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Revised Structure and Synthesis of Flavolipin

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Abstract: The proposed structure of natural flavolipin was revised as N-[N-[(3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine as a result of a synthetic study and biological activity tests, and its isomers were synthesized in a stereocontrolled manner. © 1998 Elsevier Science Ltd. All rights reserved.

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Introduction

A serine-containing lipid, flavolipin [1], which was isolated by Kawai *et al.* from an opportunistic pathogen, *Flavobacterium meningosepticum*, exhibits definite hemagglutination activity [1] and strongly stimulates macrophages to generate immunoregulatory substances [2,3]. However, in mice, flavolipin exhibits none of the lethal toxicity [2] that is exhibited by lipopolysaccharide. This fact suggests that flavolipin is a nontoxic immunoactivator [4]. Therefore, we tried to synthesize the proposed flavolipin (16) to determine the configuration of the natural flavolipin and to investigate the biological activities of its stereoisomers. All four stereoisomers (16SL, 16SD, 16RL and 16RD) of the proposed flavolipin were synthesized in a stereocontrolled manner from D-glucose. However, none of them was identical with the reported natural product in the analytical data [5]. As a result, our synthetic study negated the proposed structure (16) [6]. Judging from the FAB MS and ¹H NMR analyses of natural flavolipin, the proposed flavolipin is lacking in a glycine moiety. Moreover, the natural flavolipin yielded negative results in the ninhydrin test; characteristic of amino acids. Therefore, we anticipated the structure of flavolipin as N - [N - [15 - methyl - 3 - (13 - methyltetradecanoyloxy)hexadecanoyl]glycyl]serine (22) rather than such amino acids as 23, 24 and 25 in



Reagents and conditions: a) NaBH₄, EtOH, 92%; b) BnBr, NaH, DMF, 80%; c) dioxane-H₂O-1 M HCl (20:1:1), 60%; d) NaBH₄, EtOH, 94%; e) TBDPSCl, Et₃N, 79%; f) PhCH(OMe)₂, PPTS, DMF, 78%; g) Bu₄NF, THF, 98%; h) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, 93%; i) Me₂CH(CH₂)₉CH=PPh₃, THF, 63%; j) H₂, 5% Pd/C, THF; then H₂, Pd(OH)₂/C, MeOH, 65%; k) (i) NaIO₄, dioxane-H₂O (4:1); (ii) *m*-CPBA, CHCl₃; (iii) Ph₂CN₂, EtOAc, 3 steps 83%; l) Me₂CH(CH₂)₁₁COOH, DCC, DMAP, CH₂Cl₂; m) H₂, Pd(OH)₂/C, EtOH, 52% (2 steps); n) L- or D-serine benzyl ester, DCC, DMAP; o) H₂, Pd/C, 57-77% (2 steps).

Figure 1. After synthesizing each of the four stereoisomers of 18 and 22, and comparing the physical data and biological activity of natural flavolipin with those of synthetic compounds, we revised the proposed structure of natural flavolipin to N-[N-[(3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (22RL). However, this compound was already reported as WB-3559 D [7] isolated from *Flavobacterium* sp. No. 3559 and topostin D654 [8] isolated from *Flexibacter topostinus* sp. nov. Therefore, it becomes clear that flavolipin is the same compound as both WB-3559 D and topostin D654. In this paper, we describe the synthesis of flavolipin (22RL) and all its stereoisomers.

Figure 1



Results and Discussion

First, we tried to synthesize the proposed flavolipin 16 [2] via (3S)-and (3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acids (14S and 14R), both of which were obtained from the common aldehyde 1 [9,10,11].

Compound 14S was synthesized as follows. Reduction of 1 with NaBH₄, followed by benzylation of the resultant alcohol 2 with benzyl bromide and NaH yielded benzyl ether 3. Treatment of 3 with dioxane-H₂O-1 M aqueous HCl (20:1:1) gave hemiacetal 4, which was further converted to triol 5 by NaBH₄ reduction. The primary alcohol of 5 was protected as *t*-butyldiphenylsilyl ether 6, and then the remaining diol was protected as a benzylidene group by treatment with PhCH(OMe)₂ and pyridinium *p*-toluenesulfonate (PPTS) to give 7 as a single isomer. Deprotection of the silyl ether of 7 with tetrabutylammonium fluoride (TBAF), Swern oxidation of the alcohol 8, and Wittig reaction of the aldehyde 9 with 11-methyldodecyltriphenylphosphorane gave 10. Compound 10 was hydrogenated over Pd on cabon, and then Pd(OH)₂ on carbon (wet, Degussa type) to give triol 11. The vicinal diol part of 11 was oxidatively cleaved by NaIO₄, and resultant aldehyde was subjected to *m*-chloroperoxybenzoic acid oxidation. Subsequent esterification of the obtained carboxylic acid by Ph₂CN₂ gave ester 12S. Reaction of 12S with 13-methyltetradecanoic acid [7,12] using 1,3-dicyclohexylcarbodiimide (DCC) as dehydrating agent gave ester 13S. Subsequent hydrogenolysis over Pd(OH)₂ on carbon as a catalyst furnished acid 14S.

Compound 14R was obtained from aldehyde 1 according to the reported method [7,12] via compounds 12R and 13R.

Reaction of 14S and 14R with L- or D-serine benzyl ester using DCC as a dehydrating agent gave benzyl esters 15SL, 15SD, 15RL and 15RD, respectively. Hydrogenolysis of these four esters produced 16SL $([\alpha]_D^{24} = +12.8^\circ (c \ 0.13, CHCl_3))$, 16SD $([\alpha]_D^{24} = -13.7^\circ (c \ 0.18, CHCl_3))$, 16RL $([\alpha]_D^{24} = +13.3^\circ (c \ 0.14, CHCl_3))$, and 16RD $([\alpha]_D^{24} = -14.4^\circ (c \ 0.18, CHCl_3))$, respectively. However, none of these four compounds, thus synthesized in a stereocontrolled manner, was identical with the natural flavolipin [5]. Judging



Reagents and conditions: a) glycine benzyl ester, DCC, CH_2Cl_2 , 24 °C, 16 h, 70-91%; b) H_2 , Pd/C, EtOAc, 2.5 h, 71-85%.



Reagents and conditions: a) $(COCl)_2$, CH_2Cl_2 , 24 °C, 1 h, then glycine benzyl ester hydrochloride, Et_3N , CH_2Cl_2 , 24 °C, 1 h, 57-69%; b) H_2 , Pd/C, EtOAc, 2 h, 94-98%; c) L or D-serine benzyl ester, DCC, CH_2Cl_2 , 24 °C, 2 h, 70-82%; d) H_2 , Pd/C, EtOAc, 2.5 h, 50-73%.



from the physical data of natural flavolipin, it was apparent that the proposed structure of flavolipin was lacking in a glycine part.

Therefore, next, we synthesized the acyl-serylglycines 18. Treatment of 16SL, 16SD, 16RL, and 16RD with glycine benzyl ester using DCC as a condensing reagent gave the corresponding amide benzyl esters 17SL, 17SD, 17RL, and 17RD, respectively. Hydrogenolytic deprotection of each benzyl ester gave the corresponding acids 18SL ($[\alpha]_D^{24}$ -8.0° (c 0.23, CHCl₃)), 18SD ($[\alpha]_D^{24}$ +7.1° (c 0.63, CHCl₃)), 18RL ($[\alpha]_D^{24}$ -7.8° (c 0.50, CHCl₃)), and 18RD ($[\alpha]_D^{24}$ +7.6° (c 0.75, CHCl₃)). Disappointingly, none of these compounds was identical to the natural flavolipin.

Further, we tried to synthesize acyl-glycylserines 22. Compounds 14S and 14R were converted with oxalyl chloride to their corresponding acid chlorides, which were treated with glycine benzyl ester hydrochloride and Et3N to give the corresponding amides 19S and 19R. Then, hydrogenolytic deprotection of each benzyl ester of the amides gave the corresponding acids 20S [13] and 20R [8,14], which were treated with L- and D-serine benzyl esters using DCC as a condensing reagent to give the corresponding amides 21SL, 21SD, 21RL, and 21RD, respectively. Subsequent hydrogenolytic deprotection of each benzyl ester gave the corresponding acids 22SL ($[\alpha]_D^{24} + 25.6^\circ$ (c 0.38, CHCl3)), 22SD ($[\alpha]_D^{24} - 18.8^\circ$ (c 0.52, CHCl3)), 22RL ($[\alpha]_D^{24} + 18.9^\circ$ (c 0.39, CHCl3)) [15], and 22RD ($[\alpha]_D^{24} - 26.2^\circ$ (c 0.58, CHCl3)).

Fortunately, the ¹H NMR and FAB MS spectra of compounds **22SD** and **22RL** were identical to natural flavolipin as shown in Figure 2 and Figure 3.

The macrophage stimulation activity of 22RL was almost the same as that of natural flavolipin; and 22SD, however, was practically inactive. Therefore, from this synthetic study we clearly determined the structure of natural flavolipin to be N-[N-[(3R)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine [7,8].

Conclusion

Thus we could synthesize all eight isomers of flavolipin in a stereocontrolled manner and determine the correct structure of flavolipin. We are now investigating the biological activities of all the isomeric compounds of 18 and 22, and the results will be reported in due course.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and were uncorrected. ¹H NMR (270 and 400 MHz) spectra were recorded with JEOL JNM-270 and JNM-GSX 400 spectrometers using TMS as an internal standard. IR absorption spectra were determined with a IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Optical rotations were obtained by the use of a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by the Institute of Science and Technology, Inc. Separation of the compounds by column chromatography was done with silica gel 60 (230-400 mesh ASTM, E. Merck) under a slightly elevated pressure (1.2-1.5 atm) for easy elution, and the quantity of silica gel used was 50-100 times the weight charged on the column. Preparative TLC was performed on silica gel plates (Merck, Silica Gel 60 F₂₄₅). Detection involved spraying the chromatogram with a solution of 17% H₂SO₄ in water (w/w), containing ammonium molybdate (2.3%) and ceric sulfate (0.9%) (Hanessian dip), and heating the plate for several minutes at ca 180 °C. Tetrahydrofurane (THF) was distilled from LiAlH₄ and used immediately. CH₂Cl₂ was dried by being passed through an ICN Alumina B-Super I. *N,N*-Dimethylformamide (DMF) and pyridine were dried by storage over 4Å molecular sieves.

3-Deoxy-1,2-O-isopropylidene-α-D-ribose (2). To a solution of **1** [9,10,11] (4.80 g, 27.9 mmol) in 99.5% EtOH (200 ml) was added NaBH4 (1.06 g, 28.0 mmol) at 0-5 °C. After stirring for 30 min, the reaction mixture was diluted with CHCl3, washed with H2O, and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give 2 (4.45 g, 92%). IR v_{max}(KBr) 3482, 3382 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.33 (3H, s), 1.52 (3H, s), 1.77-1.90 (2H, m, containing OH), 2.01 (1H, dd, J=4.6, 13.5 Hz), 3.57 (1H, ddd, J=4.5, 7.1, 11.6 Hz), 3.90 (1H, td, J=3.2, 12.4 Hz), 4.35 (1H, m), 4.76 (1H, t, J=4.2 Hz), 5.83 (1H, d, J=3.7 Hz). EI MS *m*/z 175 (M+1). Anal. Calcd. for CgH14O4·0.1H2O (174.2+1.8): C, 54.55; H, 8.13. Found: C, 54.70; H, 8.20.

5-O-Benzyl-3-deoxy-1,2-O-isopropylidene-α-D-ribose (3). A solution of 2 (7.24 g, 41.6 mol) in DMF (80 ml) was added dropwise to a suspension of NaH (1.05 g, 43.8 mmol) at 0-5 °C. After stirring for 30 min at 24 °C, benzyl bromide (5.2 ml, 43.7 mmol) was added to this mixture, which was stirred for 16 h at 24 °C. The reaction mixture was diluted with EtOAc, washed with H2O and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (9:1) gave 3 (8.81 g, 80%). IR ν_{max}(CHCl₃) 2992, 2937 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.32 (3H, s), 1.51 (3H, s), 1.77 (1H, ddd, J=4.9, 10.9, 13.4 Hz), 2.06 (1H, dd, J=4.5, 13.4 Hz), 3.55 (1H, dd, J=4.9, 10.7 Hz), 3.65 (1H, dd, J=3.6, 10.7 Hz), 4.40 (1H, m), 4.59 (2H, s), 4.73 (1H, t, J=4.2 Hz), 5.84 (1H, d, J=3.6 Hz), 7.26-7.40 (5H, m). EI MS *m/z* 264 (M⁺). Anal. Calcd. for C15H20O4 (264.3): C, 68.16; H, 7.63. Found: C, 68.15; H, 7.62.

5-0-Benzyl-3-deoxy-D-ribose (4). A solution of 3 (8.94 g, 33.8 mmol) in 1,4-dioxane (200 ml), H₂O (10 ml) and aqueous 1 M HCl (10 ml) was stirred for 3 h at 70 °C. The reaction mixture was neutralized with 1 M aqueous NaOH (10 ml), concentrated *in vacuo*, diluted with CHCl₃, washed with H₂O, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (3:1) gave 4 (4.55 g, 60%). IR v_{max} (CHCl₃) 3417, 3086, 1717 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.87-2.09 (1.35H, m), 2.25 (0.65H, ddd, *J*=5.1, 8.3, 13.5 Hz), 2.54 (0.35H, d, *J*=6.6 Hz, OH), 3.40-3.59 (1.35H, m), 3.68 (0.65H, dd, *J*=2.7, 10.1 Hz), 3.77 (0.65H, d, *J*=7.9 Hz, OH), 4.22 (0.65H, t, *J*=4.8 Hz, changed to a doublet on addition of D₂O), 4.30 (0.35H, m, changed to dd, *J*=5.2, 11.0 Hz on addition of D₂O), 4.44-4.62 (3H, m), 5.17 (0.65H, d, *J*=8.0 Hz, changed to a singlet on addition of D₂O), 5.38 (0.35H, t, *J*=4.0-5.0 Hz, changed to a doublet, *J*=4.0 Hz, on addition of D₂O), 7.26-7.39 (5H, m). MS *m/z* 225 (M⁺+1), 224 (M⁺). Anal. Calcd. for C₁₂H₁₆O4 (224.3): C, 64.27; H, 7.19. Found: C, 64.38; H, 7.11.

(2S,4R)-1-Benzyloxy-2,4,5-trihydroxypentane (5). To a solution of 4 (3.36 g, 15.0 mmol) in 99.5% EtOH (50 ml) was added NaBH4 (570 mg, 15 mmol) at 0-5 °C. After stirring for 1.5 h at 0-5 °C, the reaction mixture was quenched with 0.1 M aqueous HCl and diluted with EtOAc. The solution was washed with H2O and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with CH₂Cl₂-MeOH (20:1) gave 5 (3.18 g, 94%) as an oil. IR v_{max} (CHCl₃) 3584, 3474 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.59-1.65 (2H, m), 2.30 (1H, bs), 3.03 (1H, bs), 3.35-3.66 (5H, m), 3.96-4.12 (2H, m), 4.56 (2H, s), 7.26-7.36 (5H, m). MS *m*/z 227 (M⁺+1). Anal. Calcd. for Cl₂H₁₈O₄·0.5 H₂O (226.3+9.0): C, 61.25; H, 8.14. Found: C, 61.16; H, 8.15.

(2R,4S)-5-Benzyloxy-1-(tert-butyldiphenylsilyloxy)-2,4-dihydroxypentane (6). To a solution of 5 (300 mg, 1.33 mmol) in DMF (5 ml) were added tert-butyldiphenylsilyl chloride (0.38 ml, 1.46 mmol) and Et₃N (0.20 ml, 1.44 mmol) at 24 °C under nitrogen. After stirring for 2 h at 24 °C, the reaction

mixture was diluted with EtOAc. The solution was sequentially washed with 0.1 M HCl, H₂O, sat. NaHCO₃, and brine, dried over MgSO₄, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with hexane-EtOAc (3:1) gave 6 (488 mg, 79%) as an oil. IR v_{max} (CHCl₃) 3583, 3517 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.06 (9H, s), 1.51-1.70 (2H, m), 3.09 (1H, d, J=2.5 Hz, OH), 3.22 (1H, d, J=1.9 Hz, OH), 3.41, 3.45 (2H, AB-q, J=10.4 Hz), 3.55-3.63 (2H, m), 3.97-4.06 (2H, m), 4.55 (2H, s), 7.28-7.47 (11H, m), 7.63-7.67 (4H, m). FAB MS (positive) *m/z* 487 [M+Na]⁺. High Resolution FAB MS, Calcd. for C₂₈H₃₆O₄SiNa: 487.2280; Found: 487.2254.

(2*R*,4*S*)-2,4-[(*R*)-Benzylidenedioxy]-5-benzyloxy-1-(*tert*-butyldiphenylsilyloxy)pentane (7). To a solution of 6 (480 mg, 1.03 mmol) in DMF (10 ml) were added PhCH(OMe)₂ (5.0 ml) and pyridinium *p*-toluenesulfonate (1.0 g, 3.98 mmol) at 24 °C under nitrogen atmosphere. After stirring for 16 h at 24 °C, the reaction mixture was diluted with EtOAc. The solution was washed with sat. NaHCO3 and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with hexane-EtOAc (20:1) gave 7 (448 mg, 78%) as an oil. IR v_{max} (CHCl₃) 2961, 2932, 2861 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.06 (9H, s), 1.53 (1H, m), 3.09 (1H, d, *J*=2.5 Hz, OH), 3.22 (1H, d, *J*=1.9 Hz, OH), 3.41, 3.45 (2H, AB-q, *J*=10.4 Hz), 3.55-3.63 (2H, m), 3.97-4.06 (2H, m), 4.55 (2H, s), 7.28-7.47 (16H, m), 7.63-7.67 (4H, m). EI MS *m/z* 552 (M⁺+1), 551 (M⁺). High Resolution MS, Calcd. for C35H40O4Si: 552.2696; Found: 552.2672.

(2S,4R)-2,4-[(S)-Benzylidenedioxy]-1-benzyloxy-5-hydroxypentane (8). To a solution of 7 (440 mg, 0.80 mmol) in THF (5 ml) was added 1 M THF solution of TBAF (1.0 ml) at 24 °C under nitrogen. After stirring for 30 min at 24 °C, the reaction mixture was diluted with EtOAc. The solution was washed with H₂O and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with hexane-EtOAc (2:1) gave 8 (246 mg, 98%) as a solid. mp 53-55 °C (from EtOAc-hexane). IR v_{max}(CHCl₃) 3601, 2924, 2869 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.57-1.62 (2H, m), 2.00 (1H, broad, OH), 3.54 (1H, dd, *J*=5.0, 10.2 Hz), 3.62-3.78 (3H, m), 4.03 (1H, m), 4.15 (1H, m), 4.58, 4.62 (2H, AB-q, *J*=12.2 Hz), 5.60 (1H, s), 7.28-7.42 (8H, m), 7.48-7.54 (2H, m). MS *m/z* 314 (M⁺). Anal. Calcd. for C19H22O4 (314.4): C, 72.59; H, 7.05. Found: C, 72.31; H, 7.24.

(2R,4S)-2,4-[(*R*)-Benzylidenedioxy]-5-benzyloxypentanal (9). To a solution of oxalyl chloride (0.075 ml, 0.860 mmol) in CH₂Cl₂ (1 ml) was added dimethyl sulfoxide (0.135 ml, 1.90 mmol) at -78 °C with stirring under nitrogen. After 5 min, a solution of 8 (100 mg, 0.32 mmol) in CH₂Cl₂ (1 ml) was added to this solution at -78 °C, and the stirring was continued for 15 min, and then Et₃N (0.60 ml, 4.30 mmol) was added. After 5 min, the reaction mixture was warmed to room temperature and diluted with CH₂Cl₂. The solution was washed with H₂O and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with hexane-EtOAc (2:1) gave 9 (93 mg, 93%) as an oil. IR v_{max}(CHCl₃) 1740 cm⁻¹. 270 MHz ¹H NMR (CDCl₃) δ 1.67 (1H, td, *J*=11.6, 13.6 Hz), 1.95 (1H, td, *J*=2.8, 13.6 Hz), 3.57 (1H, dd, *J*=5.3, 9.9 Hz), 3.69 (1H, dd, *J*=5.3, 9.9 Hz), 4.16 (1H, m), 4.36 (1H, dd, *J*=2.8, 11.6 Hz), 4.60 (2H, s), 5.65 (1H, s), 7.29-7.57 (10H, m), 9.74 (1H, s). FAB MS (negative) *m/z* 311 (M-1)⁻. Anal. Calcd. for C19H₂₀O4 (312.4): C, 73.06; H, 6.45. Found: C, 73.00; H, 6.66.

(2S,4R)-2,4-[(S)-Benzylidenedioxy]-1-benzyloxy-16-methylheptadec-5(EZ)-ene (10).

To a solution of 11-methyldodecyltriphenylphosphonium chloride (1.60 g, 3.33 mmol) in THF (15 ml) was added a 1.6 M hexane solution of *n*-BuLi (2.00 ml, 3.20 mmol) at -78 $^{\circ}$ C with stirring under nitrogen. After

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stirring for 30 min at -78 °C and then for 30 min at 24 °C, this solution was poured into a solution of **9** (100 mg, 0.33 mmol) in THF (10 ml) at 24 °C, and stirring was continued for 1 h. The reaction mixture was concentrated *in vacuo*, diluted with EtOAc, washed with H₂O and brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with hexane-EtOAc (20:1) gave an *E*:*Z*=5:3 mixture of **10** (97 mg, 63%) as an oil. IR v_{max} (CHCl₃) 2955, 2928, 2856 cm⁻¹. The mixture was partially separated on a silica gel preparative TLC plate. [*E*-isomer: Rf=0.44 (benzene); *Z*-isomer: Rf=0.36 (benzene)]. 270 MHz ¹H NMR of *E*-isomer: (CDCl₃) δ 0.86 (6H, d, *J*=6.6 Hz), 1.10-1.37 (17H, m), 1.44-1.70 (2H, m), 2.29-2.47 (2H, m), 3.53 (1H, dd, *J*=4.9, 10.0 Hz), 3.68 (1H, dd, *J*=5.8, 10.0 Hz), 4.13 (1H, m), 4.53 (1H, m), 4.58, 4.62 (2H, AB-q, *J*=11.9 Hz), 5.60 (1H, s), 5.60-5.72 (2H, m), 7.28-7.39 (8H, m), 7.47-7.53 (2H, m). 270 MHz ¹H NMR of *Z*-isomer: (CDCl₃) δ 0.86 (6H, d, *J*=6.6 Hz), 1.13-1.45 (17H, m), 1.45-1.65 (2H, m), 2.07-2.17 (2H, m), 3.53 (1H, dd, *J*=4.8, 10.1 Hz), 3.68 (1H, dd, *J*=5.9, 10.1 Hz), 4.16 (1H, m), 4.59, 4.63 (2H, AB-q, *J*=12.4 Hz), 4.68 (1H, dd, *J*=7.4, 13.9 Hz), 5.44-5.59 (2H, m), 5.62 (1H, s), 7.26-7.39 (8H, m), 7.49-7.54 (2H, m). FAB MS (positive) *m/z* 501 [M+Na]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C32H46O3Na: 501.3345; Found: 501.3336.

(2S,4S)-16-Methyl-1,2,4-trihydroxyheptadecane (11). A solution of the E:Z mixture of 10 (50 mg, 0.18 mmol) in THF (10 ml) containing 5% Pd on carbon (25 mg) was hydrogenated for 1 h at 20 -25 °C and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was dissolved in MeOH (20 ml). The solution was hydrogenolyzed using Pd(OH)₂ on carbon (wet, Degussa type, 20 mg) for 1 h at room temperature. The reaction mixture was filtered through Celite, and concentrated in vacuo to give 11 (27 mg, 65%) as a powder, which was partly recrystallized from EtOAc; mp 70 °C. IR ν_{max} (CHCl₃) 3307, 2953, 2920, 2850 cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (6H, d, *J*=6.6 Hz), 1.10-1.69 (27H, m), 2.46-2.58 (1H, broad, OH), 3.49 (1H, dd, *J*=6.6, 11.3 Hz), 3.65 (1H, dd, *J*=3.3, 11.3 Hz), 3.87-3.98 (2H, m). FAB MS (positive), *m/z* 325 [M+Na]⁺.

(35)-Diphenylmethyl 15-methyl-3-hydroxyhexadecanoate (125). (i) A solution of 11 (31 mg, 0.10 mmol) in 1,4-dioxane (2 ml) and a solution of NaIO4 (111 mg, 0.52 mmol) in H₂O (0.4 ml) were mixed and stirred for 1 h at 24 °C. The reaction mixture was diluted with H₂O and extracted with CHCl₃. The solution was washed with brine, dried over MgSO4, and filtered. The filtrate was concentrated *in vacuo* to give 28 mg of (35)-15-methyl-3-hydroxyhexadecanal, which was dissolved in CHCl₃ (5 ml). (ii) To this solution was added *m*-chloroperoxybenzoic acid (28 mg, 0.16 mmol). The solution was warmed at 50 °C for 1 h with stirring in the dark to give (35)-15-methyl-3-hydroxyhexadecanoic acid and concentrated *in vacuo* to give a mixture, which was dissolved in EtOAc (5 ml). (iii) To this solution was added diphenyldiazomethane (78 mg, 0.40 mmol). This solution was warmed at 50 °C for 45 min with stirring and concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (19:1) gave a benzhydryl ester 12S (40 mg, 86%) as an oil. $[\alpha]_D^{24} + 14.5^\circ$ (c 0.2, CHCl₃). IR v_{max}(film) 3032 (broad), 2926, 2853, 1736 cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (6H, d, *J*=7.0 Hz), 1.14-1.55 (23H, m), 2.58-2.61 (3H, m), 4.01 (1H, m), 6.91 (1H, s), 7.33-7.34 (10H, m). EI MS *m/z* 452 (M⁺). Anal. Calcd. for C₃₀H44O₃·0.24H₂O (457.0): C, 78.85; H, 9.81. Found: C, 78.97; H, 9.61.

(3R)-Diphenylmethyl 15-methyl-3-hydroxyhexadecanoate (12R). The above mentioned procedures (ii) and (iii) applied to (3R)-15-methyl-3-hydroxyhexadecanal (833 mg, 3.08 mmol), which was obtained from compound (1) according to the reported method [6], gave 12R (1.261 g) in 90% yield as an oil. $[\alpha]_D^{24}$ -14.0° (c 0.2, CHCl3). EI MS m/z 452 (M⁺).

(3S)-Diphenylmethyl 15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoate (13S).

To a solution of 12S (1.12 g, 2.47 mmol) and 13-methyltetradecanoic acid (0.90 g, 3.71 mmol) in CH₂Cl₂ (25 ml) were added 1,3-dicyclohexylcarbodiimide (765 mg, 3.71 mmol) and 4-dimethylaminopyridine (453 mg, 3.71 mmol). The mixture was stirred for 16 h at room temperature and filtered through Celite. After filtration, the filter cake was washed with a small amount of EtOAc. The combined filtrate was diluted with EtOAc. The solution was washed with aq. NaHCO3 and brine, dried over MgSO4, filtered, concentrated *in vacuo*, and the residual mixture was chromatographed on a silica gel column. Elution with hexane-EtOAc (20:1) gave 13S (1.52 g, 90%) as a wax. $[\alpha]_D^{24} + 3.1^\circ$ (c 0.2, CHCl₃). IR v_{max}(film) 2926, 2855, 1742 cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.13-1.55 (44H, m), 2.05-2.15 (2H, m), 2.61-2.70 (2H, m), 5.26 (1H, m), 6.89 (1H, s), 7.29-7.33 (10H, m). EI MS *m/z* 676 (M⁺). Anal. Calcd. for C45H72O4 (677.1): C, 79.83; H, 10.72. Found: C, 79.52; H, 11.02.

(3R)-Diphenylmethyl 15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoate (13R).

The same treatment mentioned above of **12***R* (336 mg) gave **13***R* (449 mg) in 89% yield. $[\alpha]_D^{24}$ -1.1° (c 0.2, CHCl₃). MS *m/z* 676 (M⁺). Anal. Calcd. for C45H72O4·(677.1): C, 79.83; H, 10.72. Found: C, 79.53; H, 11.01.

(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acid (14S). A solution of 13S (537 mg, 0.80 mmol) in EtOH (10 ml) containing 20% Pd(OH)₂ on carbon as a catalyst (120 mg) was stirred under hydrogen atmosphere for 2 h at room temperature and filtered to give a crude mixture, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (10:1) gave 14S (369 mg, 91%) as wax. The crude mixture contained diphenylmethane, but this did not affect in next reaction. Therefore, the mixture was employed for the next reaction without chromatographic purification. [α]_D²⁴ +2.2° (c 0.5, CHCl₃). IR v_{max}(film) 3700-3000 (broad), 2926, 2855, 1740, 1713 cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, *J*=6.6 Hz), 1.11-1.63 (43H, m), 2.25-2.31 (2H, m), 2.58-2.62 (2H, m), 3.99 (1H, s), 5.21 (1H, m). Anal. Calcd. for C₃₂H₆₂O4 (510.8): C, 75.24; H, 12.23. Found: C, 74.60; H, 12.07.

(3R)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acid (14R). The same treatment as described above of 13R gave 14R in 90% yield as a wax. $[\alpha]_D^{24}$ -0.7° (c 1.0, CHCl3). Anal. Calcd. for C32H62O4 (510.8): C, 75.24; H, 12.23. Found: C, 75.01; H, 12.29.

N-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine benzyl ester (15*S*L). To a solution of 14*S* (200 mg, 0.392 mmol) in CH₂Cl₂ (2 ml) was added oxalyl chloride (250 mg). After 1 h stirring at room temperature, the reaction mixture was concentrated *in vacuo* to give an acid chloride, which was dissolved in CH₂Cl₂ (4 ml). To this solution, L-serine benzyl ester hydrochloride (137 mg, 0.591 mmol) and Et₃N (100 mg, 0.990 mmol) were added under nitrogen and stirred for 1 h at 0-5 °C. The reaction mixture was concentrated *in vacuo* and dissolved in EtOAc. The solution was washed with brine, dried over MgSO4, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (3:1) gave 15*S*L (174 mg, 65%) as a wax. $[\alpha]_D^{24}$ +10.5° (c 0.23, CHCl₃). IR v_{max}(film) cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, *J*=6.6 Hz), 1.11-1.72 (45H, m), 2.25-2.33 (2H, m), 2.48-2.52 (2H, m), 3.94 (2H, d, *J*=3.3 Hz), 4.64 (1H, td, *J*=3.3, 7.1 Hz, changed to a triplet on addition of D₂O), 5.21 (1H, m), 5.23 (2H, s), 6.64 (1H, d, *J*=7.1 Hz, NH), 7.36 (5H, bs). FAB MS (positive) *m/z* 688 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C42H74NO6, 688.5516; Found, 688.5513. *Anal.* Calcd. for C42H73NO6·(688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.03; H, 10.65; N, 2.04.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine benzyl ester (15SD). The same treatment as described above of 14S with D-serine benzyl ester hydrochloride gave 15SD . $[\alpha]_D^{24}$ -9.8° (c 0.23, CHCl3). FAB MS (positive) m/z 688 [M+H]⁺. High Resolution FAB MS (positive) m/z: Calcd. for C42H74NO6, 688.5516; Found, 688.5562. Anal. Calcd. for C42H73NO6 (688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.44; H, 10.74; N, 2.09.

N-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine benzyl ester (15*R*L). The same treatment as described above of 14*R* with L-serine benzyl ester hydrochloride gave 15*R*L in 62% yield as a wax. $[\alpha]_D^{24}$ +10.0° (c 0.19, CHCl3). 270 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, J=6.6 Hz), 1.11-1.70 (45H, m), 2.27-2.33 (2H, m), 2.50 (2H, d, J=5.8 Hz), 3.91-4.04 (2H, broad), 4.66 (1H, td, J=3.4, 6.8 Hz, changed to a triplet on addition of D₂O), 5.17 (1H, quintet, J=6.2 Hz), 5.22 (2H, s), 6.57 (1H, d, J=7.0 Hz, NH), 7.36 (5H, bs). FAB MS (positive) *m*/*z* 688 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C42H74NO6, 688.5516; Found, 688.5516. Anal. Calcd. for C42H73NO6 (688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.22; H, 10.73; N, 2.07.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine benzyl ester (15RD). The same treatment as described above of 14R with D-serine benzyl ester hydrochloride gave 15RD as a wax. $[\alpha]_D^{24}$ -12.5° (c 0.24, CHCl3). FAB MS (positive) m/z 688 [M+H]⁺. High Resolution FAB MS (positive) m/z: Calcd. for C42H74NO6, 688.5516; Found, 688.5580. Anal. Calcd. for C42H73NO6·(688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.61; H, 10.75; N, 2.04.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine (16SL). A solution of 15SL (156 mg, 0.227 mmol) containing 10% Pd on carbon (20 mg) as a catalyst in EtOAc (3 ml) was stirred under hydrogen atmosphere at room temperature for 2 h. After filtration, the solution was concentrated *in vacuo* to give 16SL (135 mg, 99%) as a wax. $[\alpha]_D^{24}$ +12.8° (c 0.1, CHCl3). 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.66 (44H, m), 2.31 (2H, t, J=7.5 Hz), 2.48-2.63 (2H, m), 3.25-3.28 (2H, broad, OH x 2), 3.88 (1H, dd, J=2.9, 11.7 Hz), 4.08 (1H, m), 4.55 (1H, m), 5.24 (1H, m), 6.89 (1H, d, J=6.6 Hz, NH). FAB MS (positive) *m*/*z* 620 [M+Na]⁺, 598 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C35H68NO6, 598.5047; Found, 598.5057. *Anal.* Calcd. for C35H67NO6 (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.37; H, 11.27; N, 2.38.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine (16SD).

Compound 15SD was hydrogenolyzed as described above to give 16SD in 97% yield as a wax. $[\alpha]_D^{24}$ -13.7° (c 0.18, CHCl3). 270 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.63 (44H, m), 2.31 (2H, t, J=7.5 Hz), 2.55 (2H, d, J=5.6 Hz), 2.59-2.70 (2H, broad, OHx2), 3.88 (1H, d, J=10.1 Hz), 4.10 (1H, d, J=10.4 Hz), 4.56 (1H, s), 5.18 (1H, t, J=6.1 Hz), 6.83 (1H, d, J=5.8 Hz, NH). FAB MS (positive) *m*/z 620 [M+Na]⁺, 598 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C35H68NO6, 598.5047; Found, 598.5069. *Anal.* Calcd. for C35H67NO6 (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.57; H, 11.23; N, 2.41.

N-[(3R)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine (16RL).

Compound 15*RL* was hydrogenolyzed as described above to give 16*RL* in 95% yield as a wax. $[\alpha]_D^{24}$ +13.3° (c 0.14, CHCl₃). ¹H NMR spectra of 16*SD* and 16*RL* were identical. FAB MS (positive) *m/z* 620 [M+Na]⁺, 598 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C35H68NO6, 598.5047; Found, 598.5071. Anal. Calcd. for C35H67NO6 (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.05; H, 11.37; N, 2.27.

N-[(3R)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine (16RD).

Compound 15RD was hydrogenolyzed as described above to give 16RD in 99% yield as a wax. $[\alpha]_D^{24}$ -14.4° (c 0.18, CHCl3). ¹H NMR spectra of 16RD and 16SL were identical. FAB MS (positive) *m/z* 620 [M+Na]⁺, 598 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C35H68NO6, 598.5047; Found, 598.5071. Anal. Calcd. for C35H67NO6 (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.25; H, 11.33; N, 2.30.

N-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-seryl]glycine benzyl ester (17*SL*). To a solution of 16*SL* (56 mg, 0.094 mmol) and glycine benzyl ester (31 mg, 0.188 mmol) in CH₂Cl₂ (30 ml) was added DCC (39 mg, 0.188 mmol) under nitrogen. The mixture was stirred for 16 h at room temperature, concentrated *in vacuo*, and diluted with EtOAc. The solution was washed with aq. NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (1:1) gave 17*SL* (63 mg, 90%) as a wax. IR v_{max} (KBr) 3303, 2954, 2923, 2852, 1744, 1727, 1641 cm⁻¹. 270 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, *J*=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, s), 1.51 (2H, septet, *J*=6.6 Hz), 1.55-1.68 (4H, m), 2.26-2.31 (2H, m), 2.43-2.51 (2H, m), 3.11-3.21 (1H, broad m, OH), 3.61 (1H, m), 3.98-4.16 (3H, m), 4.47 (1H, m), 5.18 (2H, s), 5.20 (1H, m), 6.71 (1H, d, *J*=7.2 Hz, NH), 7.18 (1H, t, *J*=5.7 Hz, NH), 7.32-7.40 (5H, m). FAB MS (positive) *m/z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C44H76N2O7Na, 767.5550; Found, 767.5552. *Anal.* Calcd. for C44H76N2O7 (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 70.73; H, 10.46; N, 3.78.

N-[*N*-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine benzyl ester (17SD). Compound 16SD was treated as described above to give 17SD in 70% yield as a wax. $[\alpha]_D^{24}$ +12.4° (c 0.50, CHCl3). IR ν_{max} (KBr) 3299, 2954, 2922, 2852, 1727, 1640 cm⁻¹. 270 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, *J*=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, s), 1.51 (2H, septet, *J*=6.6 Hz), 1.55-1.62 (4H, m), 2.26-2.31 (2H, m), 2.46-2.49 (2H, m), 3.17-3.23 (1H, broad, OH), 3.62 (1H, m), 3.99-4.15 (3H, m), 4.50 (1H, m), 5.17-5.22 (3H, m), 6.72 (1H, d, *J*=7.3 Hz, NH), 7.25 (1H, t, *J*=5.7 Hz, NH), 7.32-7.40 (5H, m). FAB MS (positive) *m*/*z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C44H77N2O7, 745.5731; Found, 745.5716. Anal. Calcd. for C44H76N2O7 (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 71.14; H, 10.44; N, 3.76.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-seryl]glycine benzyl ester (17*R*L). Compound 16*R*L was treated as described above to give 17*R*L in 70% yield as a wax. $[\alpha]_D^{24}$ -16.4° (c 0.50, CHCl3). IR and ¹H NMR spectra of 17*R*L and 17SD were identical. FAB MS (positive) *m*/*z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C44H77N2O7, 745.5731; Found, 745.5711. Anal. Calcd. for C44H76N2O7 (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 70.97; H, 10.39; N, 3.78.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine benzyl ester (17*R*D). Compound 16*R*D was treated as described above to give 17*R*D in 91% yield as a wax. IR and ¹H NMR spectra of 17*S*L and 17*R*D were identical. FAB MS (positive) *m/z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C44H76N2O7Na, 767.5550; Found, 767.5510. Anal. Calcd. for C44H76N2O7 (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 70.77; H, 10.30; N, 3.87.

N-[N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-seryl]glycine(18SL).A solution of 17SL (16 mg, 0.021 mmol) containing 10% Pd on carbon (16 mg) as a catalyst in

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EtOAc (3 ml) was stirred under hydrogen atmosphere at room temperature for 2.5 h. The reaction mixture was filtered through Celite and concentrated *in vacuo* to give **18SL** (10 mg, 71%) as a wax, which was a 3:1 mixture of amide rotational cis-trans isomers. $[\alpha]_D^{24}$ -8.0° (c 0.23, CHCl3). IR v_{max}(KBr) 3350, 3309, 2955, 2922, 2852, 1722, 1699, 1621 cm⁻¹. 400 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.56-1.65 (4H, m), 2.29 (2H, t, J=7.1-7.8 Hz), 2.52 (2H, d, J=5.9 Hz), 3.45 (2H, bs, OH and COOH), 3.68 (1H, dd, J=4.6, 11.3 Hz, serine CHOH), 3.96-4.10 (3H, m, glycine CH₂N and serine CHOH), 4.59 (1H, m, changed to a triplet on addition of D₂O, J=4.2 Hz, serine CH-N), 5.18 (1H, quintet, J=5.8-6.8 Hz, CH-OCO), 7.17 (3/4H, d, J=7.4 Hz, serine CONH), 7.22 (1/4H, d, J=7.6 Hz, serine CONH), 7.56 (3/4H, t, J=5.3 Hz, glycine CONH), 7.60 (1/4H, t, J=5.3 Hz, glycine CONH). FAB MS (positive) *m*/*z* 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C37H71N2O7, 655.5261; Found, 655.5267.

N-[*N*-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine (18SD). Compound 17SD was hydrogenolyzed as described above to give 18SD in 84% yield as a wax, which was a 3:1 mixture of amide rotational cis-trans isomers. $[\alpha]_D^{24}$ +7.1° (c 0.63, CHCl3). IR v_{max}(KBr) 3354, 3056, 2954, 2921, 2851, 1738, 1723, 1664 cm⁻¹. 400 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.55-1.65 (4H, m), 2.26-2.32 (2H, m), 2.47-2.57 (2H, m), 3.63-3.70 (1H, m, CHOH), 3.94-4.11 (3H, m, CHOH, CH₂N), 4.58 (1H, m, changed to a triplet on addition of D₂O, J=4.2 Hz, serine CHN), 5.15-5.26 (1H, m, CHOCO), 7.20 (1/4H, d, J=7.4 Hz, serine CONH), 7.29 (3/4H, d, J=7.7 Hz, serine NH), 7.59 (1/4H, t, J=5.3 Hz, glycine CONH), 7.64 (3/4H, t, J=5.4 Hz, glycine NH). FAB MS (positive) m/z 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) m/z: Calcd. for C37H71N2O7, 655.5261; Found, 655.5254

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-seryl]glycine (18*R*L). Compound 17*R*L was hydrogenolyzed as described above to give 18*R*L in 84% yield as a wax, which was a 3:1 mixture of amide rotational cis-trans isomers. $[\alpha]_D^{24}$ -7.8° (c 0.50, CHCl3). IR and ¹H NMR were identical with those of 18SD. FAB MS (positive) *m*/*z* 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C37H71N2O7, 655.5261; Found, 655.5255. Anal. Calcd. for C37H70N2O7 (655.0): C, 67.85; H, 10.77; N, 4.28. Found: C, 67.47; H, 10.72; N, 4.27.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine (18*R* D). Compound 17*R* D was hydrogenolyzed as described above to give 18*R* D in 85% yield as a wax, which was a single amide isomer. $[\alpha]_D^{24}$ +7.6° (c 0.75, CHCl3). IR v_{max}(KBr) 3350, 3308, 2955, 2922, 2852, 1735 (shoulder), 1721, 1699, 1621, 1576 cm⁻¹. 400 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, *J*=6.6 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, *J*=6.6 Hz), 1.56-1.65 (4H, m), 2.29 (2H, t, *J*=7.1-7.8 Hz), 2.52 (2H, d, *J*=5.9 Hz), 3.45 (2H, broad, OH, COOH), 3.68 (1H, dd, *J*=4.6, 11.3 Hz, CHOH), 3.96-4.10 (3H, m, CHOH, CH₂N), 4.55-4.59 (1H, m, *J*=4.3 Hz, serine CHN), 5.18 (1H, quintet, *J*=5.8-6.8 Hz, CH-OCO), 7.18 (1H, d, *J*=7.4 Hz, serine CONH), 7.56 (1H, d, *J*=5.3 Hz, glycine NH). FAB MS (positive) *m/z* 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C37H70N2O7Na, 677.5081; Found, 677.5073. Anal. Calcd. for C37H70N2O7 (655.0): C, 67.85; H, 10.77; N, 4.28. Found: C, 67.47; H, 10.84; N, 4.44.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine benzyl ester (19S). To a solution of 14S (400 mg, 0.783 mmol) in CH₂Cl₂ (4 ml) was added oxalyl chloride (500 mg, 3.94 mmol). After 1 h stirring at room temperature, the reaction mixture was concentrated *in vacuo* to give an acid chloride, which was dissolved in CH₂Cl₂ (4 ml). To this solution, glycine benzyl ester hydrochloride (236 mg, 1.175 mmol) and Et₃N (200 mg, 1.980 mmol) were added under nitrogen atmosp[here and stirred for 1 h at 0-5 °C. The reaction mixture was concentrated *in vacuo* and dissolved in EtOAc. The solution was washed with brine, dried over MgSO4, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (9:1) gave 19S (294 mg, 57%) as a wax. IR ν_{max} (film) 3310, 2954, 2921, 2852, 1737, 1729, 1640, 1549 cm⁻¹. 400 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.57-1.66 (4H, m), 2.30 (2H, t, J=7.5-7.8 Hz), 2.47-2.57 (2H, m), 4.07 (2H, d, J=5.1 Hz), 5.16 (1H, m), 5.19 (2H, s), 6.26 (1H, t, J=4.9 Hz, NH), 7.34-7.40 (5H, m). FAB MS (positive) *m/z* 680 [M+Na]^{+,} 658 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C41H72NO5, 658.5410; Found, 658.5388. *Anal*. Calcd. for C41H71NO5 (658.0): C, 74.84; H, 10.88; N, 2.13. Found: C, 74.72; H, 11.00; N, 2.24.

N-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine benzyl ester (19*R*). The same reaction as described above of 14*R* gave 19*R* in 69% as a wax. $[\alpha]_D^{24}$ +0.2° (c 0.50, CHCl₃). IR and ¹H NMR spectra of 19*R* and 19*S* were identical. FAB MS (positive) *m*/*z* 680 [M+Na]⁺, 658 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C4₁H7₁NO₅Na, 680.5230; Found, 680.5235. Anal. Calcd. for C4₁H7₁NO₅ (658.0): C, 74.84; H, 10.88; N, 2.13. Found: C, 74.73; H, 10.60; N, 2.31.

N-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (20S). A solution of 19S (253 mg, 0.38 mmol) containing 10% Pd on carbon (30 mg) as a catalyst in EtOAc (6 ml) was stirred under hydrogen at room temperature for 2 h. The reaction mixture was filtered through Celite and concentrated *in vacuo* to give 20S (205 mg, 94%) as a wax. IR v_{max} (film) 3363, 2955, 2919, 2850, 1724, 1627, 1549 cm⁻¹. 400 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.56-1.67 (4H, m), 2.31 (2H, t, J=7.5-7.7 Hz), 2.49-2.59 (2H, m), 4.07 (2H, d, J=5.2 Hz), 5.16 (1H, quintet, J=5.5-6.9 Hz, CHOCO), 6.41 (1H, t, J=5.2 Hz, NH). FAB MS (positive) *m*/z 590 [M+Na]⁺, 568 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C34H66NO5, 568.4941; Found, 568.4958. Anal. Calcd. for C34H65NO5 (567.9): C, 71.91; H, 11.54; N, 2.47. Found: C, 71.90; H, 11.66; N, 2.53.

N-[(3R)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (20R). The same hydrogenolysis as described above of 19R gave 20R in 98% yield. IR and ¹H NMR spectra of 20R and 20S were identical. FAB MS (positive) m/z 590 [M+Na]⁺, 568 [M+H]⁺. High Resolution FAB MS (positive) m/z: Calcd. for C34H66NO5, 568.4941; Found, 568.4944.

N-[*N*-[(3S)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine benzyl ester (21SL). To a solution of 20S (80 mg, 0.14 mmol) and L-serine benzyl ester (82 mg, 0.42 mmol) in CH₂Cl₂ (4 ml) was added 1,3-dicyclohexylcarbodiimide (87 mg, 0.42 mmol) under nitrogen. The mixture was stirred for 2 h at room temperature, concentrated *in vacuo*, and then diluted with EtOAc. The solution was washed with aq. NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (1:1) gave 21SL (80 mg, 76%) as a wax. $[\alpha]_D^{24}$ +25.0° (c 0.5, CHCl₃). IR v_{max}(KBr) 3398, 3330, 2954, 2923, 2852, 1738, 1727, 1707(w), 1657, 1637, 1615 (w), 1538 cm⁻¹. 400 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, s), 1.51 (2H, septet, J=6.6 Hz), 1.55-1.63 (4H, m), 2.31 (2H, t, J=7.5-7.8 Hz), 2.47 (2H, d, J=6.2 Hz), 3.35 (1H, dd, J=5.8, 7.4 Hz, OH), 3.88-4.12 (4H, m), 4.72 (1H, td, J=3.6, 7.8 Hz), 5.21 (1H, m, CHOCO), 5.22 (2H, s), 6.49 (1H, t, J=5.5 Hz, NH), 7.17 (1H, d, J=7.7 Hz, NH), 7.33-7.38 (5H, m). FAB MS (positive) *m*/z 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C44H77N2O7, 745.5731; Found, 745.5722. Anal. Calcd. for C44H76N2O7 (745.1): C, 70.93; H, 10.28; N, 3.76. Found: C, 70.63; H, 10.00; N, 3.81.

N-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-D-serine benzyl ester (21*S*D). The same procedure as described above of 20*S* and D-serine benzyl ester gave 21*S*D in 70% yield as a wax. $[\alpha]_D^{24}$ -10.6° (c 0.5, CHCl₃). IR ν_{max} (KBr) 3319, 2954, 2922, 2852, 1733, 1661, 1637, 1549 cm⁻¹. 400 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.17 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.56-1.63 (4H, m), 2.30 (2H,t, J=7.5 Hz), 2.48 (2H, d, J=5.9 Hz), 3.18 (1H, t, J=5.8 Hz, OH), 3.94-4.05 (4H, m), 4.70 (1H, td, J=3.4, 7.6 Hz), 5.15 (1H, quintet, J=5.8 Hz, CHOCO), 5.21 (2H, s), 6.48 (1H, t, J=5.4 Hz, glycine NH), 7.05 (1H, d, J=7.6 Hz, serine NH), 7.33-7.39 (5H, m). FAB MS (positive) *m*/*z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C44H77N₂O7, 745.5731; Found, 745.5734. Anal. Calcd. for C44H76N₂O7 (745.1): C, 70.93; H, 10.28; N, 3.76. Found: C, 70.79; H, 10.36; N, 3.80.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine benzyl ester (21*R*L). The same procedure as described above of 20*R* and L-serine benzyl ester gave 21*R*L in 72% yield as a wax. $[\alpha]_D^{24}$ +10.2° (c 0.5, CHCl3). IR and ¹H NMR spectra of 21*R*L and 21SD were identical. FAB MS (positive) *m*/z 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C44H77N2O7, 745.5731; Found, 745.5720. Anal. Calcd. for C44H76N2O7 (745.1): C, 70.93; H, 10.28; N, 3.76. Found: C, 71.02; H, 10.29; N, 3.81.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-D-serine benzyl ester (21*R*D). The same procedure as described above of 20*R* and D-serine benzyl ester gave 21*R*D in 82% yield as a wax. $[\alpha]_D^{24}$ -24.0° (c 0.5, CHCl3). IR and ¹H NMR spectra of 21*S*L and 21*R*D were identical. FAB MS (positive) *m*/*z* 767 [M+Na]⁺, 745 [M+H]⁺. High Resolution FAB MS (positive) *m*/*z*: Calcd. for C44H77N2O7, 745.5731; Found, 745.5725. *Anal.* Calcd. for C44H76N2O7 (745.1): C, 70.93; H, 10.28; N, 3.76. Found: C, 71.27; H, 10.15; N, 3.81.

N-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (22*SL*). A solution of 21*SL* (32 mg) in EtOAc (4 ml) containing 10% Pd on carbon (32 mg) was hydrogenolized under hydrogen for 2.5 h at room temperature. The solution was filtered through Celite and concentrated *in vacuo* to give 22*SL* (14 mg, 50%) as a wax. $[\alpha]_D^{24}$ +25.6° (c 0.38, CHCl3). IR v_{max}(KBr) 3371, 3335, 3280, 3073 (w), 2955, 2918, 2851, 1746, 1683, 1644 cm⁻¹. 400 MHz ¹H NMR: (CDCl₃) δ 0.86 (12H, d, *J*=6.6 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, *J*=6.6 Hz), 1.55-1.65 (4H, m), 2.30 (2H,t, *J*=7.5 Hz), 2.51 (2H, d, *J*=5.5 Hz), 3.88-4.12 (4H, m, CH₂OH, CH₂N), 4.61 (1H, broad s), 5.21 (1H, quintet, *J*=5.8-6.2 Hz, CHOCO), 5.3-5.8 (2H, broad, OH, COOH), 7.18 (1H, broad s, NH), 7.60 (1H, broad s, NH). FAB MS (positive) *m/z* 699 [M+2Na-H]⁺, 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C37H70N2O7Na, 677.5081; Found, 677.5092.

N-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-D-serine (22*S*D). The same hydrogenolysis as described above of 21*S*D gave 22*S*D in 73% yield as a wax. $[\alpha]_D^{24}$ -18.8° (c 0.52, CHCl3). IR v_{max}(KBr) 3318, 3079 (w), 2955, 2922, 2852, 1723, 1681, 1659, 1629, 1545 cm⁻¹. 400 MHz ¹H NMR: (CDCl3) δ 0.86 (12H, d, J=6.6 Hz), 1.12-1.20 (4H, m), 1.25 (34H, bs), 1.51 (2H, septet, J=6.6 Hz), 1.55-1.65 (4H, m), 2.30 (2H, t, J=7.5 Hz), 2.46-2.57 (2H, m), 3.88-4.12 (4H, m, CH₂OH, CH₂N), 4.63 (1H, broad s, CHN), 5.18 (1H, quintet, J=5.6-5.9 Hz, CHOCO), 5.60 (2H, broad, OH, COOH), 7.16 (1H, broad s, glycine NH), 7.62 (1H, d, J=6.9 Hz, serine NH). FAB MS (positive) *m/z* 699 [M+2Na-H]⁺, 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C37H71N2O7, 655.5261; Found, 655.5271.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (22*R*L). The same hydrogenolysis as described above of 21*R*L gave 22*R*L in 70% yield as a wax. $[\alpha]_D^{24}$ +18.9° (c 0.39, CHCl3). IR and ¹H NMR spectra of 22*R*L and 22*S*D were identical. And 500 MHz ¹H NMR and FAB MS data of 22*R*L and natural flavolipin were identical. FAB MS (positive) *m/z* 699 [M+2Na-H]⁺, 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m/z*: Calcd. for C37H70N2O7Na, 677.5081; Found, 677.5090. The macrophage stimulation activity of 22*R*L was almost the same as that of natural flavolipin, whereas 22*S*D was practically inactive.

N-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-D-serine (22*R*D). The same hydrogenolysis as described above of 21*R*D gave 22*R*D in 47% yield as a wax. $[\alpha]_D^{24}$ -26.2° (c 0.58, CHCl3). IR and ¹H NMR spectra of 22*S*L and 22*R*D were identical. FAB MS (positive) *m*/z 699 [M+2Na-H]⁺, 677 [M+Na]⁺, 655 [M+H]⁺. High Resolution FAB MS (positive) *m*/z: Calcd. for C37H71N2O7, 655.5261; Found, 655.5266.

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References and Notes

- [1] Kawai, Y.; Yano, I.; Kaneda, K. Eur. J. Biochem. 1988;171:73-80.
- [2] Kawai, Y.; Akagawa, K. Infection and Immunity. 1989;57:2086-2091.
- [3] Kawai, Y.; Kamoshita, K.; Akagawa, K. FEMS Microbiology Lett. 1991;83:127-130.
- [4] Kawai, Y.; Kaneda, K.; Morisawa, Y.; Akagawa, K. Infection and Immunity. 1991;59:2560-2566.
- [5] Shiozaki, M.; Deguchi, N.; Mochizuki, T.; Nishijima, M. Tetrahedron Lett. 1996;37:3875-3876.
- [6] Shiozaki, M.; Deguchi, N.; Ishikawa, T.; Haruyama, H.; Kawai, Y.; Nishijima, M. Tetrahedron Lett. 1998;39:4497-4500.
- [7] Uchida, I.; Yoshida, K.; Kawai, Y.; Takasa, S.; Itoh, Y.; Tanaka, H.; Kohsaka, M.; Imanaka, H. J. Antibiotics. 1985;38:1476-1486.
- [8] Nemoto, T.; Ojika, M.; Takahata, Y.; Andoh, T.; Sakagami, Y. Tetrahedron. 1998;54:2683-2690.
- [9] Freudenberg, K.; Wolf, A. Ber. 1927;60:232-238.
- [10] Barton, DHR.; McCombie, SW. J. Chem. Soc. Perkin Trans I. 1975:1574-1585.
- [11] Murray, DH.; Prokop, J. J. Pharm. Sci. 1965;54:1468-1473.
- [12] Uchida, I.; Yoshida, K.; Kawai, Y.; Takase, S.; Itoh, Y.; Tanaka, H.; Kosaka, M.; Imanaka, H. Chem. Pharm. Bull. 1985;33:424-427.
- [13] Compound (20S) has been reported as belonging to the N-type calcium channel blockers from a marine bacterium, Cytophaga sp. SANK 71996. Morishita, T.; Sato, A.; Hisamoto, M.; Oda, M.; Matsuda, K.; Ishii, A.; Kodama, K. J. Antibiotics. 1997;50:457-468.
- [14] Compound (20R) was already reported in reference [7].
- [15] Yoshida, K.; Iwami, M.; Umehara, Y.; Nishikawa, M.; Uchida, I.; Kohsaka, M.; Aoki, H.; Imanaka, H. J. Antibiotics. 1985;38:1469-1475.