Poststatin, a New Inhibitor of Prolyl Endopeptidase

IV. The Chemical Synthesis of Poststatin

MAKOTO TSUDA, YASUHIKO MURAOKA*, MACHIKO NAGAI and TOMIO TAKEUCHI

Institute of Microbial Chemistry, M. C. R. F.,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

TAKAAKI AOYAGI

Department of Hygienic Chemistry, Showa College of Pharmaceutical Sciences,
3-Chome Higashitamagawagakuen, Machida-shi, Tokyo 194, Japan

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Total synthesis of poststatin was achieved by both liquid phase and solid phase methods. In both methods, the (2*R*,3*S*)-3-amino-2-hydroxyvaleric acid moiety was incorporated into protected pentapeptides, and was oxidized to (*S*)-3-amino-2-oxovaleric acid (postine). Deprotection of the oxidized pentapeptides gave a specimen identical with natural poststatin in physico-chemical properties and prolyl endopeptidase inhibitory activity.

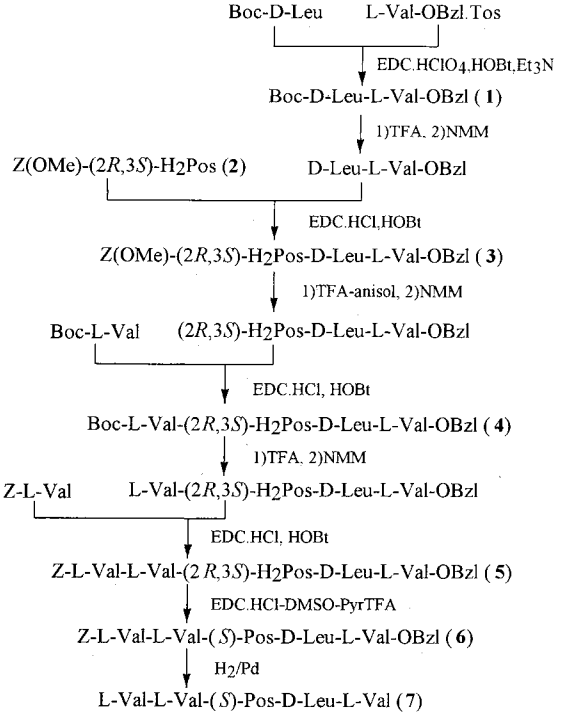
Poststatin is an inhibitor of prolyl endopeptidase ($IC_{50}=0.03\,\mu g/ml$) isolated from a culture filtrate of *Streptomyces viridochromogenes* MH534-30F3[1]. Its structure and absolute stereochemistry was elucidated as L-Val-L-Val-(*S*)-3-amino-2-oxovaleryl-D-Leu-L-Val in the previous paper[2,3].

Synthetic inhibitors specific for prolyl endopeptidase reported by YOSHIMOTO in 1977[4] and WILK in 1983[5], were peptidyl chloromethyl ketones or peptide aldehydes such as benzyloxycarbonyl (abbreviated as Z)-Gly-Pro-CH_2Cl or Z-Pro-prolinal which contain pyrrolidine groups derived from proline. On the other hand, poststatin includes a 3-amino-2-oxovaleric acid residue (named as postine,[1] abbreviated as Pos) and neither pyrrolidine nor aldehyde in its structure. Because of its interesting biological activities and unique structure, we attempted to establish the synthetic method which could be applied for studies on structure-activity relationships and the development of effective protease inhibitors described in the following papers[6,7]. We report herein the total synthesis of poststatin by liquid phase and solid phase synthesis. The key feature of these syntheses is assembling the peptide containing a 3-amino-2-hydroxyvaleric acid (abbreviated as H_2Pos) residue and then oxidation of it to Pos moiety.

Chemistry

The liquid phase synthesis of poststatin was achieved by following the procedure shown in scheme 1. Boc-D-Leu-L-Val benzyl ester (**1**) was synthesized (97.8%) by conventional liquid phase synthesis. The (2*R*,3*S*)-3-(*p*-methoxybenzyloxycarbonyl)amino-2-hydroxyvaleric acid (abbreviated as Z(OMe)-H_2Pos, (**2**)) was prepared as described in previous paper[3]. In the following steps of the synthesis, the *N*-protected amino

Scheme 1. Liquid phase synthesis of poststatin.



Tos = *p*-toluensulfonate, NMM = *N*-methylmorpholine
PyrTFA = pyridinium trifluoroacetate

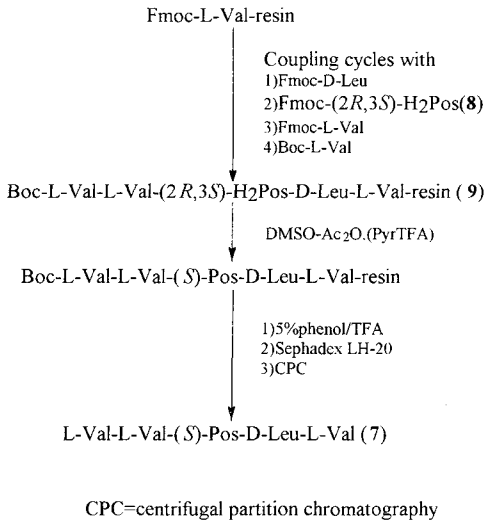
acids were coupled to the growing chain in a stepwise fashion using water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (abbreviated as EDC) and 1-hydroxybenzotriazol (abbreviated as HOBt). The coupling steps were monitored for completion using ninhydrin and HBr-ninhydrin visualization on TLC. For the coupling reaction the *N*-protected peptides were cleaved by trifluoroacetic acid in the presence or absence of anisole. The yield obtained for the *N*-protected peptides as intermediates were 85.6% for Z(OMe)-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**3**), 74.1% for Boc-L-Val-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**4**), and 88.4% for Z-L-Val-L-Val-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**5**), based on the appropriate amine components.

Selective oxidation of the (2*R*,3*S*)-H_2Pos moiety of **5** to a (*S*)-Pos moiety was performed by the Pfitzner-Moffatt method[8] to give Z-L-Val-L-Val-(*S*)-Pos-D-Leu-L-Val-OBzl (**6**), (66.4%). The reaction was monitored by TLC using 2,4-dinitrophenylhydrazine and HBr-ninhydrin visualization. Catalytic reduction of **6** with palladium-black in AcOH - MeOH - H_2O (6 : 3 : 1) gave L-Val-L-Val-(*S*)-Pos-D-Leu-L-Val (**7**) as a colorless powder, which was purified first by adsorption resin (Diaion HP-20, Mitsubishi Chemical Industries Ltd.) and then by gel filtration on Sephadex LH-20 to give poststatin as a colorless powder.

The solid phase synthesis of poststatin is outlined in scheme 2. The starting *N*-9-fluorenylmethyloxycarbonyl (abbreviated as Fmoc) valyl *p*-alkoxybenzyl ester resin (Kokusan Chemical Works, Ltd.) provided a substitution level of 0.28 mmol/0.5 g. Optically active Fmoc-(2*R*,3*S*)-H_2Pos (**8**) was prepared from (2*R*,3*S*)-H_2Pos by the reaction with 9-fluorenylmethyl chloroformate[9]. A cycle for the incorporation of each amino acid residue into the growing peptide chain consisted of the following operations[10]: (1) *N*,*N*-Dimethylformamide (abbreviated as DMF), 3 × 1 minute (2) 20% piperidine in DMF, 2 × 3 minutes and 1 × 20 minutes (3) DMF, 3 × 1 minute (4) *N*-methyl-2-pyrrolidone (abbreviated as NMP) 3 × 1 minute (5) coupling, three equivalent of Fmoc-amino acid, three equivalent of HOBt in NMP and three equivalent of diisopropylcarbodiimide (abbreviated as DIPCI), 2 hours (6) NMP, 3 × 1 minute.

Oxidation of the H_2Pos residue in Boc-pentapeptide resin (**9**) to a Pos residue was achieved by application of the Albright-Goldman method[11] to solid phase synthesis consisting of the following operations: (1) DMSO, 3 × 1 minute (2) oxidation, acetic anhydride (0.4 ml) and pyridinium trifluoroacetate (106 mg) in DMSO (6.5 ml), overnight (3) DMF, 3 × 1 minute (4) MeOH, 3 × 1 minute (5) drying over P_2O_5. The completed pentapeptide, poststatin, was cleaved from the dried resin and the Boc-protecting group was removed simultaneously by treatment with trifluoroacetic acid - phenol (19 : 1) (2 × 1 hour). A brown residue obtained after solvent evaporation was purified by gel filtration on Sephadex LH-20 in MeOH followed by centrifugal partition chromatography[12] to give the poststatin as a white powder. The identity of this synthetic compound (**7**) with a natural poststatin, was established by TLC, MS and ^{1}H NMR spectra, elemental analysis, and/or optical rotation and HPLC.

Scheme 2. Solid phase synthesis of poststatin.



CPC=centrifugal partition chromatography

Experimental

General

Melting points were determined on a micro melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively with a JEOL JNM-GX400 spectrometer. FAB-MS spectra were measured on a JEOL JMS-SX102 mass spectrometer. TLC was carried out on Merck precoated silica gel 60F_{254} plates, or precoated RP-18F_{254} plates.

The high-performance liquid chromatography system consisted of a Waters Assoc. Model M-6000A pump, Model 440 UV detector operating at 254 nm and a Model U6K injector. The centrifugal partition chromatography system consisted of a Sanki Engineering Ltd. Model NMF centrifuge at 1000 rpm revolutional speed, Model LBP-V pump at flow rate of 2.4 ml/minute, Model UVIS 200 detector operating at 254 nm and Model FCU-V injector. The two phase solvent system was composed of 1-BuOH - AcOH - H_2O (100 : 1 : 100), which was equili-

brated in a separatory funnel at room temperature and the layers were separated before use. The upper layer was used as stationary phase and the lower layer was used as mobile phase in desending mode.

Boc-D-Leu-L-Val-OBzl (**1**)

To an ice-cold solution of Boc-D-leucine hydrate (0.749 g), valine benzyl ester *p*-toluenesulfonate (1.252 g) and HOBt (0.811 g) in CH_2Cl_2 (15 ml) was added triethylamine (0.504 ml) and 97% EDC·$HClO_4$ (1.108 g), and the resulting solution was chilled in an ice bath for 2 hours. Stirring was continued for 4 hours at room temperature, and water (30 ml) and 1% aq citric acid (5 ml) were added to the mixture. After the separated crystals were filtered off, the organic layer was separated, and washed with water (20 ml), saturated aq $NaHCO_3$ (20 ml) and water (10 ml), and dried (Na_2SO_4). Evaporation of the solvent gave crude crystals. The product was dissolved in EtOAc and subjected to silica gel column chromatography with EtOAc - hexane (5 : 2) to give **1** as a white solid, 1.233 g (97.8%): mp 82~83°C; $[\alpha]_D^{29}$ +35.1° (*c* 1.0, $CHCl_3$); Rf 0.54 (CH_2Cl_2 - MeOH, 40 : 1); FAB-MS *m/z* 421 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.89, 0.92 (3H, 3H, d, d, each $J=7.0$ Hz, CH_3 (Val)), 0.93, 0.94 (3H, 3H, d, d, each $J=6.4$ Hz, CH_3 (Leu)), 1.44 (9H, s, Boc), *ca.* 1.45 (1H, m, overlapping, β-C*Ha*Hb (Leu)), 1.62~1.76 (2H, m, β-CHa*Hb* (Leu), γ-CH (Leu)), 2.19 (1H, m, β-CH (Val)), 4.14 (1H, m, α-CH (Leu)), 4.57 (1H, dd, $J=4.2$, 9.2 Hz, α-CH (Val)), 4.79 (1H, br, NH), 5.13, 5.19 (2H, ABq, $J=12.0$ Hz, CH_2Ph), 6.68 (1H, br, NH), 7.29~7.40 (5H, m, Ph).

Z(OMe)-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**3**)

A solution of **1** (305.3 mg) in TFA (3 ml) was stirred at room temperature for 40 minutes. The solution was evaporated, and the residue was coevaporated twice with toluene (each 3 ml). To the residue was added **2** (227.7 mg) and HOBt (147.1 mg) in DMF (2.5 ml). *N*-methylmorpholine (86 μl) and EDC·HCl (181.0 mg) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 6 hours. The mixture was diluted with EtOAc (25 ml), and was washed with 4% aq $NaHCO_3$, 10% aq citric acid and saturated aq NaCl (each 20 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1~50 : 1) to give **3** as colorless crystals, 372.6 mg (85.6%): mp 115~115.5°C; $[\alpha]_D^{25}$ +45.5° (*c* 1.0, $CHCl_3$); Rf 0.22 (CH_2Cl_2 - MeOH, 30 : 1); FAB-MS *m/z* 600 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.80~1.04 (15H, m, $CH_3\times5$), 1.51~1.80 (5H, m, β-CH_2 (Leu), γ-CH (Leu), CH_2 (H_2Pos)), 2.19 (1H, m, β-CH (Val)), *ca.* 3.78 (1H, m, β-CH (H_2Pos)), 3.80 (3H, s, CH_3O), 4.12 (1H, dd, $J=6.4$, 3.0 Hz, α-CH (H_2Pos)), 4.49 (1H, m, α-CH (Leu)), 4.52 (1H, dd, $J=8.4$, 4.8 Hz, α-CH (Val)), 4.61 (1H, br d, $J=6.4$ Hz, OH), 4.98 (2H, s, CH_2Ph(OMe)), 5.08, 5.18 (2H, ABq, $J=12.4$ Hz, CH_2Ph), 5.31 (1H, br d, $J=8.4$ Hz, NH (H_2Pos)), 6.79 (1H, br d, $J=8.4$ Hz, NH (Val)), 6.87 (2H, m, *Ph*(OMe)), 7.07 (1H, br d, $J=8.4$ Hz, NH (Leu)), 7.22~7.40 (7H, m, *Ph*(OMe), Ph).

Boc-L-Val-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**4**)

4 was obtained, in a manner similar to that described in the preparation of **3**, by coupling reaction of trifluoroacetate salt of deproteced **3** (0.285 mmol) with Boc-Val (65.4 mg, 0.301 mmol). The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1~100 : 3) to give **4** as colorless crystals, 292.4 mg (74.1%): mp 167~168°C; $[\alpha]_D^{26}$ +35.7° (*c* 1.0, $CHCl_3$); Rf 0.28 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 635 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.80~1.03 (21H, m, $CH_3\times7$), 1.44 (9H, s, Boc), 1.52~1.90 (5H, m, β-CH_2 (Leu), γ-CH (Leu), CH_2 (H_2Pos)), 2.11 (1H, m, β-CH (Val)), 2.19 (1H, m, β-CH (Val)), 3.87 (1H, m, β-CH (H_2Pos)), 3.93 (1H, dd, $J=5.8$, 8.5 Hz, α-CH (Val)), 4.10 (1H, dd, $J=3.4$, 7.0 Hz, α-CH (H_2Pos)), 4.36 (1H, m, α-CH (Leu)), 4.58 (1H, dd, $J=5.0$, 8.6 Hz, α-CH (Val)), 5.09 (1H, br, NH (Val)), 5.10, 5.20 (2H, ABq, $J=12.2$ Hz, CH_2Ph), 5.36 (1H, br, OH), 6.98 (1H, br d, $J=8.6$ Hz, NH (Val)), 7.04 (1H, br d, $J=8.4$ Hz, NH (H_2Pos)), 7.20 (1H, br d, $J=8.4$ Hz, NH (Leu)), 7.30~7.40 (5H, m, Ph).

Z-L-Val-L-Val-(2*R*,3*S*)-H_2Pos-D-Leu-L-Val-OBzl (**5**)

5 was obtained, in a manner similar to that described in the preparation of **3**, by coupling reaction of trifluoroacetate salt of deprotected **4** (0.461 mmol) with Z-Val (121.5 mg, 0.484 mmol). The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1~40 : 1) to give **5** as colorless crystals, 312.6 mg (88.4%): mp 222~223°C; $[\alpha]_D^{27}$ +9.2° (*c* 1.0, $CHCl_3$); Rf 0.33 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 768 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.76~1.00 (27H, m, $CH_3\times9$), 1.37 (1H, m, C*Ha*Hb (H_2Pos)), 1.53~*ca.* 1.75 (3H, m, overlapping, β-CH_2 (Leu), γ-CH (Leu)), 1.78 (1H, m, CHa*Hb* (H_2Pos)), 1.93~2.10 (2H, m, β-$CH\times2$ (Val)), 2.17 (1H, m, β-CH (Val)), 4.13~4.27 (2H, m, $CH\times2$ (H_2Pos)), 4.41~4.64 (4H, m, α-CH (Val$\times3$, Leu)), 5.06, 5.15 (2H, ABq, $J=12.0$ Hz, CH_2Ph), 5.06, 5.09 (2H, ABq, $J=12.0$ Hz, CH_2Ph), 5.49 (1H, br s, OH), 5.86 (1H, br, NH (Val)), 7.09 (1H, br, NH (Val)), 7.22 (1H, br, NH (H_2Pos)), *ca.* 7.22~7.39 (10H, m, overlapping, $Ph\times2$), 7.45 (1H, br d, $J=7.8$ Hz, NH (Leu)), 8.09 (1H, br, NH (Val)).

Z-L-Val-L-Val-(*S*)-Pos-D-Leu-L-Val-OBzl (**6**)

A mixture of **5** (308.3 mg), pyridinium trifluoroacetate (40.2 mg), EDC·HCl (231.4 mg), anhydrous DMSO (3 ml) was stirred at room temperature for 24 hours. The reaction mixture was diluted with EtOAc (30 ml), and the mixture was washed with water (30 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2 - MeCN (20 : 3~20 : 7) to give **6**, 207.5 mg. This **6** was purified by Sephadex LH-20 with 0.3% AcOH - MeOH to give **6** as crystals, 204.2 mg (66.4%): mp 201~202°C; $[\alpha]_D^{23}$ +9.5° (*c* 1.0, $CHCl_3$); Rf 0.41

(CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 766 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.77~1.04 (27H, m, $CH_3\times9$), 1.50~1.85 (4H, m, β-CH_2 (Leu), γ-CH (Leu), C*Ha*Hb (Pos)), 2.00 (1H, m, CHa*Hb*Pos)), 2.05~2.25 (3H, m, β-CH×3 (Val)), 4.06 (1H, m, α-CH (Val)), 4.32 (1H, dd, J=7.4, 8.6 Hz, α-CH (Val)), 4.47~4.63 (2H, m, α-CH×2 (Val, Leu)), 5.08, 5.16 (2H, ABq, J=12.0 Hz, CH_2Ph), 5.10, 5.11 (2H, ABq, J=12.4 Hz, CH_2Ph), 5.34 (1H, ddd, J=4.8, 8.2, 8.2 Hz, CH (Pos)), 5.57 (1H, br d, J=8.6 Hz, NH (Val)), 6.69 (1H, br d, J=8.6 Hz, NH (Val)), 6.74 (1H, br d, J=9.1 Hz, NH (Val)), 6.86 (1H, br d, J=8.2 Hz, NH (Pos)), 7.25~7.40 (10H, m, Ph×2), 7.48 (1H, br d, J=8.8 Hz, NH (Leu)); ^{13}C NMR ($CDCl_3$) δ 195.5 (*C*OCONH) was obserbed.

Poststatin (L-Val-L-Val-(*S*)-Pos-D-Leu-L-Val) (**7**)

To a solution of **6** (203.1 mg) in AcOH-MeOH-H_2O (6:3:1, 30 ml) was added palladium-black catalyst (12.4 mg). The mixture was hydrogenated at room temperature in a hydrogen atmosphere for 27 hours. The catalyst was filtered off, evaporation of the solvent gave white powder. The product was purified by Diaion HP-20 with 5% aq MeOH-50% aq MeOH (containing 0.3% AcOH) to give powder of **7**, 122.6 mg. The product was rechromatographed on a column of Sephadex LH-20 with 0.3% AcOH-MeOH elution to give epimer-containing powder 83.3 mg and pure **7**, 33.3 mg. (23.2%): mp 182~184°C; $[\alpha]_D^{28}$ +15.1° (*c* 0.62, AcOH) [lit. $[\alpha]_D^{20}$ +13.9° (*c* 0.5, AcOH)][1]; Rf 0.59 (RP-18F_{254} plate, 5% AcOK and 1% citric acid-MeOH, 3:2); FAB-MS *m/z* 542 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 0.70~0.95 (27H, m, $CH_3\times9$), 1.40~1.67 (3H, m, β-C*Ha*Hb (Leu), γ-CH (Leu), C*Ha*Hb (Pos)), 1.61 (1H, m, β-CHa*Hb* (Leu)), 1.76 (1H, m, CHa*Hb* (Pos)), 1.86~2.10 (3H, m, β-CH×3 (Val)), 3.20 (1H, m, α-CH (Val)), 4.06 (1H, m, α-CH (Val)), 4.27 (1H, m, α-CH (Val)), 4.43 (1H, m, α-CH (Leu)), 4.95 (1H, m, CHCOCO), 7.98 (1H, br d, J=8.4 Hz, NH (Val)), 8.10 (1H, br d, J=8.9 Hz, NH (Val)), 8.36 (1H, d, J=6.6 Hz, NH (Pos)), 8.56 (1H, d, J=8.7 Hz, NH (Leu)). HR-MS (FAB) *m/z* 542.3549 (calcd for $C_{26}H_{48}N_5O_7$ $(M+H)^+$; 542.3554);

Anal Calcd for $C_{26}H_{47}N_5O_7\cdot1.25H_2O$:
C 55.35, H 8.84, N 12.41, O 23.39.
Found:
C 55.48, H 8.67, N 12.12, O 23.36.

Synthetic **7** was identical with the natural poststatin in every respects including prolyl endopeptidase inhibitory activity.

Fmoc-(2*R*,3*S*)-H_2Pos (**8**)

To the solution of (2*R*,3*S*)-H_2Pos (0.759 g) in dioxane (10 ml) and aq $NaHCO_3$ (1.199 g of $NaHCO_3$ in 20 ml of water) was added slowly with stirring and ice bath cooling a solution of 9-fluorenylmethyl chloroformate (1.623 g) in dioxane (10 ml). The mixture was stirred at room temperature for 2 hours, diluted with water (300 ml), and extracted twice with ether (150 ml and 75 ml). The aqueous layer was cooled in an ice bath, acidified with 6N HCl to pH 1.5, and extracted thrice with EtOAc (each 50 ml). The combined extracts were washed with saturated aq NaCl (50 ml), and dried (Na_2SO_4). Crude **8** obtained after evaporation was crystallized with EtOAc-hexane (1:1) to give **8** as colorless crystals, 0.999 g, mp 160~161.5°C. From the filtrate was collected crude amorphous solid of **8** (0.548 g), and the solid was purified by silica gel column chromatography with $CHCl_3$-MeOH-AcOH (95:5:1) to give **8** as a white solid, 0.307 g. The first crops were suspended in EtOAc (4 ml) at 50°C for 40 minutes, added hexane (4 ml), and collected by filtration to give colorless crystals of **8**, 0.866 g: mp 162~162.5°C; $[\alpha]_D^{26}$ −30.2° (*c* 1.0, MeOH); Rf 0.32 ($CHCl_3$-MeOH-AcOH, 90:10:5); FAB-MS *m/z* 356 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 0.96 (3H, t, J=7.4 Hz, CH_3), 1.69 (2H, m, CH_2), 3.25 (br, COOH, OH), 4.01 (1H, m, C*H*NH), 4.18 (1H, dd, J=6.6, 6.6 Hz, ArC*H*Ar), 4.24 (1H, br s, C*H*OH), 4.39 (1H, dd, J=6.6, 10.6 Hz, C*Ha*HbOCO), 4.43 (1H, dd, J=6.6, 10.6 Hz, CHa*Hb*OCO), 5.13 (1H, d, J=9.6 Hz, NH), 7.29 (2H, m, aromatic protons), 7.38 (2H, m, aromatic protons), 7.54 (2H, m, aromatic protons), 7.74 (2H, m, aromatic protons).

Solid Phase Synthesis of Poststatin (**7**)

Fmoc-Val-resin (0.5 g, 0.28 mmol) was placed in the peptide synthesis flask and the solid phase synthesis was carried out with 6.5 ml portions of solvents. In each cycle, three-equivalent of amino acid derivative, HOBt and DIPCI were used. Thus, Fmoc-D-Leu (297.2 mg), Fmoc-(2*R*,3*S*)-H_2Pos (**8**) (298.9 mg), Fmoc-L-Val (285.2 mg), and Boc-L-Val (182.7 mg) were succesively coupled to the resin. To the resultant pentapeptide resin was added acetic anhydride (0.4 ml) and pyridinium trifluoroacetate (106 mg) in DMSO (6 ml), and the mixture was stirred overnight. The oxidated pentapeptide resin was washed with DMSO (6.5 ml), thrice with DMF (each 6.5 ml), and thrice with MeOH (each 6.5 ml) and dried over P_2O_5. To liberate the peptide from the resin, the resin was stirred in TFA (6.5 ml) containing 5% (w/v) phenol for 1 hour, and removed the resin particles by filtration. This operation was carried out again, and the resin was washed thrice with TFA (6.5 ml). The combined filtrates and washings were concentrated and gel chromatographed on a colum of Sephadex LH-20 with MeOH elution. Evaporation of the active eluate gave **7** as slightly colored solid, 192.2 mg. The product was purified by centrifugal partition chromatography with two phase solvent system as described above to give white powder of **7** as trifluoroacetate, 149.3 mg: mp 189~190°C (dec);

Anal Calcd for $C_{26}H_{47}N_5O_7\cdot CF_3COOH$:
C 51.29, H 7.39, N 10.68.
Found:
C 51.27, H 7.50, N 10.57.

FAB-MS and ^1H NMR spectra of this synthetic **7** was identical with the poststatin trifluoroacetate prepared from natural poststatin. TLC (BuOH-AcOH-H_2O

(4 : 1 : 1) and BuOH - MeOH - H_2O (4 : 1 : 2) on silica gel plate, and 1% aq citric acid and 5% aq AcOK - acetonitrile (65 : 35) on reversed phase RP-18 plate; Rydon-Smith visualization) and the retention time in HPLC (column, Capcell pak C18, 150 × 4.6 mm i.d., Shiseido Co., Ltd; eluent, 1% aq citric acid and 0.65% aq AcOK - acetonitrile - 1-PrOH (18 : 1 : 3, v/v); flow rate, 0.6 ml/minute; temperature; 25°C) was also agreed with that of natural poststatin.

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