Lipase-Catalyzed Enantiomeric Resolution of Ceramides†

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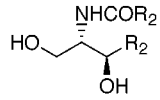
Lipase-catalyzed enantiomeric kinetic resolution of ceramides related to C_{16}-sphinganine and C_{18}-sphingenine is described. Two hydroxy groups in readily available racemic *N*-stearoyl-*erythro*-C_{16}-sphinganine were acetylated, and several kinds of lipases were screened for the hydrolysis of this substrate. Among them, a *Burkholderia cepacia* lipase (SC lipase A, Sumitomo Chemical Co., Ltd.) showed the highest reactivity and enantioselectivity. The rate of hydrolysis and selectivity were greatly affected by some additives. Especially, the combined use of a detergent, Triton X-100, and the solid support, Florisil, for immobilization showed the highest enantioselectivity (E = ca. 170), although the reaction rate turned low. Introduction of a double bond into the substrate (*N*-stearoyl-*erythro*-C_{18}-sphingenine) also retarded the hydrolysis. By utilizing the preferential hydrolysis of the acetate on the primary hydroxy group, another advantageous feature of this enzyme-catalyzed reaction, the resulting product could directly be used as the glycosyl acceptor for cerebroside synthesis.

Introduction

A wide range of physiological activity as well as the requirement for biochemical studies and medicinal applications of sphingoids[1,2] involving sphingosines, ceramides (*N*-acylsphingenines, e.g. **1**, *N*-acylsphinganines, e.g. **2**, Figure 1), and cerebrosides has prompted the development of chemical syntheses of these compounds in diastereomerically[3−5] and enantiomerically[6−12] well-defined forms.

In this paper, we present a new entry to enantiomerically enriched forms of ceramides: the lipase-catalyzed kinetic resolution of the racemic forms of ceramides and the precursors, readily available by straightforward syntheses.[3,4] This approach provides both enantiomers, which would potentially be subjected to a further wide range of biological evaluation.



1a: R^1 = (*E*)-CH=CH$C_{13}H_{27}$, R^2 = $C_{17}H_{35}$
(*N*-stearoyl-C_{18}-*erythro*-sphingenine)

2a: R^1 = $C_{13}H_{27}$, R^2 = $C_{17}H_{35}$
(*N*-stearoyl-C_{16}-*erythro*-sphinganine)

Figure 1. Ceramides: *N*-acylsphingenines and *N*-acylsphinganines.

Results and Discussion

Our first attempt was a lipase-catalyzed enantioselective acylation of a secondary amine.[13] The racemic substrate (±)-(4*R**,5*S**)-**4a** with a sphinganine (saturated) side-chain was obtained by a catalytic hydrogenation of (±)-(4*R**,5*S**)-**3**, which was prepared according to Grob's nitroaldol synthesis[3a,cf.10g] (Scheme 1). To our disappointment, a *Candida antarctica* lipase-catalyzed acylation[13] (lipase B, Novo Nordisk Co., SP525) of the amino group of **4a**, however, was very slow. Only under forced conditions, at elevated temperature and reduced

† The experimental part of this work was taken from the forthcoming M.S. thesis of M. B. (1999) and the B.S. thesis of M. T. (1996).

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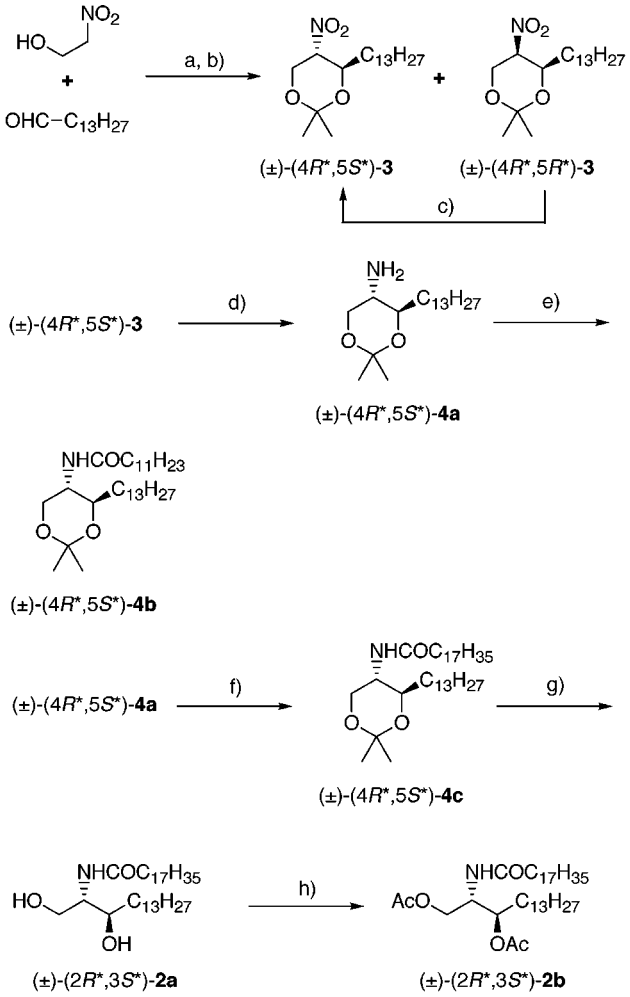
pressure,[14] the desired C−N bond formation occurred and the acylated product **4b** was obtained in a 22% yield. In the absence of lipase, the reaction did not proceed. This lipase-catalyzed amide formation is an interesting tool for ceramide synthesis, because an *N*-acyl group could be introduced under neutral conditions without any condensing reagent other than the lipase; however, the product **4b** was racemic and further enantiomeric resolution was required.

Then we switched to another approach, an enantioselective hydrolysis of the primary *O*-acyl group, discriminating the stereochemistry on the adjacent carbon atom.[15] Indeed, in the case of the *O*,*O*′-diacetylated substrate **2b** (Scheme 1), its structure is closely related to a triacyl glycerol, and the problem in enantioselective hydrolysis is looked upon as the site (*sn*-1 or *sn*-3) specificity of lipases,[16] if we see the two long side-chains and the primary acetyl group as the three fatty acid ester moieties of triacyl glycerol.

Scheme 1. Preparation of Racemic Substrates for Lipase-Catalyzed Reactions and Attempted Enantioselective *N*-Acylation



a) KOH, MeOH; b) *p*-TsOH, 2,2-dimethoxypropane, acetone; c) Et_3N, reflux; d) HCO_2NH_4, Pd-C, EtOH; e) *Candida antarctica* lipase, $C_{11}H_{23}CO_2Me$, 60 °C, 8 mm Hg; f) $C_{17}H_{35}COCl$ / THF-aq CH_3CO_2Na; g) *p*-TsOH, MeOH; h) Ac_2O, py, DMAP.

From the screening of several kinds of lipases on hydrolyzing **2b**, a *Burkholderia cepacia* lipase (SC lipase A, Sumitomo Chemical Co., Ltd.)[17] was found as the most

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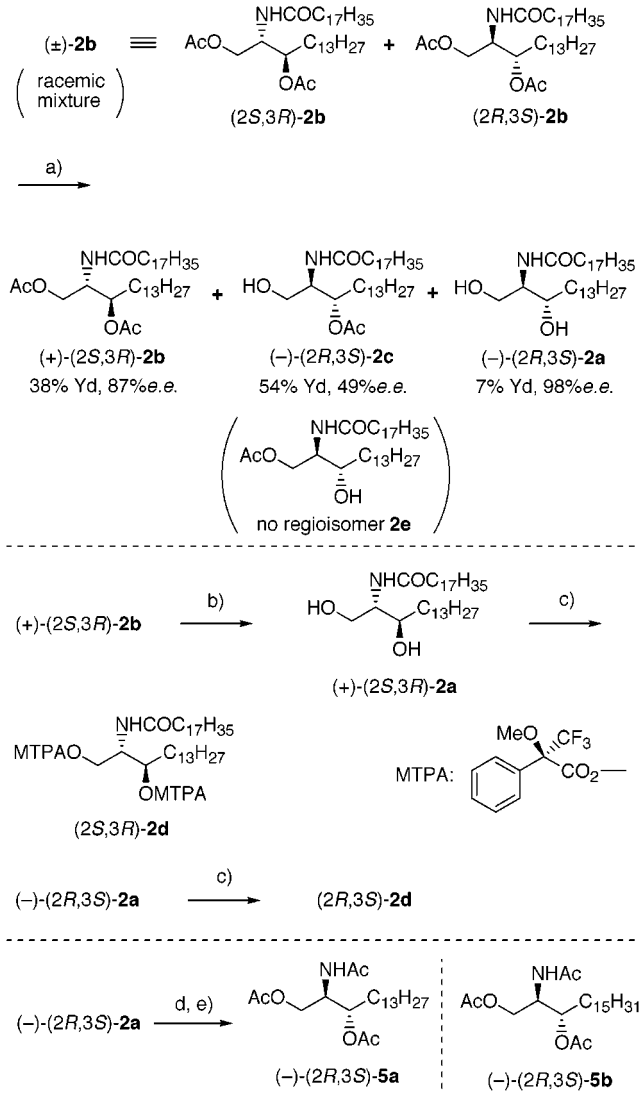
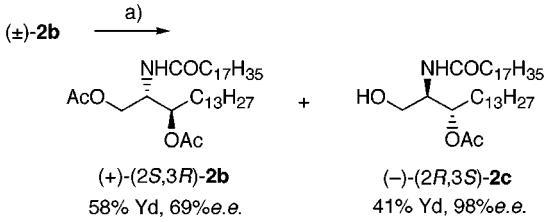
Scheme 2. Hydrolysis with Native SC Lipase A



a) SC lipase A, decane–0.1 M buffer, pH 7.1, 30 °C, 3 days; b) KOH, EtOH–H_2O; c) (*R*)-MTPA acid, DCC, DMAP, CH_2Cl_2; d) 2 N HCl, reflux; e) Ac_2O, py, DMAP.

potent candidate. The treatment of **2b** with the lipase gave a mixture of three products, unreacted (+)-**2b** (38%), (−)-monoacetate **2c** (54%), and (−)-diol **2a** (7%) (Scheme 2). The enantiomeric excesses (ee's) of the products were determined after conversion to the bis-MTPA esters[18] **2d**, by the HPLC analyses as well as ^{1}H NMR measurements. The absolute configuration of (−)-**2a** was determined as (2*R*,3*S*), by comparing its sign of rotation of a derivative (−)-**5a** with that of an analogue, (−)-**5b**, whose absolute configuration was unambiguously established.[9e] From these results, the stereochemical course of the reaction was figured out as depicted in Scheme 2. SC lipase A preferentially hydrolyzed a nonnatural (2*R*,3*S*) form of ceramide diacetate. In this case, the primary acetate of (2*R*,3*S*)-**2b** was predominantly hydrolyzed to give monoacetate (2*R*,3*S*)-**2c**, and subsequently a part of (2*R*,3*S*)-**2c** was slowly hydrolyzed to diol (2*R*,3*S*)-**2a**, as shown in Table 1. On the other hand, the hydrolysis of a natural (2*S*,3*R*) form of **2b** stopped at the stage of monoacetate (2*S*,3*R*)-**2c**. In both cases, it was noteworthy that no regioisomer **2e** was observed in the mixture. A sequential kinetic resolution[19] provided both enantiomers of ceramides: natural enantiomer as diacetate (87% ee), and nonnatural enantiomer as diol (98% ee).

Scheme 3. Hydrolysis with Immobilized SC Lipase A



a) immobilized SC lipase A, decane–0.1 M buffer, pH 7.1, 30 °C, 4 days.

Although the resolution worked well as above, there remained a serious technical problem in this lipase-catalyzed reaction. Due to the crystalline and hydrophobic nature of the substrate **2b**, the hydrolysis only proceeds in a two-phase system between a buffer and a hydrophobic solvent, decane.[cf.20] As the hydrolysis took place and the products formed, the mixture turned into an emulsion caused by lipase protein as well as the products themselves, which resulted in a seriously intractable situation at the step of extractive workup.

To avoid this laborious isolation procedure, the use of an immobilized form of lipase[14] was attempted. As expected, the extraction was very much facilitated after the enzyme protein was readily removed by simple filtration at the beginning of workup. Moreover, the distribution of the products dramatically changed compared with the results obtained by using unimmobilized (native) SC lipase A. The hydrolysis stopped at the stage of monoacetate: (+)-**2b** (58% yield, 69% ee) and (−)-**2c** (41% yield, 98% ee) were obtained, as shown in Scheme 3.

As already described, the hydrolysis proceeds in two steps with native SC lipase A. The enantiomeric ratio[21] of each step (E_1, E_2) was 9 and 31, respectively (Table 1, entry 1). A *Pseudomonas cepacia* lipase (lipase PS, Amano pharmaceutical Co., entry 2) showed a marked contrast, a larger E_1 value (30) compared with the selectivity in the second step (E_2 = 4). The use of immobilized lipase not only caused lowering of the reaction rate, but also a great change in E_1 from 9 to 168 (entry 3); thus, we became interested in this change of selectivity.[22] The immobilized form[14,23] of SC lipase A consists of an enzyme protein, a detergent, Triton X-100 (poly(ethylene glycol) octylphenyl ether), and a solid support, Florisil (magnesium silicate). The separate use of two additives (entries 4 and 5) revealed the selectivity mostly depended upon the detergent (entry 5, E_1 = 170).

(17) A patent on this enzyme has been applied for by Sumitomo Chemical Co., Ltd.; Japanese Patent Application Number: 08-344076. For correspondence, Dr. Satoshi Mitsuda, Biotechnology Laboratory, Sumitomo Chemical Co., Ltd., 4-2-1 Takatsukasa, Takarazuka 665-0051, Japan.

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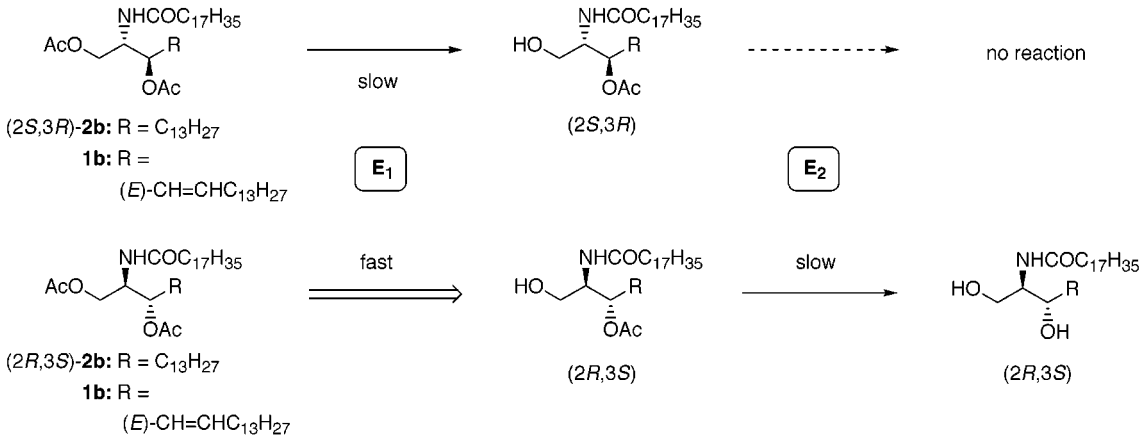
Table 1. Effect of Additives on the Selectivity



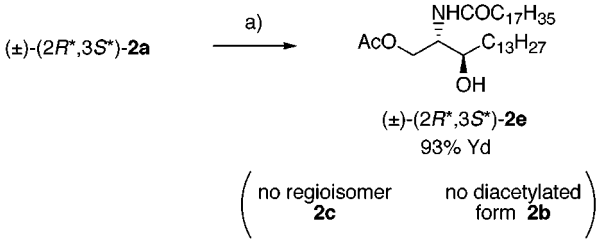
entry	substrate	lipase	additive	time (d)	E_1	E_2
1	**2b**	*B. cepacia* (SC lipase A)	none	3[a]	9	31
2	**2b**	*P. cepacia* (Amano PS)	none	5.5[b]	30	4
3	**2b**	SC lipase A	Florisil, Triton X-100	4[a]	168	–
4	**2b**		Florisil	3[a]	58	–
5	**2b**		Triton X-100	4[a]	170	–
6	**1b**	SC lipase A	none	4[c]	5	62
7	**1b**		Florisil	4[c]	12	–
8	**1b**		Triton X-100	no reaction[c]		
9	**1b**		Florisil, Triton X-100	no reaction[c]		

[a] Enzyme/substrate = 1/10 (w/w). [b] Enzyme/substrate = 4/1 (w/w). [c] Enzyme/substrate = 1/3 (w/w).

As mentioned above, the treatment of **2b** with lipase afforded the nonnatural enantiomer of diol **2a**, and this result suggested to us a possibility of the lipase-catalyzed kinetic resolution of the intermediate, monoacetate (±)-**2c** itself. To our surprise, it was revealed that the hydrolysis **2c** was extremely slow, and almost no diol **2a** was observed. This result suggested that the reactivity and the selectivity depended on the aggregation and orientation of the substrate under the reaction interface, which was greatly affected by the addition of detergent (Triton X-100). The successful enzymatic hydrolyses proceeded in a two-phase solution as a complex micelle consisting of the starting diacetate, monoacetate, and diol.

Next, our attention was directed to the lipase-catalyzed reaction in a homogeneous organic solution. The acylation of hydroxy groups was attempted in a solvent system of vinyl acetate−tetrahydrofuran, which had successfully been applied in lipase-catalyzed transesterification of long-chain α-hydroxy acid.[24] SC lipase A-catalyzed acylation proceeded smoothly to provide a completely regioselective acylation on the primary hydroxy group (Scheme 4); however, the product **2e** was racemic. Even worse, lipase PS lost such regioselectivity: the product was a mixture of 1-*O*-acetylated form **2e** and its regioisomer, 3-*O*-acetylated form **2c** (87:13).

Scheme 4. Transesterification with Immobilized SC Lipase A



a) immobilized SC lipase A, vinyl acetate-THF, 30 °C, 22 h

The results obtained so far encouraged us to attempt the kinetic resolution of ceramide diacetate of sphingenine type **1b** which was prepared as shown in Scheme 5.[4a,b,25] The introduction of a double bond to the substrate surprisingly retarded[cf.26] the progress of hydrolysis. The native SC lipase A exhibited similar E_1 and E_2 values to this substrate compared with **2b**, respectively (entries 1 and 6). The addition of Florisil enhanced the value of E_1 to some extent (12, entry 7). On the other hand, Triton X-100 completely suppressed the hydrolysis (entry 8). The distribution of the products starting from (±)-**1b** is described in Scheme 5.

(22) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836−6841. Kitaguchi, H.; Itoh, I.; Ono, M. *Chem. Lett.* **1990**, 1203−1206. Itoh, T.; Ohira, E.; Takagi, Y.; Nishiyama, S.; Nakamura, K. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 624−627. Okabe, M.; Sun, R.-C.; Zenchoff, G. B. *J. Org. Chem.* **1991**, *56*, 4392−4397. Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 4941−4944. Tsukube, H.; Betchaku, A.; Hiyama, Y.; Itoh, T. *J. Chem. Soc., Chem. Commun.* **1992**, 1751−1752. Brevet, J.-L.; Mori, K. *Synthesis* **1992**, 1007−1012.; Hirose, Y.; Kariya, K.; Sasaki, I.; Kurono, Y.; Ebiike, H.; Achiwa, K. *Tetrahedron Lett.* **1992**, *33*, 7157−7160. Secundo, F.; Riva, S.; Carrea, G. *Tetrahedron: Asymmetry* **1992**, *3*, 267−280. Moore, A. N. J.; Dutton, P. J.; Zahalka, H. A.; Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1995**, *117*, 5677−5686. Hansen, T. V.; Waagen, V.; Partali, V.; Anthonsen, H. W.; Anthonsen, T. *Tetrahedron: Asymmetry* **1995**, *6*, 499−504. Kinoshita, M.; Ohno, A. *Tetrahedron* **1996**, *52*, 5397−5406. Ohtsuka, Y.; Katoh, O.; Sugai, T.; Ohta, H. *Bull. Chem. Soc., Jpn.* **1997**, *70*, 483−491. Review: Kvittingen, L. *Tetrahedron* **1994**, *50*, 8253−8274. Itoh, T.; Takagi, Y.; Tsukube, H. *J. Mol. Catal. B: Enzymol.* **1997**, *3*, 259−270.

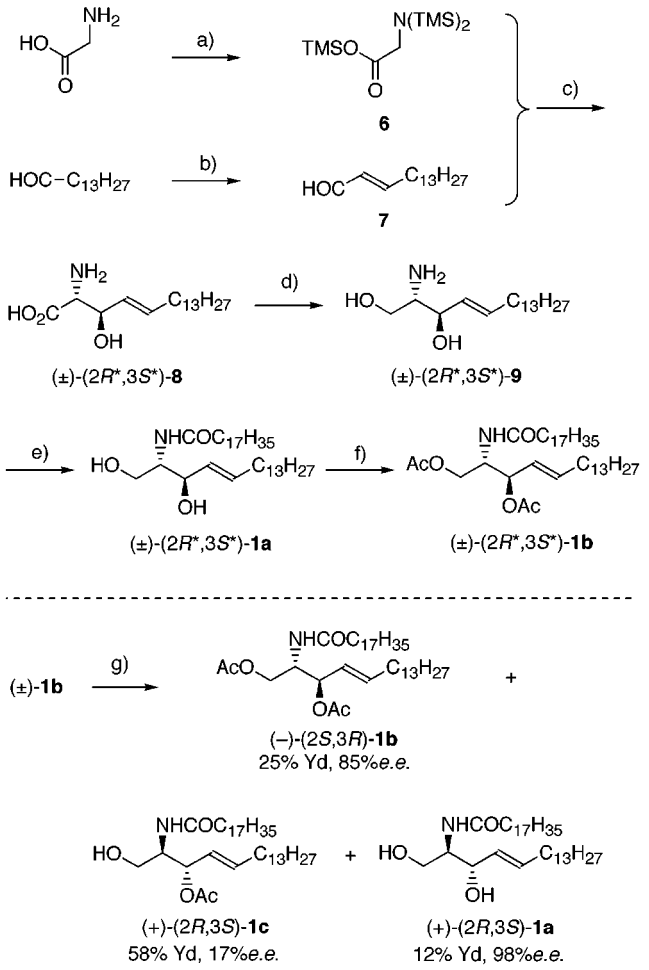
(23) Yamada, H.; Sugai, T.; Ohta, H.; Yoshikawa, S. *Agric. Biol. Chem.* **1990**, *54*, 1579−1580.

(24) Sugai, T.; Ohta, H. *Tetrahedron Lett.* **1991**, *32*, 7063−7064.

(25) Nakagawa, Hino and co-workers reported the synthesis[3c] of racemic ceramide **1b** via Grob's nitroaldol synthesis. In our case, however, the contamination of *threo*-isomer was observed at the stage of the reduction of nitro group to amino group with $LiAlH_4$, probably due to the epimerization caused by basicity of the reagent prior to the reduction. From this reason, we synthesized **1b** essentially according to the Schmidt's procedure,[4a,b] with some modifications as described in the Experimental Section.

(26) An example of the low reactivity of the sphingenine-related substance was reported very recently.[12c]

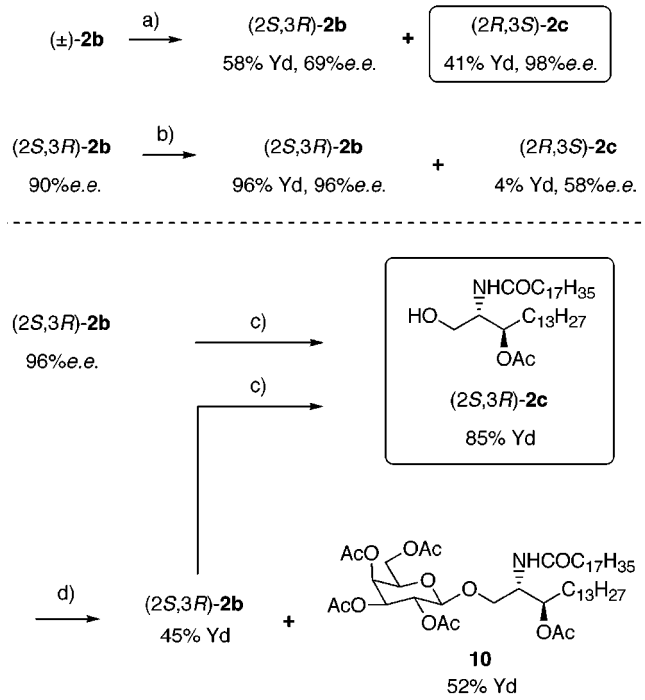
Scheme 5. Hydrolysis of Ceramide Diacetate with Double Bond with SC Lipase A



As we stated above, an advantageous feature of this lipase-catalyzed hydrolysis is the remarkable regioselectivity. Scheme 6 illustrates the preparation of both natural and nonnatural enantiomers of ceramide with a liberated primary alcohol, which would be appropriate for cerebroside syntheses.[27] First, nonnatural (2*R*,3*S*)-**2c** was obtained (41% yield, 98% ee) by immobilized SC lipase A-catalyzed hydrolysis of (±)-**2b** leaving a partially enantiomerically enriched form of (2*S*,3*R*)-**2b**. The ee of the recovered (2*S*,3*R*)-**2b** could be enhanced (96% ee) by the repetition of hydrolysis with the same immobilized lipase, while the contaminated (2*R*,3*S*)-isomer was thoroughly hydrolyzed to the corresponding monoacetate. Then, native SC lipase A-catalyzed hydrolysis in a low enantioselective but a highly regioselective manner was applied to (2*S*,3*R*)-**2b**, to afford the natural form of monoacetate (2*S*,3*R*)-**2c** in 85% yield. This monoacetate was effectively glycosylated with tetra-*O*-acetyl-α-galactosyl trichloroacetimidate to give **10** in 52% yield. In this glycosylation reaction so far, the migration of the acetyl group from the glycosyl donor to the acceptor had been

(27) (a) Tanahashi, E.; Murase, K.; Shibuya, M.; Igarashi, Y.; Ishida, H.; Hasegawa, A.; Kiso, M. *J. Carbohydr. Chem.* **1997**, *16*, 831−858. (b) Kiso, M.; Nakamura, A.; Hasegawa, A. *J. Carbohydr. Chem.* **1987**, *6*, 411−422.

Scheme 6. Both Enantiomers of Monoacetate and the Synthesis of Galactosyl Ceramide



problematic; indeed, the diacetate **2b** was obtained in 45% as the major byproduct. Our achievement in developing the regioselective hydrolysis using SC lipase A, however, cleanly solved this problem, as **2b** was readily converted back to the glycosyl acceptor, monoacetate **2c**.

Conclusion

SC Lipase A-catalyzed enantiomeric kinetic resolution of ceramides related to C_{16}-sphinganine and C_{18}-sphingenine was achieved. The rate of hydrolysis and the selectivity were greatly affected by the additive, especially the detergent, Triton X-100. By combining the enantioselective and the preferential hydrolysis of the acetate on the primary hydroxyl group, the protected forms of both enantiomers of ceramide acetate with free primary alcohol became available, which is a new entry to glycosyl ceramide synthesis.

Experimental Section

All mps were uncorrected. IR spectra were measured as KBr disks on a Jasco IRA-202 and FT/IR-410 spectrometer unless otherwise stated. ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 270 MHz on a JEOL JNM EX-270 spectrometer unless otherwise stated. Mass spectra were recorded on Hitachi M-80B spectrometer at 70 eV. Optical rotations were recorded on a Jasco DIP 360 polarimeter. Wako Gel B-5F and silica gel 60 K070-WH (70−230 mesh) of Katayama Chemical Co. were used for preparative TLC and column chromatography, respectively.

***erythro*-2,2-Dimethyl-5-nitro-4-tridecyl-1,3-dioxane (±)-(4*R**,5*S**)-3 and *threo*-2,2-Dimethyl-5-nitro-4-tridecyl-1,3-dioxane (±)-(4*R**,5*R**)-3.** According to the reported procedure,[3f] a mixture of (±)-(4*R**,5*S**)-**3** (37%) and (±)-(4*R**,5*R**)-**3** (38%) was prepared starting from 2-nitroethanol and tetradecanal.

(±)-(4*R,5*S**)-3:** R_f 0.51 [silica gel, hexanes−ethyl acetate (10:1)], ^{1}H NMR δ 0.88 (3H, t, J = 6.5 Hz, 13′-CH_3), 1.18 (22H, m), 1.38 and 1.52 [total 6H, each 3H, s, $O_2C(CH_3)_2$], 1.56 (2H, m, 1′-CH_2), 4.09 (1H, dd, J = 5.6, 12.0 Hz, 6-eqH), 4.18 (1H, m, 4-H), 4.22 (1H, dd, J = 7.0, 12.0 Hz, 6-axH), 4.44 (1H, ddd, J = 5.6, 7.0, 9.0 Hz, 5-H). Its NMR spectrum was identical with that reported previously.[3f]

(±)-(4*R,5*R**)-3:** R_f 0.16 [silica gel, hexanes−ethyl acetate (10:1)], ^{1}H NMR δ 0.87 (3H, t, J = 6.6 Hz, 13′-CH_3), 1.25 (22H, m), 1.44 and 1.48 [total 6H, each 3H, s, $O_2C(CH_3)_2$], 1.56 (2H, m, 1′-CH_2), 4.06 (1H, dt, J = 3.3, 6.6 Hz, 4-H), 4.19 (1H, dd, J = 4.6, 13.0 Hz, 6-axH), 4.35 (1H, dd, J = 3.0, 13.0 Hz, 6-eqH), 4.51 (1H, ddd, J = 3.0, 3.3, 4.6 Hz, 5-H). A solution of (±)-(4*R**,5*R**)-**3** (4.92 g, 14.3 mmol) in triethylamine (100 mL) was stirred under reflux overnight.[3c] After confirming a satisfactory conversion by TLC analysis, the reaction was quenched by adding 2 N hydrochloric acid. The mixture was extracted with ethyl acetate, and the organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified with a silica gel column chromatography (100 g) to give the *erythro*-isomer of **3** (4.46 g, 91%).

***erythro*-5-Amino-2,2-dimethyl-4-tridecyl-1,3-dioxane (±)-(4*R**,5*S**)-4a.** The acetonide (±)-(4*R**,5*S**)-**3** (2.07 g, 6.03 mmol) was dissolved in ethanol (80 mL), and palladium on charcoal (10%, 300 mg) and ammonium formate (13 g, 0.21 mol) were added. After stirring for 3 h at room temp, the disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (9:1)]. The mixture was filtered, and the residue was washed with a mixture of ethanol−ethyl acetate. The combined filtrate and washings were made alkaline by adding 1 N potassium hydroxide solution and extracted with ethyl acetate. The extract was washed with brine, dried over sodium sulfate, and concentrated in vacuo to give **4a** (1.89 g, 99%), IR ν_{max} 3380, 3270, 2950, 2850, 1660, 1610, 1460, 1380, 1265, 1200, 1165, 1070, 1010, 870, 815, 720, 665 cm^{-1}; ^{1}H NMR δ 0.87 (3H, t, J = 6.6 Hz, 13′-CH_3), 1.25 (22H, m), 1.38 and 1.44 [total 6H, each 3H, s, $O_2C(CH_3)_2$], 1.53 (2H, m, 1′-CH_2), 2.65 (1H, ddd, J = 5.3, 9.6, 9.6 Hz, 5-H), 3.40 (1H, m, 4-H), 3.47 (1H, dd, J = 9.6, 11.0 Hz, 6-axH), 3.82 (1H, dd, J = 5.3, 11.0 Hz, 6-eqH). This was employed for the next step without further purification.

***erythro*-2,2-Dimethyl-5-(lauroylamino)-4-tridecyl-1,3-dioxane (±)-(4*R**,5*S**)-4b.** A mixture of **4a** (46.7 mg, 0.149 mmol), methyl laurate (1.80 g), and immobilized *Candida antarctica* lipase[14] (100 mg) was stirred at 60 °C under a reduced pressure (8 mmHg) for 20 h. The mixture was directly charged on a silica gel column (5 g). Elution with hexane afforded the recovery of methyl laurate. Further elution with hexanes−ethyl acetate (1:2) afforded **4b** (16.1 mg, 22%) with the recovery of the starting material. Either **4b** or the recovered **4a** showed no optical rotation. **4b**: ^{1}H NMR δ 0.87 (6H, t, each 3H, J = 6.6 Hz, 13′-CH_3, 12"-CH_3), 1.25 (40H, m), 1.38 and 1.42 [total 6H, each 3H, s, $O_2C(CH_3)_2$], 1.58 (2H, m, 1′-CH_2), 2.16 (2H, t, J = 7.4 Hz, 2"-CH_2), 3.52 (2H, m, 6-axH, 4-H), 3.89 (1H, m, 5-H), 3.91 (1H, dd, J = 5.0, 8.8 Hz, 6-eqH), 5.33 (1H, d, J = 8.6 Hz, NHCO).

***erythro*-2,2-Dimethyl-5-(stearoylamino)-4-tridecyl-1,3-dioxane (±)-(4*R**,5*S**)-4c.** To a solution of **4a** (2.99 g, 9.53 mmol) in THF (50 mL) was added an aqueous sodium acetate solution[cf.7j] (30 mL, 50%) and subsequently under vigorous stirring was added portionwise stearoyl chloride (3.73 g, 12.3 mmol, 1.3 equiv). The mixture was further vigorously stirred overnight under Ar at room temp. The disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (12:1)]. The reaction mixture was poured into brine and extracted with THF three times. The organic layer was successively washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified with a silica gel column chromatography (270 g). Elution with hexanes−ethyl acetate (20:1 to 1:2) and subsequently with chloroform−methanol (20:1) afforded **4c** (4.75 g, 86%). Recrystallization from ethyl acetate gave **4c** (4.62 g, 84%) as needles, mp 65.0 °C, IR ν_{max} 3275, 3175, 2920, 2850, 1640, 1550, 1465, 1430, 1380, 1370, 1250, 1200, 1165, 1105, 1080, 1050, 1020, 980, 960, 930, 885, 860, 840, 760, 720, 700, 560, 520 cm^{-1}; ^{1}H NMR δ 0.88 (6H, t, each 3H, J = 6.6 Hz, 13′-CH_3, 12"-CH_3), 1.10−1.72 (54H, m), 1.38 and 1.42 [total 6H, each 3H, s, $O_2C(CH_3)_2$], 2.17 (2H, t, J = 7.4 Hz, 2"-CH_2), 3.46−3.57 (2H, m), 3.82−3.96 (2H, m), 5.28 (1H, d, J = 8.6 Hz, NHCO). Anal. Found: C, 76.31; H, 13.38; N, 2.49. Calcd for $C_{37}H_{73}NO_3$: C, 76.62; H, 12.69; N, 2.41%.

***erythro*-2-(Stearoylamino)-1,3-hexadecanediol (±)-(2*R**,3*S**)-2a.** A mixture of **4c** (4.49 g, 7.74 mmol), methanol (40 mL), and a catalytic amount of *p*-TsOH was stirred overnight under Ar at room temp. The disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (12:1)]. The mixture was concentrated in vacuo. The residue was diluted with chloroform and neutralized by the addition of saturated aqueous sodium hydrogen carbonate solution. After removing insoluble materials by filtration, the organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to give **2a** (4.14 g, 99%) as a solid. This was recrystallized from chloroform−methanol to give **2a** (4.05 g, 97%) as needles, mp 100.5−101.5 °C, IR ν_{max} 3450, 3375, 3300, 3100, 2940, 2860, 1640, 1550, 1465, 1380, 1280, 1260, 1220, 1200, 1180, 1130, 1110, 1070, 1050, 1030, 940, 850, 725, 700, 580 cm^{-1}; ^{1}H NMR δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.20−1.70 (54H, m), 2.23 (2H, t, J = 7.5 Hz), 2.61 (1H, d, J = 6.3 Hz), 2.73 (1H, dd, J = 4.0, 6.6 Hz), 3.70−3.88 (3H, m), 4.01 (1H, dt, J = 11.2, 3.6 Hz), 6.35 (1H, d, J = 7.3 Hz, NHCO). HRMS found: 520.5056. Calcd for $C_{34}H_{66}NO_2$ ($M^+ - 1 - H_2O$): 520.5089.

***erythro*-3-Acetoxy-2-(stearoylamino)hexadecyl Acetate (±)-(2*R**,3*S**)-2b.** A mixture of **2a** (1.09 g, 2.02 mmol), anhydrous pyridine (10 mL), acetic anhydride (5 mL), and a catalytic amount of DMAP was heated for a while to give a homogeneous mixture and further stirred overnight under Ar at room temp. The disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (15:1)]. The mixture was diluted with water (40 mL), cooled with an ice-bath, and filtered. The residue on the filter was washed successively with water, cooled hexane, 0.1 N hydrochloric acid, and water. The solid was dried in vacuo to give **2b** (1.23 g, 98%) as a solid. To the residue was added silica gel (2 g), and the residue was completely dissolved with a small portion of chloroform−methanol, subsequently adding a small portion of hexane. The mixture was charged on a silica gel column (15 g). Elution with hexanes−ethyl acetate (6:1 to 0:1) to chloroform−methanol (30:1) afforded **2b** (1.20 g, 95%) as a solid. This was recrystallized from hexanes−ethyl acetate to give **2b** (1.08 g, 86%) as needles, mp 90.0−91.0 °C, IR ν_{max} 3300, 3080, 2920, 2850, 1730, 1640, 1550, 1465, 1430, 1370, 1260, 1230, 1180, 1130, 1110, 1080, 1065, 1045, 1025, 980, 960, 940, 920, 880, 750, 725, 690, 600 cm^{-1}; ^{1}H NMR δ 0.88 (6H, t, each 3H, J = 6.3 Hz), 1.15−1.70 (54H, m), 2.05 (3H, s), 2.07 (3H, s), 2.17 (2H, t, J = 7.3 Hz), 4.04 (1H, dd, J = 4.0, 11.6 Hz), 4.25 (1H, dd, J = 5.9, 11.6 Hz), 4.40 (1H, dddd, J = 3.7, 5.9, 5.9, 8.9 Hz), 4.89 (1H, dt, J = 5.9, 5.9 Hz), 5.80 (1H, d, J = 8.9 Hz, NHCO). HRMS found: 503.5063. Calcd for $C_{34}H_{65}NO$ ($M^+ - 2AcOH$): 503.5063.

Lipase-Catalyzed Hydrolysis of 2b (with native SC lipase A). A mixture of (±)-**2b** (298 mg, 0.478 mmol) and decane (3 mL) was heated at 100 °C for a while to give a homogeneous mixture. To the mixture was added 0.1 M phosphate buffer solution (pH 7.1, 30 mL), and after cooling to room temp, SC lipase A (Sumitomo Chemical. Co., Ltd., 30 mg)[17] was added and the suspension was stirred for 72 h at 30 °C. The progress of the hydrolysis was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (15:1), R_f: **2b**, 0.78; **2c**, 0.34; **2a**, 0.20]. The mixture was diluted with chloroform. After the organic layer was separated, the aqueous layer was thoroughly extracted with chloroform. The combined extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The NMR spectrum of the crude product indicated that it was a mixture of

diacetate **2b**, monoacetate **2c**, and diol **2a**, judging from the signals of amide protons. To the mixture was added silica gel (1 g), and the product was completely dissolved with a small portion of chloroform−methanol, subsequently adding a small portion of hexane. The mixture was charged on a silica gel column (10 g). Elution with hexanes−ethyl acetate (8:1 to 5:1) afforded (+)-(2*S*,3*R*)-**2b** (113 mg, 38%) as a solid, $[\alpha]^{22}_D$ = +9.85 (*c* 1.20, chloroform). A small portion of this was hydrolyzed and converted to the corresponding bis (*R*)-MTPA ester (2*S*,3*R*)-**2d** in the following manner.[28] DCC (41.2 mg, 200 μmol), (*R*)-MTPA acid (32.8 mg, 140 μmol), and DMAP (8.6 mg, 35 μmol) were dissolved in dry dichloromethane (500 μL). A mixture of (+)-(2*S*,3*R*)-**2a** (4.0 mg, 7.4 μmol) and a half portion of the reagent solution (250 μL) as above was stirred for 2 h at room temp. The progress of esterification was confirmed by TLC analyses [silica gel, developed with chloroform−methanol (15:1) and hexanes−ethyl acetate (4:1)]. Hexane was added to the mixture, and they were charged onto a silica gel column. Elution with hexanes−ethyl acetate (1:0 to 8:1) afforded crude **2d**. Further purification by preparative TLC (hexanes−ethyl acetate 10:1, developed five times, R_f 0.4−0.6) afforded **2d** (5.8 mg, 81%) as colorless oil. It was confirmed that an absolute 1:1 mixture of the diastereomers of **2d** was obtained from (±)-**2a** through this operation. The ee of **2a** [from (2*S*,3*R*)-**2b**] was estimated from HPLC analysis and the ^{1}H NMR spectrum of **2d**. HPLC (column, Senshu Pak PEGASIL silica 60-5; solvent, hexane−tetrahydrofuran = 15:1; flow rate 1.0 mL/min): t_R = 25.1 min [(2*R*,3*S*), 6.3%], 28.1 min [(2*S*,3*R*), 93.7%]. Accordingly, the ee of (2*S*,3*R*)-**2b** was estimated to be 87%. The ^{1}H NMR spectrum (400 MHz, JEOL JNM α-400, $CDCl_3$) of a diastereomeric mixture of **2d** prepared from (±)-**2a** was as follows: δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.00−1.80 (54H, m), 1.99 [1H, t, J = 7.6 Hz (2*S*,3*R*)], 2.00 [1H, t, J = 7.6 Hz (2*R*,3*S*)], 3.47 [1.5 H, br s (2*R*,3*S*)], 3.48 [1.5 H, br s (2*S*,3*R*)], 3.52 [1.5H, br s (2*S*,3*R*)], 3.53 [1.5H, br s (2*R*,3*S*)], 4.08 [0.5H, dd, J = 6.9, 11.5 Hz (2*R*,3*S*)], 4.24 [0.5H, dd, J = 5.9, 11.5 Hz (2*S*,3*R*)], 4.31 [0.5H, dd, J = 3.6, 11.5 Hz (2*S*,3*R*)], 4.31 [0.5H, dd, J = 4.0, 11.5 Hz (2*R*,3*S*)], 4.46 [0.5H, m (2*R*,3*S*)], 4.52 [0.5H, m (2*S*,3*R*)], 5.13 [0.5H, dt, J = 5.9, 5.9 Hz (2*S*,3*R*)], 5.13 [0.5H, dt, J = 5.8, 5.8 Hz (2*R*,3*S*)], 5.26 [0.5H, d, J = 8.9 Hz (2*R*,3*S*)], 5.33 [0.5H, d, J = 8.9 Hz (2*S*,3*R*)], 7.35−7.55 (10H, m). Judging from the amide protons at δ 5.26 for (2*R*,3*S*)-**2d** (ca. 6%) and δ 5.33 for (2*S*,3*R*)-**2d** (ca. 94%), the ee of (2*S*,3*R*)-**2b** was estimated to be ca. 88%, and this value was in good accordance with that which was concluded from the HPLC analysis.

Recrystallization from hexanes−ethyl acetate afforded (+)-(2*S*,3*R*)-**2b** as needles, mp 99.5−100.5 °C, $[\alpha]^{22}_D$ = +9.87 (*c* 1.01, chloroform). Its IR and NMR spectra were identical with those of racemate. Anal. Found: C, 73.17; H, 12.56; N, 2.37. Calcd for $C_{38}H_{73}NO_5$: C, 73.14; H, 11.79; N, 2.24%.

Further elution of the column [hexanes−ethyl acetate (3:1)] afforded (−)-(2*R*,3*S*)-**2c** (150 mg, 54%) as a solid, $[\alpha]^{23}_D$ = −6.47 (*c* 1.13, chloroform); ^{1}H NMR δ 0.88 (6H, t, each 3H, J = 6.7 Hz), 1.18−1.78 (54H, m), 2.13 (3H, s), 2.20 (2H, t, J = 7.6 Hz), 2.95 (1H, dd, J = 5.5, 8.1 Hz), 3.59 (2H, m), 4.04 (1H, m), 4.81 (1H, dt, J = 4.8, 7.7 Hz), 6.17 (1H, d, J = 8.6 Hz, NHCO). A small portion of this was hydrolyzed and converted to (2*R*,3*S*)-**2d**, and based on the HPLC analysis and NMR spectrum of MTPA ester, the ee of (2*R*,3*S*)-**2c** was estimated to be 49%.

Further elution of the column [hexanes−ethyl acetate (1:1), subsequently chloroform−methanol (30:1)] afforded (−)-(2*R*,3*S*)-**2a** (17.5 mg, 7%) as a solid, $[\alpha]^{22}_D$ = −5.71 [*c* 1.15, chloroform−methanol (9:1)]. Based on the NMR spectrum and HPLC analysis of MTPA ester, the ee of (2*R*,3*S*)-**2a** was estimated to be 98%.

Recrystallization of (−)-(2*R*,3*S*)-**2a** as stated above from hexanes−ethyl acetate afforded (−)-(2*R*,3*S*)-**2a** as needles, mp 101.0−102.0 °C, $[\alpha]^{24}_D$ = −5.92 [*c* 1.03, chloroform−methanol (9:1)]; IR ν_{max} 3450, 3375, 3300, 3100, 2940, 2860, 1640, 1550, 1465, 1380, 1280, 1260, 1220, 1200, 1180, 1130, 1110, 1070, 1050, 1030, 940, 850, 725, 700, 580 cm^{-1}. Its NMR spectrum was identical with that of racemate with an exception of the chemical shift of OH signals. Anal. Found: C, 75.39; H, 13.47; N, 2.71. Calcd for $C_{34}H_{69}NO_3$: C, 75.64; H, 12.88; N, 2.59%.

A recrystallized sample of (+)-(2*S*,3*R*)-**2b** was hydrolyzed to **2a**. Recrystallization the sample as stated above from hexanes−ethyl acetate afforded (+)-(2*S*,3*R*)-**2a** as needles, mp 103.0−104.5 °C, $[\alpha]^{23}_D$ = +6.09 [*c* 1.00, chloroform−methanol (9:1)]. Its IR and NMR spectra were identical with those of (−)-(2*R*,3*S*)-**2a**. Anal. Found: C, 75.34; H, 13.66; N, 2.71. Calcd for $C_{34}H_{69}NO_3$: C, 75.64; H, 12.88; N, 2.59%.

Hydrolysis of (±)-**2b** with other lipases was carried out in the same manner. Results with *Pseudomonas cepacia* lipase (Amano PS) were listed in Table 1. *Candida antarctica* (Novo SP525), *Aspergillus niger* (Amano A), and pig pancreatic (Sigma) lipases gave no hydrolyzed products.

Lipase-Catalyzed Hydrolysis of 2b (with immobilized SC lipase A). A mixture of (±)-**2b** (1.01 g, 1.61 mmol) and decane (10 mL) was heated at 100 °C for a while to give a homogeneous mixture. To the mixture was added 0.1 M phosphate buffer solution (pH 7.1, 100 mL), and after cooling to room temp, the immobilized form of SC lipase A (1.0 g)[14] was added, and the suspension was stirred for 4 days at 30 °C. Filtration with suction and extraction as stated above afforded a crude product, and its NMR spectrum showed only the mixture of diacetate **2b** and monoacetate **2c**, but no diol **2a**. Purification of the mixture as stated above gave (+)-(2*S*,3*R*)-**2b** (580 mg, 58%) as a solid, $[\alpha]^{21}_D$ = +7.66 (*c* 1.02, chloroform). Based on the HPLC analysis and NMR spectrum of MTPA ester, the ee of (2*R*,3*S*)-**2b** was estimated to be 69%.

On the other hand, monoacetate (2*R*,3*S*)-**2c** (381 mg, 40%) was obtained, $[\alpha]^{22}_D$ = −12.42 (*c* 0.99, chloroform). Based on the HPLC analysis and NMR spectrum of MTPA ester, the ee of (2*R*,3*S*)-**2c** was estimated to be 98%.

Recrystallization of (−)-(2*R*,3*S*)-**2c** as stated above from hexanes−ethyl acetate afforded (−)-(2*R*,3*S*)-**2c** as needles, mp 80.5−81.0 °C, $[\alpha]^{24}_D$ = −12.64 (*c* 0.87, chloroform); IR ν_{max} 3470, 3320, 3080, 2930, 2850, 1730, 1720, 1640, 1550, 1470, 1370, 1240, 1200, 1180, 1160, 1120, 1070, 1030, 980, 960, 720, 700, 640, 600, 590, 480 cm^{-1}. Its NMR spectrum was identical with stated above **2c**. Anal. Found: C, 74.07; H, 13.09; N, 2.65. Calcd for $C_{36}H_{71}NO_4$: C, 74.30; H, 12.30; N, 2.41%.

Determination of the Absolute Configuration. A mixture of (−)-(2*R*,3*S*)-**2a** (57.9 mg, 0.11 mmol) and 2 N hydrochloric acid (3 mL) was heated overnight under Ar at 110 °C. The disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (15:1)]. The mixture was neutralized by the addition of aqueous ammonia solution and extracted with chloroform. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. To the residue were added excess amounts of acetic anhydride, pyridine, and a catalytic amount of DMAP, and the resulting mixture was stirred for 5 h at room temp. The progress of the acetylation was monitored by a TLC analysis [silica gel, developed with hexanes−ethyl acetate (1:3)]. The conventional workup afforded a crude product, which was purified with a silica gel column (1.5 g). The charge of the crude sample was stated as above. Elution with hexanes−ethyl acetate (8:1 to 1:3) gave **5a** (40.9 mg, 96%) as a solid, $[\alpha]^{23}_D$ = −17.8 (*c* 0.98, chloroform) [lit.[9e] $[\alpha]^{23}_D$ = −16.5 (*c* 1.2, chloroform) for (2*R*,3*S*)-**5b**, $[\alpha]^{24}_D$ = +16 (*c* 1.0, chloroform) for (2*S*,3*R*)-**5b**]. This was recrystallized from hexanes−ethyl acetate to give **5a** (34.0 mg, 73%) as needles, mp 97.0−97.5 °C, IR ν_{max} 3300, 3250, 2900, 2850, 1730, 1630, 1570, 1460, 1440, 1370, 1330, 1280, 1240, 1130, 1120, 1070, 1050, 1020, 880, 720, 700, 550, 520 cm^{-1}; ^{1}H NMR δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.00−1.70 (24H, m), 2.04 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 4.05 (1H, dd, J = 4.0, 11.5 Hz), 4.25 (1H, dd, J = 5.9, 11.5 Hz), 4.40 (1H, dddd, J = 4.0, 5.3, 5.9, 9.2 Hz), 4.89 (1H, dt, J = 5.3, 7.6 Hz), 5.85 (1H, d, J = 9.2 Hz, NHCO). Anal. Found: C, 66.03; H, 10.97; N, 3.66. Calcd for $C_{22}H_{41}NO_5$: C, 66.13; H, 10.34; N, 3.51%.

(28) For the preparation of bis MTPA esters, the use of DCC and MTPA acid was essential. In the case of the conventional MTPA chloride, a significant amount of byproduct whose long chain acyl group had migrated from amino group to hydroxyl groups made the precise estimation of ee of the products impossible.

***erythro*-3-*O*-Acetyl-2-(stearoylamino)-1,3-hexadecanediol (±)-(2*R**,3*S**)-2c.** This was essentially prepared according to the published procedure[27b] starting from (±)-(2*R**,3*S**)-**2a**. Intermediates: *erythro*-2-(stearoylamino)-1-*O*-trityl-1,3-hexadecanediol [mp 82.0−83.0 °C, IR ν_{max} 3533, 3291, 2920, 2851, 1655, 1631, 1543, 1468, 1450, 1082, 745, 706, 633, 475 cm^{-1}; ^1H NMR δ 0.88 (6H, t, each 3H, J = 6.8 Hz), 1.0−1.7 (54H, m), 2.24 (2H, t, J = 7.6 Hz), 2.97 (1H, br), 3.25 (1H, dd, J = 3.3, 9.9 Hz), 3.52 (1H, dd, J = 3.0, 9.9 Hz), 3.59 (1H, m), 3.95 (1H, dt, J = 3.8, 7.8 Hz), 6.30 (1H, d, J = 8.3 Hz, NHCO), 7.2−7.4 (15H, m). Anal. Found: C, 81.41; H, 10.72; N, 1.79. Calcd for $C_{53}H_{83}NO_3$: C, 81.38; H, 10.69; N, 1.79%.] and *erythro*-3-*O*-acetyl-2-(stearoylamino)-1-*O*-trityl-1,3-hexadecanediol [mp 93.0−94.0 °C, IR ν_{max} 3280, 2919, 2850, 1741, 1639, 1542, 1467, 1449, 1372, 1229, 1079, 1035, 764, 746, 702, 633 cm^{-1}; ^1H NMR δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.0−1.7 (54H, m), 1.90 (3H, s), 2.12 (2H, t, J = 7.6 Hz), 3.16 (1H, dd, J = 4.3, 9.6 Hz), 3.27 (1H, dd, J = 3.6, 9.6 Hz), 4.30 (1H, m), 5.05 (1H, dt, J = 4.6, 7.2 Hz), 5.73 (1H, d, J = 9.3 Hz, NHCO), 7.2−7.4 (15H, m). Anal. Found: C, 80.42; H, 10.36; N, 1.71. Calcd for $C_{55}H_{85}NO_4$: C, 80.14; H, 10.39; N, 1.70%.]

The final product was a mixture of **2c** and 1-*O*-acetylated regioisomer **2e** (83:17). Purification by silica gel chromatography and recrystallization as stated above afforded (±)-**2c** (53%) as needles. Mp 79.5−80.0 °C. Its IR and NMR spectra were identical with (−)-**2c** as described above. Anal. Found: C, 74.46; H, 12.57; N, 2.47. Calcd for $C_{36}H_{71}NO_4$: C, 74.30; H, 12.30; N, 2.41%. The detailed spectrum and physical properties of the regioisomer **2e** were described bellow.

Lipase-Catalyzed Acetylation of 2a (with immobilized SC lipase A). A mixture of **2a** (30.2 mg, 0.056 mmol), THF (dried over molecular sieves 4A and passed through a neutral alumina column, 1.7 mL), vinyl acetate (freshly distilled, 1.0 mL) and immobilized SC lipase A (30.0 mg) was stirred for 22 h at 30 °C. The progress of the reaction was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (15:1)]. R_f of the product (0.40) was slightly larger than that of **2c** (0.34) as described above. The reaction mixture was filtered by suction, and the filtrate was concentrated in vacuo. The NMR spectrum of the crude product showed no signals of regioisomer **2c** and diacetate **2b**. The mixture was completely dissolved with a small portion of chloroform, subsequently adding a small portion of hexane. The mixture was charged on a silica gel column (1.5 g). Elution with hexanes−ethyl acetate (7:1 to 2:1) afforded **2e** (30.1 mg, 93%) as a solid. This product showed no optical rotation. This was recrystallized from hexanes−ethyl acetate to give **2e** (25.0 mg, 77%) as needles, mp 100.0−100.5 °C, IR ν_{max} 3282, 2918, 2849, 1737, 1647, 1544, 1468, 1276, 1084, 778, 455, 423 cm^{-1}; ^1H NMR δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.0−1.7 (54H, m), 2.08 (3H, s), 2.09 (1H, br), 2.20 (2H, t, J = 7.6 Hz), 3.63 (1H, dt, J = 4.3, 7.6 Hz), 4.13 (1H, m), 4.18 (1H, dd, J = 3.6, 11.2 Hz), 4.37 (1H, dd, J = 6.4, 11.2 Hz), 5.93 (1H, d, J = 7.9 Hz, NHCO). Anal. Found: C, 74.22; H, 12.47; N, 2.34. Calcd for $C_{36}H_{71}NO_4$: C, 74.30; H, 12.30; N, 2.41%.

(*E*)-2-Hexadecenal 7. This was prepared according to the published procedure[29] with a slight modification. To a suspension of $Cp_2Zr(H)Cl$ (2.00 g, 7.74 mmol, 1.6 equiv), and molecular sieves 4A (400 mg) in CH_2Cl_2 (15 mL) was added a solution of 1-ethoxyacetylene (Aldrich 50% in hexane, 1.34 g, 9.55 mmol, 2.0 equiv) in CH_2Cl_2 (2.5 mL). Stirring for 20 min under Ar at room temp gave an orange suspension. To the suspension was added a solution of tetradecanal (1.00 g, 4.71 mmol) in CH_2Cl_2 (2.5 mL) and subsequently was added $AgClO_4$ (60.2 mg, 0.29 mmol, 6.2 mol %). The mixture was further stirred for 30 min under Ar at room temp. The disappearance of the starting material was confirmed by a TLC analysis [silica gel, developed with hexanes−ethyl acetate (10:1)]. The reaction mixture was diluted with ether and poured into a saturated aqueous sodium hydrogen carbonate solution. The reaction mixture was filtered through a Celite pad and extracted with ether three times. The combined organic layer was concentrated in vacuo to a small volume, subsequently adding 2 N hydrochloric acid (90 mL). The two-phase mixture was stirred for 1 h under Ar at room temp. The mixture was extracted with ether, and the organic layer was successively washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified with a silica gel column chromatography (13 g). Elution with hexanes−ethyl acetate (100:1 to 30:1) afforded **7** (0.970 g, 87%). ^1H NMR δ 0.87 (3H, t, J = 6.6 Hz), 1.15−1.40 (20H, m), 1.50 (2H, tt, J = 6.9, 6.9 Hz), 2.33 (2H, ddt, J = 1.3, 6.9, 6.9 Hz), 6.12 (1H, ddt, J = 1.3, 7.9, 15.5 Hz), 6.85 (1H, dt, J = 6.9, 15.5 Hz), 9.50 (1H, d, J = 7.9 Hz). Its NMR spectrum was identical with that reported previously.[30]

***erythro*-(*E*)-3-Hydroxy-2-amino-4-octadecenoic Acid (±)-(2*R**,3*S**)-8.** This was prepared according to the slight modification of the published procedure.[31] To a cooled solution (0 °C) of 1.68 N *n*-butyllithium in hexane (2.70 mL, 4.53 mmol, 1.1 equiv) in freshly distilled THF (11 mL) was added distilled diisopropylamine (1.34 mL, 9.57 mmol, 2.4 equiv). The colorless solution was then stirred for 30 min at 0 °C, cooled to −78 °C, and treated dropwise with a solution of *N*,*N*-bis(trimethylsilyl)glycine trimethylsilyl ester[32] (**6**, 1.18 g, 4.04 mmol) in THF (8 mL). The resulting light-yellow reaction mixture was stirred for 1 h at −78 °C and subsequently treated with **7** (1.24 g, 5.18 mmol, 1.3 equiv) in THF (9 mL). Stirring was continued for 2 h at −78 °C and for 1 h at 0 °C. Subsequently, the solution was acidified with saturated ammonium chloride solution. The colorless solid was collected by a suction filtration, and the solid was repeatedly washed with ethyl acetate and water to give **8** (114 mg). A combined filtrate and washings as a two-phase mixture was cooled overnight at −10 °C. The light-yellow precipitates were collected by a suction filtration, and the solid was repeatedly washed with cold ethyl acetate to give **8** (1.07 g, total 1.19 g), mp 177.0−180.0 °C [lit.[4a] 192−193 °C]. This was employed for the next step without further purification.

***erythro*-(*E*)-2-Amino-4-octadecene-1,3-diol (±)-(2*R**,3*S**)-9.** A mixture of **8** (1.19 g), THF (35 mL), and lithium aluminum hydride (1.40 g, 36.9 mmol, 9.8 equiv) was stirred for 2 days under Ar at 70 °C. The reaction was quenched by adding successively water (1.4 mL), 10% sodium hydroxide solution (1.4 mL), and water (2.0 mL) with ice-cooling and further stirred for 1 h at room temp. The mixture was filtered by a suction, and the solid was repeatedly washed with chloroform−methanol and ethyl acetate. A combined filtrate and washings as a two-phase mixture were separated, and the organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified with silica gel column chromatography (25 g). Elution with chloroform−methanol (20:1 to 1:1) afforded crude **9** (632 mg) as a solid. This was recrystallized from hexanes−ethyl acetate to give **9** (469 mg, two steps 39%) as needles, mp 63.0−63.5 °C [lit.[4a] 71−73 °C], ^1H NMR δ 0.87 (3H, t, J = 6.8 Hz), 1.0−1.5 (22H, m), 2.04 (2H, dt, J = 6.6, 6.9 Hz), 2.86 (5H, m), 3.68 (2H, m), 4.07 (1H, dd, J = 5.9, 6.9 Hz), 5.45 (1H, dd, J = 6.9, 15.2 Hz), 5.74 (1H, dt, J = 6.6, 15.2 Hz). Its NMR spectrum was identical with that reported previously.[4b]

***erythro*-(*E*)-2-(Stearoylamino)-4-octadecene-1,3-diol (±)-(2*R**,3*S**)-1a.** *N*-acylation was carried out according to the procedure as described for **4a**. **1a** (99%) was prepared starting from **9**. Recrystallization from hexanes−ethyl acetate gave **1a** (95%) as needles, mp 95.0−96.0 °C [lit.[4a] 97−97.5 °C], ^1H NMR δ 0.88 (3H, t, each 3H, J = 6.8 Hz), 1.00−1.70 (52H, m), 2.05 (2H, dt, J = 6.6, 6.9 Hz), 2.23 (2H, t, J = 7.6 Hz), 2.15 (2H, br), 3.70 (1H, dd, J = 2.6, 10.6 Hz), 3.91 (1H, m), 3.95 (1H, dd, J = 4.0, 10.6 Hz), 4.32 (1H, dd, J = 4.0, 6.3 Hz), 5.52 (1H, dd, J = 6.3, 15.5 Hz), 5.78 (1H, dt, J = 6.6, 15.5 Hz), 6.26 (1H, d,

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J = 7.3 Hz). Its NMR spectrum was identical with that reported previously.[4b]

***erythro*-3-Acetoxy-2-(stearoylamino)-4-octadecenyl Acetate (±)-(2*R**,3*S**)-1b.** Acetylation was carried out according to the procedure as described for **2a**. **1b** (95%) was prepared starting from **1a**. Recrystallization from hexanes−ethyl acetate gave **1b** (89%) as needles, mp 105.0−107.0 °C, IR ν_{max} 3325, 2925, 2850, 1730, 1640, 1540, 1470, 1420, 1380, 1320, 1270, 1240, 1180, 1160, 1140, 1080, 1030, 980, 970, 920, 910, 890, 820, 800, 720, 680, 610, 600 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, each 3H, J = 6.8 Hz), 1.1−1.5 (48H, m), 1.59 (4H, m), 2.02 (2H, dt, J = 6.8, 7.3 Hz), 2.05 (3H, s), 2.06 (3H, s), 2.16 (2H, t, J = 7.6 Hz), 4.03 (1H, dd, J = 3.9, 11.5 Hz), 4.30 (1H, dd, J = 5.9, 11.5 Hz), 4.45 (1H, m), 5.27 (1H, dd, J = 5.9, 6.4 Hz), 5.38 (1H, ddt, J = 1.5, 5.9, 15.1 Hz), 5.60 (1H, d, J = 9.3 Hz), 5.79 (1H, dt, J = 6.8, 15.1 Hz). Its NMR spectrum was identical with an authentic sample derived from commercially available D-*erythro*-sphingosine. Anal. Found: C, 73.68; H, 12.02; N, 2.25. Calcd for $C_{40}H_{75}NO_5$: C, 73.91; H, 11.63; N, 2.15%.

Lipase-Catalyzed Hydrolysis of 1b (with native SC lipase A). A mixture of (±)-**1b** (152.2 mg, 0.234 mmol) and decane (1.5 mL) was heated at 100 °C for a while to give a homogeneous mixture. To the mixture was added 0.1 M phosphate buffer solution (pH 7.1, 15 mL), and after cooling to room temp, SC lipase A (30 mg) was added, and the suspension was stirred for 3 days at 30 °C. To the mixture was further added SC lipase A (20 mg), and the suspension was stirred for 1 day at 30 °C. The progress of the hydrolysis was confirmed by a TLC analysis [silica gel, developed with chloroform−methanol (15:1)]. The mixture was diluted with chloroform. After organic layer was separated, the aqueous layer was thoroughly extracted with chloroform. The combined extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The NMR spectrum of the crude product indicated that it was a mixture of diacetate **1b**, monoacetate **1c**, and diol **1a**, judging from the signals of amide protons. To the mixture was added silica gel (500 mg), and the product was completely dissolved with a small portion of chloroform, subsequently adding a small portion of hexane. The mixture was charged on a silica gel column (15 g). Elution with hexane-chloroform (1:1) and subsequently with hexanes−ethyl acetate (8:1 to 5:1) afforded (−)-(2*S*,3*R*)-**1b** (38.1 mg, 25%) as a solid, $[\alpha]^{24}_D = -8.20$ (*c* 0.84, chloroform). A small portion of this was hydrolyzed and converted to the corresponding bis (*R*)-MTPA ester (2*S*,3*R*)-**1d** in the same manner as described for **2d**. The ee of (2*S*,3*R*)-**1a** [from (2*S*,3*R*)-**1b**] was estimated from HPLC analysis and the ^1H NMR spectrum of **1d**. HPLC (same condition for **2d**): t_R = 31.3 min [(2*R*,3*S*), 7.6%], 33.0 min [(2*S*,3*R*), 92.4%]. Accordingly, the ee of (2*S*, 3*R*)-**1b** was estimated to be 85%. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of a diastereomeric mixture of **1d** prepared from (±)-**1a** was as follows: δ 0.88 (6H, t, each 3H, J = 6.6 Hz), 1.20−1.70 (52H, m), 1.98 [2H, m (2*S*,3*R*)], 2.01 [2H, m (2*R*,3*S*)], 3.47 [1.5 H, br s (2*S*,3*R*)], 3.49 [1.5 H, br s (2*R*,3*S*)], 3.51 [1.5H, br s (2*R*,3*S*)], 3.52 [1.5H, br s (2*S*,3*R*)], 4.19 [0.5H, dd, J = 5.4, 11.7 Hz (2*R*,3*S*)], 4.26 [0.5H, dd, J = 3.7, 11.7 Hz (2*R*,3*S*)], 4.33 [0.5H, dd, J = 4.2, 11.5 Hz (2*S*,3*R*)], 4.38 [0.5H, dd, J = 5.1, 11.5 Hz (2*S*,3*R*)], 4.48 [0.5H, m (2*R*,3*S*)], 4.53 [0.5H, m (2*S*,3*R*)], 5.23 [0.5H, d, J = 8.8 Hz (2*R*,3*S*)], 5.27 [0.5H, d, J = 9.3 Hz (2*S*,3*R*)], 5.28 [0.5H, dd, J = 7.8, 15.1 Hz (2*S*,3*R*)], 5.38 [0.5H, dd, J = 7.3, 7.8 Hz (2*S*,3*R*)], 5.39 [0.5H, dd, J = 3.9, 8.3 Hz (2*R*,3*S*)], 5.39 [0.5H, dd, J = 8.3, 14.7 Hz (2*R*,3*S*)], 5.80 [0.5H, dt, J = 6.8, 15.1 Hz (2*S*,3*R*)], 5.92 [0.5H, dt, J = 6.8, 14.7 Hz (2*R*,3*S*)], 7.36−7.51 (10H, m). Judging from the olefin protons (H-5) at δ 5.80 for (2*S*,3*R*)-**1d** (ca. 93%) and δ 5.92 for (2*R*,3*S*)-**1d** (ca. 7%), the ee of (2*S*, 3*R*)-**1b** was estimated to be ca. 86%.

(−)-(2*S*,3*R*)-**1b** was finally obtained as needles by recrystallization of the sample as above, mp 106.0−108.0 °C. Its IR and NMR spectra were identical with those of the racemate.

Further elution of the column [hexanes−ethyl acetate (3:1)] afforded (+)-(2*R*,3*S*)-**1c** (79.7 mg, 58%) as a solid, mp 84.0−85.0 °C, $[\alpha]^{25}_D = +3.08$ (*c* 1.15, chloroform); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, each 3H, J = 6.8 Hz), 1.1−1.4 (48H, m), 1.60 (4H, br), 2.03 (2H, dt, J = 6.8, 6.8 Hz), 2.10 (3H, s), 2.20 (2H, dt, J = 2.4, 7.8 Hz), 2.77 (1H, br), 3.65 (2H, m), 4.12 (1H, m), 5.28 (1H, dd, J = 7.3, 7.3 Hz), 5.47 (1H, ddt, J = 1.5, 7.8, 15.6 Hz), 5.77 (1H, dt, J = 6.8, 15.6 Hz), 5.95 (1H, d, J = 8.3 Hz, NHCO). Its NMR spectrum was identical with that reported previously.[27b] Based on the HPLC analysis and NMR spectrum of MTPA ester, the ee of (2*R*,3*S*)-**1c** was estimated to be 17%.

Further elution of the column [hexanes−ethyl acetate (1:1) and subsequently chloroform−methanol (30:1)] afforded (+)-(2*R*,3*S*)-**1a** (16.3 mg, 12%) as a solid, mp 95.0−96.0 °C, $[\alpha]^{22}_D = +3.08$ [*c* 0.81, chloroform−methanol (9:1)]; IR ν_{max} 3450, 3375, 3300, 3100, 2940, 2860, 1640, 1550, 1465, 1380, 1280, 1260, 1220, 1200, 1180, 1130, 1110, 1070, 1050, 1030, 940, 850, 725, 700, 580 cm^{-1}. Its NMR spectrum was identical with that of racemate. Based on the HPLC analysis and of NMR spectrum MTPA ester, the ee of (2*R*,3*S*)-**1a** was estimated to be 98%.

A sample of (−)-(2*S*,3*R*)-**1b** was hydrolyzed to (−)-(2*S*,3*R*)-**1a** as a solid, mp 95.0−97.0 °C [lit.[9c] 97−98 °C], $[\alpha]^{23}_D = -2.78$ [*c* 0.88, chloroform−methanol (9:1)] [lit.[9c] $[\alpha]^{25}_D = -3.1$ (*c* 1.1, chloroform)]. Its NMR spectrum was identical with that reported previously.[9c]

Hydrolysis of (±)-**1b** with other lipases was attempted in the same manner, however, *P. cepacia* (Amano PS), *C. antarctica* (Novo SP525), *A. niger* (Amano A), pig pancreatic (Sigma) lipases, and immobilized SC lipase A gave no hydrolyzed products.

Lipase-Catalyzed Hydrolysis of (+)-(2*S*,3*R*)-2b (with native SC lipase A). A mixture of (+)-(2*S*,3*R*)-**2b** (96% ee, 40.0 mg, 0.064 mmol) and decane (0.4 mL) was heated at 100 °C for a while to give a homogeneous mixture. To the mixture was added 0.1 M phosphate buffer solution (pH 7.1, 4.0 mL), and after cooling to room temp, SC lipase A (150 mg) was added, and the suspension was stirred for 3 days at 30 °C. To the mixture was further added SC lipase A (50 mg), and the suspension was stirred for 3 day at 30 °C. Purification as stated above afforded (+)-(2*S*,3*R*)-**2b** (10.4 mg) and (+)-(2*S*,3*R*)-**2c** (23.4 mg, 85%) as a solid. Based on the HPLC analysis and NMR spectrum of MTPA ester, the ee of (+)-(2*R*,3*S*)-**2c** was enhanced to be >99%, by the hydrolytic removal of contaminating (−)-(2*R*,3*S*)-**2b** into diol **2a**.

Recrystallization from hexanes−ethyl acetate afforded (+)-(2*S*,3*R*)-**2c** as needles, mp 79.0−81.0 °C, $[\alpha]^{22}_D = +13.08$ (*c* 1.05, chloroform). Its IR and NMR spectra were identical with those of racemate. Anal. Found: C, 74.08; H, 12.41; N, 2.37. Calcd for $C_{36}H_{71}NO_4$: C, 74.30; H, 12.30; N, 2.41%.

(2*S*,3*R*)-1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-2-(stearoylamino)-1,3-hexadecanediol (10). Glycosylation was essentially carried out according to the published procedure.[33] To a suspension of molecular sieves 4A (200 mg), (+)-(2*S*,3*R*)-**2c** (33.2 mg, 0.057 mmol), and tetra-*O*-acetyl-α-galactosyl trichloroacetimidate[34] (55.5 mg, 0.113 mmol, 2.0 equiv) in CH_2Cl_2 (0.5 mL) was added a solution of $BF_3{\cdot}OEt_2$ (14.5 μL, 0.114 mmol, 2.0 equiv) in CH_2Cl_2 (0.5 mL). The suspension was then stirred for 3 h under Ar at room temp. The progress of the reaction was confirmed by a TLC analysis [silica gel, developed with hexanes−ethyl acetate (1:1)]. The mixture was diluted with chloroform and poured into a saturated aqueous sodium hydrogen carbonate solution. The organic layer was successively washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was completely dissolved with a small portion of chloroform, and the mixture was charged on a silica gel column (1.8 g). Elution with hexanes−ethyl acetate (7:1 to 2:1) afforded (+)-(2*S*,3*R*)-**2b** (16.0 mg, 45%). Further elution of the chromatography afforded **10** (27.1 mg, 52%), $[\alpha]^{23}_D = +3.57$ (*c* 0.82, chloroform). IR (film) ν_{max} 2950, 2850, 1750, 1650, 1540, 1460, 1370, 1230, 1050 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.87 (6H, t, each 3H, J = 6.6 Hz), 1.2−1.7 (54H, m), 1.98 (3H,

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s), 2.05 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.15 (3H, s), 2.16 (2H, t), 3.59 (1H, dd, J = 4.4, 10.1 Hz), 3.91 (2H, m, Gal-6), 4.13 (2H, m, Cer-1), 4.27 (1H, m, Cer-2), 4.43 (1H, d, J = 7.7, Gal-1), 4.85 (1H, dt, J = 5.1, 8.1 Hz, Cer-3), 4.99 (1H, dd, J = 3.3, 10.4 Hz, Gal-3), 5.13 (1H, dd, J = 7.7, 10.4 Hz, Gal-2), 5.38 (1H, d, J = 3.3 Hz, Gal-4), 5.99 (1H, d, J = 9.2 Hz, NHCO). MS (70 eV); *m*/*z*(%): 910 (15), 850 (25), 654 (20), 563 (40) [M^+ − Gal4Ac − H_2O + 1], 489 (25), 330 (100), 163 (35), 43 (40). HRMS Found: 550.5202. Calcd for $C_{35}H_{68}NO_3$ (M^+ − 4AcGal − OCH_2): 550.5196.

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