Isolation and Synthesis of Novel Farnesyl Protein Transferase Inhibitors, Valinoctins A and B, from *Streptomyces* Strain MJ858-NF3

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Two novel farnesyl protein transferase inhibitors, valinoctins A (**1**) and B (**2**), were isolated from the fermentation broth of *Streptomyces* strain MJ858-NF3. The tentative structures of these compounds were elucidated from NMR and mass spectra as dipeptides consisting of valine and a novel amino acyl moiety. Four possible isomers of valinoctin A were synthesized, and the protected derivative of the appropriate compound was crystallized to give the relative stereochemistry of X-ray analysis. Since the valine residue of valinoctin A was determined to be the L-configuration by a chiral HPLC column, absolute configuration of valinoctin A was determined.

Activation of the cellular *ras* oncogene is often observed in human neoplasias. The biological role of Ras protein is dependent on its ability to associate with cellular membranes.[1] For localization at the membrane, the Ras proteins are posttranslationally modified on their carboxyl terminals. Farnesylation to cysteine residues in the carboxyl terminal CaaX box (C, cysteine; a, aliphatic amino acid; X, any amino acid) is the first step of the modifications followed by proteolysis and carboxymethylation, and the farnesylation is known to be essential for the Ras functions involved in cell growth and proliferation.[2,3] Therefore, farnesyl protein transferase (FPTase) inhibitors would be expected to suppress the oncogenic Ras functions.

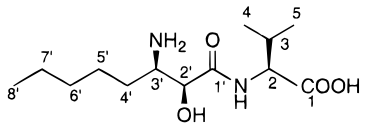
Several FPTase inhibitors such as limonene,[4] manumycin,[5] 10′-desmethoxystreptonigrin,[6] gliotoxin,[7] chaetomellic acids,[8] and pepticinnamins[9] have been reported. These natural products, however, are either nonselective, have poor cell membrane permeability, or have substantial *in vivo* toxicity. Therefore, we screened microbial secondary metabolites for FPTase inhibitors and isolated novel compounds that we named valinoctins (**1**, **2**).

Results and Discussion

Since it is known that FPTase recognizes oligopeptides including the CaaX box in the Ras protein as the substrate and transfers farnesyl groups from farnesyl pyrophosphate to the cysteine residue,[10] we employed the synthetic hexapeptide (Lys-Lys-Cys-Val-Ile-Met) as the acceptor of the farnesyl group in our assay system. We also partially purified FPTase from bovine brain.[11] The fermentation broth of the *Streptomyces* strain MJ858-NF3 inhibited the FPTase activity, and two active principles, valinoctins A (**1**) and B (**2**), were purified through chromatographic processes.

In the HRMS, **1** showed an $(M + H)^+$ ion at *m/z* 275.1973 $(M + H)^+$, indicating the molecular formula of $C_{13}H_{26}N_2O_4$ for **1**. As shown in Table 1, the ^{1}H-NMR spectrum suggested the presence of a Val residue. The ^{13}C-NMR spectrum showed 13 carbon signals that were assigned on the basis of the ^{1}H−^{13}C COSY NMR.

In the HMBC spectrum, the hydroxymethine proton at 4.23 ppm was coupled to carbons at 173.1 and 55.4 ppm and the aminomethine proton at 3.42 ppm coupled to carbons at 30.4, 26.1, 70.8 and 173.1 ppm, thus indicating the presence of an α-hydroxy-*β*-amino residue. Since the α-methine proton (4.19 ppm) of the Val residue was coupled to two carbons at 177.3 and 173.1 ppm, a peptide linkage was suggested between the amino-group of Val and the carboxyl group of the *β*-amino acid. Therefore, the structure of **1** was determined to be *N*-(3-amino-2-hydroxyoctanoyl)valine.



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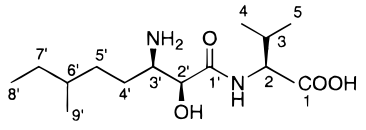

2

The molecular formula of **2** was determined to be $C_{14}H_{28}N_2O_4$ from its mass spectra. The ^{13}C-NMR and the DEPT spectra suggested the existence of 14 carbons including four Me carbons. The ^{1}H-NMR spectrum showed the structural difference between **2** and **1** to be in the aliphatic residue. The doublet Me proton at 0.92 ppm was coupled to the carbons at 33.2 ppm and 35.7 ppm in the HMBC spectrum; therefore, the structure of **2** was determined to be *N*-(6-methyl-3-amino-2-hydroxyoctanoyl)valine.

The Val residue of **1** was found to have the L-configuration by HPLC analysis of the acid hydrolysate of **1** on a chiral column (Crown Pak CR (+), 150 mm × 0.4 mm (i.d.), DAICEL, 0.4 mL/min) using $HClO_4$

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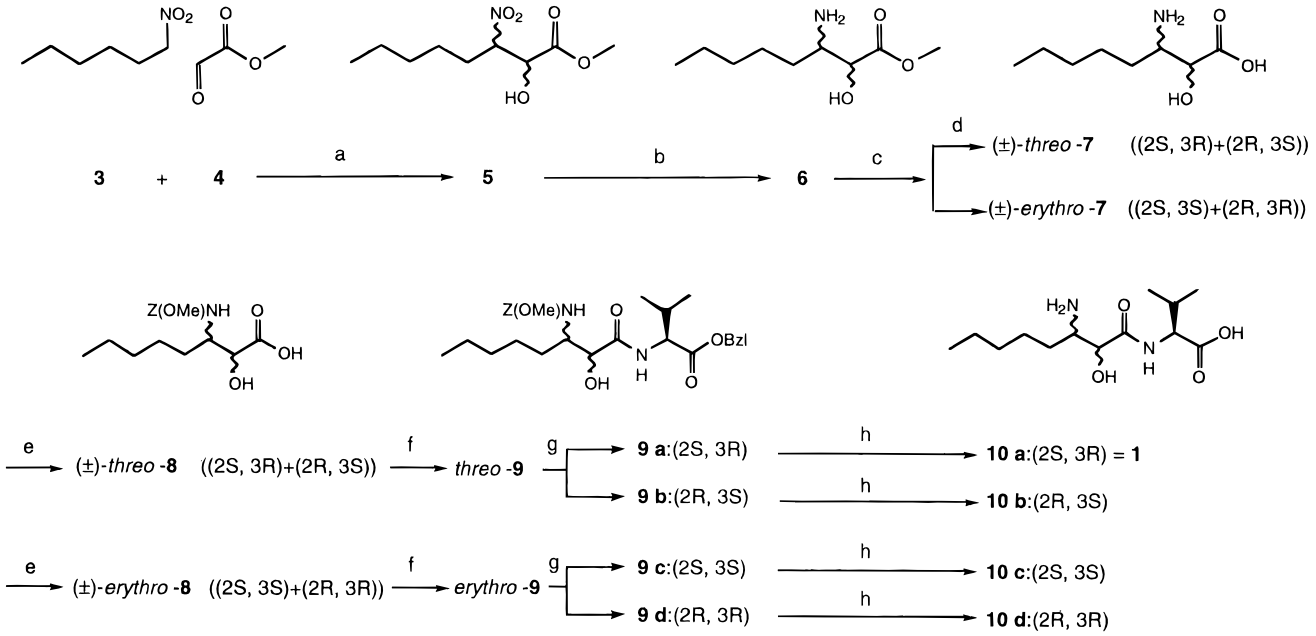
Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Valinoctins in CD_3OD[a]

1				2			
position	δ_C (multiplicity)	δ_H (multiplicity)	*J* (Hz)	position	δ_C (multiplicity)	δ_H (multiplicity)	*J* (Hz)
1	177.3 (s)			1	177.9 (s)		
2	61.0 (d)	4.19 (d)	4.9	2	61.4 (d)	4.17 (d)	4.9
3	31.9 (d)	2.23 (m)		3	32.0 (d)	2.23 (m)	
4	18.4 (q)	0.95 (d)	6.7	4	18.4 (q)	0.94 (d)	7.0
5	20.1 (q)	0.98 (d)	7.0	5	20.2 (q)	0.97 (d)	7.0
1′	173.1 (s)			1′	173.0 (s)		
2′	70.8 (d)	4.23 (d)	4.0	2′	70.8 (d)	4.23 (d)	4.0
3′	55.4 (d)	3.42 (dt)	4.0, 7.0	3′	55.9 (d)	3.38 (dt)	4.0, 6.7
4′	30.4 (t)	1.59 (m)		4′	28.1 (t)	1.62 (m)	
		1.81 (m)				1.79 (m)	
5′	26.1 (t)	1.45 (m)		5′	33.2 (t)	1.30 (m)	
						1.45 (m)	
6′	32.7 (t)[b]	1.35 (m)		6′	35.7 (d)	1.38 (m)	
7′	23.4 (t)[b]	1.35 (m)		7′	30.3 (t)	1.20 (m)	
						1.40 (m)	
8′	14.3 (q)	0.93 (t)	7.0	8′	11.6 (q)	0.91 (t)	7.3
				9′	19.3 (q)	0.92 (d)	6.4

[a] Chemical shifts were determined in ppm based on TMS as an internal standard. [b] These signal assignments may be interchangeable.

Scheme 1. Synthesis of valinoctin A (**1**) and Its Stereoisomers[a]



[a] Key: (a) NaOMe, MeOH; (b) H_2, Raney-Ni/MeOH; (c) 6 N HCl, Dowex50 (H)−NH_4OH; (d) crystallization (NH_4OH(aq)−EtOH); (e) PMZ-S, TEA, H_2O−dioxane; (f) L-valine benzyl ester, EDC, HOBt, DMF; (g) HPLC; (h) H_2, Pd/C, MeOH−AcOH−H_2O.

aqueous solution (pH 1.6) as a mobile phase. To elucidate the stereochemistry of the 3-amino-2-hydroxyoctanic acid unit **7**, we synthesized four possible stereoisomers of **1** (**10a-d**) starting with 1-nitrohexane (**3**), as shown in Scheme 1. Although valinoctin A could not be crystallized, its 4-methoxybenzyloxycarbonyl benzyl ester derivative **9a** was crystallized. The relative stereochemistry of the appropriate protected stereoisomer was determined by X-ray crystallography (Figure 1). Since the valine residue had been determined to be the L-configuration, the absolute structure of valinoctin A was determined to be (2*S*,3*R*)-***N*-(3**-amino-2-hydroxyoctanoyl)-L-valine (**10a**). **10a** gave an identical R_f value (0.58 with BuOH-MeOH−H_2O/4:1:2) and the same inhibitory activity on the enzyme. The absolute configuration of **2** is proposed to be identical with **1** because of the similar biological activity, but the 6′-configuration was not determined.

Valinoctins A (**1**) and B (**2**) inhibited bovine brain FPTase with IC_{50} values of 0.9 and 1.0 μg/mL, respectively, as shown in Figure 2. In cultured cells, however, they did not induce normal phenotypes in K-*ras*-expressing cells, even at 100 μg/mL. The cell membrane may have poor permeability toward valinoctins.

Experimental Section

General Details. Melting points were determined with a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were recorded with a Hitachi Model 260-10 spectrometer by the KBr tablet method. MS was conducted with a JEOL JMS-SX102 spectrometer. ^{1}H and ^{13}C NMR spectra for valinoctins were recorded with a JEOL JNM-A500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. ^{1}H NMR spectra for other compounds were recorded with a JEOL JNM-EX400 spectrometer at 400 MHz. TLC was carried out on Si gel plates (Kiesel gel 60F_{254}, Merck).

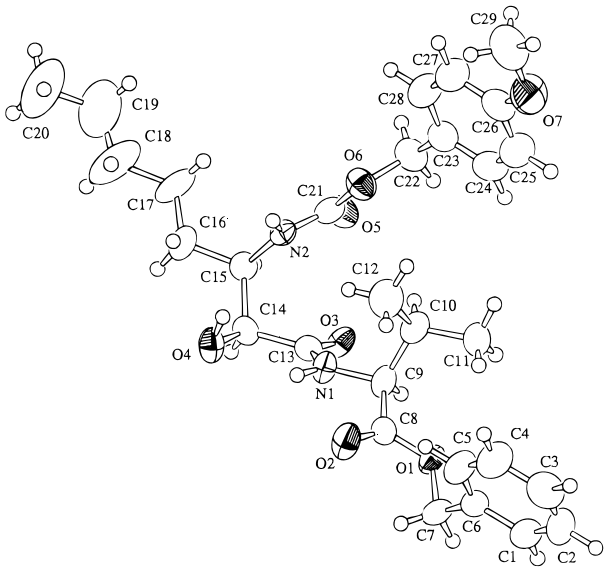


Figure 1. X-ray crystallographic analysis of PMZ-(2*S*,3*R*)-*N*-(3-amino-2-hydroxyoctanoyl)-L-valine benzyl ester (**9a**).

Preparation of FPTase. Bovine brain (50 g) was homogenized in 50 mL of buffer consisting of 50 mM Tris−HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and 0.1 mM leupeptin and then ultracentrifuged at 100 000 g for 60 min. The precipitate obtained by treatment of the supernatant with $(NH_4)_2SO_4$ at 30−50% of saturation was dissolved in 20 mL of dialyzing buffer [20 mM Tris−HCl (pH 7.5), 1 mM dithiothreitol (DTT), 20 μM $ZnCl_2$] and dialyzed against 4 L of the same buffer for 4 h and then against 4 L of fresh buffer for 15 h. The dialyzed enzyme fraction (18.5 mg protein/mL) was stored at −70 °C.

FPTase Assay. A 5 μL sample of the enzyme preparation, 11.5 μL of reaction buffer (50 mM Tris−HCl (pH 7.5), 100 μM $ZnCl_2$, 10 mM $MgCl_2$, 40 mM KCl, 1 M DTT, 1 mM PMSF, and 50 μg/mL of leupeptin) and 2.5 μL of a sample solution were mixed at 0 °C. Then, 3 μL of [^{3}H]farnesyl pyrophosphate (10 μCi/mL; New England Nuclear) and 3 μL of the hexapeptide (0.5 mg/mL, Takara Shuzo Co. Ltd., Kyoto, Japan) were added to start the reaction, and the mixture was incubated at 37 °C for 20 min. To stop the reaction, 2 μL of 250 mM EDTA was added at 0 °C. An aliquot (15 μL) of the reaction mixture was spotted onto a Si gel TLC plate and developed with PrOH−H_2O−NH_4OH (6:3:1). The chromatogram was sprayed with an enhancer (DuPont) and exposed to an XAR-5 film (Kodak) for 24 h at −70 °C.

Fermentation of Streptomyces MJ858-NF3. Taxonomic studies indicated that the producing strain belonged to the genus *Streptomyces*. The strain was deposited as *Streptomyces* strain MJ858-NF3 in the Fermentation Research Institute Agency of Industrial Science and Technology, Tsukuba, Japan (registration no. FERM P-14082). Spores of *Streptomyces* strain MJ858-NF3 were inoculated into a medium consisting of 2.0% galactose, 2.0% dextrin, 1.0% glycerol, 1.0% Bacto-soytone (Difco), 0.5% corn steep liquor, 0.2% $(NH_4)_2SO_4$, and 0.2% $CaCO_3$ (pH 7.4) and shake-cultured for 72 h at 30 °C in a 500-mL Erlenmeyer flask containing 110 mL of the medium. Therefore, 2-mL portions of the culture were inoculated into 500-mL Erlenmeyer flasks containing 110 mL of medium consisting of 2.0% soluble starch, 2.5% wheat germ, 0.1% wheat germ oil, 0.15% NaCl, 0.000 25% $CoCl_2 \cdot 6H_2O$, and 0.3% $CaCO_3$ (pH 7.4). To produce valinoctins, the suspension was fermented at 27 °C on a rotary shaker at 180 rpm for 96 h and then filtered to remove the mycelia.

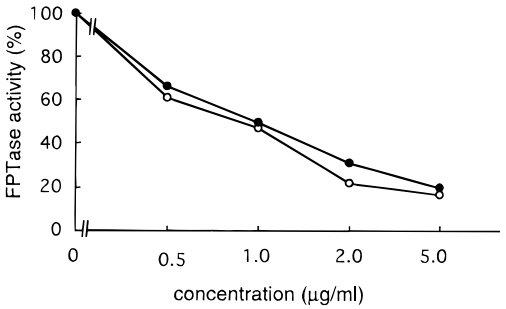


Figure 2. Inhibition of FPTase by valinoctins. Partially purified bovine brain FPTase was incubated with the hexapeptide and [^{3}H]-farnesylpyrophosphate in the presence of **1** (○) or **2** (●) for 20 min.

Isolation of Valinoctins. The filtered broth (11 L) was applied to a Dowex 50 × 4 [H^+] ion-exchange resin column (62 mm (i.d.) × 390 mm). The column was then washed with H_2O and eluted with 1 N NH_4OH. The eluant (800 mL) was extracted with the same volume of BuOH and concentrated by evaporation under reduced pressure. The precipitate (481 mg) was next dissolved in MeOH and loaded onto a Sephadex LH-20 (33 mm (i.d.) × 540 mm) gel filtration column equilibrated with MeOH, and the active fractions obtained were concentrated to dryness (178 mg). Further purification was carried out by HPLC (Capcell Pak UG120, 20 mm (i.d.) × 250 mm) using 20% CH_3CN in H_2O to give 12 mg of **1** and 11 mg of **2**.

Physicochemical Properties. **1** and **2** were obtained as white powders and were soluble in MeOH, but only slightly soluble in H_2O or in common organic solvents such as Me_2CO and EtOAc. Valinoctins A and B decomposed upon heating at 205−211 and 216−226 °C, respectively, and their UV spectra showed only end absorption. The R_f values of **1** and **2** in the solvent system BuOH−MeOH−H_2O (4:1:2) were 0.58 and 0.59, respectively.

The IR spectra of **1** and **2** were as follows: for **1**, 3390, 3250, 2970, 1675, 1580, 1545, 1400, 1275, 1155, 1110, and 775 cm^{-1}; for **2**, 3390, 3250, 2980, 1675, 1580, 1540, 1400, 1275, 1155, 1120, and 775 cm^{-1}.

Preparation of *threo*- and *erythro*-3-Amino-2-hydroxyoctanoic acid (*threo*-7 and *erythro*-8). A mixture of racemic *threo*- and *erythro*-3-amino-2-hydroxyoctanoic acid (**7**) was prepared from 1-nitrohexane (**3**) and ethyl glyoxalate (**4**) through **5** and **6** by a method previously reported in the synthesis of 3-amino-2-hydroxyvaleric acid.[12] *Threo*-**7** (less soluble isomer) was separated by recrystallization in NH_4OH−H_2O−EtOH. Crude *erythro*-**7** was recovered from the mother liquor and used in the next step without further purification. The *threo*- and *erythro*-isomers had R_f values of 0.28 and 0.40, respectively, by HPTLC (RP-18 F_{254}, Merck) in a solvent system of 5% KOAc and 1% citric acid in H_2O−CH_3CN (65:35). Because *threo*- and *erythro*-**7** were slightly soluble in H_2O, ^{1}H NMR spectra of their HCl salts were recorded.

Table 2. Positional Parameters and *B*(eq) for **9a**

atom	*x*	*y*	*z*	*B*(eq)
O(1)	0.6430(3)	0.0347(1)	0.361(1)	3.8(1)
O(2)	0.7494(4)	0.0341(2)	0.024(1)	5.1(2)
O(3)	0.9419(4)	0.0762(1)	0.730(1)	3.6(1)
O(4)	1.0672(4)	0.0601(1)	0.120(1)	4.2(1)
O(5)	1.0230(4)	0.1475(1)	0.819(1)	4.5(2)
O(6)	0.9402(4)	0.1721(1)	0.491(1)	4.4(1)
O(7)	0.6025(5)	0.2602(2)	0.103(2)	6.9(2)
N(1)	0.8834(4)	0.0662(2)	0.322(1)	3.6(2)
N(2)	1.0416(4)	0.1316(1)	0.391(1)	3.1(1)
C(1)	0.4025(6)	0.0140(2)	0.208(2)	4.3(2)
C(2)	0.3201(6)	0.0264(2)	0.086(2)	5.3(3)
C(3)	0.3276(7)	0.0472(2)	−0.123(2)	5.3(2)
C(4)	0.4184(7)	0.0560(2)	−0.213(2)	5.9(3)
C(5)	0.5002(6)	0.0435(2)	−0.100(2)	5.1(2)
C(6)	0.4941(5)	0.0226(2)	0.114(2)	3.5(2)
C(7)	0.5835(6)	0.0086(2)	0.241(2)	4.8(2)
C(8)	0.7240(5)	0.0434(2)	0.237(2)	3.3(2)
C(9)	0.7806(5)	0.0683(2)	0.401(2)	3.6(2)
C(10)	0.7448(5)	0.1045(2)	0.374(2)	3.7(2)
C(11)	0.6374(5)	0.1085(2)	0.451(2)	4.9(2)
C(12)	0.7625(7)	0.1194(2)	0.106(2)	6.3(3)
C(13)	0.9553(6)	0.0706(2)	0.495(2)	3.2(2)
C(14)	1.0596(5)	0.0702(2)	0.382(2)	3.1(2)
C(15)	1.1080(5)	0.1044(2)	0.445(1)	3.1(2)
C(16)	1.2042(5)	0.1083(2)	0.291(2)	4.3(2)
C(17)	1.2638(6)	0.1375(2)	0.377(2)	6.2(3)
C(18)	1.3492(8)	0.1423(3)	0.174(3)	10.6(5)
C(19)	1.4089(10)	0.1686(4)	0.203(3)	11.1(5)
C(20)	1.4830(9)	0.1725(4)	−0.019(3)	10.9(5)
C(21)	1.0042(6)	0.1497(2)	0.591(2)	3.7(2)
C(22)	0.8973(6)	0.1947(2)	0.680(2)	5.0(2)
C(23)	0.8200(7)	0.2135(2)	0.533(2)	4.5(2)
C(24)	0.7223(7)	0.2056(2)	0.563(2)	5.4(3)
C(25)	0.6527(7)	0.2219(2)	0.411(2)	5.9(3)
C(26)	0.6780(7)	0.2463(2)	0.238(2)	5.3(2)
C(27)	0.7741(7)	0.2545(2)	0.214(2)	6.0(3)
C(28)	0.8442(6)	0.2381(2)	0.357(2)	5.9(3)
C(29)	0.6278(8)	0.2852(3)	−0.082(2)	7.2(3)

threo-**7**: mp 243−244 °C; IR (KBr) ν max 3390, 3234, 2956, 2925, 1637, 1570, 1410, 1381, 1292, 1068 cm^{-1}; FABMS *m*/*z* $[M + 1]^+$ 176; HRMS $[M + 1]^+$ 176.1294 (calcd 176.1287, $C_8H_{18}NO_3$).

threo-**7**·HCl: ^1H NMR (D_2O, 400 MHz) δ 0.75 (3 H, t, $J = 6.8$ Hz), 1.17−1.25 (6 H, m), 1.30 (2 H, m), 1.54 (1 H, m), 1.65 (1 H, m), 3.48 (1 H, dt, $J = 3.9$, 6.8 Hz), 4.28 (1 H, d, $J = 3.9$ Hz).

erythro-**7**: FABMS *m*/*z* $[M + 1]^+$ 176; HRMS $[M + 1]^+$ 176.1306 (calcd 176.1287, $C_8H_{18}NO_3$).

erythro-**7**·HCl: ^1H NMR (D_2O, 400 MHz, signal correspond to *threo*-**7**·HCl were omitted) δ 0.74 (3 H, m), 1.15−1.36 (6 H, m), 1.52 (2 H, m), 3.57 (1 H, m), 4.37 (1 H, d, $J = 3.4$ Hz).

Synthesis of *threo*- and *erythro*-3-[(4-Methoxybenzyloxycarbonyl)amino]-2-hydroxyoctanoic Acid (*threo*-8 and *erythro*-8). *threo*-**8** and *erythro*-**8** were prepared from *threo*-**7** and crude *erythro*-**7**, respectively, by the treatment of 4-methoxybenzyl *S*-4,6-dimethylpyrimidin-2-yl thiocarbonate with Et_3N in H_2O−dioxane in the usual manner. *threo*- and *erythro*-**8** were purified by recrystallization from EtOAc−*n*-hexane.

threo-**8**: mp 74−75 °C; IR (KBr) ν max 3487, 3317, 2949, 1751, 1726, 1687, 1545, 1516, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3 H, m), 1.20−1.40 (6 H, m), 1.61 (2 H, m), 3.79 (3 H, s), 4.09 (1 H, m), 4.20 (1 H, d, $J = 1.5$ Hz), 4.57 (1 H, br s), 4.96 (1 H, d, $J = 11.7$ Hz), 5.07 (1 H, d, $J = 11.7$ Hz), 5.15 (1 H, d, $J = 9.8$ Hz), 6.86 (2H, d, $J = 8.8$ Hz), 7.26 (2 H, d, $J = 8.8$ Hz); FABMS *m*/*z* $[M]^+$ 339; HRMS $[M]^+$ 339.1660 (calcd 339.1682, $C_{17}H_{25}NO_6$).

erythro-**8**: mp 101−102 °C; IR (KBr) ν max 3467, 3354, 2956, 2931, 1732, 1693, 1680, 1518, 1304, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3 H, t, $J = 6.4$ Hz), 1.20−1.40 (6 H, m), 1.47 (1 H, m), 1.55 (1 H, m), 3.80 (3 H, s), 3.99 (1 H, m), 4.35 (1 H, d, $J = 2.0$ Hz), 5.01 (1 H, d, $J = 11.7$ Hz), 5.06 (1 H, d, $J = 11.7$ Hz), 5.10 (1 H, d, $J = 8.3$ Hz), 6.88 (2 H, d, $J = 8.8$ Hz), 7.28 (2 H, d, $J = 8.8$ Hz); FABMS *m*/*z* $[M]^+$ 339; HRMS $[M]^+$ 339.1660 (calcd 339.1682, $C_{17}H_{25}NO_6$).

Synthesis of *threo*-*N*-[3-[(4-Methoxybenzyloxycarbonyl)amino]-2-hydroxyoctanoyl]-L-valine Benzyl Ester (*threo*-9) and Separation into Diastereoisomers 9a and 9b. *threo*-**8** and L-valine benzyl ester tosylate were coupled by 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide·HCl (EDC·HCl) and 1-hydroxybenzotriazole monohydrate (HOBt) in DMF. After usual workup, the products, a mixture of **9a** and **9b**, were separated by HPLC using Capcell Pak UG120 (4.6 mm (i.d.) × 150 mm, 1 mL/min) with the solvent consisting of 45% CH_3CN, 20% MeOH, and 35% H_2O.

9a was obtained as colorless prisms (EtOAc−*n*-hexane): mp 76−77 °C; $[\alpha]^{22}_D$ −11.9° (*c* 1.0, $CHCl_3$); IR (KBr) ν max 3363, 3326, 2964, 2922, 1726, 1689, 1631, 1541, 1518, 1248 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.80 (3 H, d, $J = 6.8$ Hz), 0.85 (3 H, d, $J = 6.8$ Hz), 0.86 (3 H, m), 1.20−1.40 (6 H, m), 1.35 (1 H, m), 1.70 (1 H, m), 2.16 (1 H, m), 3.79 (3 H, s), 3.82 (1 H, m), 4.18 (1 H, dd, $J = 3.4$, 6.8 Hz), 4.56 (1 H, dd, $J = 4.9$, 9.3 Hz), 4.83 (1 H, br d, $J = 6.8$ Hz), 4.97 (2 H, s), 5.14 (1 H, d, $J = 12.2$ Hz), 5.20 (1H, d, $J = 12.2$ Hz), 5.35 (1 H, br d, $J = 8.3$ Hz), 6.86 (2 H, d, $J = 9.8$ Hz), 7.25 (1 H, m), 7.25 (2 H, d, $J = 9.8$ Hz), 7.35 (5 H, m); FABMS *m*/*z* $[M + 1]^+$ 529; HRMS $[M + 1]^+$ 529.2895 (calcd 529.2914, $C_{29}H_{41}N_2O_7$); R_f after deprotection 0.67 (EtOAc−PrOH−AcOH−H_2O, 5:5:1:3).

9b was obtained as colorless prisms (EtOAc−*n*-hexane): mp 77−78 °C; $[\alpha]^{22}_D$ +10.1° (*c* 1.0, $CHCl_3$); IR (KBr) ν max 3367, 3332, 2951, 2929, 1743, 1668, 1547, 1518, 1250, 1180, 1142 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3 H, m), 0.89 (3 H, d, $J = 6.8$ Hz), 1.20−1.40 (6 H, m), 1.71 (2 H, m), 2.20 (1 H, m), 3.79 (3 H, s), 3.80 (1 H, m), 4.20 (1 H, dd, $J = 2.9$, 5.9 Hz), 4.56 (1 H, dd, $J = 4.9$, 8.8 Hz), 4.78 (1 H, br d, $J = 5.4$ Hz), 4.99 (2 H, s), 5.09 (1 H, d, $J = 12.2$ Hz), 5.18 (1 H, d, $J = 12.2$ Hz), 5.24 (1 H, br d, $J = 8.3$ Hz), 6.86 (2 H, d, $J = 8.8$ Hz), 7.05 (1 H, br d), 7.25 (2 H, d, $J = 8.8$ Hz), 7.34 (5 H, m); FABMS *m*/*z* $[M + 1]^+$ 529; HRMS $[M + 1]^+$ 529.2899 (calcd 529.2914, $C_{29}H_{41}N_2O_7$); R_f after deprotection 0.61, (EtOAc−PrOH−AcOH−H_2O, 5:5:1:3). The absolute stereochemistry of **9a** was determined by X-ray crystallography.

Synthesis of (2*S*,3*R*)-*N*-(3-Amino-2-hydroxyoctanoyl)-L-valine (10a). An acetic acid solution of **9a** was hydrogenated over Pd black with a Parr low-pressure hydrogenation apparatus for 2 h. After filtration of the catalyst and evaporation of solvent, the product was purified by crystallization from MeOH. **10a**: mp 212−215 °C; $[\alpha]^{26}_D$ −22.9° (*c* 0.44, MeOH); ^1H NMR was identical with **1**; FABMS *m*/*z* $[M + 1]^+$ 275; HRMS $[M + 1]^+$ 275.1980 (calcd 275.1971, $C_{13}H_{26}N_2O_4$).

X-ray Crystallography. A colorless prismatic crystal of **9a** having approximate dimensions of 0.02 × 0.06 × 0.28 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite-monochromated Cu Kα radiation. Inten-

sity data were collected at a temperature of 20 °C using the $\omega - 2\theta$ scan technique to a maximum 2θ value of 140.3°. Scans of $(0.52 + 0.3 \tan \theta)°$ were made at a speed of 8.0°/min (in ω). Data reduction and application of Lorentz and polarization corrections were carried out using teXsan system software, Crystal Structure Analysis Package, Molecular Structure Corporation (1985 and 1992).

Formula $C_{29}H_{40}N_2O_7$: M 528.64, orthorhombic, space group $P2_12_12_1$, $a = 13.697(3)$ Å, $b = 40.440(3)$ Å, $c = 5.084(4)$ Å; $V = 2815(1)$ Å^3, $Z = 4$, $D_{calcd} = 1.247$ g cm^{-3}, μ(Cu Kα) = 7.27 cm^{-1}, λ(Cu Kα) = 1.5418 Å, final $R = 0.056$, 1587F with $F > 2\sigma(F)$.

The structure was solved by direct methods using SHELIX-86[13] and expanded using Fourier techniques.[14] The non-hydrogen atoms were included but not refined. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.21 and −0.21 e/Å^3, respectively.

The final atomic parameters for **9a** are shown in Table 2.[15]

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Supporting Information Available: Atomic coordinates for **9a** (5 pages). Ordering information is given on any current masthead page.

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(15) Hydrogen coordinates, thermal parameters, and bond distances and angles have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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