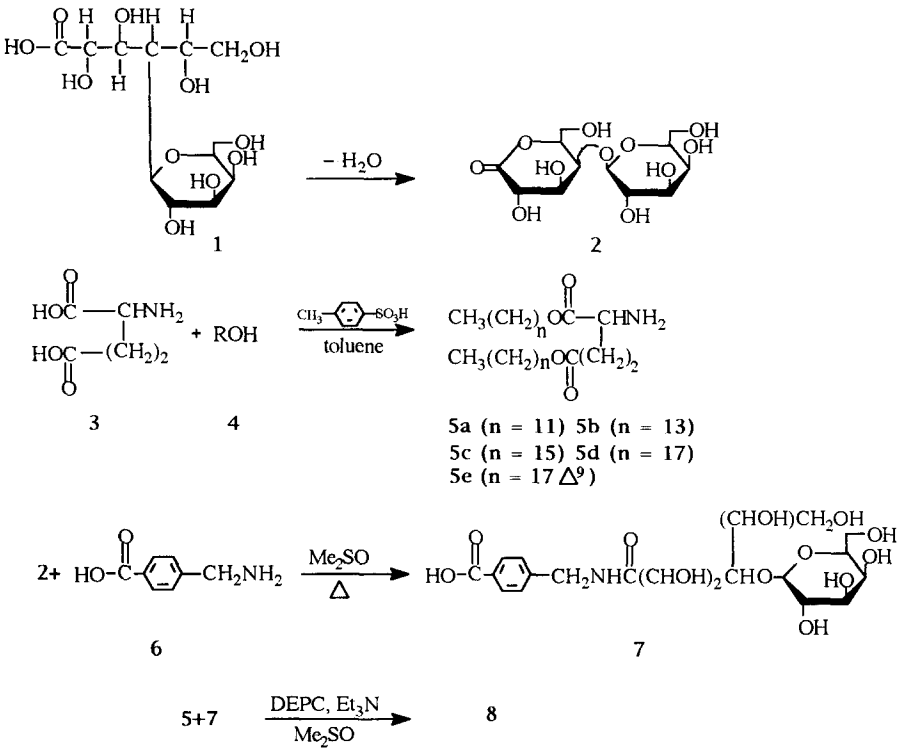
Lipase-catalyzed transesterification in organic solvents has proven to be more effective, in many instances, than the conventional esterification procedure [1]. Because of the widespread interest in nonaqueous enzymology, particularly in preparative organic synthesis, it is important to understand and control the factors that govern enzymatic catalysis in organic solvents. The method of enzymatic catalysis using lipases coated with lipids has proved to be a promising one [2–4]. Glycolipid additions are considered to be a most useful modification for these enzymes [5].

Up until now a glycolipid-containing disaccharide having only one long alkyl chain (for example, *N*-alkyllactobionamide) [6,7], as well as a monosaccharide having two long alkyl chains dialkyl *N*-glucosylglutamate (DAGG) (for example, didodecyl *N*-glucosyl glutamate; didodecyl *N*-glucosyl glutamate (DOGG) and dioleyl *N*-glucosylglutamate (DOGG) [4,5] have been synthesized. The latter was used as the glycolipid for modifying lipase for nonaqueous enzymatic catalysis. Although DDGG was considered to be a most suitable glycolipid in the esterification catalyzed by a glycolipid-coated lipase, its yield was very low, and it was difficult to obtain. Therefore, regardless of the use of these glycolipids, it would be necessary to have procedures whereby relatively large amounts of pure glycolipids could be obtained simply and quickly. In the work reported by Tsuzuki et al. [8], synthetic glycolipid DDGG and commercially available detergents such as sugar esters containing 2–7 hydroxy groups were used for modifying some lipases. Commercial detergents SE-570, SE-590 and SP-60R expressed higher reactivity than DDGG in the case of pancreatic lipase. However, since these commercial detergents were a mixture of glycolipids having a disaccharide and different longer alkyl chains, a clear understanding of the relationship between the hydrophilic structure of lipids and the lipid-coated enzyme is lacking. Moreover, information on the enantioselective transesterification catalyzed by lipid-coated lipase is limited. These interests prompted us to synthesize new glycolipids containing different hydrophilic moieties and two longer alkyl chains.

In this paper, we report the convenient and useful synthetic methods of a class of glycolipids containing amino acid residues interposed between the hydrophilic moiety and the hydrophobic double-chain segment as a hydrogen-bonding component to maintain the steric conformation of lipase in the enzymatic reactions [9]. We have investigated yields of proteins of several lipases coated with different lipids and the effects of the hydrophilic moiety in the lipids on enzymatic reactivity and enantioselectivity of lipase PS.

2. Results and discussion

Synthesis.—The synthetic route to *N*-[*p*-(dialkyl-L-glutamate-carbonyl)-benzyl]lactobionamides **8a–8e** is shown in Scheme 1. Although lactobionic acid **1** is readily converted into lactobiono-1,5-lactone (**2**), the condensation of the lactobiono-1,5-lactone with the amino group of dialkyl L-glutamates **5a–5e** is not possible, perhaps due to steric hindrance of two alkyl chains of dialkyl L-glutamate. Generally, dieth-



Scheme 1.

ylphosphorocyanidate (DEPC) [10] is considered to be the best coupling reagent between an acid and an amine, and has been used, for example, to catalyze the formation of *N*-substituted lactobionamides from amines and lactobionic acid. However, DEPC was found to have only a minor influence on this condensation reaction, as well as *N*,*N*-dicyclohexylcarbodiimide (DCC) [6]. Therefore, we have designed a simple synthetic route to prepare **8a–8e**. Aminolysis of compound **2** with compound **6** proceeds smoothly in Me_2SO, only by heating, in quantitative yield. It is not necessary to protect the carboxyl group in compound **6** in this reaction. Thus, the condensation of *p*-[(lactobionoylamino)methyl]benzoic acid (**7**) and compounds **5a–5e** by the coupling reagent DEPC is easily achieved in a one-pot procedure. Synthesis of compound **7** (**2** + **6** → **7**) and compounds **8a–8e** (**5** + **7** → **8**) can be conducted in the same solvent (Me_2SO) without separation of the intermediate from the reaction mixture. Compounds **8a–8e** can be obtained in higher yields. This method requires only a few days, and large-scale preparation is possible [11].

Differential scanning calorimetry (DSC).—In order to gain information about the phase transition of the glycolipid assembly, we performed thermodynamic measurements for **8a–8e**, **9a–9d** and **10a** with a differential scanning calorimeter. The phase-transition temperature (T_m) and the enthalpy change (ΔH) were evaluated from the endothermic peak minimum and the peak area, respectively, and they are summarized in Table 1.

Table 1
Phase-transition parameters for glycolipids in aqueous dispersions

Glycolipid	Concentration (w/w, %)	T_m (°C)	ΔH_m (kJ/mole)
8a	5.9	48.5	39.5
8b	7.1	50.5	36.5
8c	7.4	62.1	60.0
8d	5.4	64.3	50.0
8e	8.7 [a]	− 19.4	2.6
9a	6.5	29.0	40.1
9b	7.7	39.8	50.9
9c	6.1	46.6	50.5
9d	6.8	59.4 [b]	51.0
10a	5.4	58.2	46.7

[a] Aqueous ethylene glycol solution (12.5 M) was used as dispersing medium.
[b] Pre-transition was observed at about 20 °C ($\Delta H = 4.1$ kJ/mole).

Glycolipids **8a–8d** and **9a–9d** exhibit an increase with alkyl chain length for the phase-transition temperature. Glycolipid **8e** exhibits a considerably low phase-transition temperature due to the oleyl alkyl chain in the hydrophobic segment. It may be considered that this is caused by the changes of intermolecular forces among lipids. When glycolipids **8a–8d** and **9a–9d**, which contain different structures between the hydrophilic moiety and have different alkyl chain lengths, are compared, glycolipids **8a–8d** exhibit a phase-transition temperature higher than that of glycolipids **9a–9d**. The phase-transition temperature of **8a** is 19.5 °C higher than that of **9a**, for example. It is demonstrated that the number of constituent amino acid residues and the structure between the hydrophilic moiety and the hydrophobic double-chain segment greatly affects the phase-transition behavior of the aqueous dispersion. When glycolipids having identical alkyl chain length and a different hydrophilic moiety (**8a**, **9a**, and **10a**) are compared, glycolipids **8a** and **9a** containing a disaccharide express a phase-transition temperature lower than that of glycolipid **10a** having a monosaccharide.

The dependence of ΔH on the alkyl chain length and saccharide moiety is apparently complex [12]. Glycolipids **8a–8d** express odd and even phenomena for the ethaphy change at the phase-transition temperature, but glycolipids **9a–9d** display increase that trend with increasing alkyl chains. As predicted from reports in the literature [13,14], increasing alkyl chain length shifts the endothermic peak to a higher temperature, provided the number of amino acid residues is fixed. Glycolipid **8e** expresses the lowest enthalpy change at the phase-transition temperature compared to glycolipids **8a–8d**. It is obvious that the phenomena is caused by its two oleyl alkyl chains. When the glycolipids **8a**, **9a**, and **10a** containing hydrophilic moieties of different structure are compared, glycolipids **8a** and **9a** containing a disaccharide express an enthalpy change lower than that of glycolipid **10a** containing a monoaccharide. It suggests that glycolipids containing a disaccharide need less energy for phase change of assembly. The effects of glycolipid concentrations on T_m and ΔH was not investigated, because the concentration difference between the glycolipids was very small.

Table 2
The proportion (%) of proteins bound to lipase [a]

	A	AY	PS	PPL	γ-G	BSA
8a	18.6	55.7	60.6	31.5	3.9	0
8b	20.3	62.0	65.3	46.3	4.3	0
8c	30.8	95.3	69.4	58.3	5.8	0
8d	35.0	97.0	72.9	62.3	5.8	0
8e	n.d.	35.1	50.0	n.d.	n.d.	n.d. [b]
9a	17.9	40.2	63.1	31.8	12.6	0
9b	18.6	40.2	73.6	54.0	12.9	0
9c	27.6	35.1	78.0	53.8	14.0	0
9d	31.3	43.1	85.3	60.4	15.4	0
10a	9.2	30.0	56.9	22.1	2.2	0
11	n.d.	n.d.	60.0	n.d.	n.d.	n.d.

[a] Change in molar ratio of lipid to protein is as follows: lipase A 33, lipase AY 60, lipase PS 36, PPL 46, γ-G 140, BSA 62.
[b] Not determined.

Yields of proteins from lipases coated with several lipids.—Compounds **8a–8e**, **9a–9d**, **10a** and **11** (which is not a glycolipid) were used for coating lipases. Yields of proteins of four kinds of lipases (lipase A, lipase AY, lipase PS, lipase PPL) and two kinds of non-lipase proteins (γ-G, BSA) are listed in Table 2. For the all lipases, when the alkyl chain length of the glycolipids **8a–8d** and **9a–9d** increased, the yields of proteins of lipases coated with glycolipid remarkably increased. Glycolipids **8a–8d** and **9a–9d** (except **8e**) showed the yields of proteins from lipases higher than that of **10a**. Compound **11** expressed nearly the same yield of protein as did **8a** and **9a**. Glycolipids **8a–8d** expressed the yields of proteins from lipases higher than those of the glycolipids **9a–9d**. This implies that the number of amino acid residues and the structure between the identical hydrophilic moiety and the hydrophobic segment in lipids strongly affects the yields of proteins from lipases coated with lipids. The increase in yields of such proteins is important to make the best use of the lipase [8,9]. For the lipase AY, the yields of proteins from lipase coated with glycolipids **8a–8d** were shown to be the highest. On the other hand, for non-lipase protein γ-G, the yields of proteins coated with glycolipids **9a–9d** were shown to be the highest in all the glycolipids. It may be considered that glycolipids **9a–9d** are useful for the partitionnig of protein mixtures [15,16]. It was demonstrated that the yields of proteins of lipid-coated lipases were greatly affected by the glycolipid used. It was not possible to coat non-lipase BSA with all kinds of lipids. For the different origins of lipases, even if the identical glycolipid was used, the yields of proteins of the lipid-coated lipases were quite different. This implies that different lipases need to be coated with different structural glycolipids to get the best yield of lipid-coated lipases.

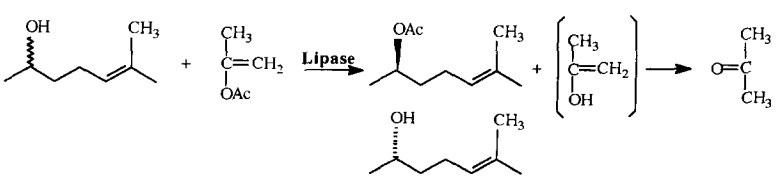
Investigation of the reactivity and enantioselectivity for lipid-coated lipase PS.—In order to investigate the reactivity of lipid-coated lipase, lipase PS was selected because it is not expensive and is commercially available. The reactivity and enantioselectivity of the lipid-coated lipase PS, using **8a–8e**, **9a–9d**, **10a** and **11** are shown in Table 3. For

Table 3
Enzymatic activities and enantioselectivities for lipase PS [a,b]

Lipid [c]	PC [d]	Activity [e]	ee_R (%)
8a	44.0	19.0	86.6
8b	36.6	20.4	84.4
8c	37.0	20.0	84.4
8d	37.2	20.2	83.9
8e	37.2	12.2	85.6
9a	28.8	19.7	87.9
9b	36.7	19.5	87.4
9c	39.0	20.7	87.6
9d	39.2	20.4	87.2
10a	39.6	19.2	88.7
11	46.3	16.3	92.2

[a] Transesterification between isopropenyl acetate and racemic sulcatol (±)-6-methylhept-5-en-2-ol catalyzed by lipid-coated PS in diisopropyl ether.
[b] The enantiomeric excess of the (*R*)-acetoxy sulcatol (ee_R) was from the enantiomeric purities (ep) of the acetylated at 27.8 to 39.5% conversion.
[c] Lipids used for coating.
[d] Protein content in complex (wt.-%).
[e] Activity [μmol h^{-1} (mg of lipase PS)$^{-1}$].

the determination of the reactivity of the lipid-coated lipase PS, the complex of lipase PS was prepared according to method described in the Section 4. Enzymatic transesterification between isopropenyl acetate and racemic sulcatol (±)-6-methylhept-5-en-2-ol in diisopropyl ether catalyzed by lipid-coated PS is shown in Scheme 2. The optimization of chemical structures **8a**, **9a**, **10a** and **11** was carried out by use of MM2 in the software Chem. 3D of Chemical Structure Markup Language in order to clearly understand the relationship between the hydrophilic structure and enzymatic specificity of lipid-coated lipase (see Figs. 1 and 2). Glycolipids **8a–8d** and **9a–9d** are almost the same as **10a** or a little better than **10a** for the reactivity of lipid-coated lipase PS, except **8e** and **11**. It was not found that the enzymatic reactivity of glycolipids **8a–8d** and **9a–9d** varied with alkyl chain length of their hydrophobic segments. It was demonstrated that the hydrophobic segment of glycolipids seldom affected the reactivity of lipid-coated lipase PS. Compound **11** expressed the lower enzymatic reactivity (about 4/5 times that of either **8a** or **9a**) in all lipids used. This indicates that the lipase PS coated by **11**, having only two hydroxy groups in its hydrophilic moiety, led to the lower



Scheme 2. Irreversible transesterification catalyzed by lipase coated with glycolipid.

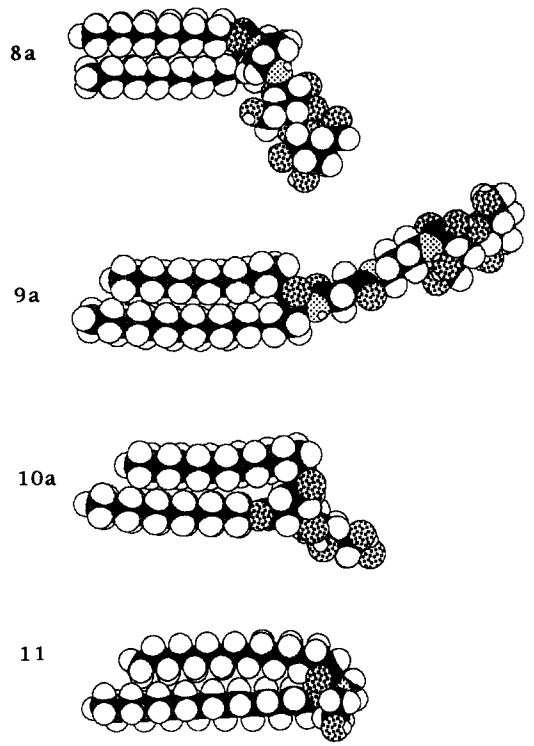


Fig. 1. MM2 calculated structures for **8a**, **9a**, **10a**, and **11**.

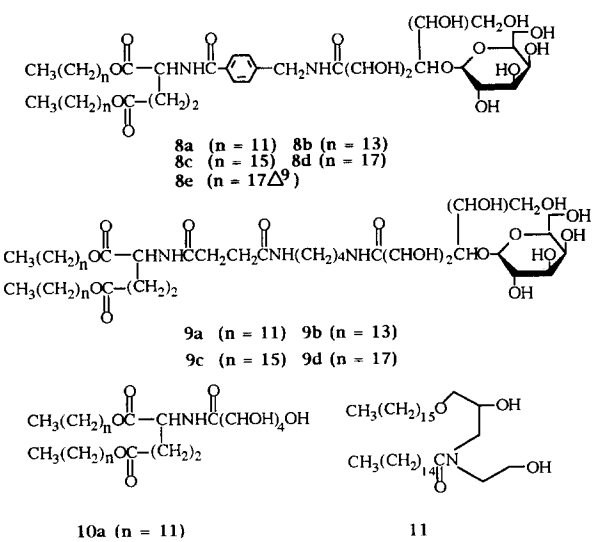


Fig. 2.

reactivity, perhaps due to its increased solubility in the organic solvent. On the other hand, the lower enzymatic reactivity of **11** is also one of the reasons why it expresses high enantioselectivity. The enantioselectivities of lipase PS coated with glycolipids **9a–9d** were better than those of **8a–8e**. These results suggest that the difference is caused by both the amino acid residues and the different structures between the hydrophilic moiety and the hydrophobic segment in the lipids. The enantioselectivities of lipase PS coated with glycolipids **8a–8d** and **9a–9d** were lower than that of glycolipid **10a**. It is demonstrated that the enantioselectivities of lipase PS coated with glycolipids **8a–8d** and **9a–9d** were dependent on the structure of the hydrophilic moiety in the glycolipids. Glycolipids **8a–8d** expressed lower the enantioselectivities for the transesterification of isopropenyl acetate, perhaps because the rigid structure between the hydrophobic segment and the hydrophilic moiety caused the change of the steric conformation of lipase PS. Although **11** expressed the lower reactivity, it exhibited the highest enantioselectivity. It suggests that the structure of the hydrophilic moiety of **11** decreases the freedom of movement of the active site of the lipases and increases the enantioselectivity, perhaps due to fewer hydroxyl groups of its hydrophilic moiety. Therefore, it is obvious that the enantioselectivity of lipid-coated lipase PS is dependent on the structure of the hydrophilic moiety of the lipids. Most of lipids used in these experiments are in the crystalline state (except **8e**) at room temperature in aqueous dispersion, but glycolipid **8e** is liquid-crystalline at room temperature and flows easily. Perhaps this is the reason why the yield of protein of lipase PS coated with glycolipid **8e** and its enzymatic reactivity are lowest.

3. Concluding remarks

Using the synthetic strategy reported herein, we have synthesized samples of *N*-[*p*-(dialkyl-L-glutamatecarbonyl)benzyl]lactobionamides in gram quantities in a chromatographically pure form. Clearly, this synthetic strategy is viable from the perspective of being relatively quick and inexpensive. This strategy can also be used to synthesize many other glycolipids containing different structures between a disaccharide and two longer alkyl chains in order to make detailed studies of their physical properties feasible (currently in progress).

The phase-transition temperature was shown to increase with two longer alkyl chain lengths of the hydrophobic segment in glycolipids. Glycolipids containing a disaccharide decreased the phase-transition temperature in comparison with glycolipids containing a monosaccharide. Glycolipids containing a disaccharide greatly improved the yields of lipases coated with glycolipids compared to glycolipids having a monosaccharide. The enzymatic enantioselectivity depended both on the structure of the hydrophilic moiety of the lipids and on the amino acid residues between the hydrophilic moiety and the hydrophobic segment of lipids.

In perspective, it may be stated that the most suitable lipids for a specified lipase, according to our experimental results. Therefore, it is indispensable to design new lipids for modification of lipases.

4. Experimental

General procedures.—Melting points (Pyrex capillary) are uncorrected. IR spectra were determined on a Hitachi IR-251 spectrophotometer. NMR spectra were taken in Me_2SO-d_6 solution using a JEOL-EX-270 instrument with reference to tetramethylsilane as an internal standard. Optical rotations were measured on a HORIBA SEPA-200 high sensitivity polarimeter. Differential scanning calorimetry (DSC) was run on a Seiko Denshi SSC 560U calorimeter. The concentration of lipase was determined on Shimadzu Spectrometer UV-1200. The composition and enantiomer ratio of the reaction mixture were monitored by gas chromatography using SUPELCO β-DEX 120 chiral capillary column (60 m) with nitrogen as carrier gas and with a flame-ionization detector. Lactobionic acid, *p*-toluenesulfonic acid monohydrate (dried under reduced pressure before use), and *p*-(aminomethyl)benzoic acid were purchased from Tokyokasei Co., Ltd. L-Glutamic acid, higher alkyl alcohols (dodecyl alcohol, tetradecyl alcohol, hexadecyl alcohol, octadecyl alcohol, and oleyl alcohol), and DEPC were purchased from Wako Co., Ltd. THF was dried over Na metal with reflux and distilled just before use. Dimethyl sulfoxide (Me_2SO) was used directly without purification. (±)-6-Methylhept-5-en-2-ol was purchased from Aldrich Chemical Co., Inc. Lipase A (*Aspergillus* sp., mw = 35,000), lipase AY (*Candida* sp., mw = 64,600), and lipase PS (*Pseudomonas* sp., mw = 31,000) were purchased from Amano Pharmaceutical Co., Ltd. Lipase PPL (EC 3.1.1.3, Porcine pancreas, mw = 49,000) was purchased from Sigma Chemical Company. BSA (Bovine Serum, mw = 67,000) was purchased from Itohamu Chemical Co., Ltd. $^{\gamma}$-G (Human Serum, mw = 150,000) was purchased from Wako Co., Ltd.

Lactobiono-1,5-lactone (**2**) was prepared from dehydration of lactobionic acid, according to ref. [17,18].

General procedure for the synthesis of glycolipids **8a–8e** is described for respective didodecyl compounds as a typical example.

Dialkyl L-*glutamate* (**5**).—Compounds **5a**, **5b**, **5c**, **5d**, and **5e** [*R* = dodecyl (*n* = 11); tetradecyl (*n* = 13); hexadecyl (*n* = 15); octadecyl (*n* = 17); oleyl (*n* = 17 Δ^9, *cis*-9-octadecyl)] were synthesized by esterification of L-glutamic acid with higher alkyl alcohols under catalysis of *p*-toluenesulfonic acid according to ref. [19].

p-*[(Lactobionoylamino)methyl]benzoic acid* (**7**).—Compound **6** (4 g, 11.76 mmol), compound **6** (1.76 g, 11.64 mmol), and 40 mL of Me_2SO were added into a 100-mL flask. The reaction mixture was heated under stirring at 130–150 °C with an oil bath for about 3 h. After the solvent Me_2SO was removed under vacuum, the residual products were dissolved in 10 mL of methanol with heating. The solution was added dropwise with a minipipet into 200 mL of chloroform under stirring. The solution was then kept in a refrigerator for 3 h. The separated precipitate was collected by filtration, and the solution was lyophilized. Yield: 5.7 g (100%). The product was identified as *p*-[(lactobionoylamino)methyl]benzoic acid (**7**) by the following spectroscopic data: ^{1}H NMR (270 MHz; Me_2SO-d_6; Me_4Si), δ (ppm): 2.51 (m, 2 H, $-CONHCH_2-$), 2.55 (s, 8 H, $-OH \times 8$), 3.0–4.47 (m, 14 H, sugar hydrogens), 7.38, 7.86 (2d, 4 H, $-C_6H_4-$), and 8.32 (s, 1 H, $-COOH$). ^{13}C NMR (270 MHz; Me_2SO-d_6; Me_4Si), δ (ppm): 60.54 ($-CONHCH_2-$), 82.98 (*C*-4 of sugar), 104.56 (*C*-1 of sugar), 126.87, 128.32, 129.07, 129.29, 129.59, 144.45 (benzene ring), 167.33 (sugar–$CONHCH_2-$), 172.59 ($-COOH$),

62.25, 68.12, 70.57, 71.32, 72.02, 73.13, 75.60, 79.06 (other carbons of sugar). Anal. Calcd for $C_{20}H_{29}NO_{13}$: C, 48.88; H, 5.95; N, 2.85. Found: C, 48.79; H, 5.91; N, 2.82.

N-*[*p-*(dialkyl*-L-*glutamatecarbonyl)benzyl]lactobionamide* (**8**).—Compound **5a** (5.43 g, 11.23 mmol) [20,21] was added to the reaction mixture containing crude **7**, without isolation from solvent Me_2SO. After that, coupling reagent DEPC (3.1 mL, 19.8 mmol) was added into the reaction mixture at 40–60 °C. The mixture was allowed to react under stirring for 3 days. After the reaction, Me_2SO was completely removed under vacuum. The resultant material was recrystallizated from acetone (100 mL). Purification of the glycolipid **8a** was carried out by silica gel column chromatography in 1:3 methanol–chloroform. Other glycolipids **8b–8e** were also prepared in the similar way. Purity of glycolipids was monitored by TLC, in 1:3 methanol–chloroform. White crystalline material gave a single spot (R_f 0.72) on TLC. The R_f value (0.72) of all glycolipids seldom changed, except for glycolipid **8e** containing two oleyl chains which had R_f 0.78. **8a**: yield 4.31 g (51.4%), mp 143–145 °C, $[\alpha]_D^{25}$ +13.3° (*c* 1.0, Me_2SO). Anal. Calcd for $C_{49}H_{84}N_2O_{16}$: C, 61.48; H, 8.85; N, 2.93. Found: C, 61.36; H, 8.83; N, 2.92. **8b**: yield, 4.89 g (54.9%), mp 146–149 °C, $[\alpha]_D^{25}$ +13.8° (*c* 1.0, Me_2SO). Anal. Calcd for $C_{53}H_{92}N_2O_{16}$: C, 62.82; H, 9.15; N, 2.77. Found: C, 62.63; H, 9.13; N, 2.76. **8c**: yield 5.79 g (61.3%), mp 150–152 °C, $[\alpha]_D^{25}$ +9.6° (*c* 0.8, 20:4:1 Me_2SO–$CHCl_3$–MeOH). Anal. Calcd for $C_{57}H_{100}N_2O_{16}$: C, 64.02; H, 9.43; N, 2.62. Found: C, 64.21; H, 9.45; N, 2.63. **8d**: yield 6.20 g (62.4%), mp 154–158 °C, $[\alpha]_D^{25}$ +4.9° (*c* 0.5, 5:4:1 Me_2SO–$CHCl_3$–MeOH). Anal. Calcd for $C_{61}H_{108}N_2O_{16}$: C, 65.09; H, 9.67; N, 2.49. Found: C, 65.30; H, 9.64; N, 2.52. **8e**: yield 4.02 g (41.7%), mp 110–115 °C, $[\alpha]_D^{25}$ +15.7° (*c* 1.0, Me_2S0). Anal. Calcd for $C_{61}H_{104}N_2O_{16}$: C, 65.33; H, 9.35; N, 2.50. Found: C, 65.15; H, 9.32; N, 2.49. **8a**: IR (KBr) (cm^{-1}): 3375 (N–H), 1735, 1680 (C=O). ^1H NMR (270 MHz; Me_2SO-d_6; Me_4Si), δ (ppm): 0.85 (t, 6 H, *J* 6.91 Hz, Me × 2), 1.23 (m, 36 H, $Me(CH_2)_9$ × 2), 1.54 (m, 4 H, $Me(CH_2)_9CH_2CH_2OOC$ × 2), 2.06 (m, 2 H, $-NHCHCH_2CH_2-$), 2.41 (m, 2 H, $-NHCHCH_2CH_2-$), 3.51–5.12 (m, 27 H, $-C_6H_4CH_2-$, $-CH_2CH_2OOC$ × 2, $-OH$ × 8, sugar hydrogens), 5.26 (m, 1 H, $-CHCH_2CH_2-$), 7.36, 7.80 (dd, 4 H, $-C_6H_4-$), 8.11 (t, 1 H, $-C_6H_4CH_2NH-$), 8.54 (d, 1 H, $-HNCOC_6H_4-$). ^{13}C NMR (270 MHz; Me_2SO-d_6; TMS), δ (ppm): 13.84 (Me × 2), 21.99–31.22 ($Me(CH_2)_{10}$ × 2, $-NHCHCH_2CH_2-$), 41.53 ($-C_6H_4CH_2-$), 51.97 ($-NHCHCH_2CH_2-$), 62.26, 63.87 ($-CH_2OH$ × 2), 73.15, 75.60 ($-CH_2OOC$ × 2), 82.88, 104.51 (sugar methine carbons), 126.66, 127.26, 131.88, 143.23 ($-C_6H_4-$), 166.45, 171.75 ($Me(CH_2)_{11}OOC$ × 2), 172.05 ($-CHNHCOC_6H_4-$), 172.54 (sugar–CONH–), 64.33, 68.12, 70.57, 71.03, 71.34, 72.05 (other carbons of sugar).

8b: IR (KBr) (cm^{-1}): 3375 (N–H), 1735, 1680 (C=O), ^1H NMR, δ: 0.86 (t, 6 H, *J* 6.83 Hz, Me × 2), 1.24 (m, 44 H, $Me(CH_2)_{11}$ × 2), 1.53 (m, 4 H, $Me(CH_2)_{11}CH_2CH_2OOC$ × 2), 2.12 (m, 2 H, $-NHCHCH_2CH_2-$), 2.40 (m, 2 H, $-NHCHCH_2CH_2$ – -), 3.50–5.12 (m, 27 H, $-C_6H_4CH_2-$,$-CH_2CH_2OOC$ × 2, $-C_6H_4CH_2-$,$-OH$ × 8, sugar hydrogens), 5.27 (m, 1 H, $-NHCHCH_2CH_2-$), 7.35, 7.80 (dd, 4 H, $-C_6H_4-$), 8.10 (t, 1 H, $-C_6H_4CH_2NH-$), 8.54 (d, 1 H, $-HNCOC_6H_4-$). ^{13}C NMR, δ: 13.84 (Me × 2), 21.99–31.21 ($Me(CH_2)_{12}$ × 2, $-NHCHCH_2CH_2-$), 41.57 ($-C_6H_4CH_2-$), 51.89 ($-NHCHCH_2CH_2-$), 62.33, 62.41 ($-CH_2OH$ × 2), 73.21, 75.58 ($-CH_2OOC$ × 2), 82.78, 104.53 (sugar methine carbons), 126.58, 127.28, 132.18, 143.42 ($-C_6H_4-$), 166.48, 171.07 ($Me(CH_2)_{11}OOC$ × 2), 172.01 ($-CHNHCOC_6H_4-$),

172.58 (sugar–CONH–), 64.23, 68.32, 70.54, 71.01, 71.38, 72.09 (other carbons of sugar).

8c: IR (KBr) (cm^{-1}): 3375 (N–H), 1735, 1680 (C=O), ^1H NMR, δ: 0.85 (t, 6 H, J 6.72 Hz, Me × 2), 1.24 (m, 52 H, $Me(CH_2)_{13}$ × 2, 1.57 (m, 4 H, $Me(CH_2)_{13}CH_2CH_2OOC$ × 2), 2.08–2.50 (m, 4 H, $-NHCHCH_2CH_2-$), 3.29–5.12 (m, 27 H, $-C_6H_4CH_2-$,$-CH_2CH_2OOC$ × 2, $-OH$ × 8, sugar hydrogens), 5.31 (m, 1 H, $-NHCHCH_2CH_2-$), 7.35, 7.78 (dd, 4 H, $-C_6H_4-$), 8.08 (t, 1 H, $-C_6H_4CH_2NH-$), 8.52 (d, 1 H, $-HNCOC_6H_4-$). ^{13}C NMR, δ: 13.85 (Me × 2), 21.96–31.19 ($Me(CH_2)_{14}$ × 2, $-NHCHCH_2CH_2-$), 40.74 ($-C_6H_4CH_2-$), 51.93 ($-NHCHCH_2CH_2-$), 62.24, 62.84 ($-CH_2OH$ × 2), 73.13, 75.58 ($-CH_2OOC$ × 2), 82.84, 104.54 (sugar methine carbons), 126.68, 127.25, 131.84, 143.21 ($-C_6H_4-$), 166.43, 171.76 ($Me(CH_2)_{11}OOC$ × 2), 172.08 ($-CHNHCOC_6H_4-$), 172.58 (sugar–CONH–), 64.21, 68.30, 71.00, 71.42, 72.01 (other carbons of sugar).

8d: IR (KBr) (cm^{-1}): 3375 (N–H), 1735, 1680 (C=O), ^1H NMR, δ: 0.86 (t, 6 H, J 6.67 Hz, Me × 2), 1.23 (m, 60 H, $Me(CH_2)_{15}$ × 2), 1.57 (m, 4 H, $Me(CH_2)_{15}CH_2CH_2OOC$ × 2), 2.09–2.50 (m, 4 H, $-NHCHCH_2CH_2-$), 3.17–5.11 (m, 27 H, $-C_6H_4CH_2-$,$-CH_2CH_2OOC$ × 2, $-OH$ × 8, sugar hydrogens), 5.30 (m, 1 H, $-NHCHCH_2CH_2-$), 7.36, 7.80 (dd, 4 H, $-C_6H_4-$), 8.11 (t, 1 H, $-C_6H_4CH_2NH-$), 8.54 (d, 1 H, $-HNCOC_6H_4-$). ^{13}C NMR δ: 13.84 (Me × 2), 22.03–31.19 ($Me(CH_2)_{16}$ × 2, $-NHCHCH_2CH_2-$), 40.37 ($-C_6H_4CH_2-$), 51.95 ($-NHCHCH_2CH_2-$), 62.12, 62.70 ($-CH_2OH$ × 2), 73.20, 75.56 ($-CH_2OOC$ × 2), 82.83, 104.57 (sugar methine carbons), 126.56, 127.32, 132.56, 143.39 ($-C_6H_4-$), 166.51, 171.09 ($Me(CH_2)_{11}OOC$ × 2), 172.03 ($-CHNHCOC_6H_4-$), 172.88 (sugar–CONH–), 64.21, 68.28, 70.51, 71.00, 71.36, 72.06 (other carbons of sugar).

8e: IR (KBr) (cm^{-1}): 3375 (N–H), 1735, 1680 (C=O), ^1H NMR, δ: 0.85 (t, 6 H, J 7.10 Hz, Me × 2), 1.23 (m, 44 H, $Me(CH_2)_5CH_2CH_2CH=CHCH_2(CH_2)_6CH_2$ × 2), 1.57 (m, 4 H, $Me(CH_2)_5CH_2$ × 2), 1.97 (d, 8 H, J 5.94 Hz, $-CH_2CH=CHCH_2$ × 2) 2.09–2.50 (m, 4 H, $-NHCHCH_2CH_2-$), 3.33–5.14 (m, 27 H, $-C_6H_4CH_2-$,$-CH_2CH_2OOC$ × 2, $-OH$ × 8, sugar hydrogens), 5.32 (m, 1 H, $-CHCH_2CH_2-$), 5.36 (t, 4 H, J 9.27 Hz, $-CH=CH$ × 2) 7.36, 7.78 (dd, 4 H, $-C_6H_4-$), 8.24 (1 H, t, $-C_6H_4CH_2NH-$), 8.64 (d, 1 H, $-HNCOC_6H_4-$). ^{13}C NMR, δ: 13.82 (Me × 2), 21.99–31.19 [$Me(CH_2)_7CH=CH(CH_2)_7$ × 2, $-NHCHCH_2CH_2-$], 40.41 ($-C_6H_4CH_2-$), 51.73 ($-NHCHCH_2CH_2-$), 62.50, 63.73 ($-CH_2OH$ × 2), 73.08, 75.50 ($-CH_2OOC$ × 2), 83.87, 104.93 (sugar methine carbons), 127.41, 127.47, 132.67, 143.12 ($-C_6H_4-$), 129.77, 130.15 ($-CH=CH$ × 2), 166.77, 171.31 ($-CH_2OOC$ × 2), 172.43 ($-CHNHCOC_6H_4-$), 172.56 (sugar–CONH–), 64.37, 68.23, 70.74, 71.80, 71.46, 72.23 (other carbons of sugar).

N-[4-(dialkyl-L-glutamatecarbonylethylcarbonylamino)-butyl]lactobionamides (**9a**–**9d**), didodecyl N-glucosylglutamate (**10a**) and N-(2-hydroxy-3-hexadecyloxypropyl)-N-2-hydroxy-ethylhexadecanamide (**11**) were prepared according to ref. [22], [2], and [23], respectively.

Differential scanning calorimetry (DSC).—For DSC, weighed quantities of glycolipids (1–2 mg) were dispersed in 50 μL of aqueous ethylene glycol (12.5 M) prepared from deionized double-distilled water by sonication. All measurements were run on a Seiko Denshi SSC 560U calorimeter at a scanning rate of 1.0 °C/min.

Preparation of glycolipid-coated lipase.—A typical preparation of lipid-coated enzymes [2,4] was as follows: 12.1 μmol of a given lipid was added into 0.1 M (pH 7.0) phosphate buffer (16 mL) of lipase (1.380 g/L) and stirred vigorously at room temperature for 4 h. After it was treated in an ultrasonic cleaner for 10 min, the solution was incubated in a refrigerator for more than 12 h. The lipase–lipid complex was collected by centrifugation and dried over in a vacuum. The complex obtained thus was insoluble in water but almost entirely soluble in organic solvent. It was stored at 4 °C in a refrigerator until use. It contained the organic solvent-soluble lipase and free glycolipid.

Analysis of lipase concentration.—Solutions (1 L) for determining the lipase concentration were prepared by addition of Coomassie Brilliant Blue G-250 (100 mg) (Fluka), phosphoric acid [85% (w/v), 10 mL], ethanol (50 mL), and distilled water. The solution was filtered with a cellulose acetate minifilter (0.45 μm) before use. The protein of lipase combined with Pigment C.B.B.G-250 displays the maximum absorption in the acidic solution, when it combines with lipase, and wave length in maximum absorption moves from 465 to 595 nm. Its absorption increases linearly with the concentration of lipase. The content of the glycolipid–lipase complex can be determined by change of the lipase concentration (Shimadzu Spectrometer UV-1200), according to the Bradford method [24].

Transesterification catalyzed by glycolipid-coated lipase PS.—The enzymatic activity of the lipid-coated lipase PS was investigated in the irreversible transesterification of racemic (±)-6-methylhept-5-en-2-ol (0.5 mol/L, 2 mmol) with isopropenyl acetate (0.5 mol/L, 2 mmol) in diisopropyl ether (4 mL) at 30 °C, by catalyses of all kinds of lipid–lipase complex. Chlorobenzene (1 mmol) was added into this reaction system as an internal standard. The disappearance of sulcatol (R) and the production of ester compound were followed by gas chromatography with a chiral capillary column of 60 m (Supelco-DEX 120).

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