STUDIES ON WB-3559 A, B, C AND D, NEW POTENT FIBRINOLYTIC AGENTS

II. STRUCTURE ELUCIDATION AND SYNTHESIS

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The structures of WB-3559 A, B, C and D, new fibrinolytic agents isolated from *Flavobacterium* sp. No. 3559, have been elucidated to be as shown in **1**, **2**, **3** and **4**, respectively, on the basis of chemical and spectroscopic evidence. Total synthesis of WB-3559 D (**4**) was achieved starting from the optically active aldehyde **14**.

WB-3559 A (**1**), B (**2**), C (**3**) and D (**4**) are new fibrinolytic agents produced by *Flavobacterium* sp. No. 3559. Taxonomy, fermentation, isolation and characterization of those compounds were described in the preceding paper.[1] In the previous communication,[2] we reported the structures of **1**, **2**, **3** and **4**, together with total synthesis of WB-3559 D (**4**). The present paper deals with a full account of that work.

Structure of WB-3559 A, B, C and D

As reported in the preceding paper,[1] the mixture of these four components was designated as WB-3559. WB-3559 A (**1**), B (**2**), C (**3**) and D (**4**) (ratios, A: B: C: D=1: 4: 1: 4) are all colorless waxes, which have almost the same Rf value on thin-layer chromatography (MeOH - H_2O, 7: 3, Rf 0.73). Each component was finally isolated by preparative HPLC.[1] With limited supplies of the isolated components, chemical reactions were performed on the mixture WB-3559.

Catalytic hydrogenation of WB-3559 over 10% Pd-C showed by liquid chromatographic analysis that the original four peaks due to WB-3559 A, B, C and D changed to two peaks corresponding to WB-3559 C and D in the ratio of 1: 4. This result implied that WB-3559 A and B contain unsaturated bonds. On treatment with sodium methoxide in absolute methanol WB-3559 provided two

	R_1	R_2
WB-3559 A (**1**)	$(CH_3)_2CH(CH_2)_{10}$	$(CH_3)_2CH(CH_2)_7CH=CHCH_2CH_2$
WB-3559 B (**2**)	$(CH_3)_2CH(CH_2)_{11}$	$(CH_3)_2CH(CH_2)_7CH=CHCH_2CH_2$
WB-3559 C (**3**)	$(CH_3)_2CH(CH_2)_{10}$	$(CH_3)_2CH(CH_2)_{11}$
WB-3559 D (**4**)	$(CH_3)_2CH(CH_2)_{11}$	$(CH_3)_2CH(CH_2)_{11}$

Scheme 1.

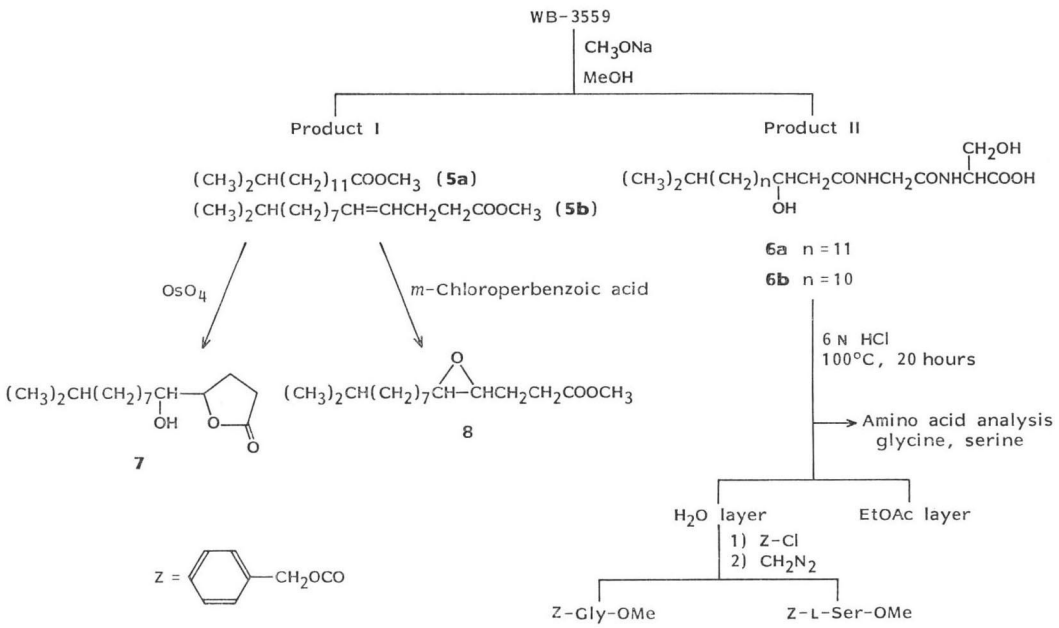
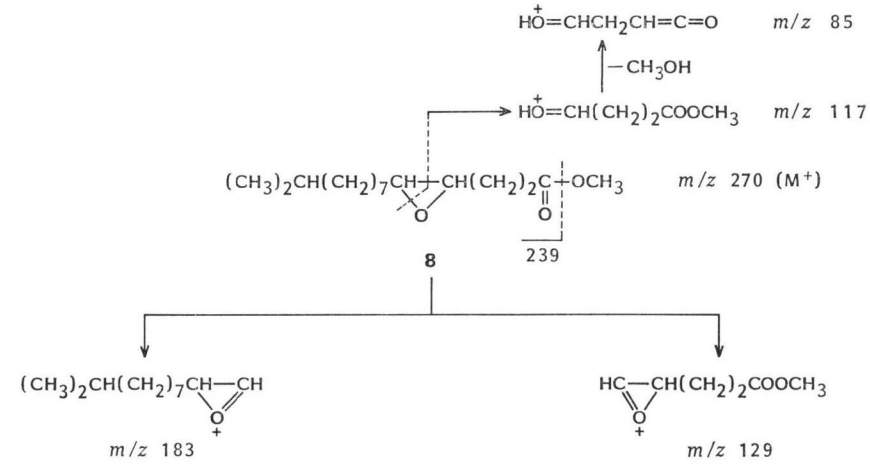


Fig. 1. The assignment of the fragmentation peaks in the EI-MS of **8**.



kinds of products which have Rf 0.9 (Product I) and 0.1 (Product II), respectively, on silica gel thin-layer chromatography developed with benzene - dioxane - acetic acid (10: 5: 1). Each product was found to be a mixture of two fatty acid methyl esters (Product I, **5a** and **5b**) and two acyl peptides (Product II, **6a** and **6b**) respectively (Scheme 1).

GC-MS analysis of product I demonstrated that it consists of two compounds in the ratio of *ca.* 1: 1, and their molecular weight are 256 (**5a**, retention time: 7 minutes) and 254 (**5b**, retention time: 7.8 minutes). By catalytic hydrogenation over 10% Pd-C, the mixture (**5a** and **5b**) converged to the former (**5a**), which was identified as methyl 13-methyltetradecanoate by comparison of the authentic

sample synthesized from 10-bromodecanoic acid *via* three steps (see Experimental). Therefore, the latter compound (**5b**) must be the dehydro-compound of **5a**. The presence and position of the double bond in **5b** were established as follows. Oxidation of the mixture (Product I) with osmium tetroxide afforded a γ-lactone [**7**, $C_{15}H_{28}O_3$ (M^+ 256); NMR ($CDCl_3$) δ 3.90 (1H, m), 4.45 (1H, m); IR ($CHCl_3$) 1765 cm^{-1}], and with *m*-chloroperbenzoic acid an epoxide (**8**). The analysis of the fragment peaks in the EI-MS of **8** (Fig. 1),[3] unequivocally determined the latter fatty acid methyl ester (**5b**) to be methyl 13-methyl-4-tetradecenoate. The foregoing experimental results suggested that WB-3559 A (**1**) and B (**2**) possess 13-methyl-4-tetradecenoic acid moiety, while WB-3559 C (**3**) and D (**4**) 13-methyl-tetradecanoic acid moiety in those molecules respectively.

On the other hand, product II obtained by methanolysis of WB-3559 was assumed to be a mixture of two compounds from its FD mass spectral data [**6a**: *m/z* 453 (M^++Na), n=11; **6b**: *m/z* 439 (M^++Na), n=10]. Acid hydrolysis (6 N HCl, 100°C, 20 hours) of product II (**6a** and **6b**) yielded glycine and serine (1:1), which were characterized by conventional amino acid analysis. After the hydrolysate was extracted with ethyl acetate, the residual aqueous solution was lyophilized and the residue was treated with carbobenzoxy chloride (Z-Cl) and subsequently with diazomethane to give Z-L-Ser-OMe ($[\alpha]_D^{20}$ +7.42° (*c* 0.7, $CHCl_3$); synthetic sample from L-serine: $[\alpha]_D^{20}$ +7.48° (*c* 1.0, $CHCl_3$)) together with Z-Gly-OMe. Consequently, all the serine molecules contained in WB-3559 should have L-configuration.

Table 1. The assignment of the signals in ^1H NMR spectrum of **9** (100 MHz, $CDCl_3$).

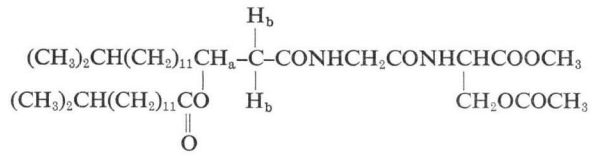
Chemical shift (ppm)	Multiplicity (*J* in Hz)	Assignment
		C |
7.12	d (8.0)	CON*H*CHC
6.70	t (4.5)	CON*H*CH_2
5.20	tt (6.0 and 6.0)	*C*HCH_2CO | –O
4.82	td (4.0 and 8.0)	NHC*H*$COOCH_3$ | CH_2COOCH_3
4.40 (2H)	d (4.0)	*C*H_2OAc
4.00 (2H)	d (4.5)	CONHC*H*$_2$CO
3.80 (3H)	s	$COOCH_3$
2.54 (2H)	d (6.0)	CHC*H*$_2$CO | –O
2.06 (3H)	s	$OCOCH_3$
0.85 (6H)	d (6.0)	*C*H_3 \>CH *C*H_3

In order to disclose the unknown structure of the acyl part in WB-3559, structure analysis was focused on the isolated component: WB-3559 D (**4**). **4** was transformed into the acetyl methyl ester derivative (**9**) by methylation with diazomethane followed by treatment with acetic anhydride in pyridine. In the ^1H NMR spectrum (100 MHz, $CDCl_3$, Fig. 2) of **9**, irradiation of a triplet triplet signal (H_a) at δ 5.20 (1H, J=6, 6 Hz) caused the decoupling of a doublet signal (H_b) at δ 2.54 (2H, J=6 Hz) into a singlet, indicating the presence of β-acyloxyacyl unit in the molecule of **9**. Further analysis of the ^1H NMR spectrum of **9** assisted by double resonance technique led to the assignments of all the signals as shown in Table 1.

The sequence of the β-acyloxyacyl unit, glycine and L-serine in the structure of WB-3559 D (**4**) was elucidated by analysis of the EI-MS of its methyl ester (**13**), derived from **4** on treatment with diazomethane. The structures of the other three components: WB-3559 A, B and C were clarified by the comparison of the mass fragment peaks in the EI-MS of their respective methyl esters **10**, **11** and **12** with those of **13**. Table 2 shows the assignment of each fragment peak, which is in good agreement with the corresponding chemical structures shown.

The foregoing experimental data and discussions enabled us to deduce the structures of WB-3559

Fig. 2. ^{1}H NMR spectrum of **9** (100 MHz, $CDCl_3$).



(9)

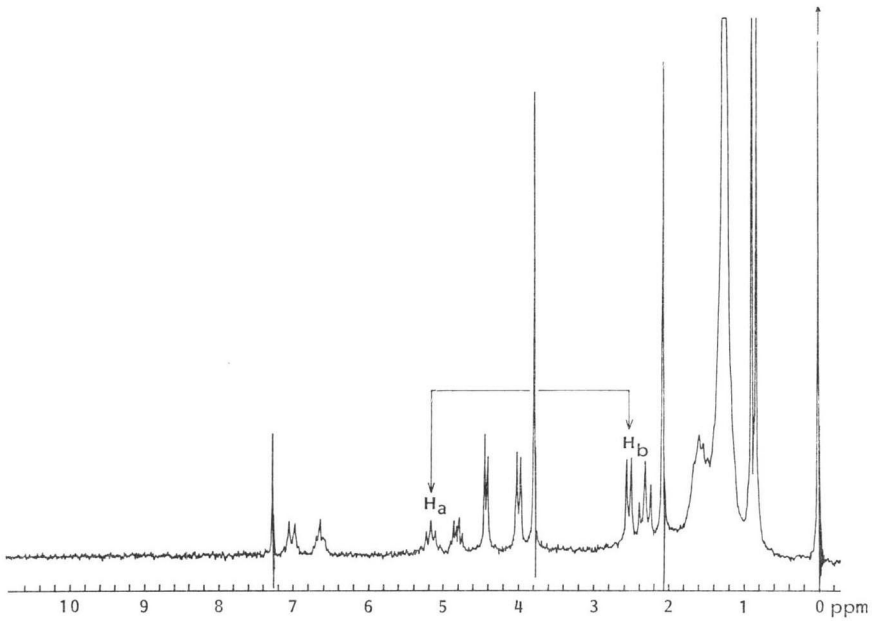
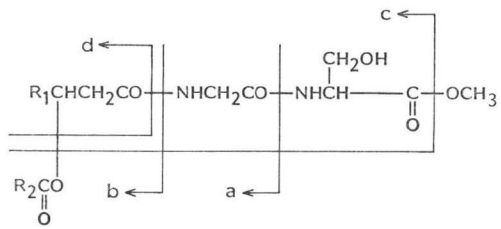


Table 2. EI mass fragmentation peaks of **10**, **11**, **12** and **13**.

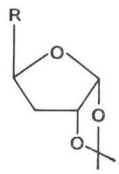


	WB-3559 A methyl ester (**10**)	WB-3559 B methyl ester (**11**)	WB-3559 C methyl ester (**12**)	WB-3559 D methyl ester (**13**)
	$R_1=(CH_3)_2CH(CH_2)_{10}$ $R_2=(CH_3)_2CH(CH_2)_7$-$CH=CHCH_2CH_2$	$R_1=(CH_3)_2CH(CH_2)_{11}$ $R_2=(CH_3)_2CH(CH_2)_7$-$CH=CHCH_2CH_2$	$R_1=(CH_3)_2CH(CH_2)_{10}$ $R_2=(CH_3)_2CH(CH_2)_{11}$	$R_1=(CH_3)_2CH(CH_2)_{11}$ $R_2=(CH_3)_2CH(CH_2)_{11}$
M^+	652	666	654	668
a	534	548	536	550
b	477	491	479	493
c	382	396	382	396
d	237	251	237	251

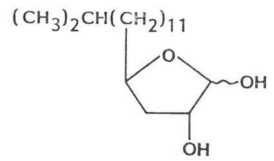
A, B, C and D as shown in **1**, **2**, **3** and **4**, respectively, except for the absolute configurations of the acyl part in those molecules. The structure of WB-3559 D (**4**) including the remaining absolute configuration was established by the total synthesis as described in the following section.

Total Synthesis of WB-3559 D

The aldehyde **14**[4] obtained from D-glucose was used as the optically active starting material. The Wittig reagent, 11-methyldodecyltriphenylphosphonium chloride (**15**), was synthesized from 1,10-dichlorodecane by the following treatment: (1) NaI/CH_3COCH_3, (2) $(CH_3)_2CHMgCl$, $CuCl_2$, LiCl, (3) $(C_6H_5)_3P$. 11-Methyldodecyltriphenylphosphorane prepared from **15** with *n*-BuLi *in situ*, was subjected to the Wittig reaction with **14** to furnish the olefine **16**. Catalytic hydrogenation of **16** over PtO_2 gave the acetonide **17** quantitatively. The 1,2-isopropylidene group of **17** was removed by heating with 0.1 N HCl in aqueous dioxane for two hours to lead to the anomeric mixture **18** in 92% yield. Treatment of **18** with sodium metaperiodate resulted in the diol cleavage to provide the linear β-hydroxyaldehyde (**19**). The conversion of **19** into the β-hydroxycarboxylic acid (**20**) was carried out in the three steps (51% overall yield): (1) protection of the alcohol with dihydropyran, *p*-toluenesulfonic acid (*p*-TsOH), (2) oxidation of the aldehyde with Ag_2O into the carboxylic acid, (3) deprotection of the tetrahydropyranyl group with 5% citric acid. The acid **20** thus synthesized showed $[\alpha]_D^{23}$ −12.0° (*c* 1.0, $CHCl_3$). The specific rotation of the methyl ester of **20** prepared by treatment with diazomethane, was $[\alpha]_D^{20}$ −12.6° (*c* 0.1, $CHCl_3$). The negative sign of the specific rotation in $CHCl_3$[5] of the methyl ester is consistent with the (*R*) configuration at the β-carbon atom of the β-hydroxycarboxylic acid **20**. As acylations of the alcohol of the key intermediate **20** was not successful, the *N*-hydroxysuccinimide ester of **20** was first condensed with glycine benzyl ester to lead to the *N*-acylglycine deriva-



14 R=CHO
16 R=CH=CH$(CH_2)_9CH(CH_3)_2$
17 R=$(CH_2)_{11}CH(CH_3)_2$



18

$(CH_3)_2CH(CH_2)_{10}P^+(C_6H_5)_3Cl^-$

15

$(CH_3)_2CH(CH_2)_{11}CHCH_2CHO$ (OH)

19

$(CH_3)_2CH(CH_2)_{11}\underset{\displaystyle OR_1}{\overset{(R)}{C}}HCH_2\underset{\displaystyle O}{C}R_2$

20 R_1=H, R_2=OH
21 R_1=H, R_2=$NHCH_2COOCH_2C_6H_5$
22 R_1=$CO(CH_2)_{11}CH(CH_3)_2$, R_2=$NHCH_2COOCH_2C_6H_5$
23 R_1=$CO(CH_2)_{11}CH(CH_3)_2$, R_2=$NHCH_2COOH$
4 R_1=$CO(CH_2)_{11}CH(CH_3)_2$, R_2=$NHCH_2CONH\overset{(S)}{C}HCOOH$ (CH_2OH)

tive **21**, and then acylation of **21** with 13-methyltetradecanoyl chloride was effected to give **22** (overall yield 64% from **20**). 13-Methyltetradecanoic acid[6] was synthesized as follows: (1) Wittig condensation of isobutyraldehyde and 10-carboxydecyltriphenylphosphorane in DMSO-THF, 56% yield, (2) hydrogenation over Pd-black at 3.5~4.0 atm, 84% yield. After hydrogenation of **22** over Pd-black in acetic acid, the resulting *N*-acylglycine **23** was finally coupled with L-serine by active ester procedure to afford *N*-[*N*-[15-methyl-3(*R*)-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (**4**), $[\alpha]_D^{22}$ +19.5° (*c* 0.3, $CHCl_3$), which was all identical with the natural WB-3559 D.[1]

The total synthesis of WB-3559 D thus achieved, disclosed the (*R*) configuration of the assymetric center in the acyl part of the natural product (**4**). The analogy of the $[\alpha]_D$ values among WB-3559 A, B, C and D allowed the assignment of the absolute configurations in the acyl part of these four natural products to be all (*R*) configuration.

Experimental

The IR spectra were recorded with a Jasco IRA-2 spectrophotometer. ^1H NMR spectra were measured on a Jeol PMX-60 or a Jeol PS-100 spectrophotometer, and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; br, broad; m, multiplet). EI-MS and FD-MS spectra were recorded using a Hitachi M-80 mass spectrometer and a Jeol JMS-D-300 mass spectrometer, respectively. Melting points were measured with a Yanagimoto microscope hot-stage apparatus and are uncorrected. Preparative thin-layer chromatography (TLC) was carried out on a Merck Silica gel F254 pre-coated plate, Art 5744.

Catalytic Hydrogenation of WB-3559

A solution of WB-3559 (10 mg) in MeOH (2 ml) was hydrogenated over 10% Pd-C (10 mg) under H_2 at atmospheric pressure. After removal of the catalyst by filtration, the filtrate was evaporated to give a residue (9 mg), which was subjected to liquid chromatography on the conditions used in the preceding paper,[1] showing that the products contain WB-3559 C and D in the ratio of 1:4.

Methanolysis of WB-3559

To a solution of WB-3559 (190 mg) in MeOH (8 ml) was added a solution of 28% CH_3ONa in MeOH (150 μl) and the resulting solution was stirred at room temp for 2 hours. The reaction mixture was poured into ice water and the mixture was acidified with 1 N HCl, extracted with EtOAc and ether. The extracts were combined, washed with H_2O, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was purified by preparative TLC, eluted with a mixture of benzene - dioxane - acetic acid (10: 5: 1) to give product I (oil, Rf 0.9, 62 mg) and product II (powder, Rf 0.1, 109 mg).

GC-EI-MS of product I showed that it consists of two compounds in the ratio of *ca.* 1:1, and their molecular weights are 256 (**5a**, retention time: 7 minutes) and 254 (**5b**, retention time: 7.8 minutes).

The GC-MS conditions are as follows. Instrument: Shimadzu QP 1000. Capillary column GC column: fused silica ULBON HR-52 (0.32 mm i.d. ×25 m); temperatures: column 160°C isothermal, injector 220°C; carrier gas: He, 1 ml/minute. MS ion source temp: 250°C, separator temp: 250°C; electron energy: 20 eV.

The product II was shown to be a mixture of two acyl peptides (**6a** and **6b**) from the following physical data: IR (Nujol) 3250, 2950, 2850, 1640 cm^{-1}; NMR (CD_3OD) δ 0.85 (6H, d, J=7 Hz), 1.0~1.6 (21~23H, m), 2.38 (2H, d, J=6 Hz), 3.6~4.0 (5H, m), 4.36 (1H, m); FD-MS *m/z* 453 (M^++Na, **6a**), 439 (M^++Na, **6b**).

Catalytic Hydrogenation of the Mixture of 5a and 5b

The mixture (9 mg) of **5a** and **5b** obtained by methanolysis of WB-3559, was dissolved in a mixture of MeOH (1 ml) and EtOAc (1 ml). This solution was hydrogenated over 10% Pd-C (10 mg) under H_2 at atmospheric pressure for 5 hours. After removal of the catalyst by filtration, the filtrate was evaporated to give a residue, which was purified by preparative TLC, developed with a mixture of

EtOAc - hexane (9: 1) to afford methyl 13-methyltetradecanoate (**5a**, 8 mg) as an oil: IR ($CHCl_3$) 2920, 2850, 1725, 1465, 1440 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (6H, d, J=7 Hz), 1.0~1.45 (19H, m), 1.45~1.80 (3H, m), 2.30 (2H, t, J=7 Hz), 3.67 (3H, s); EI-MS m/z 256 (M^+).

γ-Lactone (**7**)

The mixture (30 mg) of **5a** and **5b** obtained by methanolysis of WB-3559, was dissolved in ether (2 ml). To this solution was added OsO_4 (25 mg), and the mixture was stirred at room temp under a nitrogen atmosphere overnight. After removal of the solvent, the black residue was dissolved in pyridine (1 ml), and then a solution of $NaHSO_3$ (140 mg) in H_2O (1 ml) was added. The resulting mixture was stirred at room temp for 5 hours, followed by addition of H_2O (10 ml) and by extraction with EtOAc (10 ml×2). The EtOAc solution was washed with dil HCl, H_2O and dried over $MgSO_4$ and then evaporated *in vacuo* to give a residue (29 mg), which was purified by preparative TLC, eluted with MeOH - $CHCl_3$ (1: 20) to afford **7** (10 mg) and **5a** (14 mg) as oils. Compound **7**: IR ($CHCl_3$) 3600, 3450, 2940, 2850, 1765, 1180 cm^{-1}; NMR ($CDCl_3$) δ 0.86 (6H, d, J=7 Hz), 1.0~1.7 (14H, m), 2.0~2.65 (6H, m), 3.9 (1H, m), 4.45 (1H, m); EI-MS m/z 256 (M^+).

Epoxide **8**

The mixture (20 mg) of **5a** and **5b** obtained by methanolysis of WB-3559, was dissolved in CH_2Cl_2 (1 ml). To this solution was added *m*-chloroperbenzoic acid (18 mg) and the resulting mixture was stirred at room temp for 3 hours. The reaction mixture was poured into H_2O (10 ml) and extracted with $CHCl_3$ (10 ml×2). The $CHCl_3$ solution was washed with aq $NaHCO_3$, H_2O, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by preparative TLC, eluted with ether - heptane (3: 7) to afford **8** (6 mg) and **5a** (9 mg) as oils.

Compound **8**: NMR ($CDCl_3$) δ 0.85 (6H, d, J=7 Hz), 1.0~2.0 (17H, m), 2.45 (2H, t, J= 7Hz), 2.91 (2H, m), 3.68 (3H, s); EI-MS m/z 270 (M^+), 239, 183, 129, 117, 85.

Acid Hydrolysis of the Mixture of **6a** and **6b**

The mixture (45 mg) of **6a** and **6b** obtained by methanolysis of WB-3559, was dissolved in 6 N HCl (7 ml). The resulting solution was heated at 100°C for 21 hours. After cooled, the solution was diluted with H_2O (20 ml) and extracted with EtOAc (15 ml×2). The residual aqueous solution was lyophilized to give a residue (15 mg), which was shown to contain glycine and serine (*ca.* 1: 1) by amino acid analysis. This residue was dissolved in 1 N NaOH (1 ml) and then carbobenzoxy chloride (100 mg) was added at 0°C. After the resulting mixture was stirred at 0°C for 3 hours, the reaction mixture was poured into ice water, and extracted with ether to remove excess carbobenzoxy chloride. The residual aqueous solution was acidified with 1 N HCl, extracted with EtOAc, washed with brine, and evaporated to dryness. The residue (14 mg) was purified by preparative TLC, eluted with EtOAc - $CHCl_3$ (1: 4) to give Z-Gly-OMe (5 mg) and Z-L-Ser-OMe (6 mg), which were all identical with those of authentic samples.

Z-Gly-OMe: NMR ($CDCl_3$) δ 3.72 (3H, s), 3.94 (2H, d, J=6 Hz), 5.12 (2H, s), 5.40 (1H, br), 7.35 (5H, m); EI-MS m/z 127 (M^+).

Z-L-Ser-OMe: NMR ($CDCl_3$) δ 3.75 (3H, s), 3.95 (2H, d, J=5 Hz), 4.40 (1H, m), 5.10 (2H, s), 5.80 (1H, br), 7.35 (5H, m); EI-MS m/z 153 (M^+); $[\alpha]_D^{20}$ +7.42° (*c* 0.7, $CHCl_3$), synthetic $[\alpha]_D^{20}$ +7.48° (*c* 1.0, $CHCl_3$).

Methyl Ester Derivative (**10**) of WB-3559 A

To a solution of WB-3559 A (5 mg) in methanol (1 ml) was added an ethereal solution of excess diazomethane. The resulting solution was kept in a refrigerator overnight, and then evaporated to dryness to give the methyl ester derivative (**10**, 5 mg) of WB-3559 A as a powder: IR ($CHCl_3$) 3400, 2930, 2850, 1725, 1660 cm^{-1}; EI-MS m/z 652 (M^+).

Methyl Ester Derivative (**11**) of WB-3559 B

WB-3559 B (5 mg) was methylated with diazomethane according to similar manner to the preparation of compound **10** to give the methyl ester derivative (**11**, 4 mg) of WB-3559 B as a powder: IR ($CHCl_3$) 3400, 2920, 2850, 1720, 1650 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (12H, d, J=6 Hz), 1.2~1.8

(36H, m), 2.0 (2H, m), 2.3~2.5 (6H, m), 3.77 (3H, s), 3.95 (4H, m), 4.60 (1H, m), 5.20 (1H, m), 5.35 (2H, m); EI-MS *m/z* 666 (M^+).

Methyl Ester Derivative (**12**) of WB-3559 C

WB-3559 C (13 mg) was methylated with diazomethane according to similar manner to the preparation of compound **10** to give the methyl ester derivative (**12**, 10 mg) of WB-3559 C as a powder: IR ($CHCl_3$) 3400, 2920, 2850, 1725, 1660 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (12H, d, J=6 Hz), 1.0~1.8 (42H, m), 2.30 (2H, m), 2.50 (2H, m), 3.78 (3H, s), 3.95 (4H, m), 4.60 (1H, m), 5.20 (1H, m); EI-MS *m/z* 654 (M^+).

Methyl Ester Derivative (**13**) of WB-3559 D

WB-3559 D (18 mg) was methylated with diazomethane according to similar manner to the preparation of compound **10** to give the methyl ester derivative (**13**, 14 mg) of WB-3559 D as a powder: IR ($CHCl_3$) 3400, 2920, 2850, 1725, 1660 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (12H, d, J=6 Hz), 1.0~1.8 (46H, m), 2.30 (2H, d, J=7 Hz), 2.45 (2H, d, J=7 Hz), 3.77 (3H, s), 3.95 (4H, m), 4.60 (1H, m), 5.20 (1H, m); EI-MS *m/z* 668 (M^+).

Acetyl Methyl Ester Derivative (**9**) of WB-3559 D

A solution of **13** (12 mg) and Ac_2O (0.5 ml) in pyridine (1 ml) was allowed to stand overnight at room temp. The reaction mixture was evaporated off using high vacuum pump to give a residue (14 mg), which was purified by preparative TLC, eluted with acetone - $CHCl_3$ (1: 9) to afford the acetyl methyl ester derivative (**9**, 13 mg) of WB-3559 D as an oil: NMR, see Table 1; EI-MS *m/z* 710 (M^+).

11-Methyldodecyl Chloride

A mixture of 1,10-dichlorodecane (32 g), NaI (23 g) and acetone (200 ml) was refluxed for 4 hours. The cooled mixture was filtered to remove the resultant NaCl and the filtrate was evaporated under reduced pressure. To the residue was added H_2O (100 ml) and the mixture was extracted with ether (50 ml×2). The extracts were washed with aq $Na_2S_2O_3$, H_2O and then dried over $MgSO_4$. After removal of the solvent, the residual oil was distilled under reduced pressure to give 1-chloro-10-iododecane (12 g, bp 140°C/2.5 mm) as a colorless oil.

To a suspension of Mg (1 g) in dry THF (20 ml) was added isopropyl chloride (3.6 ml), and the mixture was refluxed for 30 minutes. After cooled, the resulting Grignard reagent was slowly added to a stirred solution of 1-chloro-10-iododecane (12 g), LiCl (68 mg) and $CuCl_2$ (54 mg) in THF (20 ml) at 5°C. The mixture was stirred at the same temp for 1 hour under argon atmosphere, and poured into ice-water, and then extracted with ether. The organic solution was washed with 1 N HCl, water and dried over $MgSO_4$. Removal of the solvent gave an oil, which was distilled under reduced pressure to afford 11-methyldodecyl chloride (5 g) as a colorless oil: bp 106°C/0.6 mm; NMR ($CDCl_3$) δ 0.86 (6H, d, J=7 Hz), 1.20~1.80 (19H, m), 3.52 (2H, t, J=7 Hz).

11-Methyldodecyltriphenylphosphonium Chloride (**15**)

A mixture of 11-methyldodecyl chloride (2.32 g) and triphenylphosphine (2.8 g) was heated at 140°C for 12 hours. After cooled, the resulting solid was triturated with benzene and then ether to give a powder of 11-methyldodecyltriphenylphosphonium chloride (4.5 g), which was used for the next Wittig reaction without further purification.

(3a*R*,5*S*,6a*R*)-2,2-Dimethyl-5-(12-methyl-1-tridecenyl)furo-[2,3-d][1,3]dioxole (**16**)

To a solution of 11-methyldodecyltriphenylphosphonium chloride (2 g) in anhydrous THF (30 ml), was added a solution (2.6 ml, 1.56 M solution) of *n*-butyllithium in *n*-hexane slowly, and the resulting red-orange solution was stirred at room temp for 1 hour under dry nitrogen atmosphere. To this solution was added a freshly prepared solution of 1,2-*O*-isopropylidene-3-deoxy-D-*erythro*-pentodialdofuranose[4] (693 mg) in anhydrous THF (5 ml), and the mixture was stirred at ambient temp for 30 minutes and then at 50°C for 5 minutes under dry nitrogen atmosphere. After the solvent of the reaction mixture was removed, H_2O (30 ml) was added to the residue and the mixture was extracted

with *n*-pentane (20 ml×2). The extract was washed with brine, dried over $MgSO_4$ and evaporated to leave an oil, which was chromatographed on silica gel, eluted with $CHCl_3$ to give *cis* and *trans* mixture of **16** (0.88 g) as an oil: IR ($CHCl_3$) 2920, 2850, 1650, 1460, 1380, 1370, 1160, 1060, 1040, 1010 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (6H, d, J=7 Hz), 1.17~1.73 (25H, m), 1.97~2.28 (2H, m), 4.70 (1H, t, J=4 Hz), 4.83~5.67 (3H, m), 5.80 (1H, d, J=4 Hz); EI-MS m/z 338 (M^+).

(3a*R*,5*S*,6a*R*)-2,2-Dimethyl-5-(12-methyltridecyl)furo-[2,3-d][1,3]dioxole (**17**)

A solution of **16** (0.78 g) in EtOH (20 ml) was hydrogenated over PtO_2 (0.3 g) under H_2 at atmospheric pressure for 3 hours. After removal of the catalyst by filtration, the filtrate was evaporated to leave an oil, which was chromatographed on silica gel, eluted with $CHCl_3$ to give **17** (0.8 g) as an oil: IR ($CHCl_3$) 2920, 2850, 1460, 1380, 1370, 1210, 1010 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (6H, d, J=7 Hz), 1.32 (3H, s), 1.52 (3H, s), 1.12~1.60 (24H, m), 2.08 (1H, dd, J=14, 4 Hz), 4.16 (1H, m), 4.68 (1H, t, J=4 Hz), 5.78 (1H, d, J=4 Hz); EI-MS m/z 340 (M^+).

(2*RS*,3*R*,5*R*)-5-(12-Methyltridecyl)tetrahydrofuran-2,3-diol (**18**)

To a solution of **17** (370 mg) in dioxane (4.2 ml) was added H_2O (1.2 ml) and 1 N HCl (0.6 ml). The mixture was stirred at 70°C for 2 hours and then neutralized with 1 N NaOH. The resulting mixture was concd to dryness *in vacuo* to give a residue, which was diluted with H_2O and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, evaporated to dryness to leave an oil, which was purified by preparative TLC, developed with MeOH - $CHCl_3$ (1:9) to afford **18** (300 mg) as an oil: IR ($CHCl_3$) 3400, 2920, 2850, 1460, 1380, 1360, 1020 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (6H, d, J=7 Hz), 1.23~1.66 (26H, m), 2.00 (1H, m), 4.23~4.50 (2H, m), 5.40 (1H, m); FD-MS m/z 301 (M^++1).

15-Methyl-3(*R*)-hydroxyhexadecanal (**19**)

To a stirred solution of **18** (370 mg) in dioxane (10 ml) was added a solution of $NaIO_4$ (1.3 g) in H_2O (2 ml), and the mixture was stirred at room temp for 2 hours under argon atmosphere. The reaction mixture was concd to dryness *in vacuo* to leave a residue, to which H_2O was added. The mixture was extracted with $CHCl_3$, and extract was washed with brine, dried over $MgSO_4$ and then evaporated to give crude **19** (300 mg) as an oil: IR ($CHCl_3$) 3400, 2920, 2850, 2720, 1715, 1460, 1380, 1360, 1180 cm^{-1}; EI-MS m/z 270 (M^+).

15-Methyl-3(*R*)-hydroxyhexadecanoic Acid (**20**)

To a stirred solution of **19** (300 mg) and catalytic amount of *P*-TsOH·H_2O in anhydrous dioxane (4 ml) was added dihydropyran (1 ml) and the mixture was stirred at ambient temp for 1 hour under argon atmosphere. Removal of the solvent *in vacuo* left an oil, which was dissolved in ether. The solution was washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$, and then evaporated to give crude 15-methyl-3(*R*)-tetrahydropyranyloxyhexadecanol (310 mg) as an oil, which was dissolved in EtOH (4 ml). To this solution were added a solution of $AgNO_3$ (830 mg) in H_2O (0.8 ml) and a solution of KOH (1.3 g) in H_2O (2 ml). The heterogeneous mixture was stirred at room temp for 4 hours under argon atmosphere. The reaction mixture was diluted with H_2O and extracted with ether. The residual aqueous solution was acidified with 5% citric acid and extracted with $CHCl_3$. The chloroform solution was washed with brine, dried over $MgSO_4$ and evaporated to leave an oil, which was purified by preparative TLC on silica gel, developed with MeOH - $CHCl_3$ (1:4) to afford **20** (162 mg) as a powder: $[\alpha]_D^{23}$ −12.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3100, 2920, 2850, 1700, 1460, 1380, 1360 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (6H, d, J=7 Hz), 1.17~1.60 (23H, m), 2.50 (2H, d, J=6 Hz), 4.00 (1H, m), 6.77~7.00 (2H, br); FD-MS m/z 287 (M^++1).

Methyl 15-Methyl-3(*R*)-hydroxyhexadecanoate

To a solution of **20** (20 mg) in MeOH (2 ml) was added an ethereal solution of excess diazomethane. The solution was kept in a refrigerator overnight and then evaporated to dryness to give methyl 15-methyl-3(*R*)-hydroxyhexadecanoate (21 mg) as an oil: $[\alpha]_D^{20}$ −12.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 3550, 2920, 2850, 1720, 1460, 1440, 1175 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (6H, d, J=7 Hz), 1.00~1.80 (23H, m), 2.40 (2H, d, J=6 Hz), 2.80 (1H, br), 3.67 (3H, s), 4.00 (1H, m); EI-MS m/z 301 (M^++1).

15-Methyl-3(*R*)-hydroxyhexadecanoic Acid Succinimide Ester

To a solution of **20** (107 mg) and *N*-hydroxysuccinimide (48 mg) in a mixture of dioxane (2 ml) and EtOAc (2 ml), was added a solution of dicyclohexylcarbodiimide (78 mg) in EtOAc (0.5 ml) at 0°C. The mixture was stirred at room temp overnight, and the solvent was removed *in vacuo* to leave an oil, which was dissolved in EtOAc. The insoluble residue was filtered off, and the filtrate was washed with H_2O, brine and dried over $MgSO_4$. The EtOAc solution was evaporated to give a residue, which was treated with ether - EtOAc (1: 1). The insoluble residue was removed by filtration and the filtrate was evaporated *in vacuo* to afford 15-methyl-3(*R*)-hydroxyhexadecanoic acid succinimide ester (142 mg) as an oil: IR ($CHCl_3$) 3500, 2920, 2850, 1820, 1780, 1730, 1460, 1360, 1060 cm^{-1}; NMR ($CDCl_3$) δ 0.87 (6H, d, J=7 Hz), 1.10~1.63 (23H, m), 2.66~2.95 (6H, m), 4.07 (1H, m).

Benzyl *N*-(15-Methyl-3(*R*)-hydroxyhexadecanoyl)glycinate (**21**)

To a suspension of glycine benzyl ester *p*-toluenesulfonic acid salt (195 mg) in THF (2 ml) was added triethylamine (81 μl), and the mixture was stirred at room temp for 30 minutes. To this solution was added a solution of 15-methyl-3(*R*)-hydroxyhexadecanoic acid succinimide ester (74 mg) in THF (1 ml). The resulting solution was stirred at room temp for 2 hours, and then the solvent was removed *in vacuo* to leave a residue, which was dissolved in EtOAc. The EtOAc solution was washed with 1 N HCl, 5% Na_2CO_3, brine, and dried over $MgSO_4$. Removal of the solvent to dryness to afford **21** (80 mg) as an oil: IR ($CHCl_3$) 3400, 2920, 2850, 1730, 1660, 1520, 1380, 1080 cm^{-1}; NMR ($CDCl_3$) δ 0.90 (6H, d, J=7 Hz), 1.10~1.60 (23H, m), 2.50 (2H, d, J=7 Hz), 4.10 (1H, m), 4.20 (2H, d, J=5 Hz), 5.25 (2H, s), 6.40 (1H, m), 7.40 (5H, s); FD-MS m/z 434 (M^++1).

(10-Carboxydecyl)triphenylphosphonium Bromide

A mixture of 11-bromoundecanoic acid (13.3 g) and triphenylphosphine (13 g) was stirred at 85°C for 1 hour. After cooled, the resulting solid was dissolved in a hot solution of $CHCl_3$ - EtOH (20: 1). To the cooled mixture was added ether and the precipitate was collected by filtration. After this operation was repeated twice, the resulting powder was dried up by using vacuum pump to give (10-carboxydecyl)triphenylphosphonium bromide (24 g) as a powder: IR (Nujol) 3480, 3250, 1700, 1440, 1110, 760, 740, 720 cm^{-1}.

13-Methyl-11-tetradecenoic Acid

(10-Carboxydecyl)triphenylphosphonium bromide (6.85 g) and redistilled isobutyraldehyde (1.3 g) were dissolved in a mixture of dry THF (26 ml) and dry DMSO (34 ml). The resulting solution was added over a period of 45 seconds to NaH (1.3 g, from which the mineral oil had been previously removed) at 0~5°C under a nitrogen atmosphere. After being stirred for 2 hours at 5~10°C and then for 20 hours at room temp, the mixture was diluted with H_2O, acidified to pH 3 with 30% phosphoric acid, and extracted with *n*-hexane. The hexane extract was concd to an oil, taken up in benzene and extracted with 5% NaOH and the alkali washings were combined and washed with benzene. After acidification with 30% phosphoric acid, the mixture was extracted with ether and the ethereal extract was washed with H_2O, dried over $MgSO_4$, and evaporated to give the *cis*, *trans* mixture of 13-methyl-11-tetradecenoic acid (1.75 g) as an oil: IR (film) 3600~2400, 2940, 2870, 1710 cm^{-1}; NMR ($CDCl_3$) δ 0.95 (6H, d, J=6 Hz), 1.20~1.80 (14H, m), 1.87~2.70 (5H, m), 5.13~5.32 (2H, m), 10.0 (1H, br s); FD-MS m/z 241 (M^++1).

13-Methyltetradecanoic Acid[6]

A solution of 13-methyl-11-tetradecenoic acid (1 g) in EtOH (30 ml) was hydrogenated over Pd-black (200 mg) under H_2 at 3.5~4.0 atm for 3 hours. After removal of the catalyst by filtration, the filtrate was evaporated to give 13-methyltetradecanoic acid (0.85 g), which was crystallized from acetone: mp 53°C; IR (KBr) 2920, 2850, 1700, 1465, 1430, 1405 cm^{-1}; FD-MS m/z 243 (M^++1).

Methyl 13-Methyltetradecanoate

To a solution of 13-methyltetradecanoic acid (100 mg) in MeOH (5 ml) was added an ethereal solution of excess diazomethane. The resulting solution was kept in a refrigerator overnight and then

evaporated to dryness to give a residue, which was purified by preparative TLC, developed with a mixture of EtOAc - hexane (9: 1) to afford methyl 13-methyltetradecanoate (105 mg) as an oil: IR ($CHCl_3$) 2920, 2850, 1725, 1465, 1440 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (6H, d, J=7 Hz), 1.0~1.45 (19H, m), 1.45~1.80 (3H, m), 2.30 (2H, t, J=7 Hz), 3.67 (3H, s); EI-MS m/z 256 (M^+).

Benzyl *N*-[15-Methyl-3(*R*)-(13-methyltetradecanoyloxy)hexadecanoyl]glycinate (**22**)

To a solution of 13-methyltetradecanoic acid (252 mg) in dry benzene (5 ml) was added thionyl chloride (1 ml) and the mixture was refluxed for 90 minutes. After cooled, the mixture was concd *in vacuo* to give crude 13-methyltetradecanoyl chloride, to which was added a solution of **21** (80 mg) in dry pyridine (2 ml). The resulting mixture was stirred at 80°C for 2 hours and then the solution was evaporated to leave an oil which was dissolved in EtOAc. The EtOAc solution was washed with 1 N HCl, brine and dried over $MgSO_4$, and then evaporated to give an oil, which was purified by preparative TLC, eluted with MeOH - $CHCl_3$ (1: 99) to afford **22** (90 mg) as an oil: IR ($CHCl_3$) 3450, 2950, 2900, 1740, 1680, 1520, 1480, 1200 cm^{-1}; NMR ($CDCl_3$) δ 0.93 (12H, d, J=7 Hz), 1.23~1.77 (44H, m), 2.37 (2H, t, J=7 Hz), 2.60 (2H, d, J=7 Hz), 4.13 (2H, d, J=5 Hz), 5.23 (1H, m), 5.27 (2H, s), 6.37 (1H, m), 7.40 (5H, s); EI-MS m/z 657 (M^+).

N-[15-Methyl-3(*R*)-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (**23**)

A solution of **22** (86 mg) in AcOH (10 ml) was hydrogenated over Pd-black (20 mg) under H_2 at atmospheric pressure for 2 hours. After removal of the catalyst by filtration, the filtrate was evaporated to give **23** (75 mg) as an oil: IR ($CHCl_3$) 3200, 2950, 2900, 1730, 1670, 1480, 1380, 1360, 1260, 1100 cm^{-1}; NMR ($CDCl_3$-CD_3OD) δ 0.90 (12H, d, J=7 Hz), 1.15~1.80 (44H, m), 2.35 (2H, t, J= 7 Hz), 2.60 (2H, d, J=7 Hz), 4.10 (2H, s), 5.25 (1H, m); FD-MS m/z 568 (M^++1).

N-[*N*-[15-Methyl-3(*R*)-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (**4**)

To a solution of **23** (75 mg) and *N*-hydroxysuccinimide (17 mg) in a mixture of dioxane (2 ml) and EtOAc (2 ml) was added a solution of dicyclohexylcarbodiimide (29 mg) in EtOAc (2 ml) at 0°C. The mixture was stirred at room temp overnight, and then concd to dryness under reduced pressure to leave a residue, to which EtOAc was added. The insoluble residue was filtered off and the filtrate was evaporated to give crude succinic ester of **22**, which was dissolved in DMF (1 ml). To this solution was added a solution of L-serine (70 mg) in a mixture of H_2O (1.5 ml), DMF (2 ml) and Et_3N (93 μl). The resulting mixture was stirred at room temp for 4 hours, and then diluted with H_2O, acidified to pH 3 with 1 N HCl, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 0.1 N HCl, dried over $MgSO_4$, and evaporated to leave an oil, which was purified by preparative TLC on silica gel, eluted with benzene - dioxane - AcOH (10: 5: 1) to afford *N*-[*N*-[15-methyl-3(*R*)-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine (**4**) (50 mg) as a colorless wax: $[\alpha]_D^{22}$ +19.5° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) 3350, 2950, 2870, 1725, 1660 cm^{-1}; NMR ($CDCl_3$-CD_3OD) δ 0.85 (12H, d, J= 6 Hz), 1.10~1.50 (40H, m), 1.60 (4H, m), 2.32 (2H, t, J=7 Hz), 2.54 (2H, d, J=6 Hz), 3.92 (4H, m), 4.53 (1H, m), 5.20 (1H, m).

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