Poststatin, a New Inhibitor of Prolyl Endopeptidase

III. Optical Resolution of 3-Amino-2-hydroxyvaleric Acid and Absolute Configuration of Poststatin

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(Received for publication September 27, 1995)

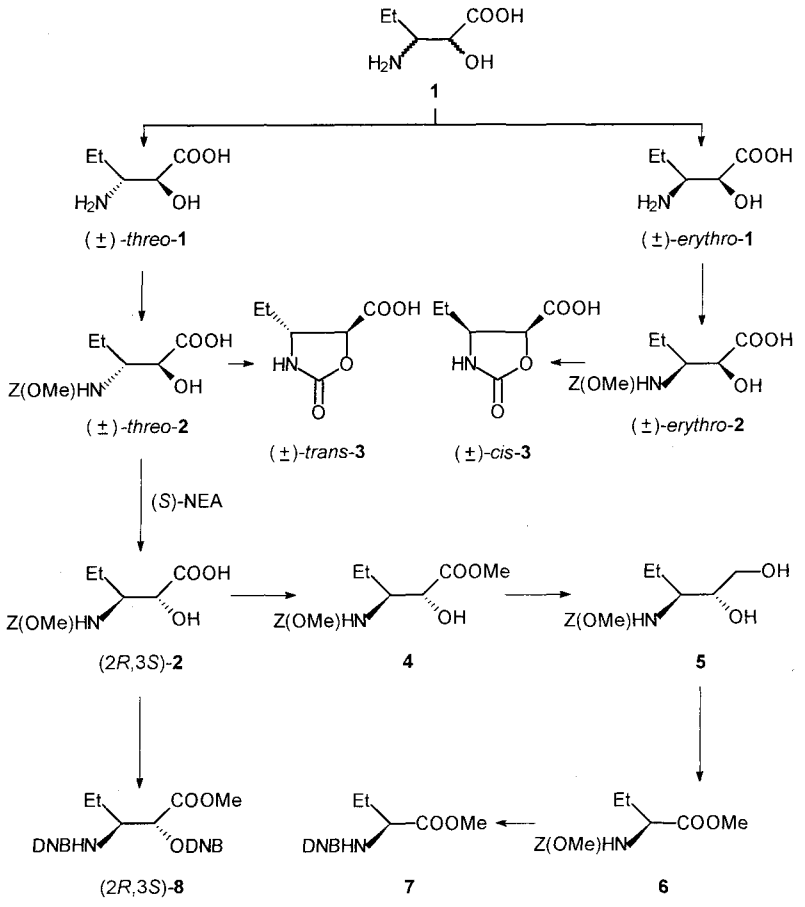
3-Amino-2-hydroxyvaleric acid was prepared, and separated into its diastereomers. The relative stereochemistry was determined by ^{1}H NMR in their oxazolidone derivatives. The *threo*-isomer was resolved by (*S*)-1-(1-naphthyl)ethylamine in the *N*-(*p*-methoxybenzyloxycarbonyl) derivative. The absolute configuration of (−)-*threo*-3-(*p*-methoxybenzyloxycarbonyl)amino-2-hydroxyvaleric acid was confirmed to be 2*R*,3*S*. The absolute configuration of 3-amino-2-oxovaleric acid in poststatin was confirmed to be *S* by comparison of the four stereoisomers of methyl *N*,*O*-bis(3,5-dinitrobenzoyl)-3-amino-2-hydroxyvalerate derived from 3-amino-2-hydroxyvaleric acid and that derived from 3-amino-2-oxovaleryl moiety of poststatin by means of HPLC with chiral column.

In the preceding paper we described the structure of poststatin, a new inhibitor of prolyl endopeptidase[1]. Poststatin is a pentapeptide containing a 3-amino-2-oxovaleric acid named postine (abbreviated as Pos) residue, but the absolute configuration of the postine moiety was left unsolved. In this paper we describe the method and result of determination of the absolute configuration of the postine moiety. We prepared optically active *threo*-3-amino-2-hydroxyvaleric acid and determined the absolute configuration of it. Moreover, we converted optically active *threo*- and racemic *erythro*-3-amino-2-hydroxyvaleric acid into methyl esters of *N*,*O*-bis(3,5-dinitrobenzoyl) derivatives to determine the retention times of these four stereoisomers on HPLC using a chiral column. We compared them with the specimen derived from poststatin. The technique described in this paper requires a smaller amount of sample than the measurement of the specific rotation.

A diastereomeric mixture of 3-amino-2-hydroxyvaleric acid (**1**) was prepared in good yield from diethyl tartrate in four steps[2,3]. The determination of absolute configuration of this compound is outlined in Scheme 1. Fractional crystallization of **1** with water and ethanol afforded crystals of the less soluble isomer which was later assigned to *threo*-**1**. The more soluble isomer (*erythro*-**1**) was recovered from the mother liquor. Both diastereomers were converted into their 2-oxazolidone derivatives (*trans*- and *cis*-**3**) respectively by alkali treatment of their *N*-(*p*-methoxybenzyloxycarbonyl) derivatives (*threo*- and *erythro*-**2**). The coupling constant of the vicinal methine protons of the 2-oxazolidone derived from the less soluble diastereomer was 4.8 Hz, while that of the diastereomer derived from the more soluble diastereomer, was 9.0 Hz (Table 1). These coupling constants were consistent with those of 2-oxazolidone derived from α-amino-β-hydroxy acids reported by FUTAGAWA *et al.*[4]. These results suggested that the configuration of less soluble **1** should be *threo*-(2*S**,3*R**).

Optical resolution of racemic *threo*-**1** was achieved by salt formation of its *N*-(*p*-methoxybenzyloxycarbonyl) derivative (*threo*-**2**). The reason that we used a *p*-methoxybenzyloxycarbonyl group for *N*-protection is that it can be removed by either acid treatment or hydrogenation procedures which are expected to be used in the following synthetic studies. Among the synthetic resolving agents tested (optically pure 1-phenylethylamine, 1-(*p*-tolyl)ethylamine, and 1-(1-naphthyl)ethylamine), only 1-(1-naphthyl)ethylamine (NEA) was able to form crystalline diastereomeric salts with *threo*-**2**. (*S*)-NEA gave a crystalline salt with (−)-*threo*-**2**. The absolute configuration of the resolved enantiomer was determined by the following procedure. Treatment of (−)-*threo*-**2** with trimethylsilyldiazomethane[5] gave its methyl ester (**4**). Reduction of **4** by sodium borohydride in mixed solvents of tetrahydrofuran-methanol[6] afforded *threo*-3-(*p*-methoxybenzyloxycarbonyl)amino-1,2-

Scheme 1.



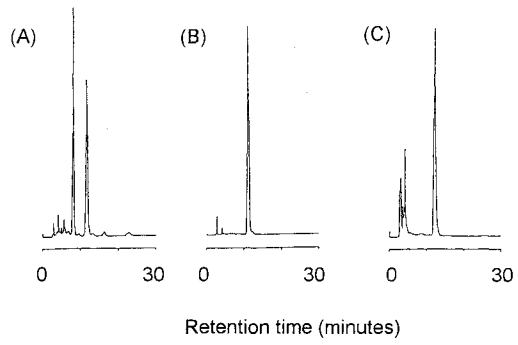
Z(OMe) = *p*-methoxybenzyloxycarbonyl, DNB = 3,5-dinitrobenzoyl, NEA = 1-(1-naphthyl)ethylamine

Table 1. Chemical shifts and coupling constants of 4-ethyl-2-oxooxazolidine-5-carboxylic acid (**3**) in CD_3OD.

	trans-**3**	*cis*-**3**
	ppm split (*J* in Hz)	ppm split (*J* in Hz)
CH_3	0.98 t (7.4)	0.97 t (7.4)
CH_2	1.69 dq (7.4, 6.2)	1.22-1.90 m
4-H	3.78 dt (4.8, 6.2)	4.05 ddd (9.0, 9.0, 4.5)
5-H	4.68 d (4.8)	5.13 d (9.0)

Fig. 1. HPLC chromatograms of methyl 2-(3,5-dinitrobenzoyl)aminobutyrate.

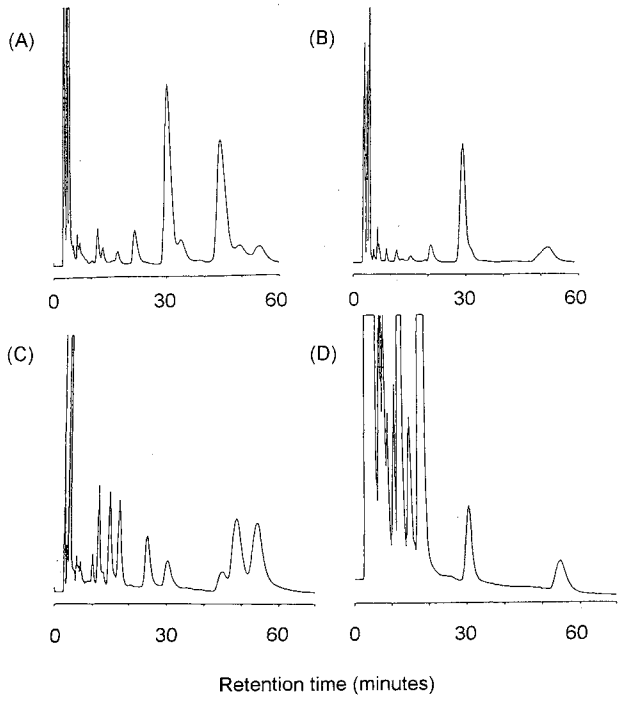
(A) Authentic (*RS*), (B) authentic (*S*), (C) derived from (−)-3-amino-2-hydroxyvaleric acid.



pentanediol (**5**). This diol gave methyl 2-(*p*-methoxybenzyloxycalbonyl)aminobutyrate (**6**) by periodate oxidation, followed by bromine oxidation of the methyl hemiacetal formed in aqueous methanolic solution[7]. After deprotection of the amino group by trifluoroacetic acid and anisol, methyl 2-(3,5-dinitrobenzoyl)aminobutyrate (**7**) was prepared by treatment with 3,5-dinitrobenzoyl chloride and triethylamine. Then, the crude solution of **7** was directly determined in HPLC with a chiral column without further purification[8]. The retention time of **7** derived from (−)-*threo*-**2** was about 11 minutes, while (±)-**7** synthesized from DL-2-aminobutyric acid showed two separated peaks at 8 and 11 minutes, and (*S*)-**7** synthesized from L-(*S*)-2-amino-

Fig. 2. HPLC chromatograms of methyl *N,O*-bis(3,5-dinitrobenzoyl)-3-amino-2-hydroxyvalerate.

(A) Racemic *threo*, (B) *threo*-(2*R*,3*S*), (C) racemic *erythro*, (D) derived from poststatin.



butyric acid showed a main peak at 11 minutes (Fig. 1). These results indicated that the configuration of C3 of (−)-*threo*-**2** was *S*. Therfore, the absolute configuration of (−)-*threo*-**2** is 2*R*,3*S*, and that of its (+)-enantiomer is 2*S*,3*R*.

In order to determine the absolute configuration of the postine moiety of poststatin, we prepared methyl *N,O*-bis(3,5-dinitrobenzoyl)-3-amino-2-hydroxyvalerate (**8**) from (±)-*threo*-**1**, (±)-*erythro*-**1** and (−)-*threo*-**1** derived by treatment of (−)-*threo*-**2** with trifluoroacetic acid and anisol. Treatment of each trifluoroacetic acid salt of **1** with 3,5-dinitrobenzoyl chloride and triethylamine (acylation) then trimethylsilyldiazomethane (esterification) gave three specimens of **8** respectively. Racemic *threo*-**8** was characterized to have the expected structure. The other reaction mixtures were directly characterized by HPLC with a chiral column. The chromatogram of (±)-*threo*-**8** showed two main peaks, at 30 and 44 minutes. On the other hand, (2*R*,3*S*)-**8** obtained from (2*R*,3*S*)-**2** showed one peak at 30 minutes. The (±)-*erythro*-**8** showed two main peaks distinguishable clearly at about 49 and 54 minutes. A diastereomeric mixture of **1** was obtained from poststatin by reduction with sodium cyanoborohydride, followed by acidic hydrolysis[1]. In a manner similar to that described above the diastereomeric mixture of **8** was obtained from this specimen. Its chromatogram showed two peaks at about 30 (identical to (2*R*,3*S*)-**8**) and 54 minutes (Fig. 2). Because the configuration at C3 of this specimen should not be changed in the reaction sequence, and that asymmetric center at C2 is formed by hydride reaction of carbonyl group, these diastereoisomers should differ from one another in the configuration at C2. Thus the configuration of C3 of postine moiety which derived from poststatin is *S*. These results indicate the peaks at 30, 44, 49 and 54 minutes correspond to the (2*R*,3*S*), (2*S*,3*R*), (2*R*,3*R*) and (2*S*,3*S*) isomers. Therefore, the structure of poststatin is L-Val–L-Val–(*S*)-Pos–D-Leu–L-Val.

Experimental

General Methods

Melting points were determined on a micro melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded at 400 and 90 MHz with a JEOL JNM-GX400 and Varian EM-390 spectrometers, respectively. SI-MS and FAB-MS spectra were measured on a Hitachi M-80H, and JEOL JMS-SX102 mass spectrometers, respectively. TLC was carried out on Merck precoated silica gel $60F_{254}$ plates and solvent systems are A) $CHCl_3$ - MeOH - AcOH (90 : 10 : 5), B) $CHCl_3$ - EtOAc - AcOH - EtOH (90 : 15 : 10 : 10). The high-performance liquid chromatography system consisted of a Waters Assoc. Model M-6000A pump, Model 440 UV detector operating at 254 nm and Model U6K injector. The chiral column used was a SUMIPAX OA-1000 (250 × 4 mm i.d., Sumitomo Chemical Co. Ltd.) and chromatograms were recorded on a Shimadzu Chromatopac C-R2AX at chart speed of 2 mm/minute. The mobile phase, *n*-hexane - 1,2-dichloroethane - EtOH (300 : 120 : 30, v/v), was run at flow rate of 1.0 ml/minute at ambient temperature.

Separation of *threo*- and *erythro*-3-Amino-2-hydroxyvaleric Acid (*threo*-**1** and *erythro*-**1**)

To a solution of diastereomeric 3-amino-2-hydroxyvaleric acid[3] (4.78 g) in hot water (40 ml) was added EtOH (40 ml). The solution was allowed to stand at room temperature for 4 hours. The precipitated crystals were collected by filtration and washed thrice with water - EtOH (1 : 1, each 3 ml), and dried to give *threo*-**1**, 2.28 g. The product was recrystallized from water - EtOH (1 : 1): mp 236~239°C (dec). After the combined filtrate and washings were decolorized by activated charcoal (0.21 g), the filtrate was evaporated and dried to give *erythro*-**1**, 2.45 g: ^{1}H NMR (D_2O) (*threo*-**1**) δ 1.02 (3H, t, J=7.2 Hz, CH_3), 1.65 (1H, ddq, J=7.2, 7.2, 14.4 Hz, C*Ha*Hb), 1.76 (1H, ddq, J=7.2, 7.2, 14.4 Hz, CHa*Hb*), 3.35 (1H, ddd, J=3.8, 7.2, 7.2 Hz, β-CH), 4.10 (1H, d, J=3.8 Hz, α-CH);

(*erythro*-**1**) δ 0.98 (3H, t, $J=7.2$ Hz, CH_3), 1.63 (2H, dq, $J=7.2, 7.2$ Hz, CH_2), 3.46 (1H, dt, $J=3.8, 7.2$ Hz, β-CH), 4.19 (1H, d, $J=3.8$ Hz, α-CH).

threo-3-(*p*-Methoxybenzyloxycarbonyl)amino-2-hydroxyvaleric Acid ((±)-*threo*-**2**)

A mixture of *threo*-**1** (1.80 g), *p*-methoxybenzyl *S*-4,6-dimethylpyrimidin-2-ylthiocarbonate (4.52 g), water (7.4 ml), dioxane (7.4 ml) and triethylamine (2.84 ml) was stirred at room temperature for 19 hours. To the reaction mixture was added water (20 ml), and unreacted carbonate was extracted twice with EtOAc (each 20 ml). The aqueous layer was cooled to 0°C and adjusted to pH 2 by addition of a 5 N hydrochloric acid, and extracted with EtOAc (once 20 ml, twice 10 ml). The combined organic layer was washed thrice with ice cold 5% hydrochloric acid (each 15 ml), and twice with saturated aq NaCl (each 15 ml) and dried over Na_2SO_4 and filtered. Evaporation of the solvent gave (±)-*threo*-**2**, 2.83 g (70.4%). Recrystallization from EtOAc-*n*-hexane (1 : 1) gave crystals: mp 117°C; ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t, $J=7.2$ Hz, CH_3), 1.68 (2H, m, CH_2), 3.40 (2H, br, OH, COOH), 3.81 (3H, s, CH_3O), 4.00 (1H, m, β-CH), 4.24 (1H, br d, α-CH), 4.99, 5.07 (2H, ABq, $J=11.6$ Hz, $-CH_2-$), 5.12 (1H, d, $J=9.9$ Hz, NH), 6.87 (2H, m, aromatic protons), 7.27 (2H, m, aromatic protons).

Anal Calcd for $C_{14}H_{19}NO_6$:
C 56.56, H 6.44, N 4.71, O 32.29.
Found:
C 56.69, H 6.42, N 4.52, O 32.39.

erythro-3-(*p*-Methoxybenzyloxycarbonyl)amino-2-hydroxyvaleric Acid ((±)-*erythro*-**2**)

(±)-*erythro*-**2** was obtained in a similar manner described above in 2.86 g (71.0%) yield from *erythro*-**1** (1.81 g). ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, $J=7.2$ Hz, CH_3), 1.63 (2H, m, CH_2) 2.82 (2H, br, OH, COOH), 3.81 (3H, s, CH_3O), 3.89 (1H, m, β-CH), 4.37 (1H, br s, α-CH), 4.92~5.17 (3H, m, NH, $-CH_2-$), 6.89 (2H, m, aromatic protons), 7.29 (2H, m, aromatic protons).

cis-4-Ethyl-2-oxooxazolidine-5-carboxylic Acid (*cis*-**3**)

To *erythro*-**2** (152.5 mg) was added 0.5 N aqueous sodium hydroxide (2.0 ml) and the mixture was stirred at room temperature for 5 hours. Next, the reaction mixture was washed twice with ether (each 2 ml), acidified with 1 N hydrochloric acid (1.0 ml) and evaporated. The residue was dissolved in a small amount of MeOH and purified by TLC with solvent A to give a white solid of *cis*-**3**, 25.5 mg (31.2%): SI-MS *m/z* 160 $(M+H)^+$. NMR data are shown in Table 1.

trans-4-Ethyl-2-oxooxazolidine-5-carboxylic Acid (*trans*-**3**)

trans-**3** (68.2 mg) was prepared from *threo*-**2** (250.3 mg) in an analogous procedure to that used for *cis*-**3**: SI-MS *m/z* 160 $(M+H)^+$. NMR data are shown in Table 1.

Optical Resolution of *threo*-3-(*p*-Methoxybenzyloxycarbonyl)amino-2-hydroxyvaleric Acid

A mixture of racemic *threo*-**2** (743.2 mg) and (*S*)-1-(1-naphthyl)ethylamine (432.0 mg) was dissolved in EtOH (2.5 ml) by external heating. The solution was allowed stand at room temperature for 1 hour. The precipitated salt was collected by filtration and dried in a desiccator containing P_2O_5. Recrystallization of the salt from EtOH was repeated twice. The diastereomeric salt was obtained in 40.0% yield: $[\alpha]_D^{27}$ −10.1° (*c* 1.0, MeOH); mp 155.5~157.5°C. The salt (285.2 mg) was treated with 0.5 N hydrochloric acid (1.6 ml) and extracted thrice with EtOAc (each 3.0 ml). The combined extracts were washed with 0.5 N hydrochloric acid (2.0 ml) and saturated aq NaCl (4.0 ml) dried (Na_2SO_4) and evaporated to give (2*R*,3*S*)-**2** in a quantitative yield: $[\alpha]_D^{27}$ −37.0° (*c* 1.8, MeOH); mp 89~92°C.

Methyl (2*R*,3*S*)-3-(*p*-Methoxybenzyloxycarbonyl)-amino-2-hydroxyvalerate (**4**)

To a stirred solution of (2*R*,3*S*)-**2** (86.0 mg) in MeOH (0.6 ml) and toluene (2.0 ml) was added 10% trimethylsilyldiazomethane in hexane (426.7 mg) at room temperature. The mixture was stirred for 30 minutes at room temperature. TLC of the mixture with solvent B showed a single spot of (2*R*,3*S*)-**4** at Rf 0.71 (*cf.* (2*R*,3*S*)-**2**: Rf 0.33). The solution was evaporated to give a clear oil of crude **4**, 103 mg.

(2*R*,3*S*)-3-(*p*-Methoxybenzyloxycarbonyl)amino-1,2-pentanediol (**5**)

To a refluxing mixture of **4** (103 mg) and sodium borohydride (27.8 mg) in THF (1.2 ml) was added MeOH (0.23 ml) by using a syringe over a period of 1 hour. After the mixture was refluxed for an additional 1 hour, water (2 ml) was added to the mixture and the organic layer was evaporated. The aqueous layer was extracted with $CHCl_3$ for six times (each 2 ml). TLC of the combined extracts with solvent B showed a spot of **5** at Rf 0.56. The solution was evaporated to give an oil of crude **5**, 90.9 mg.

Methyl (*S*)-2-(*p*-Methoxybenzyloxycarbonyl)aminobutyrate (**6**)

To an ice-cold solution of crude **5** (90.9 mg) in MeOH (1 ml) and water-MeOH (2 : 1, 2 ml) was added sodium metaperiodate (66.7 mg) in one portion with stirring. A white solid ($NaIO_3$) separated after a few minutes. TLC of the reaction mixture with solvent B give a single spot of (*S*)-2-(*p*-methoxybenzyloxycarbonyl)aminobutanal at Rf 0.77. After 30 minutes, solid $NaHCO_3$ (116.7 mg) was added to the suspension, followed by dropwise addition of bromine (0.035 ml) over 30 minutes at room temperature. Stirring was continued for an additional 1 hour and excess bromine was quenched by addition of solid $Na_2S_2O_3 \cdot 5H_2O$ (55.5 mg). After the precipitates were filtered off, MeOH was evaporated from the filtrate and water (2 ml) was added to the residue. The aqueous layer

was extracted thrice with $CHCl_3$ (each 2 ml) and dried (Na_2SO_4). TLC of the solution with solvent B showed a spot of **6** at Rf 0.86 which was agreed with that of authentic compound derived from 2-aminobutyric acid. Evaporation of the solvent gave an oil of **6**, 104.7 mg.

Methyl (*S*)-2-(3,5-Dinitrobenzoyl)aminobutyrate (**7**)

Crude **6** (104.7 mg) was dissolved in anisol (0.1 ml) and TFA (0.5 ml) and stirred at room temperature for 30 minutes. The solution was evaporated, and the residue was coevaporated twice with toluene (each 2 ml). To the residue was added 3,5-dinitrobenzoyl chloride (200.8 mg), dry THF (5 ml) and triethylamine (0.15 ml), and the mixture was maintained at 70°C for 30 minutes with stirring. After evaporation of the solvent, EtOAc (10 ml) was added to the residue, and the solution was washed with 1 N hydrochloric acid (5 ml), saturated aq NaCl (5 ml), saturated aq $NaHCO_3$ (5 ml) and saturated aq NaCl (5 ml), and was dried (Na_2SO_4). The solution of **7** in EtOAc was directly determined in HPLC without further purification.

Methyl (*S*)- and (*RS*)-2-(3,5-Dinitrobenzoyl)aminobutyrate

To a solution of L- or DL-2-aminobutyric acid (10.0 mg) in MeOH (5 ml) was added thionyl chloride (0.1 ml) with stirring at 0°C. The mixture was gently refluxed for 50~90 minutes. After evaporation of the solvent, the residue was acylated with 3,5-dinitrobenzoyl chloride (30 mg), and triethylamine (3 drops) by the similar procedure used for **7**. The solution of (*S*)- or (*RS*)-methyl 2-(3,5-dinitrobenzamide)butyrate thus obtained in EtOAc was directly determined in HPLC without further purification.

Methyl *threo*-*N*,*O*-bis(3,5-Dinitrobenzoyl)-3-amino-2-hydroxyvalerate ((±)-*threo*-**8**)

Racemic *threo*-**1** (50.7 mg) was dissolved in TFA (1.5 ml) and excess TFA was coevaporated twice with toluene (each 2 ml). To the residue was added dry THF (20 ml), 3,5-dinitrobenzoyl chloride (263.4 mg) and triethylamine (0.5 ml), and the mixture was heated at 70°C with stirring for 1 hour. After evaporation of the solvent, 1 N hydrochloric acid (10 ml) was added to the residue, and the solution was extracted thrice with EtOAc (each 10 ml). The combined extracts were dried (Na_2SO_4) and evaporated. A solution of 10% trimethylsilyldiazomethane in hexane (0.6 ml) was added to the residue in toluene (2.5 ml) and MeOH (0.5 ml). The mixture was kept at room temperature for 30 minutes with stirring. After removal of the solvent, EtOAc (25 ml) was added to the residue and washed with saturated aq $NaHCO_3$ (15 ml) and saturated aq NaCl (15 ml), and dried (Na_2SO_4). Evaporation of the solvent and purification by TLC (silica gel, dichloromethane - MeOH (80 : 1), Rf 0.46) gave (±)-*threo*-**8**: FAB-MS (+) *m*/*z* 536 $(M+1)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (3H, t, $J=7.3$ Hz, CH_3), 1.83 (1H, m, C*Ha*Hb), 1.96 (1H, m, CHa*Hb*), 3.85 (3H, s, $COOCH_3$), 4.83 (1H, m, β-CH), 5.53 (1H, d, $J=2.9$ Hz, α-CH), 6.85 (1H, d, $J=9.3$ Hz, NH), 8.98 (2H, m, aromatic protons), 9.20 (3H, m, aromatic protons), 9.29 (1H, m, aromatic proton).

Racemic Methyl *erythro*-*N*,*O*-bis(3,5-Dinitrobenzoyl)-3-amino-2-hydroxyvalerate ((±)-*erythro*-**8**)

Racemic *erythro*-**1** (10.9 mg) was dissolved in TFA (0.3 ml) and excess TFA was coevaporated twice with toluene (each 2 ml). Using this TFA salt, (±)-*erythro*-**8** was obtained by the acylation and esterification in a manner similar to that described in the preparation of (±)-*threo*-**8**. The resulting solution of racemic *erythro*-**8** in EtOAc was directly determined in HPLC without further purification.

Methyl (2*R*,3*S*)-*N*,*O*-bis(3,5-Dinitrobenzoyl)-3-amino-2-hydroxyvalerate ((2*R*,3*S*)-**8**)

(2*R*,3*S*)-**2** (10.7 mg) was disolved in a mixture of anisol (0.1 ml) and TFA (0.5 ml) and stirred at room temperature for 40 minutes. Using this TFA salt, (2*R*,3*S*)-**8** was obtained by the acylation and esterification in a manner similar to that described in the preparation of (±)-*threo*-**8**. The resulting solution of (2*R*,3*S*)-**8** in EtOAc was directly determined in HPLC without further purification.

Diastereomeric Mixture of Methyl *N*,*O*-bis(3,5-Dinitrobenzoyl)-3-amino-2-hydroxyvalerate from Poststatin

To the solution of diastereomeric mixture of 3-amino-2-hydroxyvaleric acid derived from poststatin[1] (0.7 mg) in THF (0.4 ml) and TFA (3 μl) was added 3,5-dinitrobenzoyl chloride (1.2 mg) and triethylamine (20 μl), and the mixture was maintained at 70°C for 1 hour with stirring. After evaporation of the solvent, 1 N hydrochloric acid (0.1 ml) was added to the residue, and the solution was extracted five times with EtOAc (each 0.2 ml). The combined extracts were dried (Na_2SO_4) and evaporated. The solution of 10% trimethylsilyldiazomethane in hexane (10 μl) was added to the residue in toluene (50 μl) and MeOH (10 μl). The mixture was kept at room temperature for 30 minutes with stirring. The solution of diastereomeric mixture of **8** was directly determined in HPLC without further purification.

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