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Chemo-enzymatic short-step total synthesis of symbioramide

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Abstract—A concise synthesis of symbioramide, a marine-origin ceramide from a common starting material, methyl (±)-*trans*-2,3-epoxyoctadecanoate, in a convergent manner was achieved. The key step is the direct lipase-catalyzed coupling reaction between methyl (2*R*,3*E*)-2-hydroxy-3-octadecenoate and non-protected (±)-*erythro*-dihydrosphingosine, giving natural (2*S*,3*R*,2′*R*)-symbioramide and its (2*R*,3*S*,2′*R*)-isomer in 38% and 37% yield, respectively. The optically active β,γ-unsaturated α-hydroxyester was prepared by $Mg(ClO_4)_2$-mediated isomerization of epoxide and the subsequent lipase PS-catalyzed kinetic resolution.

Symbioramide (**1a**),[1] a naturally occurring marine ceramide as a Ca^{2+}-ATPase activating factor as well as the antileukemic substance against L1210 murine leukemia cells, consists of the D-*erythro*-dihydrosphingosine [(2*S*,3*R*)-**2**] and β,γ-unsaturated α-hydroxycarboxylic acid (2*R*,3*E*)-**3a**. Three groups have been successful in chemical synthesis of **1a** and related stereoisomers.[2–4]

The present synthetic strategy has the following feature. Both the dihydrosphingosine and the long-chain β,γ-unsaturated α-hydroxycarboxylic acid moiety are derived from the common intermediate, an α,β-epoxycarboxylic ester (**4**). Toward this end, the isomerization of the α,β-epoxy ester (±)-**4**[5] into β,γ-unsaturated α-hydroxyester **5a** is the first task (Fig. 1).

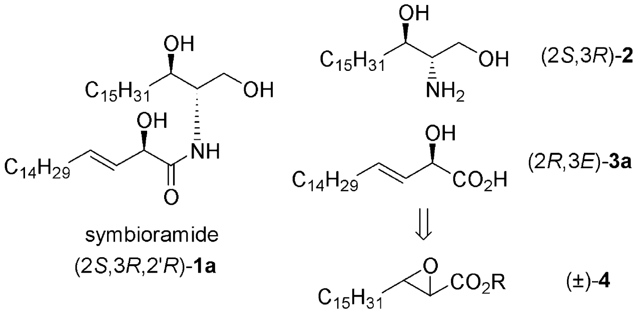

Figure 1.

Table 1. Isomerization of epoxy ester to β,γ-unsaturated α-hydroxyester

Entry	Lewis acid	Temp (°C)	Yield (%)
1	$LiClO_4$	110	32
2	$Zn(ClO_4)_2$	110	23
3	$In(ClO_4)_3$	70	29
4	$Mg(ClO_4)_2$	110	61
5	LiOTf	110	No reaction
6	$Mg(OTf)_2$	110	No reaction
7	$Zn(OTf)_2$	110	21

All of the reaction were performed in toluene.

So far, some perchlorates[6] had been reported for similar transformations, and indeed, $LiClO_4$ promoted the desired reaction (Table 1). Among Lewis acids, whose counteranions have low nucleophilicity, $Mg(ClO_4)_2$[7] yielded the best result (61%). The reaction was stopped within 2 h at 110 °C in toluene,[8] to avoid the further decomposition of **5a** under prolonged incubation. The product **5a**, obtained as an inseparable mixture of isomers (*E*/*Z* = 4:1, Scheme 1), was once oxidized to the α,β-unsaturated ketone [(*E*)-**6**,[9] 94%] with Dess–Martin reagent. Then, the ketoester was reduced to afford the original hydroxyester **5a** with a variety of reducing reagents. In most cases, however, some unexpected basic conditions caused re-isomerization of double bond. The use of L-Selectride[10] in Et_2O/hexane (10:1) at

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Keywords: Symbioramide; Dihydrosphingosine; α-Hydroxy acid; Lipase; Aminolysis.

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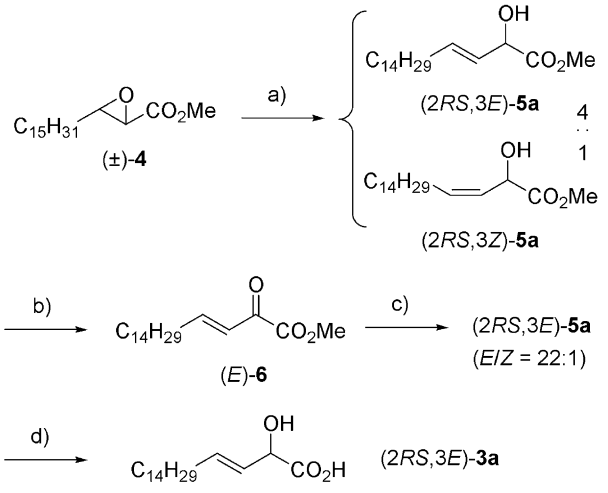
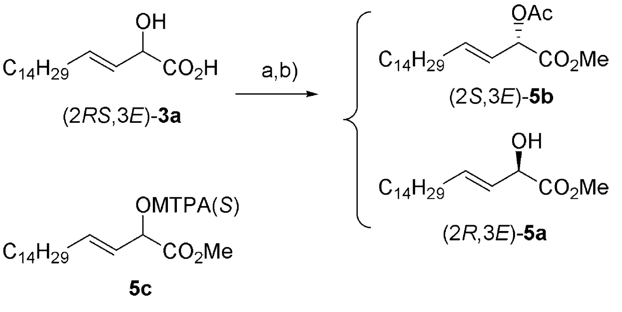


Scheme 1. Reagents and conditions: (a) $Mg(ClO_4)_2$, MS3A, toluene, 110 °C, 61%; (b) Dess–Martin periodinane, CH_2Cl_2, 94%; (c) L-Selectride, Et_2O/hexane (10:1), −78 °C, 75%; (d) Chirazyme L-2, phosphate buffer (0.1 M, pH 7.0), 30 °C, quant.

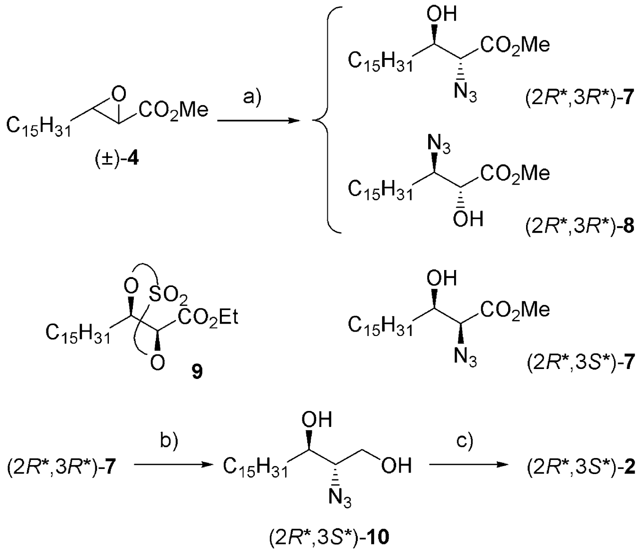
−78 °C, gave the highest ratio (*E*/*Z* = 22:1) of the product (75%).[11] The ester was hydrolyzed (quant.) under as mild as neutral conditions by the treatment with *Candida antarctica* lipase (Chirazyme L-2) in buffer, and the recrystallization of the product provided pure (2*RS*,3*E*)-**3a** (Scheme 1).[12]

The enantiomeric resolution was successful by applying vinyl acetate and *Pseudomonas cepacia* lipase (Amano PS).[13] The subsequent CH_2N_2 treatment enabled separation of the two products, to give (2*S*,3*E*)-**5b** (49%, 90.3% ee) and (2*R*,3*E*)-**5a** (40%, 97.0% ee), respectively (Scheme 2).[14] The enantiomeric ratio (*E* value) was 86.

Next, the epoxide (**4**), was treated with NaN_3 (5.0 equiv) and AcOH (2.5 equiv), α-azide (2*R**,3*R**)-**7** and β-azide (2*R**,3*R**)-**8** (quant. 6:1) formed, and pure **7**[15] (81%) was accessible by recrystallization of the crude product. α-Substitution was confirmed with an authentic product[16] derived from a cyclic sulfate **9**. If this epoxide ring-opening reaction was performed with NH_4Cl instead of AcOH, an undesired α-epimer (2*R**,3*S**)-**7** was accompanied (10%). Further transformation of pure **7** yielded the (±)-*erythro*-dihydrosphingosine (2*R**,3*S**)-**2**[17] (84%) in Scheme 3.



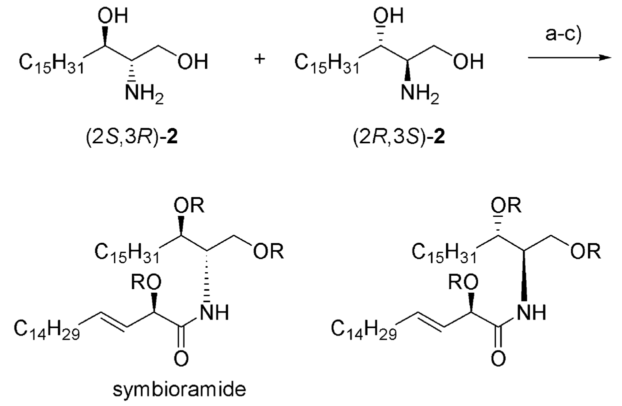
Scheme 2. Reagents and conditions: (a) Amano PS, vinyl acetate, THF, 30 °C; (b) CH_2N_2/Et_2O, CH_2Cl_2, 49% for (2*S*,3*E*)-**5b**, 40% for (2*R*,3*E*)-**5a**.



Scheme 3. Reagents and conditions: (a) NaN_3, AcOH, DMF, 50 °C, then recrystallization, 81% for (2*R**,3*R**)-**7**; (b) $LiBH_4$, THF, rt, 92%; (c) H_2, Pd–C, EtOH–AcOEt, rt, 90%.

At the stage of the two segments in hand, an unprecedented direct enzyme-mediated coupling in preparative scale was tried. *C. antarctica* lipase[18]-catalyzed acylation of (±)-**2** with (2*R*,3*E*)-**5a** (1.5 equiv) worked under the conditions at high temperature (65 °C)[19] and reduced pressure (80 Torr),[20] to ensure high specific activity of enzyme, and to remove concomitantly formed MeOH.

A tertiary alcohol,[21] 3-methyl-3-pentanol, was effective solvent, which has no effect on the enzyme-catalyzed reaction. The reaction proceeded in non-enantioselective manner, as both enantiomers of **2** were entirely consumed. Acetylation of the crude product afforded



	R	(2*S*,3*R*,2′*R*)	(2*R*,3*S*,2′*R*)
1a	H	Rf 0.34	0.43
b	Ac	0.43	0.33
c	*p*-BrC_6H_4CO	0.48	0.58

Scheme 4. Reagents and conditions: (a) (2*R*,3*E*)-**5a** (1.5 equiv), Chirazyme L-2, $(CH_3CH_2)_2C(OH)CH_3$, 65 °C, 80 Torr; (b) Ac_2O, pyridine; (c) Et_3N, MeOH, 38% for (2*S*,3*R*,2′*R*)-**1a**, 37% for (2*R*,3*S*,2′*R*)-**1a**.

natural (2*S*,3*R*,2′*R*)-**1b** and its (2*R*,3*S*,2′*R*)-isomer, (total 75%), which were well separated on chromatography. Each was deacetyated (quant.) to give symbioramide (**1a**) and the unnatural diastereomer (Scheme 4). Their relative configurations were undoubtedly confirmed as tris *p*-bromobenzoates (2*S*,3*R*,2′*R*)-**1c** and (2*R*,3*S*,2′*R*)-**1c**, respectively.[22]

In conclusion, natural (2*S*,3*R*,2′*R*)-symbioramide (**1a**) and (2*R*,3*S*,2′*R*)-isomer was synthesized by lipase-catalyzed coupling between (±)-dihydrosphingosine (**2**) and (*R*)-β,γ-unsaturated α-hydroxyester **5a**, both of which were derived from the common intermediate, methyl (±)-*trans*-2,3-epoxyoctadecanonate (**4**). The number of steps involved in all of the convergent routes totaled only 12.

Acknowledgements

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8. To a mixture of $Mg(ClO_4)_2$ (9.1 g, 30.6 mmol), MS3A (13.9 g) in dry toluene (150 mL) was added a soln of **4** (8.7 g, 27.8 mmol) in toluene (50 mL). The mixture was stirred under reflux for 2 h and then quenched by water (100 mL). Conventional workup and purification by SiO_2 (400 g, hexane/AcOEt = 10:1) to give **5a** (5.5 g, 61%, *E*/*Z* = 4:1) as colorless oil. The *E*/*Z* ratio was estimated by comparing ^{1}H NMR: (*E*) δ 5.47 (dd); (*Z*) δ 5.35–5.41 (m).
9. Mp 54.0–55.5 °C; IR 2918, 2852, 1741, 1703, 1628, 1471, 1275, 1146, 1093, 982, 717 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, *J* 6.9 Hz), 1.23–1.39 (m, 24H), 1.43–1.49 (m, 2H), 2.28 (ddt, 2H, *J* 1.5, 6.8, 6.8 Hz), 3.86 (s, 3H), 6.63 (dt, 1H, *J* 15.9, 1.5 Hz), 7.18 (dt, 1H, *J* 15.9, 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 14.21, 22.75, 27.78, 29.25, 29.41, 29.54, 29.66, 29.70, 29.71 (×3), 29.74, 32.00, 33.22, 52.90, 124.86, 155.50, 162.61, 182.81; Anal. Calcd for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61. Found: C, 73.15; H, 11.52.
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11. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* 6.8 Hz), 1.23 (br s, 24H), 2.03 (dt, 2H, *J* 7.0, 7.0 Hz), 2.87 (br s, 1H), 3.77 (s, 3H), 4.58 (d, 1H, *J* 5.9 Hz), 5.47 (dd, 1H, *J* 5.9, 15.1 Hz), 5.86 (dt, 1H, *J* 15.1, 7.0 Hz).
12. A mixture of **5a** (*E*/*Z* = 22:1, 3.1 g, 9.9 mmol), phosphate buffer (pH 7.0, 0.1 M, 100 mL) and Chirazyme L-2 (c–f, 680 mg) was stirred vigorously at 30 °C for 34 h. The mixture was diluted with water, and pH was adjusted to 4 by 1 M HCl, and mixed with AcOEt. The conventional workup gave the product (3.1 g, quant.) as white solid, and recrystallization from acetone gave **3a** (2.9 g, 98%) as colorless fine needles, mp 96.0–96.2 °C; IR 3450, 2916, 2850, 1747, 1471, 1246, 1105, 1061, 964, 889, 652 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, *J* 6.8 Hz), 1.16–1.25 (m, 24H), 1.29–1.39 (m, 2H), 2.00 (dt, 2H, *J* 7.1, 7.1 Hz), 4.62 (d, 1H, *J* 6.4 Hz), 5.49 (dd, 1H, *J* 15.3, 6.4 Hz), 5.87 (dt, 1H, *J* 15.3, 7.1 Hz). ^{13}C NMR (DMSO-d_6) δ 13.96, 22.14, 28.59, 28.77, 28.91, 29.10 (×6), 31.35, 31.54, 70.78, 128.02, 131.87, 174.28; Anal. Calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48. Found: C, 72.52; H, 11.39. The hydrolysis proceeded in non-enantioselective manner. Also the epoxy ester (±)-**4** was a good substrate for Chirazyme L-2, to give the epoxy acid (quant.).
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14. To a soln of **3a** (625 mg, 2.1 mmol) in THF (7 mL) and vinyl acetate (7 mL) was Amano PS (2.0 g), and the mixture was stirred at 30 °C for 4 days. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was diluted with CH_2Cl_2 (2 mL), and was added CH_2N_2/Et_2O to give a mixture of (2*R*,3*E*)-**5a** and (2*S*,3*E*)-**5b**, which was purified by SiO_2 (100 g, hexane/AcOEt = 8:1) to give (2*R*,3*E*)-**5a** (260 mg, 40%, 97% ee) and (2*S*,3*E*)-**5b** (363 mg, 49%, 90% ee) as colorless oil. (2*R*,3*E*)-**5a**: $[\alpha]_D^{20}$ −42.4 (*c* 0.50, $CHCl_3$) [Ref. 2 $[\alpha]_D^{19}$ −44.7 (*c* 0.257, $CHCl_3$), Ref. 10 $[\alpha]_D^{20}$ −45.3 (*c* 0.70, $CHCl_3$)]. (2*S*,3*E*)-**5b**: $[\alpha]_D^{20}$ +48.8 (*c* 1.17, $CHCl_3$); IR 2924, 2854, 1751, 1437, 1373, 1228, 1049, 968 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, *J* 6.8 Hz), 1.23 (m, 22H), 1.32–1.40 (m, 2H), 2.05 (dt, 2H, *J* 7.5, 7.5 Hz), 2.12 (s, 3H), 3.72 (s, 3H), 5.35

(d, 1H, *J* 7.5 Hz), 5.50 (dd, 1H, *J* 15.4, 7.5 Hz), 5.91 (dt, 1H, *J* 15.4, 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.08, 20.68, 22.66, 28.52, 29.08, 29.33, 29.38, 29.54, 29.61 (×2), 29.64, 31.88, 32.26, 52.41, 73.30, 121.53, 138.26, 169.58, 170.14; Anal. Calcd for $C_{19}H_{38}O_4$: C, 71.14; H, 10.80. Found: C, 70.95; H, 10.69. The ees were estimated by the ^{1}H NMR spectra of MTPA esters **5c**. (2R,2′S)-**5c**: ($CDCl_3$) δ 2.06 (dt, 1H, *J* 6.8, 6.8 Hz, H-5), 3.55 (s, 3H, MTPA-OCH_3), 3.73 (s, 3H, CO_2CH_3), 5.58 (m, 2H, H-2, H-3), 5.97 (m, 1H, H-4); (2S,2′S)-**5c**: δ 2.02 (dt, 1H, *J* 7.0, 7.0 Hz, H-5), 3.64 (s, 3H, MTPA-OCH_3), 3.77 (s, 3H, CO_2CH_3), 5.51 (d, 1H, *J* 15.1, 7.3 Hz, H-3), 5.58 (d, 1H, *J* 7.3 Hz, H-2), 5.86 (dt, 1H, *J* 15.1, 7.0 Hz, H-4).

15. A mixture of **4** (1.0 g, 3.2 mmol), NaN_3 (1.0 g, 16.0 mmol), AcOH (640 μL, 11.2 mmol) in dry DMF (31 mL) was stirred at 50 °C for 24 h. Water (30 mL) was added and extracted with Et_2O. The conventional workup, chromatography (SiO_2, 100 g, hexane/AcOEt = 8:1), and recrystallization from hexane to give pure (2R*,3R*)-**7** (920 mg, 81%) as colorless needles, mp 41.0–41.5 °C; IR 3459, 2920, 2850, 2111, 1743, 1468, 1438, 1207, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, *J* 6.8 Hz), 1.23 (br s, 26H), 1.50 (m, 2H), 2.24 (d, 1H, *J* 5.4 Hz), 3.81 (s, 3H), 3.91 (m, 1H) 3.95 (d, 1H, *J* 5.9 Hz); ^{13}C NMR ($CDCl_3$) δ 14.07, 22.66, 25.34, 29.33, 29.46, 29.53, 29.63, 29.66 (×6), 31.89, 33.00, 52.71, 66.25, 71.92, 169.46; Anal. Calcd for $C_{19}H_{37}N_3O_3$: C, 64.19; H, 10.49; N, 11.82. Found: C, 64.22; H, 10.43; N, 11.73. (2R*,3R*)-**8**: ^{1}H NMR δ 3.09 (d, 1H, *J* 5.3 Hz), 3.50 (dt, 1H, *J* 10.3, 3.4 Hz), 4.31 (dd, *J* 5.3, 3.4 Hz); (2R*,3S*)-**7**: ^{1}H NMR δ 2.00 (d, 1H, *J* 6.8 Hz), 4.04 (m, 1H); ^{13}C NMR δ 52.82, 65.92, 72.31, 169.74.
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17. **10**: colorless fine needles, mp 48.0–50.0 °C; IR 3357, 2919, 2850, 2103, 1467, 1265, 1049, 759 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, *J* 6.8 Hz), 1.23 (br s, 26H), 1.49–1.53 (m, 2H), 2.25 (m, 2H), 3.40 (ddd, 1H, *J* 4.9, 4.9, 4.9 Hz), 3.75 (m, 1H), 3.87 (dd, 1H, *J* 1.5, 4.9 Hz), 3.88 (dd, 1H, *J* 1.5, 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.66, 25.57, 29.33, 29.46, 29.51, 29.54, 29.63, 29.66 (×5), 31.89, 33.70, 62.45, 66.78, 72.58; Anal. Calcd for $C_{18}H_{37}N_3O_2$: C, 66.01; H, 11.39; N, 12.83. Found: C, 65.77; H, 11.31; N, 12.61. (2R*,3S*)-**2**: mp 62.5–63.0 °C; IR 3354, 3256, 2920, 2848, 1468, 1261, 1020, 802, 723 cm^{-1}; ^{13}C NMR (pyridine-d_5) δ 14.26, 22.93, 26.63, 29.60, 29.91, 29.98 (×5), 30.08, 30.22, 32.11, 34.26, 35.09, 58.47, 64.63, 73.82; Anal. Calcd for $C_{18}H_{39}NO_2$: C, 71.70; H, 13.04; N, 4.65. Found: C, 71.52; H, 13.11; N, 4.54. Its NMR spectra were identical with those reported previously: So, R. C.; Ndonye, R.; Izmirian, D. P.; Richardson, S. K.; Guerrera, R. L.; Howell, A. R. *J. Org. Chem.* **2004**, *69*, 3233–3235.
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22. A soln of **2** (60.2 mg, 0.20 mmol), (2R,3E)-**5a** (93.4 mg, 0.30 mmol), and 3-methyl-3-pentanol (3.5 mL) was added Chirazyme L-2 (250 mg) and stirred at 65 °C under 80 Torr for 4 h. The reaction mixture was concentrated in vacuo, and acetylated in the usual manner. After workup, the residue was purified by preparative TLC (hexane/AcOEt = 2:1) to give (2S,3R,2′R)-**1b** (53.4 mg, 38%) and (2R,3S,2′R)-**1b** (52.1 mg, 37%). (2S,3R,2′R)-**1b**: prisms from hexane–AcOEt, R_f 0.43 [hexane/AcOEt (2:1)]; $[\alpha]_D^{20}$ −7.2 (*c* 0.58, $CHCl_3$) [Ref. 3 $[\alpha]_D^{21}$ −5.09 (*c* 1.36, $CHCl_3$)]; mp 80.5–81.0 °C (Refs. 1 and 2 75–78 °C, Ref. 3 78–79 °C); IR 3313, 2850, 1736, 1664, 1541, 1468, 1385, 1269, 1039, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H), 1.23–1.39 (m, 50H), 2.02 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.16 (s, 3H, Ac), 4.01 (dd, 1H, *J* 2.9, 11.2 Hz, H-1), 4.28 (dd, 1H, *J* 6.8, 11.2 Hz, H-1), 4.32–4.36 (m, 1H, H-2), 4.88 (dt, 1H, *J* 7.8, 5.1 Hz, H-3), 5.46 (d, 1H, *J* 7.3 Hz, H-2′), 5.49 (dd, 1H, *J* 7.3, 13.8 Hz, H-3′), 5.88 (dt, 1H, *J* 13.8, 6.8 Hz, H-4′), 6.51 (d, 1H, *J* 8.3 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 14.11, 20.75, 20.95, 22.67, 25.32, 28.66, 29.22, 29.35, 29.45, 29.56, 29.59, 29.64, 29.69 (×17), 31.40, 31.91, 32.29, 50.68, 62.14, 73.81, 74.50, 122.80, 137.84, 168.53, 169.38, 170.86, 171.27. Anal. Calcd for $C_{42}H_{77}NO_7$: C, 71.24; H, 10.96; N, 1.98. Found: C, 71.24; H, 10.88; N, 1.93. Its ^{1}H NMR spectrum was identical with those reported previously.[1–3] (2R,3S,2′R)-**1b**: fine needles from hexane–AcOEt, R_f 0.33; $[\alpha]_D^{20}$ −28.3 (*c* 0.61, $CHCl_3$); mp 82.5–83.0 °C; IR 3323, 2848, 1747, 1664, 1548, 1465, 1373, 1226, 1041, 976 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, *J* 6.8 Hz), 1.23–1.36 (m, 50H), 2.03 (s, 3H, Ac), 2.05 (s, 3H, Ac), 2.15 (s, 3H, Ac), 4.08 (dd, 1H, *J* 4.2, 11.6 Hz, H-1), 4.19 (dd, 1H, *J* 6.8, 11.6 Hz, H-1), 4.36 (m, 1H, H-2), 4.82 (dt, 1H, *J* 8.3, 4.3 Hz, H-3), 5.45 (d, 1H, *J* 7.3 Hz, H-2′), 5.49 (dd, 1H, *J* 7.3, 14.4 Hz, H-3′), 5.88 (dt, 1H, *J* 14.4, 7.0 Hz, H-4′), 6.52 (d, 1H, *J* 8.8 Hz, NH); ^{13}C NMR ($CDCl_3$) δ 14.11, 20.77, 20.91, 22.67, 25.37, 28.64, 29.20, 29.31, 29.35, 29.45, 29.53, 29.58, 29.68 (×17), 31.57, 31.91, 32.31, 50.52, 62.22, 74.42, 74.55, 122.86, 138.03, 168.46, 169.40, 171.03, 171.11. Anal. Calcd for $C_{42}H_{77}NO_7$: C, 71.24; H, 10.96; N, 1.98. Found: C, 71.16; H, 10.91; N, 1.92. Each was treated with Et_3N–MeOH for deacetylation. (2S,3R,2′R)-**1a**: white solid, R_f 0.34 [$CHCl_3$/MeOH (10:1)]; $[\alpha]_D^{19}$ +5.3 (*c* 0.32, $CHCl_3$) [Ref. 1 $[\alpha]_D^{22}$ +5.8 (*c* 1, $CHCl_3$), Ref. 3 $[\alpha]_D^{19}$ +3.6 (*c* 0.31, $CHCl_3$)]; mp 112–113 °C (Ref. 1 105–107 °C, Ref. 3 115.0–116.5 °C); ^{1}H NMR δ 0.87 (t, 6H, *J* 6.8 Hz), 1.23 (br s, 50H), 1.36 (m, 2H), 2.07 (dt, 2H, *J* 7.2, 7.2 Hz, H-5′), 2.38 (m, 2H, OH), 2.98 (br s, 1H, OH), 3.74–3.83 (m, 3H, H-1, H-2, H-3), 4.00 (dd, 1H, *J* 3.4, 11.2 Hz, H-1), 4.51 (d, 1H, *J* 7.2 Hz, H-2′), 5.56 (dd, 1H, *J* 7.2, 15.5, Hz, H-3′), 5.89 (dt, 1H, *J* 15.5, 7.2 Hz, H-4′), 6.94 (d, 1H, *J* 7.2 Hz, NH); HRMS (FAB^{+}): calcd for $C_{36}H_{71}O_4NNa$: (M+Na^{+}): 604.5281; found: *m*/*z* 604.5308. Its NMR spectrum was identical with those reported previously.[1–4] (2R,3S,2′R)-**1a**: white solid, R_f 0.43; $[\alpha]_D^{20}$ −36.1 (*c* 0.15, $CHCl_3$); [Ref. 3 $[\alpha]_D^{18}$ +42.9 (*c* 1.5, $CHCl_3$) for (2S,3R,2′S)-**1a**]; mp 108–109 °C; [Ref. 3 99.5–100.5 °C for (2S,3R,2′S)-**1a**]; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, *J* 6.8 Hz), 1.25 (br s, 50H), 1.48 (m, 2H), 2.08 (dt, 2H, *J* 7.2, 7.2 Hz, H-5′), 2.33 (m, 1H, OH), 2.94 (br s, 1H, OH), 3.62 (m, 1H, OH), 3.72–3.83 (m, 3H, H-1, H-2, H-3), 4.01 (dd, 1H, *J* 3.2, 11.5 Hz, H-1), 4.51 (d, 1H, *J* 7.2 Hz, H-2′), 5.55 (dd, 1H, *J* 7.2, 15.1 Hz, H-3′), 5.89 (dt, 1H, *J* 15.1, 7.2 Hz, H-4′), 6.89 (d, 1H, *J* 7.2 Hz, NH). HRMS (FAB^{+}): calcd for $C_{36}H_{71}O_4NNa$: [M+Na^{+}]: 604.5281; found: *m*/*z* 604.5264. Its NMR spectrum was identical with that reported previously.[3] (2S,3R,2′R)-**1c**: white solid, R_f 0.48 [hexane/AcOEt (5:1)]; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, *J* 6.8 Hz), 1.21–1.39 (m, 50H), 1.75 (m, 1H, H-4), 1.85 (m, 1H, H-4), 2.03 (dt, 2H, *J* 7.1,7.1 Hz, H-5′), 4.42 (dd, 1H, *J* 4.0, 11.7, Hz, H-1), 4.54 (dd, 1H, *J* 5.4, 11.7, Hz, H-1), 4.59 (m, 1H, H-2), 5.22 (dt, 1H, *J* 8.3, 4.3 Hz, H-3), 5.62 (dd, 1H, *J* 7.1, 15.3 Hz, H-3′), 5.73 (d, 1H, *J* 7.3 Hz), 5.96 (dt, 1H, *J* 15.3, 6.8 Hz, H-4′), 7.02 (d, 1H, *J* 8.3 Hz, NH), 7.37, 7.46, 7.59, 7.57, 7.68, 7.84 (each d, *J* 8.4 Hz, 12H, aromatic H). (2R,3S,2′R)-**1c**: white solid, R_f 0.58; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 6H, *J* 6.8 Hz), 1.23–1.39 (m, 50H), 1.78 (m, 2H, H-4), 1.89 (dt, 2H, *J* 7.0, 7.0 Hz, H-5′), 4.46 (dd, 1H, *J* 4.2, 11.4 Hz, H-1), 4.56 (dd, 1H, *J* 7.3, 11.4 Hz, H-1), 4.65 (m, 1H, H-2), 5.17 (dt, 1H,

J 7.8, 4.2 Hz, 3-H), 5.49 (dd, 1H, *J* 7.0, 15.3 Hz, H-3′), 5.73 (d, 1H, *J* 7.0 Hz, H-2′), 5.88 (dt, 1H, *J* 15.3, 7.0 Hz, H-4′), 6.88 (d, 1H, *J* 8.8 Hz, NH), 7.49 (m, 6H, aromatic H), 7.66, 7.75, 7.84 (each d, *J* 8.5 Hz, 6H, aromatic H). NMR spectrum was identical with that for (2*S*,3*R*,2′*S*)-**1c**.[3]