Sulfobacins A and B, Novel von Willebrand Factor Receptor Antagonists

II. Structural Elucidation

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Sulfobacins A and B are novel von Willebrand factor (vWF) receptor antagonists produced by *Chryseobacterium* sp. NR 2993. The structures of sulfobacins A and B have been determined to be (2*R*,3*R*)-3-hydroxy-2-[(*R*)-3-hydroxy-15-methylhexadecanamido]-15-methylhexadecanesulfonic acid and (2*R*,3*R*)-3-hydroxy-15-methyl-2-[13-methyltetradecanamido]-hexadecanesulfonic acid, respectively, by various 2D NMR experiments and by methanolysis. The absolute configurations of the sulfobacins were determined by a modified MOSHER's method. The structures are related to sulfonolipids, major components of the cell envelope of gliding bacteria of the genus *Cytophaga*.

While screening for novel von Willebrand factor (vWF) receptor antagonists, we discovered sulfobacins A (**1**) and B (**2**) in the culture broth of *Chryseobacterium* sp. NR 2993. The compounds showed potent inhibitory activity against the binding of vWF to glycoprotein (GP) Ib/IX receptors. Their production, isolation, characterization and biological activities are reported in the preceding paper[1]. In this paper, we report on the structural elucidation of the sulfobacins.

Results

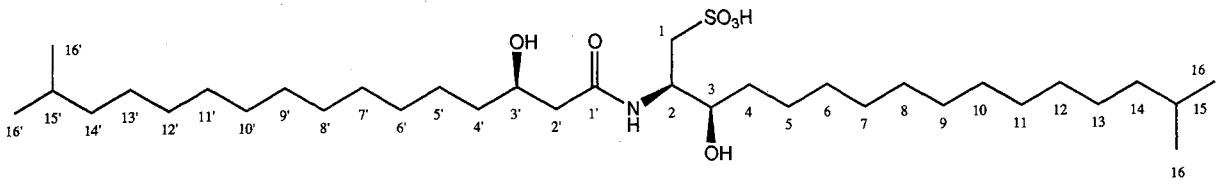
Sulfobacin A

Planar Structure

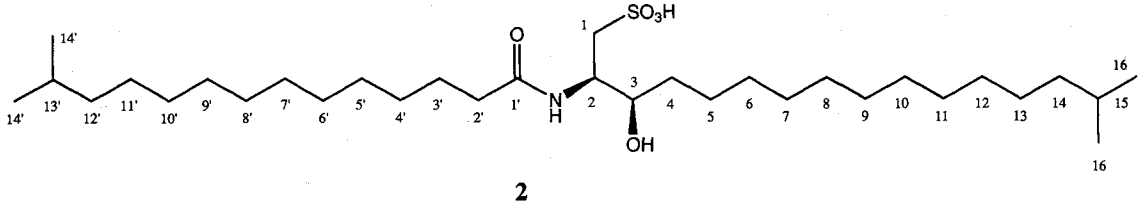
The molecular formula of **1** was determined to be $C_{34}H_{69}NO_6S$ from HRFAB-MS data (Calcd for $C_{34}H_{68}NO_6S$: 618.4768, Found: *m/z* 618.4766 $(M-H)^-$). The presence of an S atom was confirmed by ion chromatography. The IR spectral data of **1** suggested the presence of sulfonic acid (1200 and 1070 cm^{-1}) and amide (1660 and 1560 cm^{-1}) functional groups. The positive color obtained from the reactions with pinacryptol yellow[2] and Rydon-Smith reagents also supported the presence of the sulfonic acid and amide functional groups, respectively.

The structure of **1** was mainly determined based on various NMR experiments. The ^1H and ^{13}C NMR spectral data of the compound in DMSO-d_6 are shown in Table 1. The ^{13}C NMR spectrum of **1** revealed the presence of thirty-four carbons, which were assigned to four methyl, twenty-four methylene, five methine and one quaternary carbon by DEPT experiments. Interpretation of the ^1H NMR and ^1H-^1H COSY spectra of **1** revealed the following partial structures: $-C14H_2-C15H-(C16H_3)_2$, $-C14'H_2-C15'H-(C16'H_3)_2$, $-C1H_2-C2H(NH)-C3H(OH)-$ and $-C2'H_2-C3'H(OH)-C4'H_2-$. Intense signals at δ_H 1.22 (38H) suggested the presence of long alkyl chain(s). Since cross peaks were observed between the intense signals and 3-H (δ_H 3.46) and between the intense signals and 4'-H (δ_H 1.37), the two terminals of the alkyl chain(s) were connected to positions 3 and 4'. Other terminals of the methylene chain(s) were also connected to isobutyl groups, because cross peaks were observed between the signals at δ_H 1.22 and methylene proton signals at δ_H 1.14, assigned to 14-H and 14'-H of the isobutyl groups, in the ^1H-^1H COSY spectra. These results revealed that there were two methylene chains and that the chains were located between position 3 and one isobutyl group and between position 4' and the other isobutyl group. The location of the methylene chains and isobutyl groups was confirmed by ^1H-^{13}C long range couplings obtained by the HMBC experiments on **1** (Fig. 2). The carbonyl carbon C-1' (δ_C 170.2) was located between C-2' and 2-NH because of the ^1H-^{13}C long range couplings between C-1' and the protons 2-H, 2-NH and 2'-H. Judging from the ^{13}C (δ_C 51.8) and ^1H (δ_H 2.73) chemical shifts of the methylene at position 1, the methylene was assigned to be adjacent to the sulfonic acid, indicated by the IR spectral data and the color reaction. To determine the length of the methylene

Fig. 1. Structures of sulfobacins A (**1**) and B (**2**).



1



2

Table 1. ^{1}H and ^{13}C NMR spectral data for **1** and ^{1}H NMR spectral data for **2** (in DMSO-d_6).

	1		**2**
Position	δ_C a	δ_H b	δ_H b
1	51.8 (t)	2.73 (d, J=8.3)	2.62 (dd, J = 14.2, 4.4) 2.79 (dd, J = 14.2, 5.9)
2	51.1 (d)	3.92 (m)	3.84 (m)
3	72.0 (d)	3.46 (m)	3.51 (m)
4	33.4 (t)		
5	25.5 (t) [c]		
6			
7			
8			
9	29.2~29.4 (t) x 14	1.22 (m)	1.23 (m)
10			
11			
12			
13	26.9 (t) [d]		
14	38.5 (t) [e]	1.14 (m)	1.14 (m) [h]
15	27.4 (d) [f]	1.49 (m)	1.48 (m) [i]
16	22.6 (q) x 2 [g]	0.84 (d, J = 6.8)	0.84 (d, J = 6.8) [j]
2-NH	-	7.68 (d, J = 8.3)	7.58 (d, J = 8.3)
3-OH	-	4.80 (d, J = 5.4)	4.83 (d, J = 5.4)
1'	170.2 (s)	-	
2'	44.8 (t)	2.11 (dd, J = 10.8, 5.9) 2.13 (dd, J = 10.8, 5.4)	2.02 (t, J = 7.3)
3'	67.6 (d)	3.76 (m)	1.40 (m)
4'	36.6 (t)	1.37 (m)	
5'	25.2 (t) [c]		
6'			
7'			
8'			1.23 (m)
9'	29.2~29.4 (t) x 14	1.22 (m)	
10'			
11'			
12'			1.14 (m) [h]
13'	26.9 (t) [d]		1.48 (m) [i]
14'	38.5 (t) [e]	1.14 (m)	0.84 (d, J = 6.8) [j]
15'	27.4 (d) [f]	1.49 (m)	
16'	22.6 (q) x 2 [g]	0.84 (d, J = 6.8)	
3'-OH	-	4.66 (d, J = 4.4)	

a: ^{13}C NMR spectrum was recorded at 100 MHz. Multiplicities were determined by DEPT experiments.

b: ^{1}H NMR spectrum was recorded at 400 MHz. Multiplicities and coupling constants (Hz) are in parentheses.

c: These assignments can be interchanged.

d, e, f, g, h, i and j: These signals were overlapping.

Fig. 2. Proton networks (bold lines) obtained by ^1H-^1H COSY experiments and ^1H-^{13}C long range couplings (arrows) obtained by HMBC experiments on sulfobacin A (**1**).

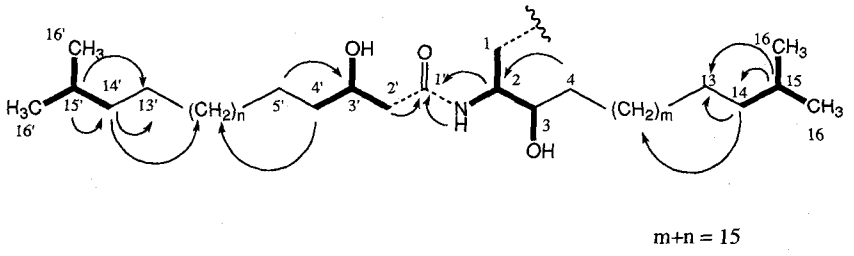
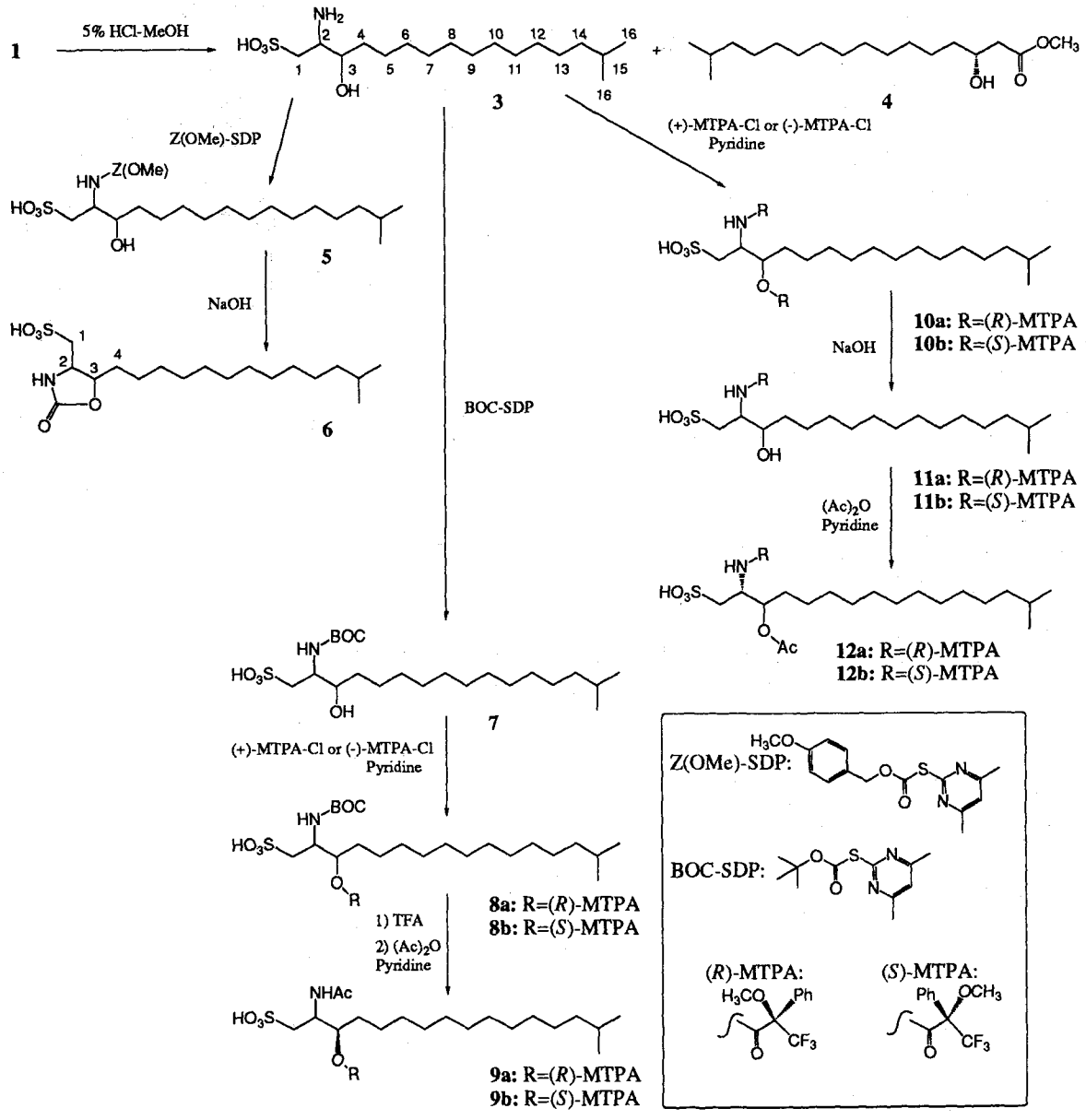


Fig. 3. Conversion of sulfobacin A (**1**).



chains, **1** was treated with methanolic hydrochloric acid to yield the aminosulfonic acid **3** and methyl ester of the fatty acid **4** (Fig. 3).

The molecular formula of **4** was determined to be $C_{18}H_{36}O_3$ from the HREI-MS data (Calcd for $C_{18}H_{36}O_3$: 300.2664, Found: *m/z* 300.2684 (M^+); Calcd for $C_{18}H_{34}O_2$: 282.2559, Found: *m/z* 282.2569 ($M-H_2O)^+$). Compound **4** was identified to be methyl

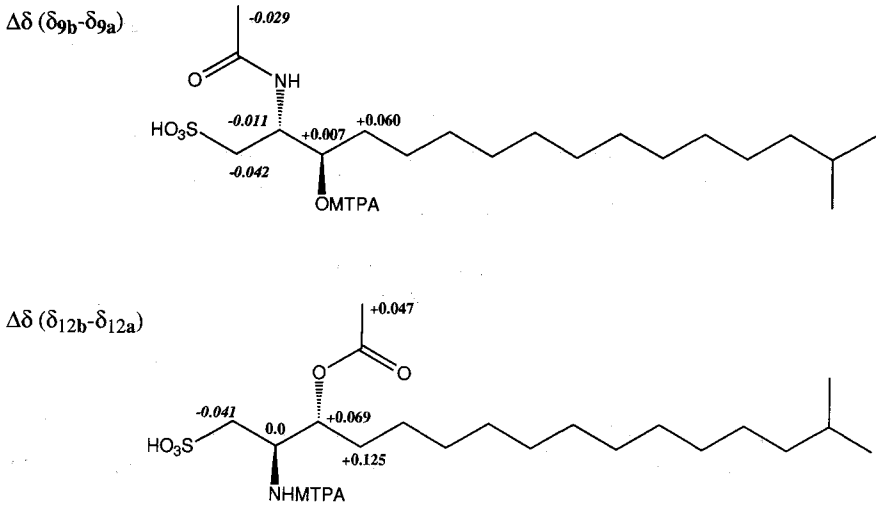
3-(*R*)-hydroxy-15-methylhexadecanoate, since its optical rotation data, $[\alpha]_D^{20}$ $-12.7°$ (*c* 0.52, $CHCl_3$), and ^1H NMR spectral data were identical to that reported for the compound[3]. This result meant that the number n, as defined by the equation m+n=15 in Fig. 2, was 7. Therefore, the number m was calculated to be 8, and **3** was suggested to be 2-amino-3-hydroxy-15-methylhexadecanesulfonic acid. The structure of **3** was further supported by color reactions, interpretation of the IR spectral data, the HRFAB-MS and ^1H NMR spectral data. The positive color obtained from the reactions with pinacryptol yellow[2] and ninhydrin suggested the presence of the sulfonic acid and primary amino functional groups, respectively. The IR spectrum of **3** also suggested the presence of the sulfonic acid functional group (1210 and 1050 cm^{-1}). The comparison of ^1H NMR spectral data of **3** with those of **1** revealed the following differences; 1) the proton signals of the 2-H (δ_H 3.22, 1H) and the 2-NH_2 (δ_H 7.42, 2H) of **3** were observed up-field from those of the 2-H (δ_H 3.92, 1H) and the 2-NH (δ_H 7.68, 1H) of **1**, respectively; 2) the other proton signals of **3** were not shifted from those of **1**, except for those of 1-H (δ 2.50, 2.76), 3-H (δ 3.60) and 3-OH (δ 5.08) of **3**. This meant that compound **3** was *N*-deacyl derivative of **1**. The other proton signals were assigned by comparing the chemical shifts and multiplicities with those of compound **1** as follows; δ_H 0.85 (6H, d, J=6.6 Hz, 16-H), 1.14 (2H, m, 14-H), 1.24 (18H, br, 5-H~13-H), 1.35 (2H, m, 4-H), 1.50 (1H, m, 15-H). Therefore, the structure of sulfobacin A was determined to be **1** except for the absolute configurations of positions 2 and 3 as shown in Fig. 1.

Stereochemistry

Although the exciton chirality method is efficient in determining the absolute configuration of various diols and their related compounds[4], this method is applicable to only *threo*-1,2-diol and *threo*-1,2-aminoalcohol in the case of acyclic systems. Therefore, to determine whether the configuration between the positions 2 and 3 was *threo* or *erythro*, we converted **3** to the oxazolidone **6** by the treatment of **3** with 4-methoxybenzyl *S*-(4,6-dimethylpyrimidin-2-yl)-thiocarbonate (Z(OMe)-SDP) followed by cyclization with 0.5 N NaOH as shown in Fig. 3[5]. TSUDA *et al.*[5] reported that the coupling constants $J_{4\sim5}$ of cyclic oxazolidone derivatives of *threo*- and *erythro*-3-amino-2-hydroxyvaleric acid were 4.8 and 9.0 Hz, respectively. Since the coupling constant between 2-H and 3-H of **6** was 7.8 Hz, the relative configuration between 2-NH_2 and 3-OH was suggested to be *erythro*. Furthermore, this *erythro* configuration was confirmed by an NOE between the 1-CH_2 and 4-CH_2 of **6**.

Since it was not possible to determine the absolute configuration of the acyclic *erythro*-1,2-aminoalcohol (**3**) by the exciton chirality method, we applied the modified MOSHER's method[6~8] to its MTPA esters **9a** and **9b** and its MTPA amides **12a** and **12b**. They were prepared by the reactions as outlined in Fig. 3. Since the bulky Boc group might change the conformation of the MTPA ester group to an abnormal one, compounds **8a** and **8b**† were converted to the acetyl derivatives **9a** and **9b**. Compounds **11a** and **11b**, insoluble in $CDCl_3$, were converted to the

Fig. 4. $\Delta\delta$ ($\delta_{9b}-\delta_{9a}$) and $\Delta\delta$ ($\delta_{12b}-\delta_{12a}$) values (ppm) obtained from ^1H NMR spectral data for the MTPA esters **9a** and **9b** and the MTPA amides **12a** and **12b** in $CDCl_3$.



† The absolute configuration at position 3 predicted by the comparison of the NMR spectral data of **8a** and **8b** was also 3*R*.

acetyl derivatives **12a** and **12b**, soluble in $CDCl_3$, because modified MOSHER's method requires the use of $CDCl_3$ as a solvent. The detailed analysis of the NMR spectral data of these MTPA derivatives revealed significant and systematic differences between the proton chemical shifts of these MTPA derivatives (Fig. 4). When the molecular models of **9a** and **9b** with the 3*R* configurations were constructed, the $\Delta\delta$ ($\delta_{9b}-\delta_{9a}$) values for the protons oriented toward the left side of the MTPA plane were all negative, while those oriented toward the right side of the plane were all positive as shown in Fig. 4. These data indicated the *R* configuration at position 3, according to modified MOSHER's method. In a similar manner, the configuration of the position 2 was also determined to be *R* (Fig. 4). The total structure of **1** was thus determined to be (2*R*,3*R*)-3-hydroxy-2-[(*R*)-3-hydroxy-15-methylhexadecanamido]-15-methylhexadecanesulfonic acid as shown in Fig. 1.

Sulfobacin B

The molecular formula of **2** was determined to be $C_{32}H_{65}NO_5S$ from the HRFAB-MS data (Calcd for $C_{32}H_{64}NO_5S$: 574.4505, Found: *m/z* 574.4496 $(M-H)^-$). The IR spectral data of **2** were almost identical to those of **1** and suggested the presence of sulfonic acid (1220 and 1060 cm^{-1}) and amide (1655 and 1550 cm^{-1}) functional groups. The positive color obtained from the reactions with pinacryptol yellow[2] and Rydon-Smith reagents supported the presence of sulfonic acid and amide functional groups, respectively.

The comparison of ^1H NMR spectral data of **2** with those of **1** in Table 1 revealed the following differences. 1) The proton signals of 3′-OH (δ_H 4.66, 1H, d, $J=4.4$ Hz) and 3′-H (δ_H 3.76, 1H, m) observed on **1** were not on **2**. 2) The proton signals of the 2′-H of **2** was observed up-field (δ_H 2.02) from those of the 2′-H of **1** (δ_H 2.11 and 2.13). This means that the *N*-acyl group of **2** was different from that of **1**.

In order to determine the unknown *N*-acyl moiety and to confirm the aminosulfonate moiety, we treated **2** with methanolic hydrochloric acid to yield methyl ester of the fatty acid **13** and the aminosulfonic acid **3**. Compound **13** was identified to be methyl 13-methyltetradecanoate by comparing its ^1H NMR, IR and GC-MS spectral data with those of the authentic sample. Although the HRFAB-MS, ^1H NMR and IR spectral data of the aminosulfonic acid obtained were identical to those of the aminosulfonic acid (**3**) obtained by the methanolysis of **1**, the stereochemistry of the aminosulfonic acid obtained from **2** was obscure because of its minute solubility, hence its optical rotation data were inconclusive. Nevertheless, since their diacetyl derivatives showed the same HREI-MS and ^1H NMR spectral data and the same optical rotation, the aminosulfonic acid obtained from **2** was determined to have the same stereochemistry as that of the aminosulfonic acid (**3**) obtained from **1**. The total structure of **2** was thus determined to be (2*R*,3*R*)-3-hydroxy-15-methyl-2-[13-methyltetradecanamido]-hexadecanesulfonic acid as shown in Fig. 1.

Discussion

Sulfobacins A and B are novel vWF receptor antagonists produced by the *Chryseobacterium* sp. NR 2993. They are the first vWF receptor antagonists of microbial origin. Our structural studies revealed that the sulfobacins have the same aminosulfonic acid moiety, (2*R*,3*R*)-2-amino-3-hydroxy-15-methylhexadecanesulfonic acid, but different *N*-acyl groups. The structures of the sulfobacins are completely different from the structure of the one known vWF receptor antagonist, aurintricarboxylic acid, which is an aromatic dye[9,10]. The structures can be related to the sulfonolipids, major components of the cell envelope of gliding bacteria of the genus *Cytophage*[11]. Although the planar structures of the sulfonolipids were determined, based on the GC-MS analysis of the mixture of the sulfonolipids, any individual component was not isolated and their stereochemistry was not determined. We have isolated sulfobacins A and B individually and determined their total structures including the absolute configurations.

Experimental

General Procedures

IR spectra were measured on a Hitachi 270-30 IR or Perkin Elmer 1600 IR spectrometer. FAB-MS, HRFAB-MS and GC-MS were measured on a JEOL JMS-DX303 mass spectrometer. ^1H and ^{13}C NMR were recorded on a JMN-GSX-400 NMR spectrometer at 400 and 100 MHz, respectively, using TMS as an internal standard. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Preparative TLC was developed with the following conditions: plate: MERCK, Kieselgel $60F_{254}$; solvent: the lower layer of $CHCl_3$-MeOH-H_2O (65:30:10); detection: pinacryptol yellow[2].

Methanolysis of 1

Sulfobacin A (100 mg) was dissolved in 5% HCl-MeOH (5 ml). This solution was heated at 90°C in a sealed tube overnight. After removal of the HCl and MeOH under reduced pressure, the residue was dissolved in MeOH. The suspension was separated into the supernatant and precipitate by centrifugation. This

supernatant was concentrated and further purified by preparative TLC (Merck, Kieselgel $60F_{254}$) developed with *n*-hexane - EtOAc (3 : 1) to give **4** (28 mg) as a colorless oil. The precipitate was washed with MeOH to give **3** (49.8 mg) as a white powder. **3**: IR ν_{max} (KBr) 3360, 3160, 2920, 2850, 1210, 1180, 1050 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 0.85 (6H, d, J=6.6 Hz, 16-H), 1.14 (2H, m, 14-H), 1.24 (18H, br, 5-H ~ 13-H), 1.35 (2H, m, 4-H), 1.50 (1H, m, 15-H), 2.50 (1H, dd, J=14.7, 11.3 Hz, 1-Ha), 2.76 (1H, d, J=14.7 Hz, 1-Hb), 3.22 (1H, m, 2-H), 3.60 (1H, m, 3-H), 5.08 (1H, m, 3-OH), 7.42 (2H, br, 2-NH). **4**: $[\alpha]_D^{20}$ −12.7° (*c* 0.518, $CHCl_3$) (literature[3]) $[\alpha]_D^{20}$ −12.6° (*c* 1.0, $CHCl_3$)); IR ν_{max} (KBr) 2920, 2850, 1730, 1470, 1440, 1210, 1170 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, J=6.6 Hz), 1.15 (2H, m), 1.26 (18H, br), 1.40 (2H, m), 1.51 (1H, m), 2.41 (1H, dd, J=16.9, 8.8 Hz), 2.52 (1H, dd, J=16.9, 3.7 Hz), 2.84 (1H, br s), 3.72 (3H, s), 4.00 (1H, m).

Preparation of **5** and **6**

To a solution of **3** (30 mg) in dry triethylamine (6 ml) was added Z(OMe)-SDP (125 mg). This solution was stirred overnight at room temperature under argon. After removal of the triethylamine under reduced pressure, the residue was purified by preparative TLC to give **5** (9.3 mg) as a colorless powder. To a solution of 5 (8.0 mg) in dioxane (2 ml) was added 0.5 N NaOH (2 ml). This solution was stirred overnight at room temperature. After the reaction mixture was neutralized with 1 N HCl, the solution was concentrated to dryness under reduced pressure. The residue was extracted with *n*-BuOH at pH 7. The solvent was removed and the residue was then purified by preparative TLC to give **6** (4.4 mg) as a colorless powder. **5**: ^1H NMR (DMSO-d_6) δ 0.84 (6H, d, J=6.6 Hz), 1.14 (2H, m), 1.23 (18H, br), 1.35 (2H, m), 1.49 (1H, m), 2.64 (1H, dd, J=14.5, 3.7 Hz), 2.78 (1H, dd, J=14.5, 5.9 Hz), 3.59 (2H, m), 3.74 (3H, s), 4.71 (1H, d, J=5.9 Hz), 4.89 (1H, d, J=12.3 Hz), 4.95 (1H, d, J=12.3 Hz), 6.84 (1H, d, J=7.3 Hz), 6.90 (2H, d, J=8.8 Hz), 7.28 (2H, d, J=8.8 Hz). **6**: positive ion FAB-MS *m/z* 378 $(M+H)^+$, 400 $(M+Na)^+$; negative ion HRFAB-MS (Calcd for $C_{18}H_{34}NO_5S$: 376.2158, Found: *m/z* 376.2160 $(M-H)^-$); IR ν_{max} (KBr) 2920, 2850, 1730, 1220, 1190, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 0.88 (6H, d, J=6.6 Hz, 16-H), 1.18 (2H, m, 14-H), 1.29 (18H, br, 5-H ~ 13-H), 1.58 (1H, m, 15-H), 1.64 (2H, m, 4-H), 2.89 (1H, dd, J=13.7, 10.0 Hz, 1-Ha), 3.03 (1H, dd, J=13.7, 3.4 Hz, 1-Hb), 4.30 (1H, ddd, J=10.0, 7.8, 3.4 Hz, 2-H), 4.71 (1H, ddd, J=7.8, 7.8, 5.9 Hz, 3-H).

Preparation of **7**

A mixture of **3** (50 mg), BOC-SDP (75 mg) and triethylamine (0.5 ml) in MeOH (10 ml) was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC (Merck, Kieselgel $60F_{254}$) developed with the lower layer of $CHCl_3$ - MeOH - H_2O (65 : 15 : 10) to give **7** (22.9 mg) as a colorless powder. **7**: negative ion FAB-MS *m/z* 450 $(M-H)^-$, 350 $(M-BOC)^-$; IR ν_{max} (KBr) 3350, 2920, 2850, 1690, 1540, 1470, 1170, 1050 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (6H, d, J=6.6 Hz), 1.14 (2H, m), 1.23 (18H, br), 1.37 (9H, s), 1.41 (2H, m), 1.50 (1H, m), 2.59 (1H, dd, J=13.9, 3.7 Hz), 2.85 (1H, dd, J=13.9, 4.4 Hz), 3.54 (2H, m), 4.71 (1H, d, J=5.1 Hz), 6.49 (1H, d, J=7.3 Hz).

Preparation of **8a**

A mixture of **7** (6.0 mg) and (*S*)-(+)-MTPA chloride (10 μl) in dry pyridine (1 ml) was stirred for 1 hour at room temperature. After removal of the pyridine under reduced pressure, the residue was purified by preparative TLC to give **8a** (7.1 mg) as a colorless powder. **8a**: negative ion FAB-MS *m/z* 666 $(M-H)^-$; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, J=6.4 Hz), 1.17 (2H, m), 1.24 (20H, br), 1.38 (9H, s), 1.50 (1H, m), 2.95 (1H, m), 3.03 (1H, m), 3.52 (3H, br s), 4.34 (1H, m), 5.40 (1H, m), 7.32 (1H, m), 7.36 (2H, m), 7.52 (2H, m).

Preparation of **8b**

(*S*)-MTPA ester **8b** (2.7 mg) was obtained as a colorless powder from **7** (3.0 mg) and (*R*)-(−)-MTPA chloride (5 μl) by similar procedures for **8a**. **8b**: negative ion FAB-MS *m/z* 666 $(M-H)^-$; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, J=6.8 Hz), 1.24 (22H, m), 1.34 (9H, s), 1.51 (1H, m), 2.89 (1H, m), 3.01 (1H, m), 3.49 (3H, br s), 4.34 (1H, m), 5.35 (1H, m), 7.39 (3H, m), 7.53 (2H, m).

Preparation of **9a**

To a solution of **8a** (5.0 mg) in dichloromethane (0.9 ml) was added trifluoroacetic acid (0.1 ml). This solution was stirred for 1 hour at room temperature. The reaction mixture was concentrated under reduced pressure to give a colorless powder. To a solution of the powder in dry pyridine (1 ml) was added acetic anhydride (0.2 ml). The mixture was stirred for 5 hours at room temperature. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC to give **9a** (2.2 mg) as a colorless powder. **9a**: negative ion HRFAB-MS (Calcd for $C_{29}H_{45}NO_7F_3S$: 608.2869, Found: *m/z* 608.2831 $(M-H)^-$); ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, J=6.8 Hz, 16-H), 1.14 (2H, m, 14-H), 1.23 (18H, m, 5-H ~ 13-H), 1.51 (1H, m, 15-H), 1.538 (2H, br, 4-H), 1.934 (3H, s, $COCH_3$), 3.048 (2H, m, 1-H), 3.49 (3H, br s, OCH_3), 4.756 (1H, m, 2-H), 5.129 (1H, m, 3-H), 7.37 (3H, m, Ph), 7.52 (2H, m, Ph).

Preparation of **9b**

The compound **9b** (1.0 mg) was obtained as a colorless powder from **8b** (2.7 mg) by similar procedures for **9a**. **9b**: negative ion HRFAB-MS (Calcd for $C_{29}H_{45}NO_7F_3S$: 608.2869, Found: *m/z* 608.2880 $(M-H)^-$); ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, J=6.8 Hz, 16-H), 1.16 (2H, m, 14-H), 1.24 (18H, m, 5-H ~ 13-H), 1.51 (1H, m, 15-H), 1.598 (2H, br, 4-H), 1.905 (3H, s, $COCH_3$), 3.006 (2H, m, 1-H), 3.45 (3H, br s, OCH_3), 4.745 (1H, m, 2-H), 5.136 (1H, m, 3-H), 7.38 (3H, m, Ph), 7.49 (2H, m, Ph).

Preparation of **10a**

A mixture of **3** (8.3 mg) and (*S*)-(+)-MTPA chloride (10 ml) in dry pyridine (1 ml) was stirred for 3 hours at room temperature. After removal of the pyridine under reduced pressure, the residue was purified by preparative TLC to give **10a** (4.4 mg) as a colorless powder. **10a**: negative ion FAB-MS *m/z* 782 $(M-H)^-$; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, $J=6.8$ Hz), 1.14 (2H, m), 1.24 (18H, m), 1.40 (2H, m), 1.51 (1H, m), 2.91 (1H, m), 3.10 (1H, d, $J=13.2$ Hz), 3.33 (3H, br s), 3.36 (3H, br s), 4.67 (1H, m), 5.34 (1H, m), 7.31 (6H, m), 7.45 (4H, m).

Preparation of **10b**

The compound **10b** (2.9 mg) was obtained as a colorless powder from **3** (5.0 mg) and (*R*)-(−)-MTPA chloride (10 ml) by a similar procedure for **10a**. **10b**: positive ion FAB-MS *m/z* 805 $(M-H+Na)^+$, 828 $(M-H+2Na)^+$; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, $J=6.6$ Hz), 1.15 (2H, m), 1.23 (18H, m), 1.48 (2H, m), 1.51 (1H, m), 2.84 (1H, dd, $J=14.7$, 6.6 Hz), 3.00 (1H, dd, $J=14.7$, 4.4 Hz), 3.13 (3H, br s), 3.47 (3H, br s), 4.66 (1H, m), 5.34 (1H, m), 7.33 (6H, m), 7.52 (4H, m).

Preparation of **11a**

A solution of **10a** (4.4 mg) in MeOH (1 ml) was adjusted to pH 13 with 1 N NaOH. The solution was stirred for 2 days at room temperature. After the reaction mixture was neutralized with 1 N HCl, the solution was concentrated to dryness under reduced pressure. The residue was purified by preparative TLC to give **11a** (2.4 mg) as a colorless powder. **11a**: negative ion FAB-MS *m/z* 566 $(M-H)^-$; ^1H NMR (CD_3OD) δ 0.86 (6H, d, $J=6.6$ Hz), 1.14 (2H, m), 1.27 (18H, m), 1.46 (2H, m), 1.51 (1H, m), 3.08 (1H, dd, $J=13.8$, 2.9 Hz), 3.27 (1H, m), 3.55 (3H, s), 3.72 (1H, m), 4.20 (1H, m), 7.39 (3H, m), 7.61 (2H, m).

Preparation of **11b**

The compound **11b** (1.3 mg) was obtained as a colorless powder from **10b** (5.0 mg) by similar procedures for **11a**. **11b**: negative ion FAB-MS *m/z* 566 $(M-H)^-$; ^1H NMR (CD_3OD) δ 0.88 (6H, d, $J=6.6$ Hz), 1.14 (2H, m), 1.29 (18H, m), 1.53 (2H, m), 1.54 (1H, m), 3.09 (1H, dd, $J=14.7$, 2.9 Hz), 3.25 (1H, m), 3.35 (3H, br s), 3.83 (1H, td, $J=8.8$, 2.9 Hz), 4.21 (1H, ddd, $J=9.5$, 4.4, 2.9 Hz), 7.43 (3H, m), 7.63 (2H, d, $J=7.3$ Hz).

Preparation of **12a**

To a solution of **11a** (2.4 mg) in dry pyridine (0.5 ml) was added acetic anhydride (0.1 ml). The mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC to give **12a** (1.5 mg) as a colorless powder. **12a**: negative ion HRFAB-MS (Calcd for $C_{29}H_{45}NO_7F_3S$: 608.2869, Found: *m/z* 608.2849 $(M-H)^-$); ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, $J=6.4$ Hz, 16-H), 1.14 (2H, m, 14-H), 1.24 (18H, m, 5-H∼13-H), 1.385 (2H, m, 4-H), 1.510 (1H, m), 1.900 (3H, s, $COCH_3$), 3.092 (2H, m, 1-H), 3.46 (3H, br s, OCH_3), 4.687 (1H, m, 2-H), 5.026 (1H, m, 3-H), 7.36 (3H, m, Ph), 7.55 (2H, m, Ph), 7.55 (1H, m, NH).

Preparation of **12b**

The compound **12b** (1.0 mg) was obtained as a colorless powder from **11b** (1.3 mg) by similar procedures for **12a**. **12b**: negative ion HRFAB-MS (Calcd for $C_{29}H_{45}NO_7F_3S$: 608.2869, Found: *m/z* 608.2849 $(M-H)^-$); ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, $J=6.4$ Hz, 16-H), 1.15 (2H, m, 14-H), 1.24 (18H, m, 5-H∼13-H), 1.510 (2H, m, 4-H), 1.510 (1H, m, 15-H), 1.947 (3H, s, $COCH_3$), 3.051 (2H, m, 1-H), 3.28 (3H, br s, OCH_3), 4.687 (1H, m, 2-H), 5.095 (1H, m, 3-H), 7.30 (3H, m, Ph), 7.54 (2H, m, Ph), 7.75 (1H, d, $J=9.8$ Hz, 2-NH).

Methanolysis of **2**

Compounds **3** (5.6 mg) and **13** (2.8 mg) were obtained from **2** (20 mg) by a similar procedure for the methanolysis of **1**. **13** (Methyl 13-methyltetradecanoate): GC-MS: column, OV-1 1% (3 mm i.d. × 1 m); column temperature, 100∼300°C (30°C/minute); carrier gas, helium; flow rate, 30 ml/minute; ionization mode, EI (positive); ionization volt, 30 eV *m/z* 256 $(M)^+$, 213, 143, 87, 74; HREI-MS Calcd for $C_{16}H_{32}O_2$: 256.2402, Found: *m/z* 256.2402 $(M)^+$; IR ν_{max} (KBr) 2930, 2850, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (6H, d, $J=6.6$ Hz, 14-H), 1.15 (2H, dt, $J=6.6$, 6.6 Hz, 12-H), 1.25 (16H, m, 4-H∼11-H), 1.51 (1H, m, 13-H), 1.62 (2H, t, $J=7.3$ Hz, 3-H), 2.30 (2H, t, $J=7.3$ Hz, 2-H), 3.67 (3H, s, 1-$COOCH_3$).

Acetylation of **3**

To a solution of **3** (5.6 mg) obtained by the methanolysis of **2** in dry pyridine (2.0 ml) was added acetic anhydride (0.1 ml). The mixture was stirred overnight at room temperature. After removal of the solvent under reduced pressure, the residue was purified by preparative TLC to give 2 mg of (2*R*,3*R*)-2-acetamido-3-acetoxy-15-methylhexadecanesulfonic acid (**14**) as a colorless powder. **14**: $[\alpha]_D^{20}$ −15.4° (*c* 0.1, MeOH); negative HRFAB-MS (Calcd for $C_{21}H_{40}NO_6S$: 434.2576, Found: *m/z* 434.2570 $(M-H)^-$); IR ν_{max} (KBr) 3420, 2920, 2850, 1740, 1650, 1550, 1230, 1050 cm^{-1}; ^1H NMR (CD_3OD) δ 0.88 (6H, d, $J=6.6$ Hz, 16-H), 1.18 (2H, m, 14-H), 1.28 (18H, m, 5-H∼13-H), 1.54 (1H, m, 15-H), 1.55 (2H, m, 4-H), 1.92 (3H, s, 2-$NHCOCH_3$ or 3-$OCOCH_3$), 2.04 (3H, s, 2-$NHCOCH_3$ or 3-$OCOCH_3$), 2.94 (1H, dd, $J=14.4$, 9.8 Hz, 1-Ha), 3.01 (1H, dd, $J=14.4$, 3.4 Hz, 1-Hb), 4.47 (1H, m, 2-H), 5.13 (1H, m, 3-H). Acetylation of **3** obtained by the methanolysis of **1** also gave (2*R*,3*R*)-2-acetamido-3-acetoxy-15-methylhexadecanesulfonic acid, $[\alpha]_D^{20}$ −19.3° (*c* 0.1, MeOH).

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