WA8242A$_1$, A$_2$ and B, Novel Secretary Phospholipase A$_2$ Inhibitors Produced by *Streptomyces violaceusniger*

III. Structure Elucidation and Total Synthesis of WA8242B

SEIJI YOSHIMURA*, TAKANAO OTSUKA, SHIGEHIRO TAKASE, MASANORI OKAMOTO, SATOSHI OKADA† and the late KEIJI HEMMI

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3 Tokodai, Tsukuba, Ibaraki 300-2698, Japan

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The structure of WA8242B, a potent novel inhibitor against phospholipase A$_2$, was fully characterized by spectroscopic methods and chemical degradation. The success of total synthesis of WA8242B confirmed the structure and allowed the pharmacological study of WA8242B. The structures of WA8242A$_1$ and A$_2$ were also described.

Phospholipase A$_2$ (PLA$_2$), a rate-determining enzyme in the arachidonic acid cascade, is believed to play an important role in inflammatory reactions. Thus, PLA$_2$ inhibitors could possibly be used in the treatment of inflammatory diseases such as shock, tuberculosis and arthritis. In our quest for PLA$_2$ inhibitors from microorganisms, we found novel, potent inhibitors, WA8242A and B from the fermentation broth of *Streptomyces violaceusniger* No. 8242[1]. The structures of WA8242A and B were elucidated by spectroscopic and chemical methods. Their structures differ from each other only in chain-length of two β-hydroxy fatty acids. Lack of availability in sufficient quantities and the laborious isolation procedure precluded further pharmacological evaluation of WA8242A and B, and hence a convenient total synthesis of WA8242B was carried out.

Results and Discussion

Structure Elucidation of WA8242B

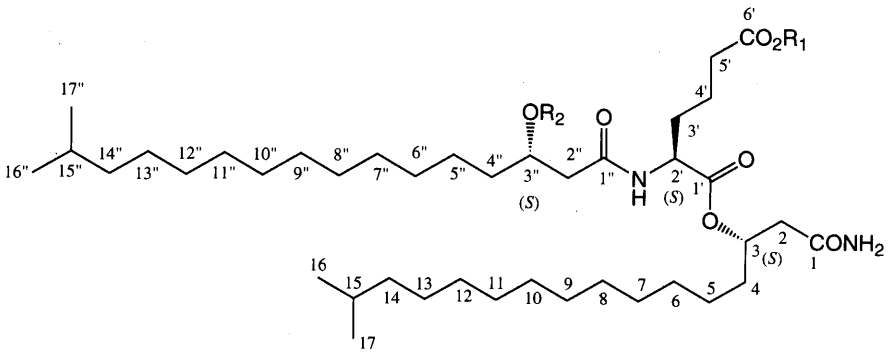
Structure elucidation studies began with WA8242B (**1**) because it was a major metabolite. The FAB-MS spectrum of **1** gave a molecular weight of 696 which was derived from a pseudomolecular ion at m/z 697 $(M+H)^+$, further supported by an ion at m/z 719 $(M+Na)^+$. The ^{13}C NMR, DEPT and ^{13}C-^1H COSY data of **1** in methanol-d_4 showed 40 carbons consisting of 4 methyl, 27 methylene, 5 methine and 4 ester or amide carbons. In the ^1H NMR spectrum in DMSO-d_6, five exchangeable protons were clearly seen at 12.08, 8.15, 7.32, 6.81 and 4.51 ppm. That is to say, totally 76 protons were observed in the ^1H NMR spectrum in DMSO-d_6. From these results and elemental analysis, the molecular formula of **1** was determined to be $C_{40}H_{76}N_2O_7$ (Calcd: C, 68.92; H, 10.99; N, 4.02. Found: C, 68.46; H, 11.29; N, 4.12). The four unsaturation units required by the molecular formula can be satisfied by the 4 carbonyl carbons, indicating an acyclic nature of **1**.

The IR absorption bands at 1725, 1715 and 1650 cm^{-1} suggested the presence of an ester, carboxylic acid and amide functionality, respectively. Complete acid hydrolysis of **1** yielded α-aminoadipic acid (Aad). Treatment of **1** with trimethylsilyldiazomethane followed by acetylation gave a mono acetylated methyl ester (**1a**, Fig. 1) (δ 3.67 (3H, s), 2.06 (3H, s); ESI-MS m/z 753 $(M+H)^+$) in which the oxymethine proton resonance had shifted from 3.95 ppm in **1** to 5.16 ppm. This proved the existence of a secondary alcohol and a carboxylic acid group.

Inspection of ^1H NMR and ^1H-^1H COSY data in DMSO-d_6 suggested the following functional groups. A weak cross-peak between 7.32 ppm (exchangeable, br s) and 6.81 ppm (exchangeable, br s) is characteristic of

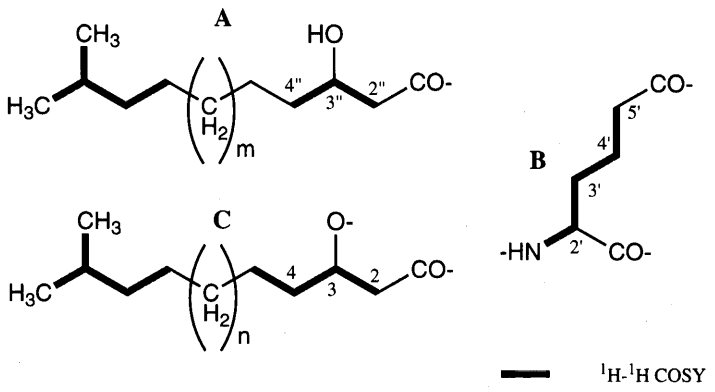
† Present address : Agricultural Chemicals Research Laboratory, Sumitomo Chemical CO., LTD., 2-1, Takatukasa 4-Chome, Takarazuka, Hyogo 665-0051, Japan

Fig. 1. Structures of WA8242B (**1**) and its derivative (**1a**).



1 $R_1=R_2=H$
1a $R_1=CH_3$, $R_2=-COCH_3$

Fig. 2. Partial structures **A**, **B** and **C** of WA8242B (**1**).



$CONH_2$. The chemical shift of 3-H (5.08 ppm) is typical for an acyloxy proton. In the high field region of the ^1H NMR spectrum, a doublet at 0.84 ppm (12H, d, $J=7$Hz) indicated the presence of two sets of isopropyl groups. ^1H NMR spectrum also showed the presence of long alkyl chains (δ 1.40~1.17 (38H, m)), that is, two long-chain iso-fatty acids. With the above functional groups in hand, a combination of ^1H-^1H COSY and ^{13}C-^1H COSY revealed the partial structures, **A**, **B** and **C** as shown in Fig. 2.

The HMBC data allowed the assembly of the sub-structures into the planar structure (Fig. 3). Correlations from 3″-H to CO-1″ (δ 171.0), 2″-H to CO-1″ and from 2′-H to CO-1″ connected **A** to **B** through the amide bond. Correlations from both 3′-H and 2′-H to CO-1′ (δ 171.4) and from 3-H to CO-1′ joined **B** and **C** together. The C-1 placement of elusive carboxamide protons was elucidated by the HMBC from $CONH_2$ to $CONH_2$, 2-H_2 to $CONH_2$ and from 3-H to $CONH_2$ and further supported by NOE cross-peak between $CONH_2$ and 2-H_2.

The remaining problem was the determination of the chain length of two β-hydroxy fatty acids. FAB-MS spectrum of **1** gave crucial information. Fragment ion peak at m/z 412 was inferred from cleavage of 1′-CO-O bond (Fig. 3). Therefore, the number of methylene, both m and n in Fig. 3 were supposed to be 7, which was confirmed by molecular weight of alkaline degradation products **4** and **5** (see Fig. 4). From the information the planar structure of **1** was elucidated. The ^1H and ^{13}C assignments were made by ^1H-^1H COSY, ^{13}C-^1H COSY and HMBC and given in Table 1.

Stereochemistry of WA8242B

The stereochemistry at C-3, C-2′, and C-3″ of **1** can not be determined by spectroscopic methods. All attempts to obtain crystals suitable for X-ray crystallography were in vain. Therefore, the absolute configurations were determined by chemical degradation as shown in Fig. 4. Hydrolysis of **1** with 6N hydrochloric acid

Fig. 3. HMBC, NOESY and FAB-MS data of WA8242B (**1**).

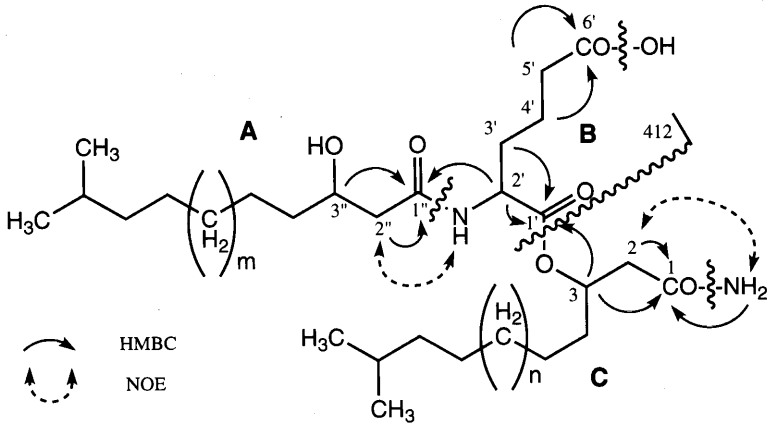


Table 1. The ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of WA8242B (**1**) in DMSO-d_6 and MeOH-d_4.

position	δ_C		δ_H	
	DMSO-d_6	MeOH-d_4	DMSO-d_6	MeOH-d_4
1	170.95 (s)	175.3 (s)		
2	39.8 (t)	41.3 (t)	2.33 (m)	2.51 (m)
			2.28 (m)	2.43 (m)
3	71.5 (d)	73.7 (d)	5.08 (m)	5.26 (m)
4	33.5 (t)	35.3 (t)	1.50 (m)	1.63 (m)
5	24.4 (t)	26.2 (t)	1.32-1.17 (34H,m)	1.33 (m)
6-11,6"-11"	28.8-29.3 (t)	30.5-30.8 (t)		1.42-1.22 (28H,m)
12,12"		31.1 (t)		
13,13"	26.8 (t)	28.6 (t)		1.32 (m)
14,14"	38.4 (t)	40.3 (t)	1.13 (m)	1.17 (m)
15,15"	27.3 (d)	29.2 (d)	1.48 (m)	1.52 (m)
16,17,16",17"	22.5 (q)	23.0 (q)	0.84 (d)	0.87 (d)
NH$_2$			7.32 (br)	
			6.81 (br)	
1'	171.4 (s)	173.1 (s)		
2'	51.7 (d)	53.8 (d)	4.14 (m)	4.36 (m)
3'	30.2 (t)	31.9 (t)	1.67 (m)	1.86 (m)
			1.56 (m)	1.71 (m)
4'	20.9 (t)	22.4 (t)	1.52 (m)	1.66 (m)
5'	33.2 (t)	34.4 (t)	2.17 (m)	2.31 (m)
6'	174.1 (s)	177.2 (s)		
NH			8.15 (d)	
1"	170.97 (s)	174.4 (s)		
2"	43.3 (t)	44.4 (t)	2.24 (m)	2.38 (m)
			2.20 (m)	
3"	67.4 (d)	69.8 (d)	3.76 (m)	3.94 (m)
4"	36.6 (t)	38.1 (t)	1.35 (m)	1.46 (m)
5"	25.0 (t)	26.6 (t)	1.36 (m)	1.49 (m)
			1.25 (m)	1.35 (m)
OH			4.51 (br)	
COOH			12.08 (br)	

afforded Aad as described above. Its stereochemistry was determined to be L using chiral column HPLC. Hydrolysis of **1** with sodium hydroxide in methanol yielded **4** and **5**. The (*S*) absolute configuration of **4** was at first inferred from its positive sign of optical rotation and finally confirmed by a comparison of **4** ($[\alpha]_D^{26}$ +18° (*c* 0.57, $CHCl_3$)) with synthetic compound **11** ($[\alpha]_D^{24}$ +19° (*c* 0.57, $CHCl_3$)). Methanolysis of compound **5**

Fig. 4. Chemical degradation of WA8242B (**1**).

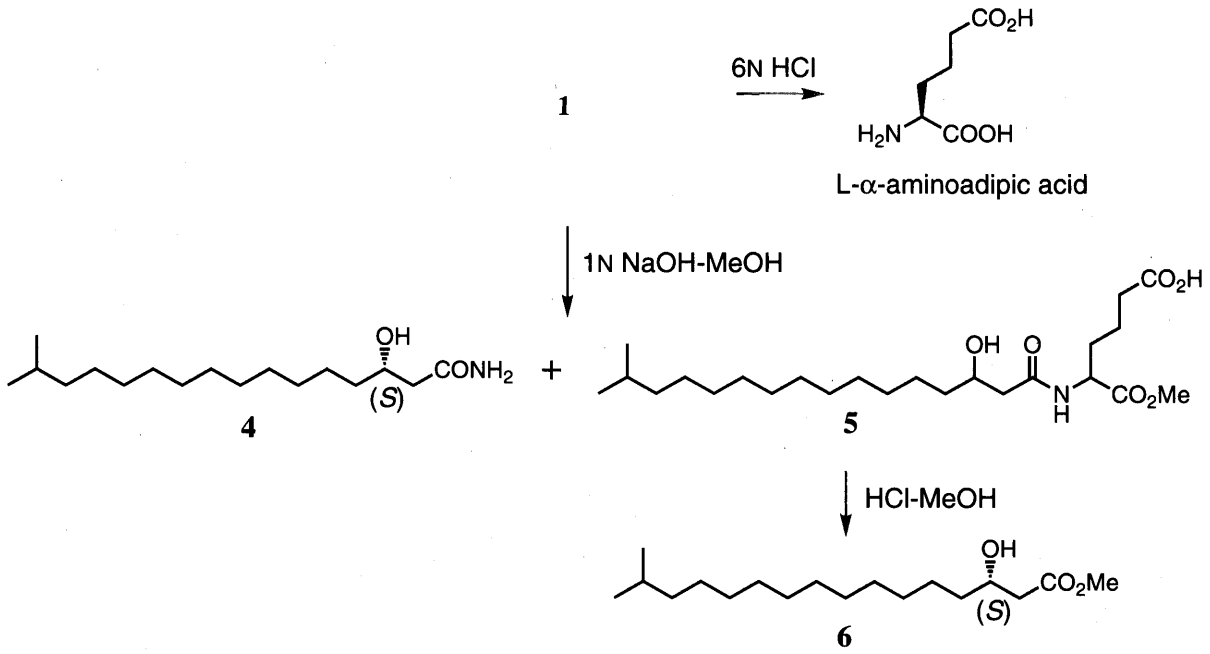
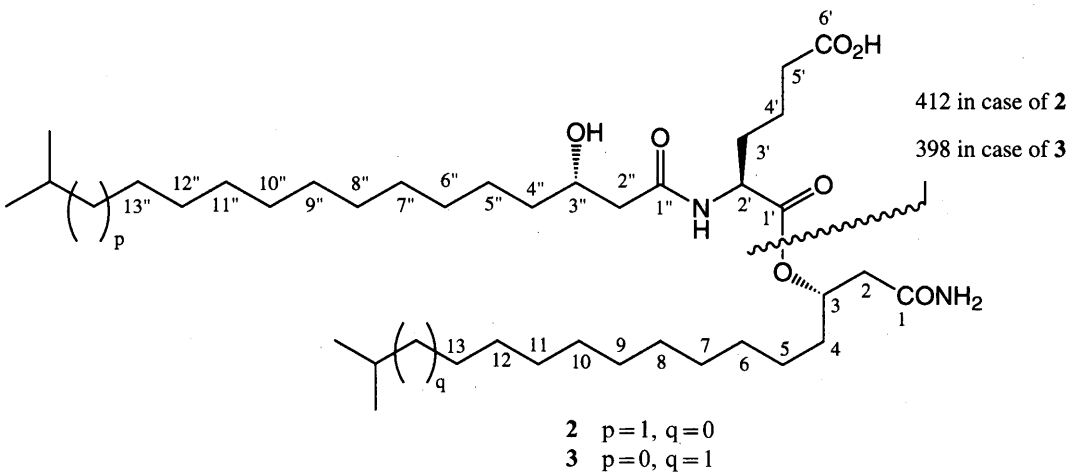


Fig. 5. Proposed Structures of WA8242A$_1$ (**2**) and A$_2$ (**3**).



with hydrogen chloride in methanol yielded **6** ($[\alpha]_D^{25}$ +10° (*c* 0.27, $CHCl_3$)), the optical antipode of the known (*R*)-compound[2] (lit. $[\alpha]_D^{20}$ −12.6° (*c* 0.26, $CHCl_3$)). Consequently, the absolute configuration of **1** was assigned as 3-(*S*), 2′-(*S*) and 3″-(*S*).

Structure Elucidation of WA8242A

The ^1H and ^{13}C NMR spectra of WA8242A are quite similar to those of WA8242B (**1**). Based on the FAB-MS, ^1H and ^{13}C NMR data, the molecular formula of WA8242A was determined to be $C_{39}H_{74}N_2O_7$, one CH_2 less than that of **1**.

WA8242A differed from WA8242B only in the length of alkyl chains. The chain lengths of the β-hydroxy fatty acids were determined by the FAB-MS data as in the case of **1**. Fragmentation ion peaks from the cleavage of CO-O bond were observed at *m/z* 412 and also *m/z* 398. This finding showed WA8242A consisted of two compounds different only in the length of two alkyl chains. The planar structures of these compounds (temporarily named A$_1$ (**2**) and A$_2$ (**3**)) were determined as shown in Fig. 5.

The stereochemistry of WA8242A was supposed as follows. The absolute configuration at C-2′ was deter-

mined to be (*S*) by the similar method to that of **1**. Both absolute configurations at C-3 and C-3″ were assigned to be (*S*) because their ^{13}C chemical shifts are in excellent agreement with those of relevant carbons in **1**. From these results, the absolute configurations of WA8242A were assigned to be 3-*S*, 2′-*S* and 3″-*S*.

Total Synthesis of WA8242B

The isolation yields of WA8242A and B were poor because they were produced in low yields and with some other related compounds by *Streptomyces* sp. Further pharmacological evaluation of **1** required a convenient preparation method, and hence a total synthesis of WA8242B was undertaken.

The synthetic strategy of WA8242B is described below. The structure of WA8242B can be divided into three compounds: two of (*S*)-3-hydroxy-15-methylhexadecanoic acid and properly protected L-α-aminoadipic acid (Aad). L-Aad was intended to be protected as its *N*-*tert*-butoxycarbonyl-ω-benzyl ester (Boc-L-Aad(OBn)) during the ester formation with the β-hydroxy fatty acid.

Boc-L-Aad(OBn) was expected to be easily obtained by utilizing amino acid chemistry. The synthesis of the optically pure β-hydroxy long-chain iso-fatty acid, however, needed more elaboration. Consequently, most synthetic efforts were focused on the preparation of the common intermediate (*S*)-isomer of the β-hydroxycarboxylic acid. There are some methods for the asymmetric synthesis of β-hydroxycarboxylic acids, baker's yeast reduction of β-ketoester, optical resolution of racemates, catalytic asymmetric reduction of β-ketoester and so on. Of these methods, baker's yeast reduction was not appropriate in this case because it was anticipated that the undesired (*R*)-isomer would be produced[3]. An optical resolution method was also inadequate because it would require more steps than other methods. As contrasted, the catalytic asymmetric reduction would be ideal because one can synthesize the required enantiomer in few steps. Catalytic asymmetric reductions, especially reductions of β-ketoesters, using chiral 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) as the chiral element have gained practical value with respect to chemical yield and optical yield in the past decade[4]. $[RuCl_2(BINAP)]_2 \cdot NEt_3$, developed by Ikariya, is an excellent catalyst, because this catalyst can be handled in common experimental apparatus by standard methods[5]. We decided to apply the asymmetric reduction of β-ketoester using Ikariya's catalyst for the preparation of the (*S*)-β-hydroxy long-chain iso-fatty acid.

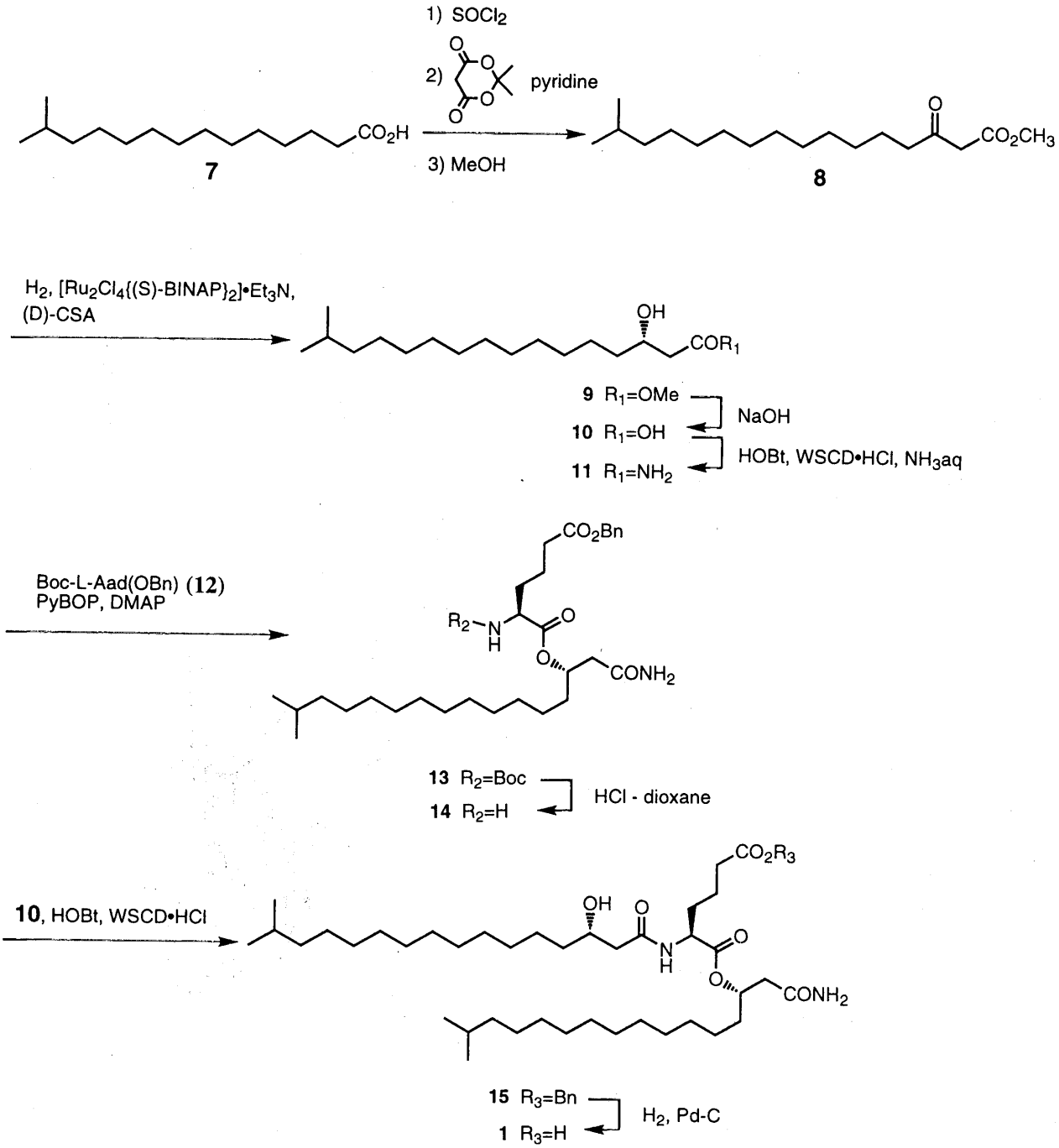
The total synthesis of WA8242B was shown in the Scheme. At first, the enantiomerically pure β-hydroxycarboxylic acid was synthesized. The substrate of the asymmetric reduction, β-ketoester **8**, was prepared in a method similar to the reported one[6]. The acid chloride from the known carboxylic acid **7**[2] was coupled with Meldrum's acid, and subsequent methanolysis gave β-ketoester **8**. Then, submission of β-ketoester **8** to asymmetric reduction using $[RuCl_2((S)\text{-BINAP})]_2 \cdot NEt_3$ and (+)-camphor-10-sulfonic acid as catalysts[5,7] under moderate pressure (5~10 atmosphere) of hydrogen furnished **9** in excellent yield (93%). The specific rotation of **9** ($[\alpha]_D^{24}$ +13° (*c* 0.27, $CHCl_3$)), opposite to that reported for (*R*)-**9** (lit. $[\alpha]_D^{20}$ −12.6° (*c* 0.26, $CHCl_3$)[2]), confirmed the (*S*) absolute configuration of **9**. The optical purity of **9** was determined to be 98% e.e. by the ^1H NMR spectra of (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) esters (**9a** and **9b**) from **9**[8]. To the best of our knowledge, this is the first success in catalytic asymmetric reduction of such a long-chain β-ketoalkanoate in high enantiomeric excess[9]. The ester **9** was hydrolyzed to give the pivotal intermediate β-hydroxycarboxylic acid **10**. The carboxylic acid **10** was converted to carboxamide **11** without protection of the 3-hydroxyl group *via* the 1-hydroxybenzotriazole (HOBt) ester.

Next, the coupling between the carboxamide **11** and the L-Aad moiety was examined. Because Boc-L-Aad(OBn) was obtained only in poor yield from expensive L-Aad, Boc-L-Aad(OBn) was prepared from L-lysine in five steps according to Sakura's method[10]. The coupling between **11** and **12** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) and HOBt resulted in low yield (25%). The yield was improved dramatically (84%) by a more powerful coupling reagent, benzotriazole-1-yloxytrispyrrolidino-phosphonium hexafluorophosphate (PyBOP) instead of WSCD.

The Boc group of **13** was removed under acidic conditions, and the resulting amine **14** was coupled with **10** using WSCD and HOBt to give compound **15**. Finally, benzyl ester **15** was transformed by hydrogenolysis to carboxylic acid **1**, whose spectra were consistent with those of natural product, WA8242B. This synthetic compound **1** inhibited group II PLA_2 with IC_{50} value of 7.6×10^{-10} M.

From the above information, the structure of **1** was concluded to be (3*S*)-3-[(2*S*)-5-carboxy-2-{(3*S*)-3-hydroxy-15-methylhexadecanoyl}aminopentanoyl]oxy-15-methylhexadecanamide. The success in synthesis may enable bulk supply for pharmacological study of WA8242B in detail. Furthermore, this synthesis could

Scheme. Total synthesis of WA8242B (**1**).



open a way to create a drug-candidate with stronger activities or better pharmacokinetics. The details of the synthetic study toward more potent PLA_2 inhibitors are now under investigation and will be reported in due course.

Experimental

Melting point (m.p.) were taken using a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 16PC FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Bruker AM400WB, DRX500, Varian Gemini300, or Mercury300 NMR spectrometer. Mass spectra were measured on a VG ZAB-SE mass spectrometer, or Micromass Platform. Amino acid analysis was performed on Hitachi amino acid analyzer system. Preparative thin-layer chromatography (TLC) was carried out on a Merck Silica gel F254 pre-coated plate, Art 5744.

WA8242A

Isolation procedure was described in the previous paper.[1] m.p. 120~123°C; $[\alpha]_D^{25}$ −9.2° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 5.28 (1H, m), 4.37 (1H, m), 3.96 (1H, m), 2.53 (1H, m), 2.44 (1H, m), 2.38 (2H, m), 2.31 (2H, m), 1.87 (1H, m), 1.78~1.10 (47H, m), 0.83 (12H, d, J=7Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 176.9, 175.3, 174.4, 173.1, 73.7, 69.8, 53.8, 41.3, 40.3, 38.1, 35.3, 34.3, 31.9, 31.1, 30.81, 30.77, 30.73, 30.61, 30.48, 29.1, 28.5, 26.6, 26.1, 23.0, 22.3; FAB-MS: *m/z* 683 $(M+H)^+$; IR (KBr, cm^{-1}): 3555, 3430, 3290, 2955, 2920, 2850, 1725, 1715, 1655, 1640, 1550, 1190.

WA8242B (**1**)

m.p. 119~123°C; $[\alpha]_D^{21}$ −10° (*c* 1.0, MeOH); ^{1}H NMR, ^{13}C NMR: see Table 1; FAB-MS: *m/z* 697 $(M+H)^+$; IR (KBr, cm^{-1}): 3550, 3435, 3290, 2955, 2920, 2850, 1725, 1715, 1655, 1640, 1555, 1190; *Anal.* Calcd for $C_{40}H_{76}N_2O_7$: C, 68.92; H, 10.99; N, 4.02. Found: C, 68.46; H, 11.29; N, 4.12.

Methyl Esterification Followed by Acetylation of WA8242B

To WA8242B (9 mg) in MeOH (2 ml) was added 10% trimethylsilyldiazomethane in dichloromethane (0.44 ml). After 30 minutes, the solution was evaporated off, and the residue was dissolved in a mixture of pyridine (0.5 ml) and acetic anhydride (0.3 ml). This mixture was allowed to stand at room temperature overnight, and evaporated off. The trace of reagents were further evaporated off with carbon tetrachloride, and the residue was purified on preparative TLC (ethyl acetate) to give **1a** (6.5 mg): $[\alpha]_D^{24}$ −2° (*c* 0.33, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.47 (1H, d, J=8 Hz), 6.02 (1H, br), 5.57 (1H, br), 5.27 (1H, m), 5.16 (1H, m), 4.50 (1H, m), 3.67 (3H, s), 2.57~2.28 (6H, m), 2.06 (3H, s), 1.94~1.44 (10H, m), 1.40~1.08 (40H, m), 0.86 (12H, d, J=7Hz); ESI-MS: *m/z* 753 $(M+H)^+$.

Hydrolysis of WA8242B with 6 N HCl

WA8242B suspension in 6 N HCl were heated at 110°C for 18 hours in evacuated sealed tubes. The solvent was evaporated, and the residue was dissolved in 0.1 N HCl. An aliquot of the solution was applied to amino acid analyzer, and CROWNPAC CR(+) (150×4.0, Daicel Chemical Industries, Ltd.). amino acid analysis: Rt 29.32 minutes (α-aminoadipic acid: 29.92 minutes), HPLC analysis (pH 2 $HClO_4$ buffer as an eluent): Rt 6.39 minutes (D-α-aminoadipic acid: 4.29 minutes, L-α-aminoadipic acid: 6.40 minutes).

Mild Hydrolysis of WA8242B with 1 N-NaOH in Methanol

To WA8242B (50 mg) was added 1 N NaOH in methanol (5 ml). The solution was allowed to stand for 15 hours at room temperature. The solvent was evaporated, and the residue was dissolved in EtOAc. This solution was washed with 1 N HCl, dried ($MgSO_4$), and evaporated. The residue was purified on preparative TLC ($CHCl_3$-MeOH (10:1)) to give **4** (11.4 mg) and **5** (15 mg).

4: $[\alpha]_D^{26}$ +18° (*c* 0.57, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.88 (1H, br s), 5.64 (1H, br s), 4.00 (1H, m), 2.46~2.25 (2H, m), 1.62~1.10 (23H, m), 0.86 (6H, d, J=7 Hz); FAB-MS: *m/z* 286 $(M+H)^+$.

5: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (1H, d, 8), 4.63 (1H, m), 4.02 (1H, m), 3.77 (3H, s), 2.50~2.30 (4H, m), 1.97~1.64 (4H, m), 1.60~1.10 (23H, m), 0.87 (6H, d, J=7 Hz); FAB-MS: *m/z* 444 $(M+H)^+$.

Acid Methanolysis of **5**

5 (15 mg) was dissolved in 10% hydrogen chloride in MeOH (2 ml), and allowed to stand for 15 hours at room temperature. The solvent was evaporated, and the residue was dissolved in ether. The ethereal solution was washed with water, dried ($MgSO_4$), and evaporated. The residue was purified on preparative TLC (EtOAc-*n*-hexane (1:5)) to give **6** (5.3 mg): $[\alpha]_D^{25}$ +10° (*c* 0.27, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00 (1H, m), 3.72 (3H, s), 2.84 (1H, d, J=4 Hz), 2.57~2.36 (2H, m), 1.60~1.10 (23H, m), 0.86 (6H, d, J=7 Hz).

Methyl 15-Methyl-3-oxohexadecanoate (**8**)

A solution of 13-methyltetradecanoic acid (**7**, 8.12 g) in thionyl chloride (4.9 ml) was heated under reflux for one hour. After concentration *in vacuo*, the excess reagent was evaporated off with toluene (20 ml × 3). The residue was added to a solution of Meldrum's acid (4.83 g) and pyridine (6.0 ml) in dichloromethane (40 ml) in an ice-water bath, and this solution was stirred at room temperature. The resulting mixture was washed with 10% hydrochloric acid (30 ml × 3) and water (30 ml). Drying over magnesium sulfate and evaporation gave viscous brownish oil. The oil was dissolved in MeOH (40 ml), and heated under reflux for 5 hours. After evaporation, the residue was purified on silica gel (90 cc) eluting with a mixture of *n*-hexane and EtOAc (10:1) to give white solid of **8** (4.45 g, 44.5%): m.p. 48°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (3H, s), 3.45 (2H, s), 2.54 (2H, t, J=7 Hz), 1.68~1.45 (3H, m), 1.40~1.10 (18H, m), 0.87 (6H, d, J=7 Hz); ESI-MS: *m/z* 297 $(M-H)^-$; IR (KBr,

cm^{-1}): 2950, 2920, 2850, 1750, 1710, 1470, 1440, 1410, 1325, 1265, 1250, 1160; *Anal.* Calcd for $C_{18}H_{34}O_3$: C, 72.44; H, 11.48. Found: C, 72.14; H, 11.63.

Methyl (*S*)-3-Hydroxy-15-methylhexadecanoate (**9**)

A stainless 100 ml-autoclave equipped with gas inlet line and pressure gauge was charge with methyl 15-methyl-3-oxohexadecanoate (**8**, 2.70 g), $[Ru_2Cl_4\{(S)\text{-BINAP}\}_2]\cdot Et_3N$ (15 mg), (+)-camphorsulfonic acid (17 mg) and degassed MeOH (10 ml) under nitrogen stream. The mixture was heated to 65°C under 5~10 atmosphere of hydrogen for 6 hours. After evaporation, the residue was purified on silica gel (60 cc) eluting with a mixture of *n*-hexane and EtOAc (10:1) to give white wax of **9** (2.54 g, 93%): $[\alpha]_D^{24}$ +13° (*c* 0.27, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.00 (1H, m), 3.72 (3H, s), 2.84 (1H, d, J=4 Hz), 2.57~2.36 (2H, m), 1.60~1.10 (23H, m), 0.86 (6H, d, J=7 Hz); ESI-MS: *m/z* 301 $(M+H)^+$; IR (KBr, cm^{-1}): 3395, 3320, 2955, 2915, 2850, 1740, 1695, 1470, 1465, 1440, 1310, 1175; *Anal.* Calcd for $C_{18}H_{36}O_3$: C, 71.95; H, 12.08. Found: C, 71.68; H, 12.42.

(*R*)- and (*S*)-MTPA Ester of **9** (**9a** and **9b**)

To a solution of **9** (3.4 mg) in pyridine (44 μl) was added (*R*)-MTPA chloride (7.4 mg) in dichloromethane (50 μl), and this mixture was allowed to stand at room temperature overnight. To the resulting mixture was added *N*,*N*-dimethylpropanediamine (2.8 mg) in dichloromethane (10 μl). After 10 minutes, the solvent was evaporated off, and the residue was purified on preparative TLC to give **9a** (5.4 mg): ^1H NMR (300 MHz, $CDCl_3$) δ 7.57~7.50 (2H, m), 7.43~7.36 (3H, m), 5.48 (1H, m), 3.66 (3H, s), 3.54 (3H, s), 2.74~2.56 (2H, m), 1.74~1.47 (3H, m), 1.35~1.10 (20H, m), 0.88 (6H, d, J=7 Hz).

9b (5.2 mg) was obtained using (*S*)-MTPA chloride from **9** (3.4 mg): ^1H NMR (300 MHz, $CDCl_3$) δ 7.56~7.49 (2H, m), 7.44~7.36 (3H, m), 5.48 (1H, m), 3.59 (3H, s), 3.53 (3H, s), 2.70~2.53 (2H, m), 1.82~1.47 (3H, m), 1.40~1.10 (20H, m), 0.88 (6H, d, J=7 Hz).

(*S*)-3-Hydroxy-15-methylhexadecanoic acid (**10**)

A mixture of methyl (*S*)-3-hydroxy-15-methylhexadecanoate (**9**, 2.36 g) and 1 N aqueous sodium hydroxide (8.6 ml) in MeOH (15 ml) was heated under reflux for one hour. After cooling, the mixture was partitioned between EtOAc (50 ml) and 1 N hydrochloric acid (15 ml). The organic phase was separated and washed with brine (20 ml). Drying over magnesium sulfate and evaporation gave white solid of **10** (2.25 g, quant.): m.p. 63°C; $[\alpha]_D^{24}$ +13° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.02 (1H, m), 2.63~2.41 (2H, m), 1.60~1.10 (23H, m), 0.86 (6H, d, J=7 Hz); ESI-MS: *m/z* 285 $(M-H)^-$; IR (KBr, cm^{-1}): 3565, 2960, 2920, 2850, 1715, 1680, 1470, 1300, 1285; *Anal.* Calcd for $C_{17}H_{34}O_3$: C, 71.28; H, 11.96. Found: C, 71.23; H, 12.34.

(*S*)-3-Hydroxy-15-methylhexadecanamide (**11**)

To a ice-cooled mixture of (*S*)-3-hydroxy-15-methylhexadecanoic acid (**10**, 600 mg) and HOBt (311 mg) in DMF (3 ml) was added WSCD·HCl (442 mg). After stirring at room temperature for 30 minutes, 28% ammonium hydroxide (153 ml) was added in an ice-water bath and the mixture was stirred at room temperature overnight. Then, the resulting mixture was diluted with water (30 ml) and extracted with EtOAc (100 ml). The organic phase was washed with 0.1 N hydrochloric acid (30 ml × 2), saturated aqueous sodium bicarbonate (30 ml) and brine (30 ml). Drying over magnesium sulfate, evaporation, and trituration in *n*-hexane (20 ml) gave white powder of **11** (535 mg, 90%): m.p. 103°C; $[\alpha]_D^{24}$ +19° (*c* 0.57, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.82 (1H, br s), 5.45 (1H, br s), 4.00 (1H, m), 2.46~2.25 (2H, m), 1.62~1.10 (23H, m), 0.86 (6H, d, J=7 Hz); IR (KBr, cm^{-1}): 3385, 3290, 3215, 2920, 2850, 1685, 1665, 1655, 1620, 1470; ESI-MS: *m/z* 284 $(M-H)^-$; *Anal.* Calcd for $C_{17}H_{35}NO_2$: C, 71.53; H, 12.36; N, 4.91. Found: C, 71.49; H, 12.43; N, 4.99.

(3*S*)-3-{(2*S*)-5-Benzyloxycarbonyl-2-(*tert*-butoxycarbonyl)aminopentanoyl}oxy-15-methylhexadecanamide (**13**)

(2*S*)-5-benzyloxycarbonyl-2-(*tert*-butoxycarbonyl)-aminopentanoic acid dicyclohexylammonium salt (**12**, 1.44 g) was partitioned between EtOAc (40 ml) and 1 N sulfuric acid (30 ml). The organic phase was washed with 1 N sulfuric acid (30 ml), water (30 ml × 2) and brine, and dried over magnesium sulfate. After evaporation, the residue was dissolved in dichloromethane (14 ml). To this mixture was added (*S*)-3-hydroxy-15-methylhexadecanamide (**11**, 700mg), PyBOP (1.40 g) and 4-dimethylaminopyridine (600 mg) with ice-cooling. The resulting mixture was stirred vigorously at room temperature overnight. After evaporation, the residue was partitioned between EtOAc (50 ml) and 0.1 N-hydrochloric acid (30 ml). The organic phase was washed with 0.1 N-hydrochloric acid (30 ml × 2), water (30 ml), saturated aqueous sodium bicarbonate (30 ml) and brine (30 ml). After drying over magnesium sulfate and evaporation,

the crude product was purified on silica gel (30 cc) eluting with a mixture of *n*-hexane and EtOAc (1 : 1) to give **13** (1.27 g, 84%): m.p. 79~81°C; $[\alpha]_D^{24}$ −1.6° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40~7.30 (5H, m), 5.92 (1H, br s), 5.32 (1H, br s), 5.25 (1H, m), 5.11 (2H, s), 5.06 (1H, m), 4.24 (1H, m), 2.48~2.36 (4H, m), 1.85~1.46 (7H, m), 1.44 (9H, s), 1.36~1.10 (20H, m), 0.86 (6H, d, J=7 Hz); ESI-MS: *m/z* 617 $(M-H)^-$; IR (KBr, cm^{-1}): 3415, 3335, 3230, 2920, 2850, 1740, 1720, 1690, 1660, 1625, 1540, 1365, 1255, 1165; *Anal.* Calcd for $C_{35}H_{58}N_2O_7$: C, 67.93; H, 9.45; N, 4.53. Found: C, 68.03; H, 9.74; N, 4.53.

(3*S*)-3-[(2*S*)-5-Benzyloxycarbonyl-2-{(3*S*)-3-hydroxy-15-methylhexadecanoyl}aminopentanoyl]oxy-15-methylhexadecanamide (**15**)

To (3*S*)-3-{(2*S*)-5-benzyloxycarbonyl-2-(*tert*-butoxycarbonyl)aminopentanoyl}oxy-15-methylhexadecanamide (**13**, 1.0 g) was added 4 N-hydrogen chloride in dioxane (2 ml) with ice-cooling. After stirring at room temperature for one hour, the solvent was evaporated off. The residue was dissolved in DMF (2 ml), and to this solution was added a solution of (*S*)-15-methyl-3-hydroxyhexadecanoic acid (**10**, 463 mg), HOBt (240 mg) and WSCD·HCl (341 mg) in DMF (3 ml), and then diisopropylethylamine (310 μl) with ice-cooling. The resulting clear mixture was allowed to stand at room temperature overnight. The mixture was diluted with EtOAc (50 ml), and washed with 0.1 N-HClaq (50 ml × 2), saturated sodium bicarbonate (30 ml × 2), and brine (30 ml). After drying over magnesium sulfate and evaporation, the crude product was triturated in diethyl ether (20 ml) to give white powder of **15** (638 mg, 50%): m.p. 112°C; $[\alpha]_D^{23}$ +6.1° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40~7.30 (5H, m), 6.58 (1H, d, J=8 Hz), 5.83 (1H, br s), 5.45 (1H, br s), 5.25 (1H, m), 5.11 (2H, s), 4.52 (1H, m), 3.97 (1H, m), 3.54 (1H, m), 2.54~2.23 (6H, m), 1.95~1.10 (46H, m), 0.86 (6H, d, J=7 Hz); ESI-MS: *m/z* 787 $(M+H)^+$; IR (KBr, cm^{-1}): 3445, 3380, 3350, 2920, 2850, 1725, 1665, 1620, 1540, 1465, 1340, 1260, 1190; *Anal.* Calcd for $C_{47}H_{82}N_2O_7$: C, 71.71; H, 10.50; N, 3.56. Found: C, 71.56; H, 10.40; N, 3.60.

(3*S*)-3-[(2*S*)-5-Carboxy-2-{(3*S*)-3-hydroxy-15-methylhexadecanoyl}aminopentanoyl]oxy-15-methylhexadecanamide (**1**)

(3*S*)-3-[(2*S*)-5-benzyloxycarbonyl-2-{(3*S*)-3-hydroxy-15-methylhexadecanoyl}aminopentanoyl]oxy-15-methylhexadecanamide (**15**, 500 mg) in a mixture of MeOH (10 ml), dioxane (10 ml) and water (1 ml) was hydrogenated on 10% palladium carbon (100 mg) under atmospheric hydrogen for 3 hours. The catalyst was filtered off with celite, and the filtrate was concentrated under reduced pressure. The residue was recrystallized in EtOAc (3 ml) to give white crystal (419 mg, 95%): m.p. 129°C; $[\alpha]_D^{23}$ −10° (*c* 1.0, MeOH); ^1H NMR (300 MHz, DMSO-d_6) δ 12.08 (1H, br s), 8.16 (1H, d, J=8 Hz), 7.34 (1H, br s), 6.83 (1H, br s), 5.07 (1H, m), 4.52 (1H, br s), 4.12 (1H, m), 3.74 (1H, m), 2.37~2.12 (6H, m), 1.72~1.06 (46H, m), 0.83 (6H, d, J=7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 171.3, 170.79, 170.74, 71.5, 67.4, 51.7, 43.3, 38.5, 36.6, 33.5, 33.2, 30.2, 29.38, 29.13, 29.04, 29.00, 28.89, 27.4, 26.9, 25.1, 24.5, 22.6, 21.0; ESI-MS: *m/z* 695 $(M-H)^-$; IR (KBr, cm^{-1}): 3445, 3215, 2955, 2920, 2850, 1730, 1700, 1675, 1650, 1530, 1470, 1190; *Anal.* Calcd for $C_{40}H_{76}N_2O_7$: C, 68.92; H, 10.99; N, 4.02. Found: C, 68.83; H, 11.02; N, 4.13.

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