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Structures of Topostins, DNA Topoisomerase I Inhibitors of Bacterial Origin

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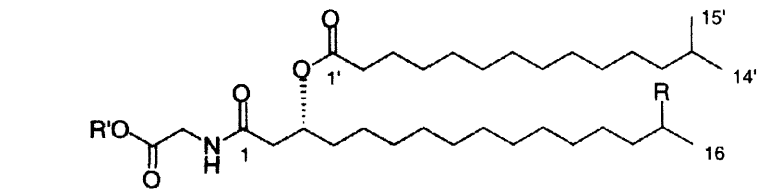
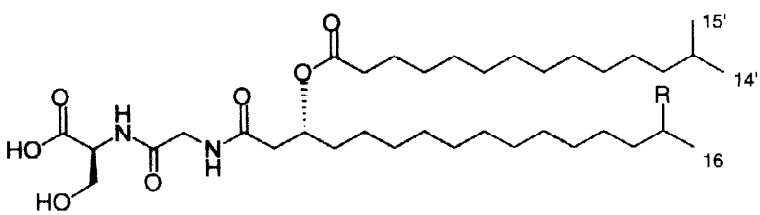
Abstract: The structures of topostins, a novel type of mammalian DNA topoisomerase I inhibitors isolated from the bacterium *Flexibacter topostinus* sp. nov., have remained unknown. Reexamination of the production of topostins resulted in the isolation of original topostins B (B553 (**1**) and B567 (**2**)) and new related compounds named topostins D (D640 (**3**) and D654 (**4**)). Their structures, including the absolute stereochemistry, were determined by spectroscopic analyses and chemical degradations. These substances are lipid-containing amino acids and peptides. © 1998 Elsevier Science Ltd. All rights reserved.

Topostins are novel inhibitors of mammalian DNA topoisomerase I (topo I) isolated from a culture broth of *Flexibacter topostinus* sp. nov.[1,2] Three topostins A1, A2, and B were isolated, and the most active compound topostin B was a 1:1 mixture of two analogues with molecular weights of 553 and 567. Because of the limited availability of the samples only the molecular weights of topostin B have been reported in the literature.[2] In order to determine the chemical structures of topostin B, Shioiri and co-workers synthesized the compounds possessing tentatively assigned structures.[3,4] However, the synthetic compounds were not identical with natural topostin B in spectroscopic comparison. Elucidation of the structures of topostins which at present are ambiguous is a challenging problem.

To determine the structures of topostins we modified the previous isolation procedure and succeeded in the isolation of a sufficient amount of pure material for analysis. In this paper we wish to describe the isolation and structural determination, including the absolute stereochemistry, of topostins B (B553 (**1**) and B567 (**2**)) and new analogues named topostins D (D640 (**3**) and D654 (**4**)).[5]



topostin B553 (**1**) , R = R' = H
5 , R = H, R' = CH_3
topostin B567 (**2**) , R = CH_3, R' = H
6 , R = R' = CH_3



topostin D640 (**3**) , R = H
topostin D654 (**4**) , R = CH_3
(= WB-3559 D)

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Isolation

The fermentation of the topostin-producing strain B-572 of *F. topostinus* sp. nov. was carried out under conditions similar to those reported previously[1] except for the use of 500-mL Sakaguchi flasks. The culture broth (4.5 L) was diluted with MeOH, and the supernatant was concentrated and extracted with Et_2O at pH 3.5. The Et_2O extract showed inhibitory activity against the camptothecin-resistant human recombinant topo I[6] and was subjected to bioassay-guided fractionation using silica gel (hexane-EtOAc-MeOH system) to afford two active fractions. The fraction eluted with hexane-EtOAc (1:1) was chromatographed repeatedly on silica gel and ODS to give a mixture of topostins B and its several analogues. The mixture was further separated by reversed-phase HPLC to give pure topostins B553 (**1**) (8.2 mg) and B567 (**2**) (2.9 mg) as well as other minor analogues. Another active fraction eluted with hexane-EtOAc-MeOH (5:10:1) in the first chromatography was separated on silica gel and ODS to give a mixture of topostins D and its several analogues. The mixture was further purified by reversed-phase HPLC to give pure topostins D640 (**3**) (41.8 mg) and D654 (**4**) (30.7 mg) as well as other minor analogues. All topostins inhibited topo I with IC_{50}'s of approximately 0.2 μM.

Table 1. NMR chemical shifts (δ) and HMBC data for **1** and **3** in $CDCl_3$.[a]

Position	**1**			**3**		
	^1H	^{13}C	HMBC	^1H	^{13}C	HMBC
Ser						
1					173.1 s	H3(Ser)
2				4.54 m	55.0 d	
3				3.85 br d (8.1)	62.3 t	
				4.02 br d (8.1)		
NH				7.75 br s		
Gly						
1		172.0 s			169.8 s	NH(Ser)
2	3.97 br s	41.4 t		3.97 br s	42.8 t	
NH	7.00 br s			7.39 br s		
fatty acid parts						
1		170.9 s	H2, H3		171.6 s	H2, H3
2	2.47 dd (14.7, 5.3)	41.4 t		2.49 dd (14.4, 5.3)	41.2 t	
	2.55 dd (14.7, 7.2)			2.60 dd (14.4, 7.4)		
3	5.18 m	71.2 d	H2	5.18 m	71.3 d	H2
4	1.60 m	34.2 t	H2	1.58 m	34.4 t	H2
5	1.2-1.4 m	25.2 t	H3	1.2-1.4 m	25.3 t	H3
6-13	1.2-1.4 m	27.4-30.0 t[b]		1.2-1.4 m	27.8-30.0 t[c]	
14	1.2-1.4 m	31.9 t	H16	1.2-1.4 m	31.9 t	H16
15	1.2-1.4 m	22.7 t	H16	1.2-1.4 m	22.7 t	H16
16	0.88 t (6.6)	14.1 q		0.87 t (6.8)	14.1 q	
1'		173.9 s	H3, H2', H3'		174.2 s	H3, H2', H3'
2'	2.28 t (7.5)	34.6 t		2.28 t (7.5)	34.5 t	
3'	1.60 m	25.0 t	H2'	1.58 m	25.1 t	H2'
4'-11'	1.2-1.4 m	27.4-30.0 t[b]		1.2-1.4 m	27.8-30.0 t[c]	
12'	1.15 m	39.1 t	H14', H15'	1.15 m	39.1 t	H14', H15'
13'	1.51 m	28.0 d		1.51 m	28.0 d	
14',15'	0.86 d (6.6)	22.7 q	H12', H13'	0.86 d (6.6)	22.6 q	H12', H13'

[a] Spectra were recorded at 400 MHz for ^1H and at 100 MHz for ^{13}C. Coupling constants in Hz are in parenthesis. [b] Signals consists of δ 27.4, 29.2, 29.3, 29.5, 29.6, 29.7, and 30.0. [c] Signals consists of δ 27.8, 29.2, 29.4, 29.5, 29.6, 29.7, and 30.0.

Structural elucidation

Our compounds **1** and **2** seemed to be the same substances as the original topostin B from comparison of their chromatographic behavior and FABMS data, which were the only information reported in the literature.[2] Thus, **1** and **2** showed negative FABMS peaks at *m/z* 552 and 566, respectively, which were the same as the values reported for topostin B. Furthermore, the ^{1}H NMR spectrum[7] of topostin B was virtually identical with that of our compounds. From these findings and the topo I inhibitory activity, we concluded that the original topostin B was a mixture of **1** and **2**.

Topostin B553 (**1**) has the molecular formula of $C_{33}H_{63}NO_5$ determined by high-resolution negative FABMS. The presence of a secondary amide group was suggested by IR bands at 3420, 1670, and 1520 cm^{-1}. IR absorption bands at 3600-2500 (br), 1730, and 1235 cm^{-1} indicate the presence of carboxyl and ester groups. Three carbonyl groups were supported by ^{13}C NMR signals at δ 170.9, 172.0, and 173.9. The strong signal at δ 1.2-1.4 in ^{1}H NMR spectrum suggested that **1** is a fatty acid derivative. ^{1}H and ^{13}C NMR signals were assigned by a ^{1}H-^{13}C COSY experiment as shown in Table 1. COSY data for **1** provided the following partial structures: C2-C5, an ethyl (C15-C16), C2'-C4', C12'-C15', and a glycine residue. The connectivity of these substructures and the three carboxyl groups was determined by HMBC data, which is summarized in Table 1. The remaining methylenes (C6-C14 and C5'-C11') should be located between C5 and C15 and between C4' and C12'. The amide linkage between the carbonyl group at C1 and the glycine residue was clear, though not evidenced by the HMBC experiment, because all other connectivities were determined as described above. The carbon-chain length of two fatty acid components and the position of the isopropyl group were clarified by derivatization experiments. Thus, methylation of **1** followed by basic methanolysis of the methyl ester **5** gave methyl 13-methyltetradecanoate and β-hydroxyamide **7**, which were identified by MS and ^{1}H NMR spectra. These findings finally disclosed the gross structure of **1**. The absolute stereochemistry of **1** was elucidated by applying the modified Mosher method.[8] The β-hydroxyamide **7** was converted to (*R*)- and (*S*)-MTPA esters, **8a** and **8b**, respectively. The calculated $\Delta\delta$ (δ_S-δ_R) values are reasonable as shown in Fig. 1, indicating the *R* configuration at the C3 position.

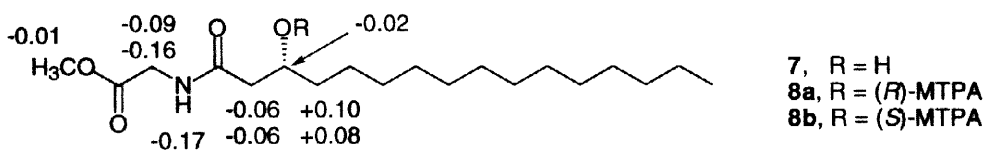


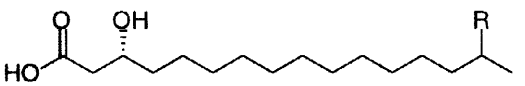
Fig. 1. Δδ Values (ppm) for the MTPA esters **8a** and **8b**.

Topostin B567 (**2**) has the molecular formula of $C_{34}H_{65}NO_5$ (high-resolution negative FABMS). The IR and NMR data for **2** are similar to those of **1**, except for the observation of the NMR signals due to an additional isopropyl group (C15-17) in place of the ethyl group (C15-16) in **1**. These NMR data coupled with the molecular formula, which is more than that of **1** by CH_2, indicated that **2** is the 15-methyl analogue of **1**. Since both **1** and **2** showed similar specific rotations, the absolute stereochemistry of **2** could be identical with that of **1**. The methyl ester **6** also showed a specific rotation similar to that of **5**. Topostin B567 (**2**) could be identical with the known bacterial substance cytolipin, the stereochemistry of which was not determined.[9]

Topostins D640 (**3**) and D654 (**4**) showed chromatographic behavior similar to those of topostins A1 and A2,[2] but their ^{1}H NMR spectra were different.[7] This fact showed that **3** and **4** were a new type of topostins.

The NMR chemical shifts and HMBC data for **3** are summarized in Table 1. On the basis of spectroscopic analyses similar to those of **1**, the gross structure of **3** was elucidated. The difference from **1** is the presence of an additional amino acid, L-serine, on the C-terminus of the glycine residue. The sequence of the dipeptide Ser-Gly was determined by correlation of NH(Ser)/C1(Gly) in the HMBC data. The absolute stereochemistry and carbon-chain length of the fatty acid components of **3** were determined as follows. Acid hydrolysis of **3** gave glycine and L-serine in the hydrophilic fraction, which were identified by chiral HPLC analysis. On the other hand, 13-methyltetradecanoic acid and (–)-3-hydroxyhexadecanoic acid (**9**) were obtained from the lipophilic fraction of the acid hydrolysate, and the absolute configuration at the C3 position of **9** was determined to be *R* from its specific rotation.[10] From these findings, topostin D640 (**3**) was proved to be the condensation product of topostin B553 (**1**) and L-serine.

The spectroscopic data for **4** are similar to those of **3**, except for the observation of the NMR signals due to an additional isopropyl group (C15-C17) in place of the ethyl group (C15-16) in **3** and the molecular formula that is more than that of **3** by CH_2. These data indicated that **4** is the 15-methyl analogue of topostin D640 (**3**) as in the case of **2**. The absolute stereochemistry was determined to be the same as that of **3**, because the acid hydrolysis of **4** afforded (–)-(*R*)-3-hydroxy-15-methylhexadecanoic acid (**10**)[11] as well as L-serine, glycine, and 13-methyltetradecanoic acid. The structure, including the absolute stereochemistry, of **4** is identical with that of the known bacterial metabolite WB-3559 D.[11,12]



9, R = H
10, R = CH_3

In this research we first determined the structures of topostins, which are known as novel mammalian DNA topoisomerase I inhibitors of a bacterial origin, and found that they were lipid-containing amino acids and peptides. Such type of compounds have been isolated from several bacteria[9,11,12] and synthesized.[11,13] Although some of them possess biological activities, topo I inhibitory activity has not been reported. Since all topostins show similar activity, the number of amino acids is not important. We and other groups found several acidic lipids such as phospholipids,[14] fatty acids,[15,16] and ceramide sulfate[17] as topo I inhibitors, indicating that both lipophilicity and anionic nature play important roles in the activity.[18]

EXPERIMENTAL

General methods. HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph. For column chromatography silica gel BW-820MH (Fuji-Silysia Co., Ltd.) or Cosmosil 75C18-OPN (Nacalai Tesque, Inc.) was used. Specific rotations were measured with a JASCO DIP-370 digital polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR-7000S. NMR spectra were recorded on a Bruker ARX400 (400 MHz) or a Bruker AMX600 (600 MHz). NMR chemical shifts were referenced to the solvent peak of δ_H 7.26 (residual $CHCl_3$) or δ_C 77.0 ppm for $CDCl_3$. Mass spectra were recorded on a JEOL JMA-2000 (EIMS), a JMS DX-705L (FABMS), or a Mstation JMS-700 (high-resolution FABMS) mass spectrometer. The matrix used in FABMS experiments was *m*-nitrobenzyl alcohol. Topo I inhibitory activity was determined by using the camptothecin-resistant human recombinant topo I[6] under the conditions reported previously.[16]

Fermentation and isolation. The strain B-572 of *F. topostinus* sp. nov., obtained from Yamanouchi Pharmaceutical Co., Ltd., was grown on the reported agar slants and inoculated into forty-five 500-mL Sakaguchi flasks containing 100 mL of the reported medium.[1] The flasks were incubated on a rotary shaker (180 rpm) at 28 °C for 4 days. The combined culture broth (4.5 L) was diluted with MeOH (9 L) and stood for one week. The supernatant was separated by centrifugation (3000 rpm, 10 min) and concentrated to an aqueous solution (1 L, pH 6.8), which was adjusted to pH 3.5 with 1 M HCl and then extracted with ether (3 x 1 L). The combined organic extracts were concentrated *in vacuo* to give a dark red oil (8.1 g), which was chromatographed on silica gel (hexane-EtOAc-MeOH, step gradient). The fraction (5.1 g) eluted with hexane-EtOAc (1:1) was chromatographed on silica gel (hexane-EtOAc-MeOH, step gradient). The fractions (760 mg) eluted with hexane-EtOAc (1:1) and hexane-EtOAc-MeOH (2:4:1) were combined and chromatographed on ODS (MeOH-H_2O, step gradient). The MeOH fraction (64 mg) was separated by silica gel TLC with $CHCl_3$-acetone-MeOH (4:1:1) to afford a mixture of topostins B and their analogues (31 mg, R_f = 0.1-0.5), which was separated by HPLC (Develosil ODS 10 (20 x 250 mm), 0.01 M NH_4OAc in 97.5% MeOH, 8 mL/min, detected at 210 nm) to afford a mixture of **1** and **2** (t_R = 28-37 min). The mixture (26 mg) was separated by recycled HPLC under the same conditions to give pure **1** (8.2 mg) and crude **2** (10 mg). The crude sample of **2** was purified by HPLC under the same conditions except for the use of 0.01 M NH_4OAc in MeCN-MeOH-H_2O (30:65:5) as a solvent to give pure **2** (2.9 mg). On the other hand, the fraction (750 mg) eluted with hexane-EtOAc-MeOH (5:10:1) in the first chromatography was separated on silica gel ($CHCl_3$-MeOH, step gradient). The fraction (446 mg) eluted with $CHCl_3$-MeOH (95:5) was chromatographed on ODS (MeOH-H_2O, step gradient). The MeOH fraction (214 mg), which consisted of topostins D and their analogues, was separated by HPLC (Develosil ODS 10 (20 x 250 mm), 0.01 M NH_4OAc in 96% MeOH, 8 mL/min) to afford crude **3** (53 mg, t_R = 28 min) and crude **4** (50 mg, t_R = 36 min). The crude sample of **3** was purified by recycle HPLC (Develosil ODS HG-5 (10 x 250 mm), 0.01 M NH_4OAc in 95% MeOH, 3 mL/min) to give pure **3** (42 mg). The crude sample of **4** was purified by recycled HPLC under the same conditions except for the use of 0.01 M NH_4OAc in 96% MeOH as a solvent to give pure **4** (31 mg).

Topostin B553 (1). Colorless powder; R_f = 0.3 (silica gel, $CHCl_3$-*i*PrOH-MeOH-NH_4OH (3:3:1:0.2)), $[\alpha]^{22}_D$ +1.5 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3600-2500 (br), 3420, 1730, 1670, 1520, 1465, and 1235 cm^{-1}; for ^1H and ^{13}C NMR data see Table 1; HRMS (negative FAB) calcd for $C_{33}H_{62}NO_5$ (M-H) 552.4628, found *m/z* 552.4601.

Topostin B567 (2). Colorless powder; R_f = 0.3 (same conditions for **1**), $[\alpha]^{22}_D$ +1.9 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) 3600-2500 (br), 3420, 1730, 1670, 1520, 1465, and 1235 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.6 Hz, 12 H), 1.15 (m, 4 H), 1.2-1.4 (m, 34 H), 1.52 (m, 2 H), 1.61 (m, 4 H), 2.30 (t, *J* = 7.5 Hz, 2 H), 2.51 (dd, *J* = 14.7 and 5.8 Hz, 1 H), 2.56 (dd, *J* = 14.7 and 7.0 Hz, 1 H), 4.07 (d, *J* = 5.1 Hz, 2 H), 5.16 (m, 1 H), and 6.39 (br t, *J* = 5.1 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.7 (q, 4 C), 25.0 (t), 25.2 (t), 27.4 (t), 28.0 (d, 2 C), 29.2 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.7 (t), 30.0 (t), 34,1 (t), 34.5 (t), 39.1 (t, 2 C), 41.3 (t), 41.4 (t), 71.2 (d), 170.5 (s), 171.7 (s), and 173.8 (s); HRMS (negative FAB) calcd for $C_{34}H_{64}NO_5$ (M-H) 566.4784, found *m/z* 566.4769.

Topostin D640 (3). Colorless powder; R_f = 0.11 (same conditions for **1**), $[\alpha]^{22}_D$ +14.2 (*c* 2.0, $CHCl_3$); IR ($CHCl_3$) 3600-2500 (br), 3345, 1730, 1655, 1520, 1465, and 1235 cm^{-1}; for ^1H and ^{13}C NMR data see Table 1; HRMS (negative FAB) calcd for $C_{36}H_{67}N_2O_7$ (M-H) 639.4948, found *m/z* 639.4964.

Topostin D654 (4). Colorless powder; R_f = 0.11 (same conditions for **1**), $[\alpha]^{22}_D$ +17.1 (*c* 2.0, $CHCl_3$); IR ($CHCl_3$) 3600-2500 (br), 3345, 1730, 1655, 1520, 1465, and 1235 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ

0.86 (d, J = 6.6 Hz, 12 H), 1.15 (m, 4 H), 1.2-1.4 (m, 34 H), 1.51 (m, 2 H), 1.58 (m, 4 H), 2.29 (t, J = 7.5 Hz, 2 H), 2.47 (dd, J = 14.5 and 5.3 Hz, 1 H), 2.60 (dd, J = 14.5 and 7.4 Hz, 1 H), 3.88 (br d, J = 10.5 Hz, 1 H), 3.97 (br s, 2 H), 4.07 (br d, J = 10.5 Hz, 1 H), 4.60 (m, 1 H), 5.18 (m, 1 H), 7.23 (br s, 1 H), and 7.63 (br d, J = 7.0 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.7 (q, 4 C), 25.0 (t), 25.3 (t), 27.5 (t), 28.0 (d, 2 C), 29.2 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 30.0 (t), 34.4 (t), 34.6 (t), 39.1 (t, 2 C), 41.3 (t), 42.9 (t), 54.8 (d), 62.4 (t), 71.4 (d), 169.6 (s), 171.5 (s), 172.6 (s), and 174.3 (s); HRMS (negative FAB) calcd for $C_{37}H_{69}N_2O_7$ (M-H) 653.5105, found *m/z* 653.5079.

Derivatization of 1. A solution of **1** (4.8 mg) in ether was treated with diazomethane in a usual manner. The crude product was chromatographed on silica gel (benzene-EtOAc (10:1, 4:1)) to give methyl ester **5** (4.8 mg, 97%) as an oil: $[\alpha]^{20}_D$ +3.1 (*c* 0.34, $CHCl_3$); IR ($CHCl_3$) 3430, 1735, 1675, 1520, 1465, 1440, 1375, 1235, and 1185 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 6.7 Hz, 6 H), 0.88 (t, J = 6.4 Hz, 3 H), 1.15 (m, 2 H), 1.2–1.4 (m, 38 H), 1.51 (m, 1 H), 1.61 (m, 4 H), 2.31 (t, J = 7.4 Hz, 2 H), 2.48 (dd, J = 14.2 and 5.3 Hz, 1 H), 2.55 (dd J = 14.2 and 6.4 Hz, 1 H), 3.76 (s, 3 H), 4.04 (d, J = 5.0 Hz, 2 H), 5.17 (m, 1 H), and 6.25 (br t, J = 5.0 Hz, 1 H); HRMS (positive FAB) calcd for $C_{34}H_{66}NO_5$ (M+H) *m/z* 568.4941, found 568.4932.

To a solution of **5** (4.4 mg) in anhydrous MeOH (0.5 mL) was added a 1.0 M solution of NaOMe in MeOH (0.05 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was neutralized with 1 M HCl (0.1 mL), diluted with water (5 mL), and extracted with EtOAc (3 x 5 mL). The combined organic phases were washed with water (2 mL), dried, and concentrated. The crude product was chromatographed on silica gel (hexane-EtOAc (50:1, 30:1) and then benzene-EtOAc (1:1, 1:2)) to give methyl 13-methyltetradecanoate (1.6 mg, 80%) and **7** (1.8 mg, 67%). Methyl 13-methyltetradecanoate: oil, MS (EI) *m/z* 256 (M^+), 225, 213, 143, 87, and 74 (base); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 6.7 Hz, 6 H), 1.15 (m, 2 H), 1.2-1.4 (m, 16 H), 1.53 (m, 1 H), 1.61 (m, 2 H), 2.30 (t, J = 7.6 Hz, 2 H), and 3.66 (s, 3 H). **7**: colorless powder; $[\alpha]^{20}_D$ –6.6 (*c* 0.14, $CHCl_3$); IR ($CHCl_3$) 3430, 1745, 1668, 1520, 1440, 1375, and 1220 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3 H), 1.2–1.4 (m, 22 H), 1.47 (m, 2 H), 2.32 (dd, J = 15.2 and 2.8 Hz, 1 H), 2.45 (dd, J = 15.2 and 8.9 Hz, 1 H), 3.77 (s, 3 H), 4.00 (m, 1 H), 4.03 (dd, J = 16.0 and 5.3 Hz, 1 H), 4.10 (dd, J = 16.0 and 5.3 Hz, 1 H), and 6.31 (br s, 1 H); HRMS (positive FAB) calcd for $C_{19}H_{38}NO_4$ (M+H) *m/z* 344.2801, found 344.2810.

Methyl Ester 6. **2** was converted to the methyl ester **6** with diazomethane under the same procedure as that in the case of **1**: oil, $[\alpha]^{20}_D$ +4.2 (*c* 0.11, $CHCl_3$); MS (positive FAB) *m/z* 604 $(M+Na)^+$ and 582 $(M+H)^+$; IR ($CHCl_3$) 3430, 1735, 1675, 1520, 1465, 1375, 1235, and 1185 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, J = 6.4 Hz, 12 H), 1.15 (m, 4 H), 1.2–1.4 (m, 34 H), 1.51 (m, 2 H), 1.60 (m, 4 H), 2.31 (t, J = 7.4 Hz, 2 H), 2.48 (dd, J = 14.0 and 5.7 Hz, 1 H), 2.55 (dd, J = 14.0 and 6.8 Hz, 1 H), 3.76 (s, 3 H), 4.04 (d, J = 5.2 Hz, 2 H), 5.17 (m, 1 H), and 6.25 (br t, J = 5.2 Hz, 1 H).

MTPA Esters 8a and 8b. A mixture of **7** (0.7 mg), (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (4.7 mg, 0.02 mmol), 4-dimetylaminopyridine (9.8 mg, 0.08 mmol), 10-camphorsulfonic acid (5.1 mg, 0.022 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.8 mg, 0.02 mmol) in anhydrous dichloromethane (0.8 mL) was stirred at room temperature for 60 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic phases were

washed with brine, dried, and concentrated. The residual oil was chromatographed on silica gel (benzene-EtOAc (3:1)) to give (*R*)-MTPA ester **8a** (0.3 mg, 26%) as an oil: MS (positive FAB) *m/z* 582 $(M+Na)^+$ and 560 $(M+H)^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.2–1.35 (m, 22 H), 1.62 (m, 1 H), 1.68 (m, 1 H), 2.56 (dd, *J* = 14.7 and 4.9 Hz, 1 H), 2.60 (dd, *J* = 14.7 and 7.1 Hz, 1 H), 3.51 (s, 3 H), 3.75 (s, 3 H), 3.90 (dd, *J* = 18.3 and 5.1 Hz, 1 H), 4.05 (dd, *J* = 18.3 and 5.4 Hz, 1 H), 5.47 (m, 1 H), 6.00 (dd, *J* = 5.4 and 5.1 Hz, 1 H), 7.39 (m, 3 H), and 7.51 (m, 2 H).

The (*S*)-MTPA ester **8b** was obtained by the same procedure: MS (positive FAB) *m/z* 582 $(M+Na)^+$ and 560 $(M+H)^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.2–1.35 (m, 22 H), 1.72 (m, 1 H), 1.76 (m, 1 H), 2.50 (dd, *J* = 15.0 and 5.1 Hz, 1 H), 2.54 (dd, *J* = 15.0 and 7.4 Hz, 1 H), 3.55 (s, 3 H), 3.74 (s, 3 H), 3.74 (dd, *J* = 18.3 and 4.7 Hz, 1 H), 3.96 (dd, *J* = 18.3 and 5.7 Hz, 1 H), 5.45 (m, 1 H), 5.83 (dd, *J* = 5.7 and 4.7 Hz, 1 H), 7.39 (m, 3 H), and 7.53 (m, 2 H).

Derivatization of 3. The solution of **3** (8.3 mg) in 6 M HCl (1 mL) was stirred at 110 °C for 20 h in an evacuated sealed tube. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic phases were concentrated, and the residual oil was chromatographed on silica gel (hexane-EtOAc (5:1, 3:1, 1:1, and then 1:2)) to give 13-methyltetradecanoic acid (3.8 mg, 100%) and **9** (0.7 mg, 20%). The aqueous phase was concentrated and dissolved in water (0.8 mL). The portion (0.002 mL) of the aqueous solution was used for HPLC analysis (CHIRALPAK WH (4.6 x 250 mm), 0.25 mM $CuSO_4$, 1.5 mL/min, 50 °C, detected at 254 nm). Glycine and L-serine were detected at t_Rs of 7.4 and 11.1 min, respectively. D-Serine as a standard was eluted at 7.8 min. 13-Methyltetradecanoic acid: oil, MS (negative FAB) *m/z* 241 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.4 Hz, 6 H), 1.15 (m, 2 H), 1.2–1.4 (m, 16 H), 1.51 (m, 1 H), 1.61 (m, 2 H), and 2.35 (t, *J* = 7.5 Hz, 2 H). **9**: colorless powder, $[\alpha]^{21}_D$ –12 (*c* 0.05, $CHCl_3$) (lit.[10] $[\alpha]^{25}_D$ –12.9 (*c* 1.3, $CHCl_3$)); MS (negative FAB) *m/z* 271 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3 H), 1.1–1.4 (m, 22 H), 1.61 (m, 2 H), 2.48 (dd, *J* = 16.6 and 8.9 Hz, 1 H), 2.59 (t, *J* = 16.6 and 3.2 Hz, 1 H), and 4.03 (m, 1 H).

Derivatization of 4. Under the same condition as in the case of **3**, **4** was subjected to acid hydrolysis to give 13-methyltetradecanoic acid and **10** from the organic fraction of the hydrolysate, and the presence of glycine and L-serine in the aqueous fraction was demonstrated by the chiral HPLC analysis. **10**: colorless powder, $[\alpha]^{21}_D$ -10 (*c* 0.05, $CHCl_3$) (lit.[12] $[\alpha]^{23}_D$ –12.0 (*c* 1.0, $CHCl_3$)); MS (negative FAB) *m/z* 285 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.7 Hz, 6 H), 1.16 (m, 2 H), 1.2–1.4 (m, 18 H), 1.4-1.6 (m, 3 H), 2.47 (dd, *J* = 16.6 and 8.9 Hz, 1 H), 2.59 (dd, *J* = 16.6 and 3.2 Hz, 1 H), and 4.02 (m, 1 H).

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