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Synthesis of Topostins B567 and D654 (WB-3559D, Flavolipin), DNA Topoisomerase I Inhibitors of Bacterial Origin

Takayuki Shioiri,* Yoshihiro Terao, Naoko Irako, and Toyohiko Aoyama

Faculty of Pharmaceutical Sciences, Nagoya City University
Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

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Abstract : Topostins B567 (**2b**) and D654 (**3b**) (WB-3559D, flavolipin) have been efficiently synthesized from 1,10-decanediol (**5**) in 11 and 13 steps, respectively, involving an asymmetric hydrogenation of the β-keto ester **14** using (*R*)-BINAP ruthenium bromide and a peptide coupling using diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$) as key steps.

Keywords: topostin; enzyme inhibitors; asymmetric reaction; coupling

Topostins were isolated from the culture broth of *Flexibacter topostinus* sp. nov., B-572, by Andoh and co-workers.[1] They have proved to be structurally novel inhibitors of mammalian DNA topoisomerase I. Topostin B comprises two components with the molecular weights of 553 and 567 in an equimolecular ratio, and was reported to be most active. Their structures have been tentatively assigned to be **1a** for topostin B-1 and / or **1b** for topostin B-2, shown in Fig. 1.[2] To establish the structure of topostin B, we synthesized[3] topostin B analogs having the proposed structures **1a** and **1b**. Although some of them proved to exhibit an inhibitory action against mammalian DNA topoisomerase I,[3b,3c] spectral comparisons of our synthesized compounds with natural topostin B have revealed that they are different from each other even in their carbon skeletons.

Topostin B-1 (1a) m+n = 25 and/or 26 **Topostin B-2 (1b)**

Fig. 1

Very recently, Ojika and co-workers[4] reinvestigated the isolation and structures of topostins, and conclusively determined the structures of original topostins B, B553 (**2a**) and B567 (**2b**), as well as new analogs named topostins D, D640 (**3a**) and D654 (**3b**), as shown in Fig. 2. Among them, the gross structure of topostin B567 was found to be identical with that of cytolipin[5] isolated from *Cytophaga johnsonae* while topostin D654 was identical with WB-3559D[6] isolated from *Flavobacterium* sp. No. 3559 and flavolipin[7] isolated from *Flavobacterium meningosepticum*. Furthermore, the western fragment of topostins B567 (**2b**) and D654 (**3b**), the (3*R*)-15-methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl group, was found to be quite

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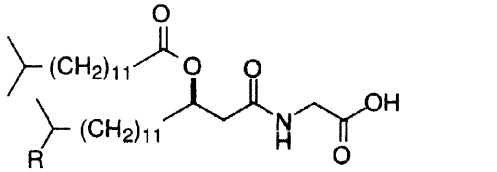
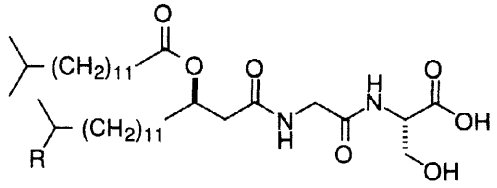
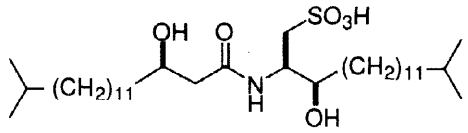
similar to the western fragment of sulfobacin A[8] (**4**, flavocristamide B[9]).[10] We now wish to report the synthesis of topostins B567 and D654. Our synthesis of D654 proved to be much more efficient than previous efforts.[6,7]



topostin B553 (2a) : R = H
topostin B567 (2b) : R = Me
(=cyclolipin)



topostin D640 (3a) : R = H
topostin D654 (3b) : R = Me
(= WB-3559D = flavolipin)

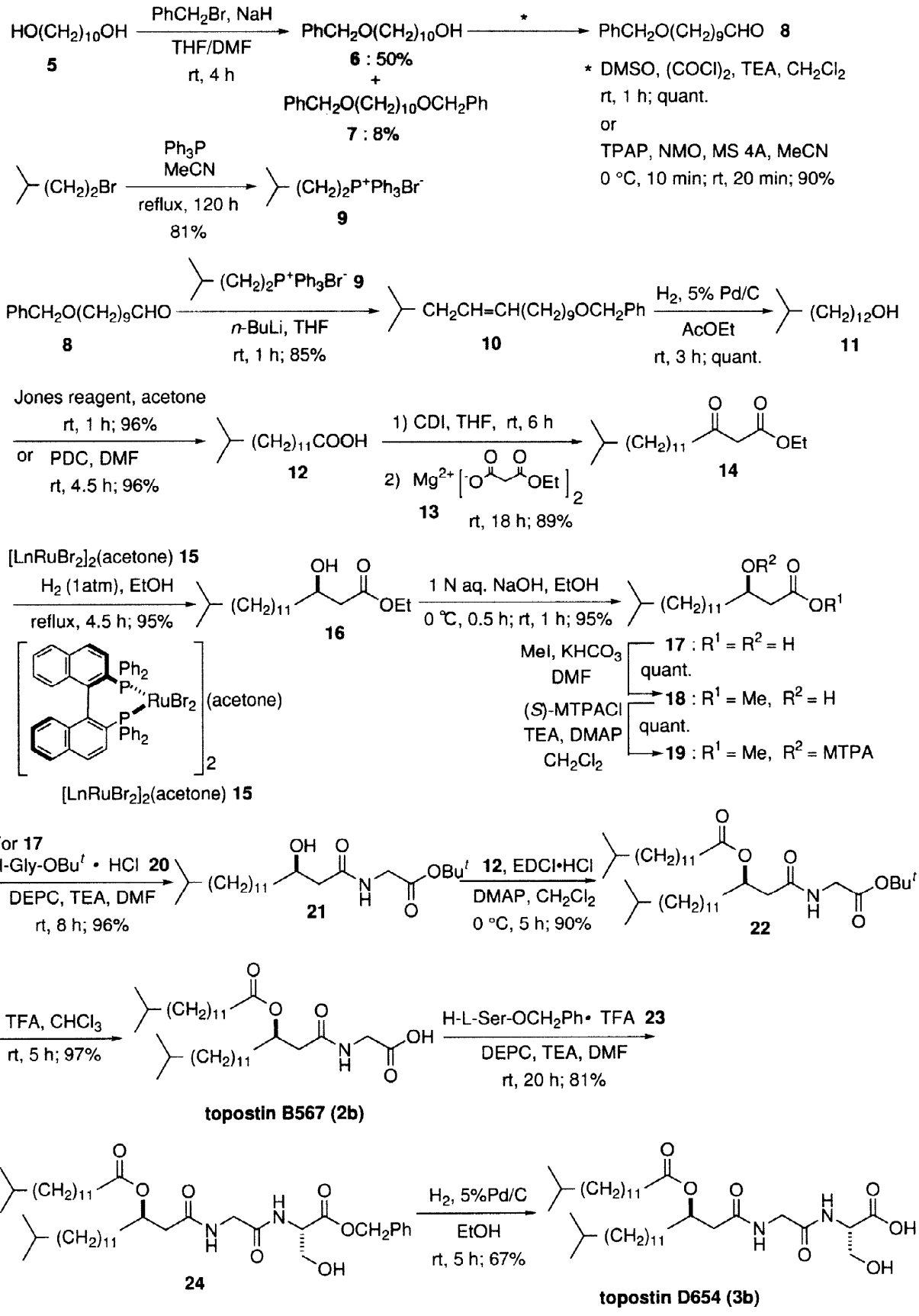


sulfobacin A (4)
(= flavocristamide B)

Fig. 2

The synthesis started from 1,10-decanediol (**5**), which was benzylated to give the monobenzyl ether **6** accompanied by a small amount of the dibenzyl ether **7**, as shown in Scheme 1. Swern oxidation of **6** or oxidation with tetra-*n*-propylammonium perruthenate (TPAP, Pr_4NRuO_4) and *N*-methylmorpholine-*N*-oxide (NMO) smoothly proceeded to give the aldehyde **8**, which underwent the Wittig reaction with the ylide derived from isoamyltriphenylphosphonium bromide (**9**) to give the olefin **10**. Catalytic hydrogenation of **10** over palladium-carbon quantitatively afforded the saturated alcohol **11**, which was oxidized with either Jones reagent or pyridinium dichromate (PDC) to give the carboxylic acid **12**. After conversion of **12** to the corresponding imidazolide with 1,1'-carbonyldiimidazole (CDI), the reaction with the magnesium enolate **13** of ethyl hydrogen malonate afforded the β-keto ester **14** in good yield.[11] Asymmetric hydrogenation of **14** smoothly proceeded by use of the ruthenium (II) catalyst **15** having (*R*)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl ((*R*)-BINAP, Ln) as a ligand[12] to give the required hydroxy ester **16**, which was saponified to give the corresponding acid **17**, whose spectral data and the specific rotation were identical with those reported,[6b] proving its absolute configuration to be *R*. The enantioselectivity of the asymmetric hydrogenation was determined by conversion of **17** to its (*S*)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivative **19** via the methyl ester **18** and measurement of the NMR spectrum, revealing that the carboxylic acid **17** was almost enantiomerically pure.

Condensation of the hydroxy acid **17** with glycine *tert*-butyl ester hydrochloride (H-Gly-OBu^t •HCl, **20**) smoothly proceeded by use of diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$)[13] in the presence of triethylamine (TEA) to give the amide **21**, which underwent the *O*-acylation with the carboxylic acid **12** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI•HCl) in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) to afford the ester **22**. Final acidic deprotection of **22** with trifluoroacetic acid (TFA) produced topostin B567 (**2b**). Furthermore, coupling of topostin B567 (**2b**) with L-serine benzyl ester trifluoroacetate (H-L-Ser-OCH_2Ph•TFA, **23**) by use of DEPC furnished the benzyl ester **24** of topostin D654, which underwent the removal of the benzyl group by catalytic hydrogenolysis to give topostin D654


HO(CH2)10OH
5
PhCH2Br, NaH
THF/DMF
rt, 4 h
PhCH2O(CH2)10OH
6 : 50%
+
PhCH2O(CH2)10OCH2Ph
7 : 8%
*
PhCH2O(CH2)9CHO 8
* DMSO, (COCl)2, TEA, CH2Cl2
rt, 1 h; quant.
or
TPAP, NMO, MS 4A, MeCN
0 °C, 10 min; rt, 20 min; 90%
(CH2)2Br
Ph3P
MeCN
reflux, 120 h
81%
(CH2)2P+Ph3Br-
9
PhCH2O(CH2)9CHO
8
(CH2)2P+Ph3Br- 9
n-BuLi, THF
rt, 1 h; 85%
CH2CH=CH(CH2)9OCH2Ph
10
H2, 5% Pd/C
AcOEt
rt, 3 h; quant.
(CH2)12OH
11
Jones reagent, acetone
rt, 1 h; 96%
or PDC, DMF
rt, 4.5 h; 96%
(CH2)11COOH
12
1) CDI, THF, rt, 6 h
2) Mg2+
13
rt, 18 h; 89%
14
[LnRuBr2]2(acetone) 15
H2 (1atm), EtOH
reflux, 4.5 h; 95%
16
1 N aq. NaOH, EtOH
0 °C, 0.5 h; rt, 1 h; 95%
MeI, KHCO3
DMF
quant.
17 : R1 = R2 = H
18 : R1 = Me, R2 = H
(S)-MTPACl
TEA, DMAP
CH2Cl2
quant.
19 : R1 = Me, R2 = MTPA
Ph2
RuBr2
(acetone)
2
[LnRuBr2]2(acetone) 15
For 17
H-Gly-OBut • HCl 20
DEPC, TEA, DMF
rt, 8 h; 96%
21
12, EDCI•HCl
DMAP, CH2Cl2
0 °C, 5 h; 90%
22
TFA, CHCl3
rt, 5 h; 97%
H-L-Ser-OCH2Ph• TFA 23
DEPC, TEA, DMF
rt, 20 h; 81%
topostin B567 (2b)
24
H2, 5%Pd/C
EtOH
rt, 5 h; 67%
topostin D654 (3b)

Scheme 1

(**3b**). Topostins B567 and D654 thus synthesized were identical with samples derived from the natural source.[4]

In summary, we have achieved an efficient synthesis of topostins B567 and D654 in 11 and 13 steps, respectively. Most of the synthetic steps, except the preparation of the monobenzyl ether **6**, proceed in greater than 90% yield. Our syntheses of topostins B567 and D654 are equivalent to the syntheses of the compounds having the gross structure of cytolipin and WB-3559D (hence flavolipin), respectively. The synthetic method is quite straightforward and will be useful for the synthesis of topostin analogs.

Experimental

Melting points were determined on a YAMATO MP-21 apparatus. Distillation was carried out using a Kugelrohr apparatus. All melting and boiling points were uncorrected. Infrared (IR) spectra were measured with a SHIMADZU FTIR-8100 spectrometer. ^{1}H and ^{13}C Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL EX-270 or α-500 spectrometer with tetramethylsilane (TMS) or $CHCl_3$ as an internal standard. Mass spectra were obtained on a JEOL SX 102A or AX 505HA spectrometer. Optical rotations were measured on a JASCO DIP-1000 automatic polarimeter. Silica gel BW-820MH or BW-200 (purchased from Fuji Silysia Chemical Co., Ltd.) was used for column chromatography. Tetrahydrofuran (THF) was dried by distillation from sodium benzophenone ketyl. Diethyl ether (Et_2O) was dried by distillation from lithium aluminum hydride. Other solvents were distilled and stored over molecular sieves MS 4A. MS 4A powder was dried at 80 °C for 12 h and then at 140 °C for 24 h under reduced pressure before use.

10-Benzyloxy-1-decanol (6). To a stirred suspension of NaH (60% oil dispersion, 1.61 g, 40.3 mmol) in DMF (30 ml) was added dropwise a solution of 1,10-decanediol (**5**) (6.99g, 40.1 mmol) in THF-DMF (40 ml-20 ml) at 0 °C under argon. The mixture was stirred at 0 °C for 2 h, and benzyl bromide (5.00 ml, 42.0 mmol) was added dropwise to the mixture at 0 °C. After stirring at room temperature for 4 h, water (100 ml) was added to the mixture at 0 °C, and the mixture was extracted with Et_2O (2×100 ml). The extracts were washed with saturated aqueous NaCl, and dried over Na_2SO_4. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-820 MH, 330 g) with hexane-EtOAc (5 : 1→1 : 1) to give the monobenzylated product **6** (5.33 g, 50%) as a colorless oil and the dibenzylated product **7** (1.07 g, 8%) as a colorless oil. For the monobenzylated product **6**, bp 180 °C / 8 mmHg. IR ν_{max} (film) : 3370, 2928, 1497, 1455, 1364, 1101, 1075, 1057, 1028, 735 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ : 1.29 (12 H, brs), 1.49-1.64 (5 H, m, 1 H exchangeable with D_2O), 3.46 (2 H, t, J = 6.6 Hz), 3.63 (2 H, t, J = 6.6 Hz), 4.50 (2 H, s), 7.27-7.38 (5 H, m). Anal. Calcd for $C_{17}H_{28}O_2$: C, 77.22 ; H, 10.67. Found : C, 76.99; H, 10.80.

The dibenzylated product **7**, mp 27 °C. IR ν_{max} (nujol) : 2926, 1455, 1364, 1120, 733 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ : 1.27 (12 H, brs), 1.34-1.63 (4 H, m), 3.46 (4 H, t, J = 6.6Hz), 4.50 (4 H, s), 7.25-7.37 (10 H, m). Anal. Calcd for $C_{24}H_{34}O_2$: C, 81.31 ; H, 9.67. Found ; C, 81.27 ; H, 9.90.

10-Benzyloxydecanal (8). **(method a)** To a solution of oxalyl chloride (3.45 ml, 39.5 mmol) in CH_2Cl_2 (25 ml) was added dropwise DMSO (3.30 ml, 46.5 mmol) in CH_2Cl_2 (10 ml) at -78 °C, and the mixture was stirred at -78 °C for 0.5 h. The monobenzylated product **6** (3.50 g, 13.2 mmol) in CH_2Cl_2 (35 ml) was added at -78 °C, and the mixture was stirred -78 °C for 0.5 h. After TEA (9.40 ml, 67.4 mmol) was added, the mixture was stirred at room temperature for 1 h. Water (70 ml) was then added, and the mixture

was extracted with $CHCl_3$ (2×150 ml). The extracts were washed with saturated aqueous NaCl, and dried over Na_2SO_4. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-820 MH, 400 g) with hexane-EtOAc (5:1) to give the aldehyde **8** (3.50 g, quant.) as a colorless oil, IR ν_{max} (film) : 2930, 1725, 1455, 1364, 1102, 735 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 1.29 (10 H, brs), 1.37-1.61 (4 H, m), 2.41 (2 H, dt, J = 7.3, 2.0 Hz), 3.64 (2 H, t, J = 6.6Hz), 4.50 (2 H, s), 7.22-7.38 (5 H, m), 9.76 (1 H, t, J = 2.0 Hz). HRMS Calcd for $C_{17}H_{26}O_2$: 262.1933. Found : 262.1932.

(method b) To a mixture of **6** (4.045 g, 15.3 mmol), NMO (3.515 g , 30.0 mmol) and MS-4A (5 g) in MeCN (100 ml) was added TPAP (351 mg, 1 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min and then at room temperature for 20 min. The mixture was filtered through a pad of celite and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820MH, 40 g) with hexane : EtOAc (5 : 1) to give **8** (3.58 g, 90%) as a pale yellow oil.

Isoamyltriphenylphosphonium bromide (9). A mixture of 1-bromo-3-methylbutane (0.605 g, 4.00 mmol) and triphenylphosphine (1.10 g, 4.19 mmol) in MeCN (11 ml) was heated at reflux for 120 h. After removal of the volatiles, a mixture of benzene and Et_2O (10:1, 11 ml) was added. The resulting white solid was collected and washed with Et_2O to give the phosphonium salt **9** (1.35g, 81%) as a white solid, which was used for the next reaction without further purification but dried at 80 °C for 12 h and then at 140 °C for 24 h under reduced pressure before use. IR ν_{max} (nujol) : 2922, 1586, 1435, 1377, 1111, 995, 743, 722 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.95 (6 H, d, J = 6.6 Hz), 1.42-1.54 (2 H, m), 1.95-2.04 (1 H, m), 3.68-3.79 (2 H, m), 7.68-7.89 (m, 15 H).

14-Benzyloxy-2-methyl-4-tetradecene (10). To a stirred suspension of the phosphonium salt **9** (8.16 g, 19.7 mmol) in THF (140 ml) at -10 °C under argon was added dropwise a solution of *n*-butyllithium (1.68 M in hexane, 15.6 ml, 26.2 mmol). The reaction mixture was stirred at -10 °C for 1 h, and then the aldehyde **8** (4.27 g, 16.3 mmol) in THF (35 ml) was added. After the mixture was stirred at -10 °C for 10 min and at room temperature for 1 h, ice water (100 ml) was added, and the mixture was extracted with Et_2O (2 ×150 ml). The extracts were washed with saturated aqueous NaCl (100 ml), and dried over $MgSO_4$. Concentration in vacuo gave the residue, which was purified by silica gel column chromatography (BW-200, 400 g) with hexane-benzene (4 : 1→1 : 1) to give the olefin **10** (4.39 g, 85%) as a colorless oil, bp 200 °C / 5 mmHg. IR ν_{max} (film) : 2926, 1455, 1102, 1028, 733 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.89 (6 H, d, J = 6.6 Hz), 1.28 (10 H, brs), 1.52-1.64 (5 H, m), 1.86-2.02 (4H, m), 3.46 (2 H, t , J = 6.6 Hz), 4.50 (2 H, s), 5.31-5.45 (2 H, m), 7.27-7.35 (5 H, m). HRMS Calcd for $C_{22}H_{36}O$: 316.2766. Found : 316.2768.

13-Methyl-1-tetradecanol (11). A mixture of the olefin **10** (1.91 g, 6.02 mmol) and 5% Pd-C (10.54 g) in EtOAc (100 ml) under an atmosphere of H_2 was stirred at room temperature for 3 h. The mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo to give the alcohol **11** (11.4 g, quant.) as colorless crystals, mp 26-27 °C (lit.[14] 26-28 °C). IR ν_{max} (film) : 3338, 2924, 1466, 1377, 1366, 1057, 722 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.26 (20 H, brs), 1.47-1.59 (4 H, m, 1 H, exchangeable with D_2O), 3.64 (2 H, t, J = 6.6 Hz). (lit.[14] 3.64 (2 H, t, J = 6.5 Hz)). Anal. Calcd for $C_{15}H_{32}O$: C, 78.88 ; H, 14.12. Found : C, 78.70 ; H, 14.18.

13-Methyltetradecanoic Acid (12) (method a) To a stirred solution of the alcohol **11** (404 mg,

1.77 mmol) in acetone (7 ml) at 0 °C was added dropwise Jones reagent (1.92 mol / L, 1.70 ml, 3.26 mmol). After stirring at room temperature for 1 h, water (50 ml) was added, and the mixture was extracted with Et_2O (80 ml). The extracts were washed with saturated aqueous NaCl, and dried over $MgSO_4$. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-200, 75 g) with hexane-EtOAc (2 : 1) to give the carboxylic acid **12** (411 mg, 96%) as a white solid, mp 46-47.5 °C (lit.[14] 44-46 °C). IR ν_{max} (nujol) : 2918, 1700, 1408, 1286, 932, 750 cm^{-1}. (lit.[14] 1700). ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.26 (18 H, brs), 1.43-1.66 (3 H, m), 2.35 (2 H, t, J = 7.4 Hz). (lit.[14] 2.35 (2 H, t, J = 7.6 Hz)). Anal. Calcd for $C_{15}H_{30}O_2$: C, 74.33 ; H, 12.47. Found : C, 73.94 ; H, 12.36.

(method b) A mixture of **11** (648 mg, 2.8 mmol) and PDC (10.65 g, 28 mmol) in DMF (40 ml) was stirred at room temperature for 4.5 h. The mixture was made alkaline with 1 N aqueous NaOH and washed with Et_2O. The aqueous layer was made acidic with 1 N aqueous $KHSO_4$ and extracted with EtOAc. The extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-820 MH, 70 g) with hexane : EtOAc (3 : 1) to give **12** (649 mg, 96%) as a white solid.

Ethyl 15-Methyl-3-oxohexadecanoate (14). To a solution of the carboxylic acid **12** (270 mg, 1.11 mmol) in THF (6 ml) was added CDI (221 mg, 1.34 mmol) and the mixture was stirred at room temperature for 6 h to form the imidazolide. Magnesium ethoxide (77 mg, 0.673 mmol) was added to a solution of ethyl hydrogen malonate (180 mg, 1.36 mmol) in THF (3 ml), and the mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure to give the magnesium enolate **13** as a yellow amorphous solid, which was used for the next reaction directly. To the magnesium salt **13** was added the above solution of the imidazolide in THF. After stirring at room temperature for 18 h, Et_2O (60 ml) was added, and the mixture was washed with 1N HCl (30 ml), water (30 ml), and saturated aqueous $NaHCO_3$ (30 ml), and dried over $MgSO_4$. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-820 MH, 35 g) with hexane-EtOAc (10 : 1) and then EtOAc alone to give the β-keto ester **14** (311 mg, 89%) as a pale yellow oil. IR ν_{max} (film) : 2924, 1748, 1717, 1418, 1235, 1159, 1034 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.16-1.25 and 1.25 (21 H, m and brs), 1.47-4.57 (3H, m), 2.53 (2 H, t, J = 7.3 Hz), 3.43 (2 H, s), 4.20 (2 H, q, J = 7.3 Hz). Anal. Calcd for $C_{19}H_{36}O_3$: C, 73.03 ; H, 11.61. Found : C, 72.77 ; H, 11.55.

(3*R*)-Ethyl 3-Hydroxy-15-methylhexadecanoate (16). To a stirred suspension of (*R*)-BINAP (41 mg, 0.066 mmol) and Ru(1,5-cyclooctadiene)(2-methylallyl)$_2$ (21 mg, 0.066 mmol) in anhydrous acetone (10 ml) under argon was added methanolic HBr (0.60 ml of a 0.29 M solution, 0.17 mmol). After stirring at room temperature for 0.5 h, the solvent was evaporated under vacuum to give the catalyst **15** as a brown solid. To the solid **15** was added a solution of the β-keto ester **14** (998 mg, 3.20 mmol) in EtOH (6.5 ml) under argon. The mixture was stirred at reflux for 4.5 h under H_2 atmosphere, and then filtered through a pad of celite. The filtrate was concentrated in vacuo to give a brown oil, which was purified by silica gel column chromatography (BW-200, 100 g) with hexane-Et_2O (2 : 1) to give the β-hydroxy ester **16** (957 mg, 95%) as a colorless oil, $[\alpha]^{23}_D$ -12.5° (c 1.02, $CHCl_3$). IR ν_{max} (film) : 3450, 2926, 1736, 1466, 1374, 1302, 1181, 1032 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.16-1.30 and 1.26 (23 H, m and brs), 1.36-1.59 (3 H, m), 2.39 (1 H, dd, J = 8.6, 16.5 Hz), 2.51 (1 H, dd, J = 16.5, 3.3 Hz), 2.92 (1 H, brs, exchangeable with D_2O), 3.99-4.00 (1 H, m), 4.18 (2 H, q, J = 7.2 Hz). Anal. Calcd for $C_{19}H_{38}O_3$: C, 72.56 ; H, 12.18. Found : C, 72.43 ; H, 12.28.

(3*R*)-3-Hydroxy-15-methylhexadecanoic Acid (17). To a solution of the β-hydroxy ester **16** (820 mg, 2.61 mmol) in EtOH (3.5 ml) at 0 °C was added dropwise 1 N aqueous NaOH (7 ml, 7.00 mmol). The mixture was stirred at 0 °C for 0.5 h, and then at room temperature for 1 h. After concentration in vacuo, the residue was acidified with 1 N HCl and extracted with Et_2O (10 ml). The extracts were dried over Na_2SO_4, and concentration in vacuo gave the β-hydroxy carboxylic acid **17** (712 mg, 95%) as a white solid, mp 46-47.5 °C. $[\alpha]^{23.1}_D$-12.0°(c 1.00, $CHCl_3$). [lit.[6b] $[\alpha]^{23.1}_D$-12.0°(c 1.0, $CHCl_3$)]. IR ν_{max}(nujol) : 3560, 2922, 1715, 1171 cm^{-1}. [lit.[6b] IR ν_{max}($CHCl_3$) : 3100, 2920, 2830, 1700, 1460, 1380, 1360cm^{-1}]. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.14-1.26 and 1.26 (20 H, m and brs), 1.44-1.56 (3 H, m), 2.47 (1 H, dd, J = 16.5, 8.4 Hz), 2.58 (1 H, dd, J = 16.5, 3.0 Hz), 4.00-4.03 (1 H, m). [lit.[6b] ^1H NMR ($CDCl_3$) δ : 0.87 (6 H, d, J = 7 Hz), 1.17-1.60 (23 H, m), 2.50 (2 H, d, J = 6 Hz), 4.00 (1 H, m)]. Anal. Calcd for $C_{17}H_{34}O_3 \cdot 1/2\ H_2O$: C, 69.11 ; H, 11.94. Found : C, 69.38 ; H, 11.92.

(3*R*)-Methyl 3-Hydroxy-15-methylhexadecanoate (18). To a solution of the β-hydroxy carboxylic acid **17** (100 mg, 0.349 mmol) in DMF (0.6 ml) was added $KHCO_3$ (70 mg, 0.7 mmol), followed by methyl iodide (0.04 ml, 0.642 mmol). The mixture was stirred at room temperature for 16.5 h. Water (20 ml) was added, and the mixture was extracted with EtOAc-benzene (2 : 1, 30 ml). The organic extracts were washed with water, 5% aqueous sodium sulfite, and saturated aqueous NaCl, then dried over $NaSO_4$. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-820 MH, 10 g) with hexane-EtOAc (10 : 1) to give the β-hydroxy methyl ester **18** (105 mg, quant.) as a colorless oil, $[\alpha]^{26.5}_D$-12.9°(c 1.00, $CHCl_3$) [lit.[6b] $[\alpha]^{20}_D$-12.6° (c 1.00, $CHCl_3$)]. IR ν_{max}(film) : 3455, 2924, 2855, 1740, 1466, 1439, 1198, 1171 cm^{-1}. [lit.[6b] IR ν_{max}(film) : 3550, 2920, 1720, 1460, 1440, 1175 cm^{-1}.] ^1H NMR ($CDCl_3$) δ : 0.87 (6 H, d, J = 6.6 Hz), 1.14-1.21 and 1.26 (18 H, m and brs), 1.43-1.54 (5 H, m) 2.41 (1 H, dd, J = 8.9, 16.5 Hz), 2.52 (1 H, dd, J = 3.6, 16.5 Hz), 2.84 (1 H, d, J = 3.6 Hz, exchangeable with D_2O), 3.27 (3 H, s), 3.99-4.00 (1 H, m) [lit.[6b] ^1H NMR ($CDCl_3$) δ : 0.85 (6 H, d, J = 7 Hz), 1.00-1.80 (23 H, m), 2.40 (2 H, d, J = 6 Hz), 2.80 (1 H, br), 3.67 (3 H, s), 4.00 (1 H, m)].

(3*R*)-Methyl 3-((*S*)-α-Methoxy-α-trifluoromethylphenylacetoxy)-15-methylhexadecanoate (19). (*S*)-MTPA ester was prepared by the method reported by D. E. Ward and C. K. Rhee.[15] Oxalyl chloride (0.08 ml, 0.917 mmol) was added to a solution of (*S*)-MTPA (33 mg, 0.136 mmol) and DMF (18 mg, 0.243 mmol) in hexane (2 ml) at room temperature. After being stirred at room temperature for 1 h, the mixture was filtered and concentrated to give (*S*)-MTPACl. To a solution of above (*S*)-MTPACl in CH_2Cl_2 (0.3 ml) was added dropwise the β-hydroxy Me ester **18** (20 mg, 0.067 mmol) in CH_2Cl_2 (0.4 ml) and then TEA (0.14 ml, 1.00 mmol) and DMAP (11 mg, 0.090 mmol) at 0 °C. After being stirred at 0 °C for 1 h and then at room temperature for 1 h, the reaction mixture was diluted with $CHCl_3$ (30 ml). The organic layer was washed with saturated aqueous $NaHCO_3$, water, and saturated aqueous NaCl, and dried over Na_2SO_4. Concentration in vacuo gave the crude product, which was purified by silica gel column chromatography (BW-200, 11 g) with hexane-Et_2O (3 : 1) to give the MTPA ester **19** (35 mg, quant.) as a colorless oil. The enantiomeric excess was determined by the two ^1H NMR signals of the methoxycarbonyl group at 3.59 ppm (*R*-form) and 3.66 ppm (*S*-form) in a ratio of 98 : 2. IR ν_{max}(film) : 2926, 2855, 1750, 1271, 1171, 1019, 717 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.16-1.25 and 1.25 (18 H, m and brs), 1.43-1.65 (5 H, m), 2.59-2.64(2 H, m), 3.53 (3 H, d, J = 0.99 Hz), 3.59 (for (*R*)-alcohol, s) and

3.66 (for (*S*)-alcohol, s) (3 H), 5.43-5.50 (1 H, m), 7.38-7.41 (m) and 7.42-7.53 (m) (5 H).

Racemic-Ethyl 3-Hydroxy-15-methylhexadecanoate (rac-16). To a solution of the β-keto ester **14** (156 mg, 0.499 mmol) in EtOH-THF (1 : 2, 1.5 ml) at 0 °C was added $NaBH_4$ (15 mg, 0.397 mmol). After the mixture was stirred at 0 °C for 1 h, 10% aqueous citric acid (10 ml) was added. After the ethanol was evaporated, the residue was basified with saturated aqueous K_2CO_3 and extracted with Et_2O (60 ml). The extracts were dried over $MgSO_4$, and concentration in vacuo gave the residue, which was purified by silica gel column chromatography (BW-820 MH, 10 g) with hexane-Et_2O (2 : 1) to give the racemic β-hydroxy ester **16** (142 mg, 90%) as a colorless oil, IR ν_{max}(film) : 3454, 2926, 1738, 1468, 1374, 1183 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.16-1.25 and 1.27 (23 H, m and brs), 1.36-1.54 (3 H, m), 2.39 and 2.51 (2 H, dd, J = 8.9, 16.5 Hz and dd, J = 3.3, 16.5 Hz), 2.91 (1 H, brs, exchangeable with D_2O), 3.99-4.00 (1 H, m), 4.18 (2 H, q, J = 7.3 Hz). The ester **16** was converted to the corresponding MTPA ester **19** as described for the (3*R*)-isomer for reference of the ^1H NMR measurement. Analysis of the MTPA ester, Calcd for $C_{28}H_{43}F_3O_5$: C, 65.10 ; H, 8.39. Found : C, 64.79 ; H, 8.49.

***N*-[(3*R*)-3-Hydroxy-15-methylhexadecanoyl]glycine *tert*-Butyl Ester (21).** To a solution of the carboxylic acid **17** (679 mg, 2.37 mmol) in DMF (30 ml) was added H-Gly-OBu^t •HCl (**20**) (486 mg, 2.9 mmol), DEPC (0.43 ml, 2.9 mmol), and then TEA (0.6 ml, 5.8 mmol) at 0 °C, and the mixture was stirred at room temperature for 8 h. The mixture was diluted with Et_2O and washed with 1 M aqueous $KHSO_4$, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200, 100 g) with hexane : EtOAc (2 : 1) to give the amide **21** (913 mg, 96%) as a white wax, $[\alpha]^{22}_D$ -10.2° (c 1.00, $CHCl_3$). IR ν_{max}($CHCl_3$) : 3350, 2926, 2855, 1730, 1651, 1556, 1468, 1370, 1215, 1157, 758 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (6 H, d, J = 6.6 Hz), 1.16-1.59 (31 H, m), 2.27 (1 H, dd, J = 8.9, 15.2 Hz), 2.42 (1 H, dd, J = 2.6, 15.2 Hz), 3.50 (1 H, brs, exchangeable with D_2O), 3.86-4.03 (3 H, m), 6.29 (1H, brs). Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13 ; H, 11.35 ; N, 3.51. Found : C, 68.90 ; H, 11.13 ; N, 3.22.

***N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine *tert*-Butyl Ester (22).** To a solution of the carboxylic acid **12** (201 mg, 0.83 mmol) in CH_2Cl_2 (5 ml) was added EDCI•HCl (230 mg, 1.2 mmol), DMAP (10 mg, 0.08 mmol) and the hydroxy amide **21** (320 mg, 0.8 mmol) at 0 °C, and the mixture was stirred at 0 °C for 5 h. The mixture was diluted with Et_2O and successively washed with H_2O, saturated aqueous $NaHCO_3$, and saturated brine, and then dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200, 50 g) with hexane : EtOAc (5 : 1) to give **22** (454 mg, 90%) as a colorless oil, $[\alpha]^{26}_D$ +0.96° (c 1.00, $CHCl_3$). IR ν_{max}(neat) : 3320, 2924, 2855, 1738, 1732, 1651, 1468, 1367, 1159, 848 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (12 H, d, J = 6.6 Hz), 1.13-1.29 (38 H, m), 1.47 (9H, s), 1.47-1.70 (6H, m), 2.31 (2H, t, J = 7.3 Hz), 2.49-2.52 (2 H, m), 3.92 (2 H, d, J = 5 Hz), 5.12-5.21 (1 H, m), 6.21 (1 H, brs). Anal. Calcd for $C_{38}H_{73}NO_5 \cdot 1/2\ H_2O$: C, 72.10 ; H, 11.78 ; N, 2.21. Found : C, 71.85 ; H, 11.65 ; N, 2.18.

***N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycine (topostin B567, 2b).** To a solution of **22** (454 mg, 0.72 mmol) in $CHCl_3$ (3 ml) was added dropwise TFA (3 ml) at 0 °C and the mixture was stirred at room temperature for 5 h. After addition of toluene, the mixture was concentrated in vacuo. This work-up was repeated three times to remove the excess of TFA completely. The residue was

purified by silica gel column chromatography (BW-200, 40 g) with $CHCl_3$: MeOH (5 : 1) to give a colorless powder. The powder was dissolved in $CHCl_3$ and the insoluble materials were filtered. The filtrate was concentrated in vacuo to give topostin B567 (**2b**) (397 mg, 97%) as a colorless powder, $[\alpha]^{24}_D$ +0.72° (c 0.4, $CHCl_3$), (Lit.[4] $[\alpha]^{22}_D$ +1.9° (c 0.25, $CHCl_3$)). IR $\nu_{max}(CHCl_3)$: 3427, 2928, 2855, 1732, 1667, 1531, 1468, 1215, 758 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (12 H, d, J = 6.6 Hz), 1.11-1.16 (4 H, m), 1.25 (34 H, brs), 1.49-1.63 (6H, m), 2.31 (2 H, t, J = 7.6 Hz), 2.47-2.61 (2 H, m), 4.07 (2 H, d, J = 5.0 Hz), 5.16 (1 H, m), 6.44 (1H, t, J = 5.0 Hz). ^{13}C-NMR ($CDCl_3$) δ : 22.6, 24.9, 25.2, 27.4, 27.9, 29.27, 29.31, 29.49, 29.54, 29.7, 29.9, 34.1, 34.5, 39.0, 41.1, 41.4, 71.2, 171.2, 172.5, 174.0. Anal. Calcd for $C_{34}H_{65}NO_5 \cdot 1/2\ H_2O$: C, 70.79 ; H, 11.53 ; N, 2.43. Found : C, 70.70 ; H, 11.31 ; N, 2.43. HRMS Calcd. for $C_{34}H_{65}NO_5$: 567.4863. Found : 567.4856.

***N*-[*N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl]-L-serine Benzyl Ester (24).** To a suspension of **2b** (267 mg, 0.47 mmol) in DMF (6 ml) was added H-L-Ser-OCH_2Ph•TFA (**23**) (139 mg, 0.71 mmol), DEPC (84 μl, 0.56 mmol), and then TEA (156 μl, 1.12 mmol) at 0 °C, and the mixture was stirred at room temperature for 20 h. The mixture was diluted with Et_2O and washed with 1 M aqueous $KHSO_4$, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200, 30 g) with hexane : EtOAc (1 : 2) to give **24** (275 mg, 81%) as a colorless wax, $[\alpha]^{24}_D$ +15.0° (c 0.50, $CHCl_3$). IR $\nu_{max}(CHCl_3)$: 3360, 2953, 2926, 2855, 1738, 1732, 1660, 1653, 1520, 1468, 1215, 1080, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (12 H, d, J = 6.6 Hz), 1.13-1.16 (4 H, m), 1.25 (34 H, brs), 1.46-1.61 (6 H, m), 2.30 (2H, t, J = 7.6 Hz), 2.48 (2 H, d, J = 5.6 Hz), 3.92-4.06 (3 H, m), 4.67-4.72 (1 H, m), 5.13-5.21 (2 H, m), 6.49 (1 H, t, J = 5.3 Hz), 7.06 (1 H, d, J = 7.3 Hz), 7.33-7.35 (5 H, m). Anal. Calcd for $C_{43}H_{76}N_2O_7$: C, 70.45 ; H, 10.45 ; N, 3.82. Found : C, 70.19 ; H, 10.18 ; N, 3.73.

***N*-[(3*R*)-15-Methyl-3-(13-methyltetradecanoyloxy)hexadecanoyl]glycyl-L-serine (topostin D654, 3b).** A mixture of **24** (262 mg, 0.36 mmol)) and 5% Pd-C (400 mg) in EtOH (18 ml) was stirred at room temperature for 5 h under H_2. The mixture was filtered through the pad of celite, and the precipitates were washed with EtOH. The combined filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (BW-200, 20 g) with $CHCl_3$: MeOH (3 : 1) to give a colorless powder. The powder was dissolved in $CHCl_3$ and the insoluble materials were filtered. The filtrate was concentrated in vacuo to give topostin D654 (**3b**) (156 mg, 67%) as a colorless powder, $[\alpha]^{26.5}_D$ +18.4° (c 0.40, $CHCl_3$), (Lit.[4] $[\alpha]^{22}_D$ +17.1° (c 2.00, $CHCl_3$)). IR $\nu_{max}(CHCl_3)$: 3345, 2953, 2926, 2855, 1728, 1651, 1531, 1468, 1215, 762 cm^{-1}. ^1H NMR ($CDCl_3$) δ : 0.86 (12 H, d, J = 6.6 Hz), 1.13-1.16 (4 H, m), 1.25 (34 H, brs), 1.46-1.59 (6 H, m), 2.29 (2 H, t, J = 7.3 Hz), 2.51 (2H, brs), 3.91-4.07 (4 H, m), 4.59 (1 H, brs), 5.18 (1 H, m), 7.15 (1 H, brs, exchangeable with D_2O), 7.63 (1 H, brs, exchangeable with D_2O). ^{13}C NMR ($CDCl_3$) : 22.6, 25.1, 25.3, 27.4, 27.9, 29.2, 29.4, 29.5, 29.6, 29.8, 30.0, 34.3, 34.6, 39.0, 41.2, 42.8, 54.9, 62.2, 71.3, 169.8, 171.6, 173.0, 174.1. Anal. Calcd for $C_{37}H_{70}N_2O_7 \cdot 1/2\ H_2O$: C, 66.93 ; H, 10.78 ; N, 4.22. Found : C, 66.78 ; H, 10.65 ; N, 4.28. FABMS : 655 $[M + H]^+$.

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