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Revised Structure and Synthesis of Flavolipin

Masao Shiozaki[a*], Noriko Deguchi[a], Takashi Mochizuki[a], Takanori Wakabayashi[a], Tomio Ishikawa[b], Hideyuki Haruyama[b], Yohko Kawai[c] and Masahiro Nishijima[c]

a) Exploratory Chemistry Research Laboratories, Sankyo Company, Limited
Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan
b) Analytical and Metabolic Research Laboratories, Sankyo Company, Limited
Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan
c) Department of Biochemistry and Cell Biology, National Institute of Infectious Diseases
Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan

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Abstract: The proposed structure of natural flavolipin was revised as *N*-[*N*-[(3*R*)-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.

Keywords: stereoisomerism; lipids; peptides; biologically active compounds

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 (**16*S*L**, **16*S*D**, **16*R*L** and **16*R*D**) 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 ^{1}H 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]seryl]glycine (**18**) or *N*-[*N*-[15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]serine (**22**) rather than such amino acids as **23**, **24** and **25** in

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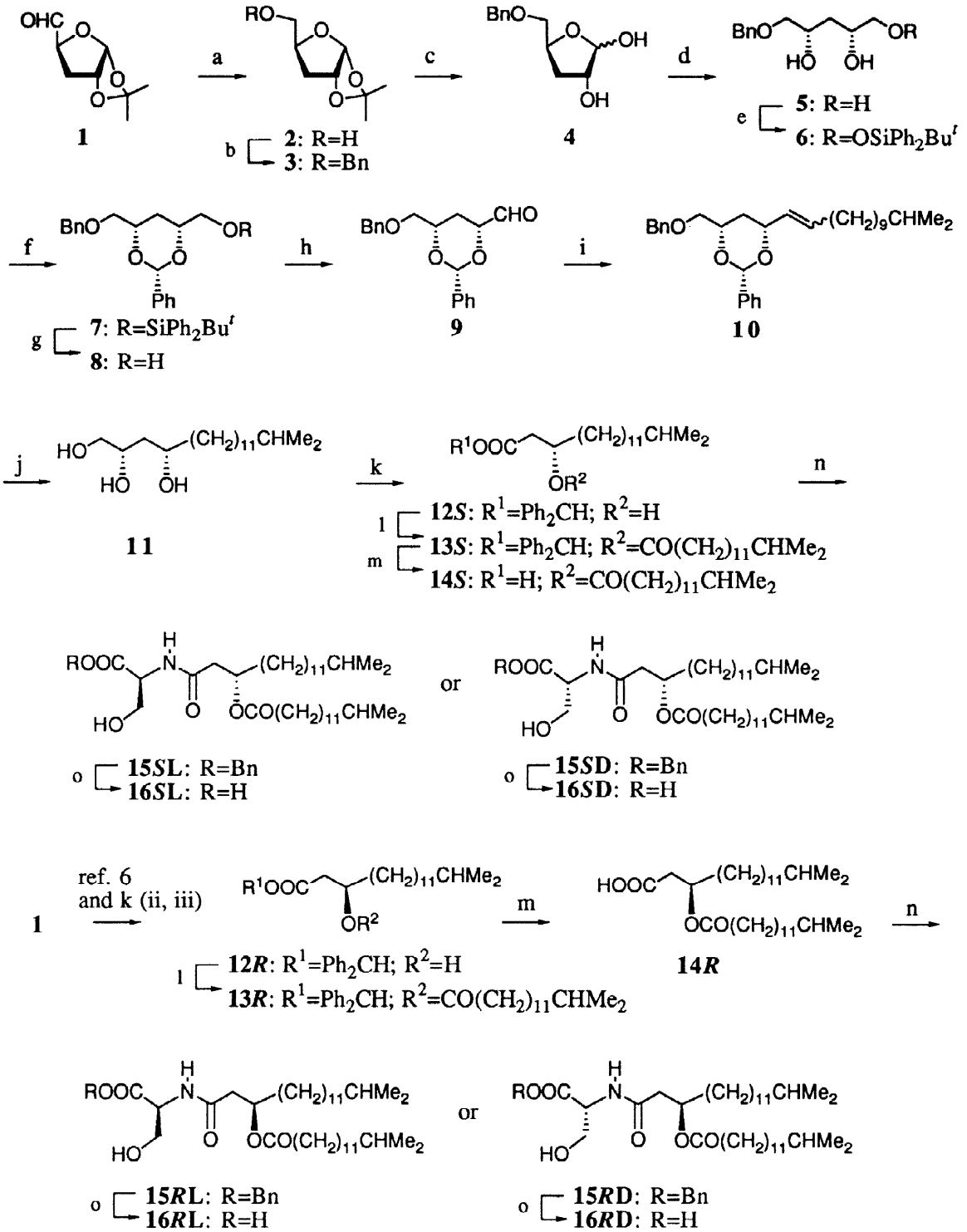
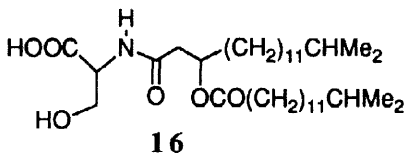
Scheme 1



Reagents and conditions: a) $NaBH_4$, EtOH, 92%; b) BnBr, NaH, DMF, 80%; c) dioxane-H_2O-1 M HCl (20:1:1), 60%; d) $NaBH_4$, EtOH, 94%; e) TBDPSCl, Et_3N, 79%; f) $PhCH(OMe)_2$, PPTS, DMF, 78%; g) Bu_4NF, THF, 98%; h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 93%; i) $Me_2CH(CH_2)_9CH{=}PPh_3$, THF, 63%; j) H_2, 5% Pd/C, THF; then H_2, $Pd(OH)_2$/C, MeOH, 65%; k) (i) $NaIO_4$, dioxane-H_2O (4:1); (ii) *m*-CPBA, $CHCl_3$; (iii) Ph_2CN_2, EtOAc, 3 steps 83%; l) $Me_2CH(CH_2)_{11}COOH$, DCC, DMAP, CH_2Cl_2; m) H_2, $Pd(OH)_2$/C, EtOH, 52% (2 steps); n) L- or D-serine benzyl ester, DCC, DMAP; o) H_2, 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



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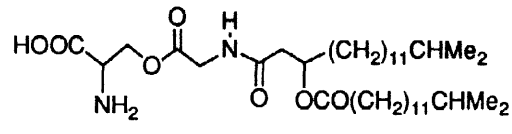

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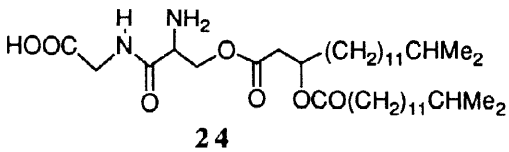

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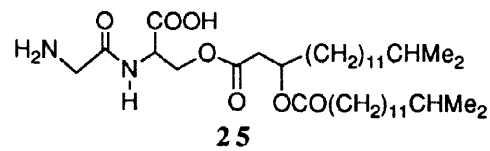


25

Results and Discussion

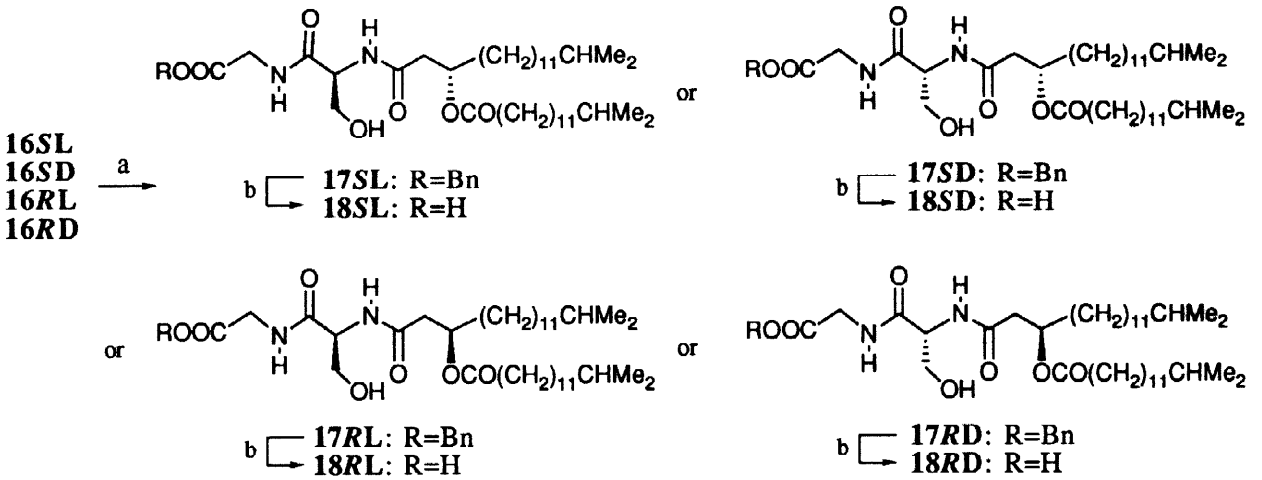
First, we tried to synthesize the proposed flavolipin **16** [2] via (3*S*)-and (3*R*)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acids (**14*S*** and **14*R***), both of which were obtained from the common aldehyde **1** [9,10,11].

Compound **14*S*** was synthesized as follows. Reduction of **1** with $NaBH_4$, followed by benzylation of the resultant alcohol **2** with benzyl bromide and NaH yielded benzyl ether **3**. Treatment of **3** with dioxane-H_2O-1 M aqueous HCl (20:1:1) gave hemiacetal **4**, which was further converted to triol **5** by $NaBH_4$ 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 $NaIO_4$, and resultant aldehyde was subjected to *m*-chloroperoxybenzoic acid oxidation. Subsequent esterification of the obtained carboxylic acid by Ph_2CN_2 gave ester **12*S***. Reaction of **12*S*** with 13-methyltetradecanoic acid [7,12] using 1,3-dicyclohexylcarbodiimide (DCC) as dehydrating agent gave ester **13*S***. Subsequent hydrogenolysis over $Pd(OH)_2$ on carbon as a catalyst furnished acid **14*S***.

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

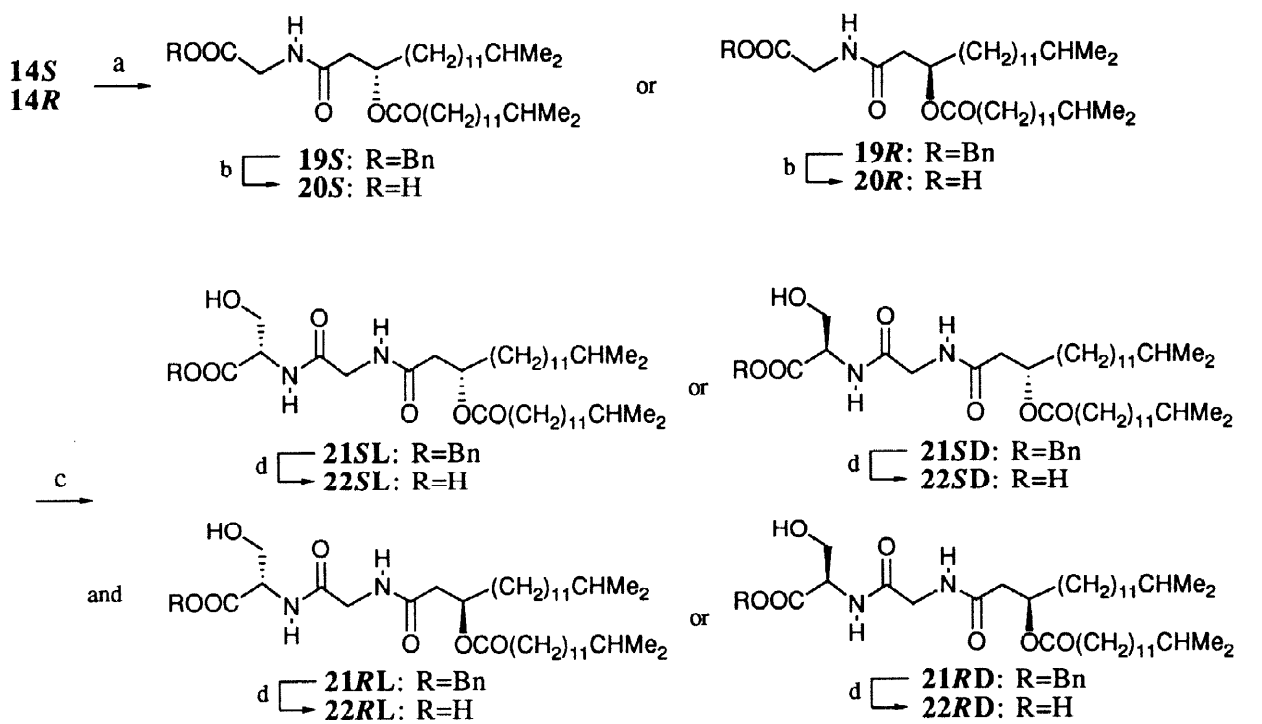
Reaction of **14*S*** and **14*R*** with L- or D-serine benzyl ester using DCC as a dehydrating agent gave benzyl esters **15*S*L**, **15*S*D**, **15*R*L** and **15*R*D**, respectively. Hydrogenolysis of these four esters produced **16*S*L** ($[\alpha]_D^{24}$ = +12.8° (c 0.13, $CHCl_3$)), **16*S*D** ($[\alpha]_D^{24}$ = -13.7° (c 0.18, $CHCl_3$)), **16*R*L** ($[\alpha]_D^{24}$ = +13.3° (c 0.14, $CHCl_3$)), and **16*R*D** ($[\alpha]_D^{24}$ = -14.4° (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

Scheme 2



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%.

Scheme 3



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%.

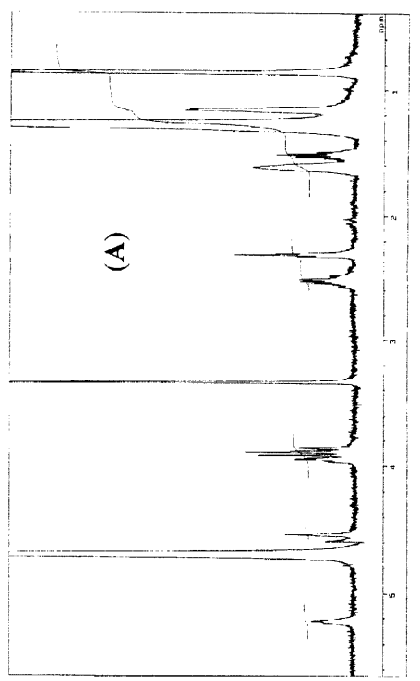


Figure 2. 400 MHz ^{1}H NMR (CD_3OD) of Natural Flavolipin (**A**) and Synthetic Flavolipin (**B**).

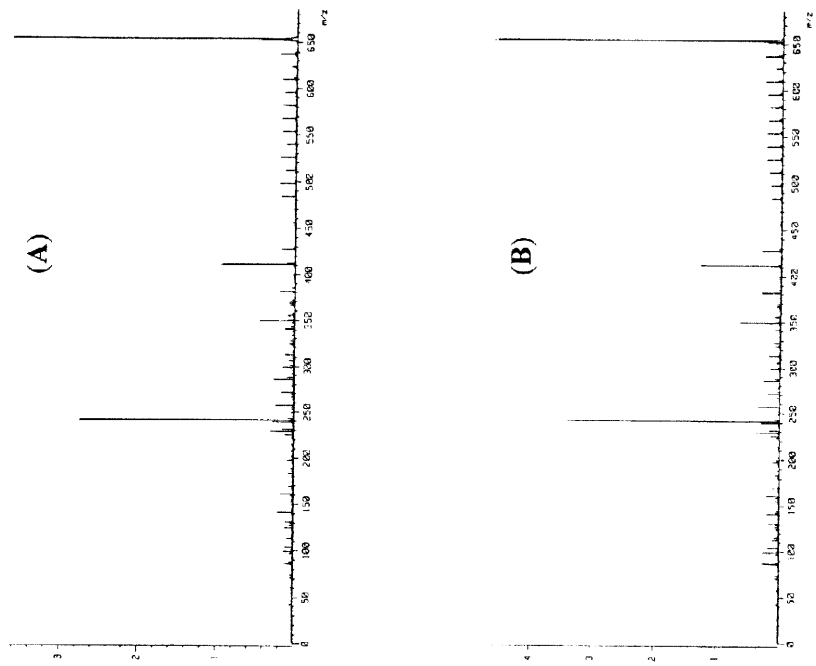


Figure 3. FAB negative MS of Natural Flavolipin (**A**) and Synthetic Flavolipin (**B**).

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 **16*S*L**, **16*S*D**, **16*R*L**, and **16*R*D** with glycine benzyl ester using DCC as a condensing reagent gave the corresponding amide benzyl esters **17*S*L**, **17*S*D**, **17*R*L**, and **17*R*D**, respectively. Hydrogenolytic deprotection of each benzyl ester gave the corresponding acids **18*S*L** ($[\alpha]_D^{24}$ -8.0° (c 0.23, $CHCl_3$)), **18*S*D** ($[\alpha]_D^{24}$ +7.1° (c 0.63, $CHCl_3$)), **18*R*L** ($[\alpha]_D^{24}$ -7.8° (c 0.50, $CHCl_3$)), and **18*R*D** ($[\alpha]_D^{24}$ +7.6° (c 0.75, $CHCl_3$)). Disappointingly, none of these compounds was identical to the natural flavolipin.

Further, we tried to synthesize acyl-glycylserines **22**. Compounds **14*S*** and **14*R*** were converted with oxalyl chloride to their corresponding acid chlorides, which were treated with glycine benzyl ester hydrochloride and Et_3N to give the corresponding amides **19*S*** and **19*R***. Then, hydrogenolytic deprotection of each benzyl ester of the amides gave the corresponding acids **20*S*** [13] and **20*R*** [8,14], which were treated with L- and D-serine benzyl esters using DCC as a condensing reagent to give the corresponding amides **21*S*L**, **21*S*D**, **21*R*L**, and **21*R*D**, respectively. Subsequent hydrogenolytic deprotection of each benzyl ester gave the corresponding acids **22*S*L** ($[\alpha]_D^{24}$ +25.6° (c 0.38, $CHCl_3$)), **22*S*D** ($[\alpha]_D^{24}$ -18.8° (c 0.52, $CHCl_3$)), **22*R*L** ($[\alpha]_D^{24}$ +18.9° (c 0.39, $CHCl_3$)) [15], and **22*R*D** ($[\alpha]_D^{24}$ -26.2° (c 0.58, $CHCl_3$)).

Fortunately, the ^1H NMR and FAB MS spectra of compounds **22*S*D** and **22*R*L** were identical to natural flavolipin as shown in Figure 2 and Figure 3.

The macrophage stimulation activity of **22*R*L** was almost the same as that of natural flavolipin; and **22*S*D**, 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 F_{245}). Detection involved spraying the chromatogram with a solution of 17% H_2SO_4 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 $LiAlH_4$ and used immediately. CH_2Cl_2 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. MeCN was dried by storage over 3Å 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 $NaBH_4$ (1.06 g, 28.0 mmol) at 0-5 °C. After stirring for 30 min, the reaction mixture was diluted with $CHCl_3$, washed with H_2O, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to give **2** (4.45 g, 92%). IR ν_{max}(KBr) 3482, 3382 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_8H_{14}O_4 \cdot 0.1H_2O$ (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 H_2O and brine, dried over $MgSO_4$, 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}($CHCl_3$) 2992, 2937 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{15}H_{20}O_4$ (264.3): C, 68.16; H, 7.63. Found: C, 68.15; H, 7.62.

5-*O*-Benzyl-3-deoxy-D-ribose (4). A solution of **3** (8.94 g, 33.8 mmol) in 1,4-dioxane (200 ml), H_2O (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 $CHCl_3$, washed with H_2O, dried over $MgSO_4$, 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 ν_{max}($CHCl_3$) 3417, 3086, 1717 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 D_2O), 4.30 (0.35H, m, changed to dd, *J*=5.2, 11.0 Hz on addition of D_2O), 4.44-4.62 (3H, m), 5.17 (0.65H, d, *J*=8.0 Hz, changed to a singlet on addition of D_2O), 5.38 (0.35H, t, *J*=4.0-5.0 Hz, changed to a doublet, *J*=4.0 Hz, on addition of D_2O), 7.26-7.39 (5H, m). MS *m/z* 225 (M^++1), 224 (M^+). *Anal.* Calcd. for $C_{12}H_{16}O_4$ (224.3): C, 64.27; H, 7.19. Found: C, 64.38; H, 7.11.

(2*S*,4*R*)-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 $NaBH_4$ (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 H_2O and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with CH_2Cl_2-MeOH (20:1) gave **5** (3.18 g, 94%) as an oil. IR ν_{max}($CHCl_3$) 3584, 3474 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{12}H_{18}O_4 \cdot 0.5\ H_2O$ (226.3+9.0): C, 61.25; H, 8.14. Found: C, 61.16; H, 8.15.

(2*R*,4*S*)-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 Et_3N (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, H_2O, sat. $NaHCO_3$, and brine, dried over $MgSO_4$, 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 $\nu_{max}(CHCl_3)$ 3583, 3517 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{28}H_{36}O_4SiNa$: 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. $NaHCO_3$ and brine, dried over $MgSO_4$, 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 $\nu_{max}(CHCl_3)$ 2961, 2932, 2861 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{35}H_{40}O_4Si$: 552.2696; Found: 552.2672.

(2*S*,4*R*)-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 H_2O and brine, dried over $MgSO_4$, 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 $\nu_{max}(CHCl_3)$ 3601, 2924, 2869 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{19}H_{22}O_4$ (314.4): C, 72.59; H, 7.05. Found: C, 72.31; H, 7.24.

(2*R*,4*S*)-2,4-[(*R*)-Benzylidenedioxy]-5-benzyloxypentanal (9). To a solution of oxalyl chloride (0.075 ml, 0.860 mmol) in CH_2Cl_2 (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 CH_2Cl_2 (1 ml) was added to this solution at -78 °C, and the stirring was continued for 15 min, and then Et_3N (0.60 ml, 4.30 mmol) was added. After 5 min, the reaction mixture was warmed to room temperature and diluted with CH_2Cl_2. The solution was washed with H_2O and brine, dried over $MgSO_4$, 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 $\nu_{max}(CHCl_3)$ 1740 cm^{-1}. 270 MHz ^1H NMR ($CDCl_3$) 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 $C_{19}H_{20}O_4$ (312.4): C, 73.06; H, 6.45. Found: C, 73.00; H, 6.66.

(2*S*,4*R*)-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 °C with stirring under nitrogen. After

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 H_2O and brine, dried over $MgSO_4$, 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 $\nu_{max}(CHCl_3)$ 2955, 2928, 2856 cm^{-1}. The mixture was partially separated on a silica gel preparative TLC plate. [*E*-isomer: R_f=0.44 (benzene); *Z*-isomer: R_f=0.36 (benzene)]. 270 MHz ^1H NMR of *E*-isomer: ($CDCl_3$) 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: ($CDCl_3$) 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 $C_{32}H_{46}O_3Na$: 501.3345; Found: 501.3336.

(2*S*,4*S*)-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 $\nu_{max}(CHCl_3)$ 3307, 2953, 2920, 2850 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $[M+Na]^+$.

(3*S*)-Diphenylmethyl 15-methyl-3-hydroxyhexadecanoate (12*S*). (i) A solution of **11** (31 mg, 0.10 mmol) in 1,4-dioxane (2 ml) and a solution of $NaIO_4$ (111 mg, 0.52 mmol) in H_2O (0.4 ml) were mixed and stirred for 1 h at 24 °C. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The solution was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo* to give 28 mg of (3*S*)-15-methyl-3-hydroxyhexadecanal, which was dissolved in $CHCl_3$ (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 (3*S*)-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 **12*S*** (40 mg, 86%) as an oil. $[\alpha]_D^{24}$ +14.5° (c 0.2, $CHCl_3$). IR ν_{max}(film) 3032 (broad), 2926, 2853, 1736 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{30}H_{44}O_3 \cdot 0.24H_2O$ (457.0): C, 78.85; H, 9.81. Found: C, 78.97; H, 9.61.

(3*R*)-Diphenylmethyl 15-methyl-3-hydroxyhexadecanoate (12*R*). The above mentioned procedures (ii) and (iii) applied to (3*R*)-15-methyl-3-hydroxyhexadecanal (833 mg, 3.08 mmol), which was obtained from compound (**1**) according to the reported method [6], gave **12*R*** (1.261 g) in 90% yield as an oil. $[\alpha]_D^{24}$ -14.0° (c 0.2, $CHCl_3$). EI MS *m/z* 452 (M^+).

(3*S*)-Diphenylmethyl 15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoate (13*S*). To a solution of **12*S*** (1.12 g, 2.47 mmol) and 13-methyltetradecanoic acid (0.90 g, 3.71 mmol) in CH_2Cl_2 (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. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated *in vacuo*, and the residual mixture was chromatographed on a silica gel column. Elution with hexane-EtOAc (20:1) gave **13*S*** (1.52 g, 90%) as a wax. $[\alpha]_D^{24}$ +3.1° (c 0.2, $CHCl_3$). IR ν_{max}(film) 2926, 2855, 1742 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{45}H_{72}O_4$ (677.1): C, 79.83; H, 10.72. Found: C, 79.52; H, 11.02.

(3*R*)-Diphenylmethyl 15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoate (13*R*). 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, $CHCl_3$). MS *m/z* 676 (M^+). *Anal.* Calcd. for $C_{45}H_{72}O_4$·(677.1): C, 79.83; H, 10.72. Found: C, 79.53; H, 11.01.

(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acid (14*S*). A solution of **13*S*** (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 **14*S*** (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. $[\alpha]_D^{24}$ +2.2° (c 0.5, $CHCl_3$). IR ν_{max}(film) 3700-3000 (broad), 2926, 2855, 1740, 1713 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{32}H_{62}O_4$ (510.8): C, 75.24; H, 12.23. Found: C, 74.60; H, 12.07.

(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoic acid (14*R*). The same treatment as described above of **13*R*** gave **14*R*** in 90% yield as a wax. $[\alpha]_D^{24}$ -0.7° (c 1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{62}O_4$ (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 CH_2Cl_2 (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 CH_2Cl_2 (4 ml). To this solution, L-serine benzyl ester hydrochloride (137 mg, 0.591 mmol) and Et_3N (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 $MgSO_4$, 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, $CHCl_3$). IR ν_{max}(film) cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 D_2O), 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 $C_{42}H_{74}NO_6$, 688.5516; Found, 688.5513. *Anal.* Calcd. for $C_{42}H_{73}NO_6$·(688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.03; H, 10.65; N, 2.04.

N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine benzyl ester (15*S*D).** The same treatment as described above of **14*S with D-serine benzyl ester hydrochloride gave **15*S*D** . $[\alpha]_D^{24}$ -9.8° (c 0.23, $CHCl_3$). FAB MS (positive) *m/z* 688 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{42}H_{74}NO_6$, 688.5516; Found, 688.5562. *Anal.* Calcd. for $C_{42}H_{73}NO_6$ (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, $CHCl_3$). 270 MHz ^1H NMR: ($CDCl_3$) 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 D_2O), 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 $C_{42}H_{74}NO_6$, 688.5516; Found, 688.5516. *Anal.* Calcd. for $C_{42}H_{73}NO_6$ (688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.22; H, 10.73; N, 2.07.

N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine benzyl ester (15*R*D).** The same treatment as described above of **14*R with D-serine benzyl ester hydrochloride gave **15*R*D** as a wax. $[\alpha]_D^{24}$ -12.5° (c 0.24, $CHCl_3$). FAB MS (positive) *m/z* 688 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{42}H_{74}NO_6$, 688.5516; Found, 688.5580. *Anal.* Calcd. for $C_{42}H_{73}NO_6$·(688.0): C, 73.32; H, 10.70; N, 2.04. Found: C, 73.61; H, 10.75; N, 2.04.

***N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine (16*S*L).** A solution of **15*S*L** (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 **16*S*L** (135 mg, 99%) as a wax. $[\alpha]_D^{24}$ +12.8° (c 0.1, $CHCl_3$). 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{35}H_{68}NO_6$, 598.5047; Found, 598.5057. *Anal.* Calcd. for $C_{35}H_{67}NO_6$ (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.37; H, 11.27; N, 2.38.

***N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine (16*S*D).** Compound **15*S*D** was hydrogenolyzed as described above to give **16*S*D** in 97% yield as a wax. $[\alpha]_D^{24}$ -13.7° (c 0.18, $CHCl_3$). 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{35}H_{68}NO_6$, 598.5047; Found, 598.5069. *Anal.* Calcd. for $C_{35}H_{67}NO_6$ (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.57; H, 11.23; N, 2.41.

***N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-L-serine (16*R*L).** Compound **15*R*L** was hydrogenolyzed as described above to give **16*R*L** in 95% yield as a wax. $[\alpha]_D^{24}$ +13.3° (c 0.14, $CHCl_3$). ^1H NMR spectra of **16*S*D** and **16*R*L** were identical. FAB MS (positive) *m/z* 620 $[M+Na]^+$, 598 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{35}H_{68}NO_6$, 598.5047; Found, 598.5071. *Anal.* Calcd. for $C_{35}H_{67}NO_6$ (597.9): C, 70.31; H, 11.30; N, 2.34. Found: C, 70.05; H, 11.37; N, 2.27.

***N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-serine (16*R*D).** Compound **15*R*D** was hydrogenolyzed as described above to give **16*R*D** in 99% yield as a wax. $[\alpha]_D^{24}$ -14.4° (c 0.18, $CHCl_3$). ^1H NMR spectra of **16*R*D** and **16*S*L** were identical. FAB MS (positive) *m/z* 620 $[M+Na]^+$, 598 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{35}H_{68}NO_6$, 598.5047; Found, 598.5071. *Anal.* Calcd. for $C_{35}H_{67}NO_6$ (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*S*L).** To a solution of **16*S*L** (56 mg, 0.094 mmol) and glycine benzyl ester (31 mg, 0.188 mmol) in CH_2Cl_2 (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. $NaHCO_3$ and brine, dried over $MgSO_4$, 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*S*L** (63 mg, 90%) as a wax. IR ν_{max}(KBr) 3303, 2954, 2923, 2852, 1744, 1727, 1641 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{44}H_{76}N_2O_7Na$, 767.5550; Found, 767.5552. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 70.73; H, 10.46; N, 3.78.

***N*-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine benzyl ester (17*S*D).** Compound **16*S*D** was treated as described above to give **17*S*D** in 70% yield as a wax. $[\alpha]_D^{24}$ +12.4° (c 0.50, $CHCl_3$). IR ν_{max}(KBr) 3299, 2954, 2922, 2852, 1727, 1640 cm^{-1}. 270 MHz ^1H NMR: ($CDCl_3$) 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 $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5716. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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, $CHCl_3$). IR and ^1H NMR spectra of **17*R*L** and **17*S*D** were identical. FAB MS (positive) *m/z* 767 $[M+Na]^+$, 745 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5711. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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 $C_{44}H_{76}N_2O_7Na$, 767.5550; Found, 767.5510. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (745.1): C, 70.91; H, 10.29; N, 3.76. Found: C, 70.77; H, 10.30; N, 3.87.

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

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, $CHCl_3$). IR ν_{max}(KBr) 3350, 3309, 2955, 2922, 2852, 1722, 1699, 1621 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, O*H* and COO*H*), 3.68 (1H, dd, *J*=4.6, 11.3 Hz, serine C*H*OH), 3.96-4.10 (3H, m, glycine C*H*$_2$N and serine C*H*OH), 4.59 (1H, m, changed to a triplet on addition of D_2O, *J*=4.2 Hz, serine C*H*-N), 5.18 (1H, quintet, *J*=5.8-6.8 Hz, C*H*-OCO), 7.17 (3/4H, d, *J*=7.4 Hz, serine CON*H*), 7.22 (1/4H, d, *J*=7.6 Hz, serine CON*H*), 7.56 (3/4H, t, *J*=5.3 Hz, glycine CON*H*), 7.60 (1/4H, t, *J*=5.3 Hz, glycine CON*H*). FAB MS (positive) *m/z* 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{71}N_2O_7$, 655.5261; Found, 655.5267.

***N*-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]-D-seryl]glycine (18*S*D).** Compound **17*S*D** was hydrogenolyzed as described above to give **18*S*D** 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, $CHCl_3$). IR ν_{max}(KBr) 3354, 3056, 2954, 2921, 2851, 1738, 1723, 1664 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, C*H*OH), 3.94-4.11 (3H, m, C*H*OH, C*H*$_2$N), 4.58 (1H, m, changed to a triplet on addition of D_2O, *J*=4.2 Hz, serine C*H*N), 5.15-5.26 (1H, m, C*H*OCO), 7.20 (1/4H, d, *J*=7.4 Hz, serine CON*H*), 7.29 (3/4H, d, *J*=7.7 Hz, serine N*H*), 7.59 (1/4H, t, *J*=5.3 Hz, glycine CON*H*), 7.64 (3/4H, t, *J*=5.4 Hz, glycine N*H*). FAB MS (positive) *m/z* 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{71}N_2O_7$, 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, $CHCl_3$). IR and ^1H NMR were identical with those of **18*S*D**. FAB MS (positive) *m/z* 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{71}N_2O_7$, 655.5261; Found, 655.5255. *Anal.* Calcd. for $C_{37}H_{70}N_2O_7$ (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, $CHCl_3$). IR ν_{max}(KBr) 3350, 3308, 2955, 2922, 2852, 1735 (shoulder), 1721, 1699, 1621, 1576 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, O*H*, COO*H*), 3.68 (1H, dd, *J*=4.6, 11.3 Hz, C*H*OH), 3.96-4.10 (3H, m, C*H*OH, C*H*$_2$N), 4.55-4.59 (1H, m, *J*=4.3 Hz, serine C*H*N), 5.18 (1H, quintet, J=5.8-6.8 Hz, C*H*-OCO), 7.18 (1H, d, *J*=7.4 Hz, serine CON*H*), 7.56 (1H, d, *J*=5.3 Hz, glycine N*H*). FAB MS (positive) *m/z* 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{70}N_2O_7Na$, 677.5081; Found, 677.5073. *Anal.* Calcd. for $C_{37}H_{70}N_2O_7$ (655.0): C, 67.85; H, 10.77; N, 4.28. Found: C, 67.47; H, 10.84; N, 4.44.

N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine benzyl ester (19*S*).** To a solution of **14*S (400 mg, 0.783 mmol) in CH_2Cl_2 (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 CH_2Cl_2 (4 ml). To this solution, glycine benzyl ester hydrochloride

(236 mg, 1.175 mmol) and Et_3N (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 $MgSO_4$, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (9:1) gave **19*S*** (294 mg, 57%) as a wax. IR ν_{max}(film) 3310, 2954, 2921, 2852, 1737, 1729, 1640, 1549 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, N*H*), 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 $C_{41}H_{72}NO_5$, 658.5410; Found, 658.5388. *Anal.* Calcd. for $C_{41}H_{71}NO_5$ (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, $CHCl_3$). 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 $C_{41}H_{71}NO_5Na$, 680.5230; Found, 680.5235. *Anal.* Calcd. for $C_{41}H_{71}NO_5$ (658.0): C, 74.84; H, 10.88; N, 2.13. Found: C, 74.73; H, 10.60; N, 2.31.

N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (20*S*).** A solution of **19*S (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 **20*S*** (205 mg, 94%) as a wax. IR ν_{max}(film) 3363, 2955, 2919, 2850, 1724, 1627, 1549 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, C*H*OCO), 6.41 (1H, t, *J*=5.2 Hz, N*H*). FAB MS (positive) *m/z* 590 $[M+Na]^+$, 568 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{34}H_{66}NO_5$, 568.4941; Found, 568.4958. *Anal.* Calcd. for $C_{34}H_{65}NO_5$ (567.9): C, 71.91; H, 11.54; N, 2.47. Found: C, 71.90; H, 11.66; N, 2.53.

N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (20*R*).** The same hydrogenolysis as described above of **19*R gave **20*R*** in 98% yield. IR and ^1H NMR spectra of **20*R*** and **20*S*** were identical. FAB MS (positive) *m/z* 590 $[M+Na]^+$, 568 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{34}H_{66}NO_5$, 568.4941; Found, 568.4944.

N*-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine benzyl ester (21*S*L).** To a solution of **20*S (80 mg, 0.14 mmol) and L-serine benzyl ester (82 mg, 0.42 mmol) in CH_2Cl_2 (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. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a mixture, which was chromatographed on a silica gel column. Elution with benzene-EtOAc (1:1) gave **21*S*L** (80 mg, 76%) as a wax. $[\alpha]_D^{24}$ +25.0° (c 0.5, $CHCl_3$). IR ν_{max}(KBr) 3398, 3330, 2954,, 2923, 2852, 1738, 1727, 1707(w), 1657, 1637, 1615 (w), 1538 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, O*H*), 3.88-4.12 (4H, m), 4.72 (1H, td, *J*=3.6, 7.8 Hz), 5.21 (1H, m, C*H*OCO), 5.22 (2H, s), 6.49 (1H, t, *J*=5.5 Hz, N*H*), 7.17 (1H, d, *J*=7.7 Hz, N*H*), 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 $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5722. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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*SD*).** The same procedure as described above of **20*S and D-serine benzyl ester gave **21*SD*** in 70% yield as a wax. $[\alpha]_D^{24}$ -10.6° (c 0.5, $CHCl_3$). IR ν_{max}(KBr) 3319, 2954,, 2922, 2852, 1733, 1661, 1637, 1549 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, O*H*), 3.94-4.05 (4H, m), 4.70 (1H, td, *J*=3.4, 7.6 Hz), 5.15 (1H, quintet, *J*=5.8 Hz, C*H*OCO), 5.21 (2H, s), 6.48 (1H, t, *J*=5.4 Hz, glycine N*H*), 7.05 (1H, d, *J*=7.6 Hz, serine N*H*), 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 $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5734. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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*RL*).** The same procedure as described above of **20*R and L-serine benzyl ester gave **21*RL*** in 72% yield as a wax. $[\alpha]_D^{24}$ +10.2° (c 0.5, $CHCl_3$). IR and ^1H NMR spectra of **21*RL*** and **21*SD*** were identical. FAB MS (positive) *m/z* 767 $[M+Na]^+$, 745 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5720. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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*RD*).** The same procedure as described above of **20*R and D-serine benzyl ester gave **21*RD*** in 82% yield as a wax. $[\alpha]_D^{24}$ -24.0° (c 0.5, $CHCl_3$). IR and ^1H NMR spectra of **21*SL*** and **21*RD*** were identical. FAB MS (positive) *m/z* 767 $[M+Na]^+$, 745 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{44}H_{77}N_2O_7$, 745.5731; Found, 745.5725. *Anal.* Calcd. for $C_{44}H_{76}N_2O_7$ (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, $CHCl_3$). IR ν_{max}(KBr) 3371, 3335, 3280, 3073 (w), 2955, 2918, 2851, 1746, 1683, 1644 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, C*H*$_2$OH, C*H*$_2$N), 4.61 (1H, broad s), 5.21 (1H, quintet, *J*=5.8-6.2 Hz, C*H*OCO), 5.3-5.8 (2H, broad, O*H*, COO*H*), 7.18 (1H, broad s, N*H*), 7.60 (1H, broad s, N*H*). FAB MS (positive) *m/z* 699 $[M+2Na-H]^+$, 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{70}N_2O_7Na$, 677.5081; Found, 677.5092.

N*-[*N*-[(3*S*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-D-serine (22*SD*).** The same hydrogenolysis as described above of **21*SD gave **22*SD*** in 73% yield as a wax. $[\alpha]_D^{24}$ -18.8° (c 0.52, $CHCl_3$). IR ν_{max}(KBr) 3318, 3079 (w), 2955, 2922, 2852, 1723, 1681, 1659, 1629, 1545 cm^{-1}. 400 MHz ^1H NMR: ($CDCl_3$) 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, C*H*$_2$OH, C*H*$_2$N), 4.63 (1H, broad s, C*H*N), 5.18 (1H, quintet, J=5.6-5.9 Hz, C*H*OCO), 5.60 (2H, broad, O*H*, COO*H*), 7.16 (1H, broad s, glycine N*H*), 7.62 (1H, d, *J*=6.9 Hz, serine N*H*). FAB MS (positive) *m/z*

699 $[M+2Na-H]^+$, 677 $[M+Na]^+$, 655 $[M+H]^+$. High Resolution FAB MS (positive) *m/z*: Calcd. for $C_{37}H_{71}N_2O_7$, 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, $CHCl_3$). 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 $C_{37}H_{70}N_2O_7Na$, 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, $CHCl_3$). 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 $C_{37}H_{71}N_2O_7$, 655.5261; Found, 655.5266.

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