Aspartic Protease Inhibitors: Expedient Synthesis of 2-Substituted Statines

Jeremy M. Travins,[†] Matthew G. Bursavich,[†] Daniel F. Veber,[#] and Daniel H. Rich[*,†,§]

[†]*Department of Chemistry and School of Pharmacy, University of Wisconsin-Madison, Madison, WI, USA 53706*

Supporting Information.

General procedures. All water-sensitive reactions were carried out under an atmosphere of argon using flame or oven dried glassware. Tetrahydrofuran (THF) was distilled from Na/benzophenone. Dichloromethane (DCM) was distilled from calcium hydride. Dimethylformamide (DMF) was stored over oven-dried molecular sieves. All other commercially available solvents and reagents were used without further purification. Lithium diisopropylamine (LDA) was prepared by adding BuLi to a –78 ˚C solution of diisopropylamine in THF. ^{1}H NMR spectra were acquired on a 300 MHz Bruker Aspect 3000 system at ambient temperature. Chemical shifts were reported in ppm (δ units) downfield from tetramethylsilane (TMS) as the internal standard except for MeOH-d_4 where solvent peaks were used as the internal standard. Solvent peaks were used as the internal standard for ^{13}C NMR chemical shifts (the $CDCl_3$ peak was set to 77 ppm in mixed solvent systems). Peptides were analyzed and/or purified on C-18 reverse-phase HPLC columns using acetonitrile/water gradients containing 0.1% trifluoroacetic acid for elution.

Boc-β-ketoester (1). To a stirred solution of carbonyldiimidazole (511 mg, 3.15 mmol) in THF (10 ml) on an ice bath was added a solution of Boc-leucine (694 mg, 3 mmol) in THF (5 mL), dropwise over 10 min. The ice bath was removed and the solution stirred for 2 h. A solution of benzyl isovalerate (1.25 g, 6 mmol) in THF (12 mL) was added dropwise over 10 min to a –78 ˚C solution of LDA (7.5 mmol) in THF (30 mL). After the solution was stirred for 1 h at –78 ˚C, the solution of Boc-Leu-im was added to the enolate over 3 min. The reaction was stirred for 30 min at –78 ˚C and quenched with AcOH. Ethyl acetate was added and the solution was extracted with citric acid (3 x 30 mL), $NaHCO_3$ (3 x 30 mL), and

brine (60 mL). Concentration of the solution followed by chromatography yielded 438 mg (35%) of impure **1** which was used without further purification. ^{1}H and ^{13}C NMR analysis revealed extra signals due to the diastereomeric β-ketoester. ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 5H), 5.0-5.15 (m, 2H), 4.81 (br d, 0.5H, *J* = 8.7 Hz), 4.70 (br d, 0.4H, *J* = 8.7 Hz), 4.32 (m, 1H), 3.74 (m, 1H), 1.15-1.85 (m,15H), 0.87 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 205.73, 205.01, 169.41, 169.29, 155.43, 135.39, 128.61, 128.55, 128.48, 128.36, 128.29, 79.89, 74.5, 67.15, 67.04, 57.81, 57.51, 54.14, 53.36, 51.6, 41.88, 40.34, 39.79, 37.35, 28.26, 26.26, 26.04, 24.77, 23.27, 22.80, 22.51, 22.11, 22.00, 21.40, 19.05, 13.67.

(2,3-*syn*)-Boc-2-isobutyl-statine benzyl esters. To a –78 ˚C solution of $Zn(BH_4)_2$[1] in Et_2O was added a solution of β-ketoester **1** (200 mg, 3 mmol) in Et_2O, dropwise over 5 min. The solution stood for 2 h during which it warmed to –15 ˚C. The solution was carefully poured into a vigorously stirred biphasic mixture of Et_2O and aq. NH_4Cl (gas evolution!). The layers were separated and the aqueous layer was extracted with Et_2O (30 mL). The combined organic layers were extracted with citric acid (3 x 20 mL), $NaHCO_3$ (3 x 20 mL), and brine (40 mL). Concentration of the solution followed by chromatography (5% to 15% EtOAc in PE) yielded 63 mg (31%) of the (2*S*, 3*S*, 4*S*) stereoisomer **2** (R_f = 0.3; 10% EtOAc in PE) and 88 mg (44%) of the (2*R*, 3*R*, 4*S*) stereoisomer **3** (R_f = 0.4; 15% EtOAc in PE).

(2*S*, 3*S*, 4*S*)-N-Boc-2-isobutyl-statine benzyl ester (2). ^{1}H NMR ($CDCl_3$): δ 7.33 (m, 5H), 5.13 (s, 2H), 4.59 (d, 1H, *J* = 8.8 Hz), 3.67 (ddd, 1H, *J* = 2.8, 7.5, 9.6 Hz), 3.45 (m, 2H), 2.65 (m, 1H), 1.70 (m, 1H), 1.57 (m, 2H), 1.41 (s, 9H), 0.87 (app dd, 12H, *J* = 3.9, 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 174.70, 156.35, 135.82, 128.50, 128.28, 128.21, 79.46, 74.50, 66.26, 51.62, 47.61, 41.12, 37.79, 28.30, 26.22, 24.71, 23.60, 22.93, 22.24, 21.77. FAB-MS: Calculated for $[C_{24}H_{39}NO_5Na]^+$: 444.2726. Found: 444.2715.

(2*R*, 3*R*, 4*S*)-N-Boc-2-isobutyl-statine benzyl ester (3). ^{1}H NMR ($CDCl_3$): δ 7.34 (s, 5H), 5.15 (d, 1H, *J* = 12.2 Hz), 5.09 (d, 1H, *J* = 12.2 Hz), 4.49 (d, 1H, *J* = 7.3 Hz), 3.67 (m, 2H), 3.34 (br s, 1H), 2.70 (m, 1H), 1.70-1.15 (m, 6H), 1.42 (s, 9H), 0.89 (app d, 6H, *J* = 5.1 Hz), 0.86 (app d, 6H, *J* = 5.9 Hz). ^{13}C NMR ($CDCl_3$): δ 175.3, 155.9, 135.5, 128.5, 128.32, 123.27, 79.4, 75.8, 51.73, 46.4, 39.7, 36.6, 28.3, 26.2, 24.7, 21.34, 21.32. FAB-MS: Calculated for $[C_{24}H_{39}NO_5Na]^+$: 444.2726. Found: 444.2746.

2,4-Dimethylphenyl isocaproate. 4-methylvaleric acid (2.32 g, 20 mmol) was added dropwise to a stirred solution of carbonyldiimidazole (3.41 g, 21 mmol) in THF (20 mL) and stirred for 2 h. A solution of 2,4-dimethylphenol (3.67 g, 30 mmol) in THF (10 mL) was added to a suspension NaH (810 mg, 32 mmol) in THF (20 mL) at 0 ˚C and stirred for 60 min. During this time, the solution became homogeneous with a light purple color. The acyl imidazole solution was added dropwise over 5 min to the sodium phenolate solution at 0 ˚C, and was stirred for 3 h. Ethyl acetate (100 mL) was added and the solution was extracted with citric acid (3 x 30 mL), $NaHCO_3$ (3 x 30 mL), and brine (60 mL). Concentration of the solution followed by chromatography (10-30% DCM in PE) yielded 2.86 g (65%) the aryl ester. ^{1}H NMR ($CDCl_3$): δ 7.05 (s, 3H), 2.61 (t, 2H, *J* = 7.7 Hz), 2.15 (s, 6H), 1.71 (m, 3H), 0.98 (d, 6H, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$): δ 171.39, 148.09, 129.91, 128.36, 125.55, 33.77, 31.93, 27.61, 22.07, 16.16. FAB-MS: Calculated for $[C_{14}H_{20}O_2Na]^+$: 220.1463; Found: 220.1466.

(2,3-*anti*)-Boc-2-isobutyl-statine 2,4-dimethylphenyl esters. A solution of 2,4-dimethylphenyl isocaproate (2.05 g, 9.30 mmol) in THF (10 mL) was added dropwise over 10 min to a –78 ˚C solution of LDA (10.23 mmol) in THF (40 mL). After the solution was stirred for 1 h at –78 ˚C, a –78 ˚C solution of Boc-leucinal[2] in THF was added dropwise over 2 min. The solution was stirred for 1 h during which the reaction warmed to –20 ˚C. The reaction was quenched with AcOH, EtOAc was added (120 mL), and the solution was extracted with citric acid (3 x 30 mL), $NaHCO_3$ (3 x 30 mL), and brine (60 mL). The solution was concentrated and the residue subjected to column chromatography to yield 910 mg (30%) of **4a** and 1.07 g (38%) of **5a** which were contaminated with Boc-leucinal (~20% and 5% respectively based on integration of the aldehyde proton). The 2-isobutyl statine aryl esters were used crude in further reactions. The aryl esters (and other 2-^{i}Bu-statine esters) showed conformers in their ^{1}H and ^{13}C NMR spectra. The conformer ratio changed based on the solvent system ($CDCl_3$, $CDCl_3$/CD_3OD mixtures, d_6-DMSO).

(2*R*, 3*S*, 4*S*)-N-Boc-2-isobutyl-statine 2,4-dimethylphenyl ester (4a). ^{1}H NMR (CD_3OD): δ 7.03 (m, 3H), 3.87 (m, 1H), 3.74 (dd, 1H, *J* = 1.1, 10.1 Hz, 2.89 (m, 1H), 2.18 (s, 6H), 1.30-1.75 (m, 15H) 0.94 (m, 12H). ^{13}C NMR (CD_3OD): δ 174.74, 157.89, 149.76, 131.68, 129.67, 126.84, 80.04, 76.12, 50.57,

49.88, 43.22, 39.01, 28.82, 27.48, 25.98, 24.41, 23.55, 22.78, 21.45, 17.29. ESI-MS: Calculated for $[C_{25}H_{41}NO_5H]^+$: 436.3063; Found:436.3059.

(2*S*, 3*R*, 4*S*)-N-Boc-2-isobutyl-statine 2,4-dimethylphenyl ester (5a). ^{1}H NMR ($CDCl_3$): δ 7.05 (m, 3H), 4.67 (d, 1H, *J* = 7.9 Hz), 3.85 (m, 1H), 3.45 (m, 2H), 2.91 (m, 1H), 2.85 (d, 1H, *J* = 6.1 Hz), 2.19 (s, 6H), 1.65-1.75 (m, 3H), 1.44 (s, 9H), 1.20-1.45 (m, 3H), 0.89 (m, 12H).^{13}C NMR ($CDCl_3$): δ 173.14, 155.61, 148.07, 130.18, 128.64, 125.85, 79.30, 75.29, 50.66, 46.75, 38.14, 37.31, 28.32, 26.11, 24.57, 23.91, 23.20, 21.81, 21.55, 16.89. FAB-MS: Calculated for $[C_{25}H_{41}NO_5H]^+$: 436.3063; Found: 436.3052.

Saponification of phenyl esters. Sodium hydroxide (2N, 5 equivalents) was added dropwise to a solution of the 2,4-dimethylphenyl ester in MeOH. The solution was stirred at rt for 18 h and was monitored by TLC. Methanolysis of the aryl ester took place within 6 h, after which the subsequent methyl ester was saponified to the carboxylic acid (precipitate formation). The reaction was quenched with aqueous acetic acid and the methanol was removed under reduced pressure. The residue was dissolved in dichloromethane and aq $NaHCO_3$. The layers were separated and the aqueous layer was extracted with DCM (3 x 10 mL). The aq layer was acidified to pH~3 with 1N HCl and extracted with DCM (5 x 15 mL). The organic layer was washed with water and brine and dried over sodium sulfate. Concentration of the solution followed by chromatography (10-25% EtOAc in PE, 1% acetic acid) yielded the N-Boc carboxylic acid.

(2*R*, 3*S*, 4*S*)-Boc-2-isobutyl-statine (4b). Yield = 71%. ^{1}H NMR (CD_3OD): δ 3.85 (m, 1H), 3.54 (d, 1H, *J* = 6.5 Hz), 2.57 (m, 1H), 1.15-1.7 (m, 15H), 0.92 (m, 12H). ^{13}C NMR: δ 178.89, 157.85, 79.93, 75.98, 50.52, 49.65, 43.05, 38.94, 28.80, 27.53, 25.92, 24.29, 23.50, 22.75, 21.59. FAB-MS: Calculated for $[C_{17}H_{32}NO_5Na_2]^+$: 376.2076. Found: 376.2078. Calculated for $[C_{17}H_{33}NO_5Na]^+$: 354.2256. Found: 354.2260.

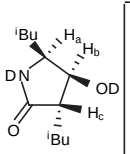
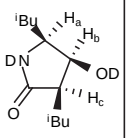
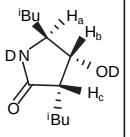
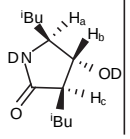
(2*S*, 3*R*, 4*S*)-Boc-2-isobutyl-statine (5b). Yield = 74%. ^{1}H NMR (CD_3OD): δ 3.62 (m, 2H), 2.55 (m, 1H), 1.66 (m, 3H), 1.43 (s, 9H), 1.27 (m, 3H), 0.92 (m, 12H). ^{13}C NMR: δ 178.52, 158.01, 82.59, 80.02, 52.02, 39.43, 38.50, 28.80, 27.37, 26.65, 24.36, 23.90, 24.67, 22.10, 21.84. FAB-MS: Calculated for $[C_{17}H_{32}NO_5Na_2]^+$: 376.2076. Found: 376.2061.

Esterification of Boc-2-isobutyl-statines. Cesium carbonate (1.05 eq) was added to a stirred solution of Boc-2-isobutyl-statine in acetone. After stirring for 5 min, benzyl bromide (2 eq) was added and the reaction was heated to 30 ˚C for 1 h. EtOAc was added and the solution was extracted with citric acid, $NaHCO_3$, and brine. Concentration of the solution followed by chromatography yielded the benzyl ester.

(2*R*, 3*S*, 4*S*)-Boc-2-isobutyl-statine benzyl ester (4c). Yield = 94%. ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 5H), 5.21 (d, 1H, *J* = 12.3 Hz), 5.11 (d, 1H, *J* = 12.3 Hz), 4.65 (d, 1H, *J* = 9.7 Hz), 3.80 (m, 1H), 3.69 (m, 1H), 2.68 (m, 2H), 1.28-1.66 (m, 15H), 0.92 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 175.65, 155.73, 135.76, 128.52, 128.23, 128.18, 79.14, 75.01, 66.37, 49.61, 47.68, 42.26, 38.12, 28.33, 26.12, 24.77, 23.56, 22.99, 22.31, 21.14. FAB-MS: Calculated for $[C_{24}H_{39}NO_5Na]^+$: 444.2726. Found: 444.2743.

(2*S*, 3*R*, 4*S*)-Boc-2-isobutyl-statine benzyl ester (5c). Yield = 95%. ^{1}H NMR ($CDCl_3$): δ 7.33 (s, 5H), 5.18 (d, 1H, *J* = 12.4 Hz), 5.11 (d, 1H, *J* = 12.4 Hz), 4.66 (d, 1H, *J* = 9.0 Hz), 3.68 (m, 2H), 3.16 (d, 1H, *J* = 7.0 Hz), 2.68 (m, 1H), 1.67 (m, 2H), 1.20-1.50 (m, 13H), 0.88 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 175.49, 155.67, 135.64, 128.45, 128.17, 79.25, 75.82, 66.37, 51.14, 45.98, 38.52, 38.05, 28.30, 25.94, 24.60, 23.80, 23.17, 21.68, 21.61. FAB-MS: Calculated for $[C_{24}H_{39}NO_5Na]^+$: 444.2726. Found: 444.2722.

Synthesis of γ–lactams. Trifluoroacetic acid was added dropwise to a 0 ˚C solution of Boc-2-isobutyl-statine ester in DCM, bringing the solution composition to 25% TFA in DCM. The solution was stirred for 1 h at 0 ˚C and the solvent was removed under reduced pressure. The residue was dissolved in methanol to give a 0.1 M solution, and 10 eq of solid $NaHCO_3$ was added. The solution was stirred for 6 h at rt. Ethyl acetate was added, the solution was filtered, and the solvent was removed under reduced

Statine	Lactam	H_a δ	H_a J_{ab}	H_b δ	H_b J_{bc}	H_c δ
2		3.69	5.0	4.09	n.o.	2.38
4a		3.60	3.7	4.22	4.8	2.46
5a		3.49	n.o.	4.07	5.3	2.50
3		3.40	5.7	3.72	6.7	2.40

pressure. The residue was analyzed by standard ^{1}H NMR spectroscopy and COSY-NMR experiments.

General peptide synthesis procedures (from Scheme 3).

(**a**) Boc removal: The substrate was dissolved in wet DCM and cooled to 0 ˚C. TFA was added until the solution composition was 50% TFA in DCM. The solution was stirred for 30 min at 0 ˚C and the solvent was removed under reduced pressure. The substrate was used crude in subsequent reactions.
(**b**) Fmoc removal: The substrate was dissolved in a solution of 20% piperidine in DMF and stood at rt for 15 min. The solvent was removed under reduced pressure and the substrate was used crude in the subsequent reactions. (**c**) Coupling reaction: The Fmoc amino acid (2.5-3 eq) was preactivated for 30 min at 0 ˚C in DMF using a combination of EDCI (1.25 eq) and HOBt (1.5 eq). At the end of the preactivation time 4 eq of DIEA was added, followed by a solution of the amine coupling partner (as the free amine or amine salt). The reaction was allowed to warm to rt and stirred for 6 h. The peptides were purified via aqueous workup and SiO_2 flash chromatography. (**d**) N-acetylation: The amine substrate was dissolved in DMF and cooled to 0 ˚C. DIEA (2 eq) was added followed by acetic anhydride (1.5 eq). The reaction was stirred for 6 h. The N-acetyl peptide was purified via aqueous workup and SiO_2 flash chromatography. Hydrogenolysis (**e**): The peptide benzyl ester was dissolved in methanol and 10% Pd-C (20 mol %) was added. The heterogeneous solution was stirred under a hydrogen atmosphere (balloon) for 8 h. The catalyst was removed via filtration through celite and the peptide acid product was purified via SiO_2 flash chromatography. (**f**) Coupling reaction: The peptide acid was preactivated for 15 min at 0 ˚C using HATU (1.1 eq), HOAt (2.2 eq), DIEA (4 eq). At the end of the preactivation time DIEA (3 eq) was added followed by the amino ester hydrochloride salt (3 eq). The reaction was stirred for 3 h at rt and were purified via aqueous workup and SiO_2 flash chromatography.

Fmoc-Lys(N_ε-Boc)-(2*R*, 3*S*, 4*S*)-2-isobutyl-statine benzyl ester (6a). ^{1}H NMR ($CDCl_3$/CD_3OD): δ 7.65 (d, 2H, *J* = 7.3 Hz), 7.49 (d, 2H, *J* = 7.0 Hz), 7.28 (t, 2H, *J* = 7.2 Hz), 7.19 (m, 7H), 6.90 (d, 1H, *J* = 9.7 Hz), 6.29 (d, 1H, *J* = 8.1 Hz), 5.24 (m, 1H), 4.99 (s, 2H), 4.24 (m, 2H), 4.33 (m, 2H), 4.08 (t, 1H, *J* = 6.8 Hz), 3.99 (m, 1H), 3.57 (d, 1H, *J* = 9.3 Hz), 2.91 (m, 2H), 2.49 (m, 1H), 1.65 (m, 1H), 1.15-1.55 (m, 20H), 0.77 (m, 12H). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 175.19, 172.19, 156.44, 156.39, 143.63, 143.44, 140.99, 140.97, 135.54, 128.14, 127.86, 127.84, 127.42, 126.79, 124.81, 124.77, 119.63, 78.94, 74.10, 66.73, 65.99, 54.86, 48.03, 46.80, 41.11, 37.72, 32.02, 39.55, 28.99, 28.03, 25.97, 24.38, 23.31, 22.56, 22.51, 21.88, 20.82. FAB-MS: Calculated for $[C_{45}H_{61}N_3O_8Na]^+$: 794.4356. Found: 794.4379.

Fmoc-Val-Lys(N_ε-Boc)-(2*R*, 3*S*, 4*S*)-2-isobutyl-statine benzyl ester (7a). ^{1}H NMR ($CDCl_3/CD_3OD$): δ 7.76 (d, 2H, *J* = 7.5 Hz), 7.72 (d, 1H, *J* = 8.8 Hz), 7.62 (d, 2H, *J* = 3.0, 7.2 Hz), 7.28-7.42 (m, 9H), 7.08 (d, 2H, *J* = 9.0 Hz), 6.33 (d, 1H, *J* = 8.8 Hz), 5.50 (m, 1H), 5.14 (d, 2H, *J* = 1.7 Hz), 4.46 (dd, 1H, *J* = 7.0, 10.3 Hz), 4.33 (m, 2H), 4.22 (m, 1H), 4.10 (m, 1H), 3.96 (t, 1H, *J* = 7.6 Hz), 3.64 (d, 1H, *J* = 9.8 Hz), 2.93 (m, 2H), 2.55 (m, 1H), 2.05 (m, 1H), 1.77 (m, 1H), 1.15-1.70 (m, 20H), 0.86 (m, 18H). ^{13}C NMR ($CDCl_3/CD_3OD$): δ175.05, 171.95, 171.54, 156.62, 156.38, 143.50, 143.38, 135.53, 128.10, 127.84, 127.39, 126.75, 124.74, 124.67, 119.61, 78.80, 74.18, 66.67, 65.96, 60.22, 53.10, 48.00, 46.80, 41.07, 39.66, 31.46, 30.76, 28.85, 27.92, 25.91, 24.26, 23.22, 22.57, 21.68, 20.73, 18.70, 17.47. FAB-MS: Calculated for $[C_{50}H_{70}N_4O_9Na]^+$: 893.5041. Found: 893.5079.

Ac-Val-Lys(N_ε-Boc)-(2*R*, 3*S*, 4*S*)-2-isobutyl-statine benzyl ester (8a). ^{1}H NMR ($CDCl_3/CD_3OD$): δ 7.91 (d, 1H, *J* = 7.9 Hz), 7.66 (d, 1H, *J* = 8.1 Hz), 7.30 (m, 5H), 7.20 (d, 1H, *J* = 9.6 Hz), 5.89 (m, 1H), 5.13 (d, 1H, *J* = 15.8 Hz), 5.08 (d, 1H, *J* = 15.8 Hz), 4.30 (m, 1H), 4.10 (m, 1H), 3.62 (d, 1H, *J* = 9.7 Hz), 2.96 (m, 2H), 2.55 (m, 1H), 1.97 (s, 3H), 1.96 (m, 1H), 1.77 (m, 1H), 1.15-1.70 (m, 21H), 0.86 (m, 18H). ^{13}C NMR ($CDCl_3/CD_3OD$): δ174.84, 171.57, 171.53, 171.39, 156.38, 135.40, 127.82, 127.60, 127.55, 78.42, 74.00, 65.66, 58.55, 52.90, 40.88, 37.38, 31.22, 30.12, 28.66, 27.58, 25.69, 23.99, 22.90, 22.53, 22.24, 21.53, 21.35, 20.38, 18.35, 17.50. FAB-MS: Calculated for $[C_{37}H_{62}N_4O_8Na]^+$: 713.4465. Found: 713.4459.

Ac-Val-Lys(N_ε-Boc)-(2*R*, *3S*, 4*S*)-2-isobutyl-statine (9a). ^{1}H NMR ($CDCl_3/CD_3OD$): δ 4.37 (dd, 1H, *J* = 6.0, 8.4 Hz), 4.17 (d, 1H, *J* = 7.0 Hz), 4.12 (m, 1H), 3.61 (d, 1H, 9.4 Hz), 3.06 (t, 2H, *J* = 6.0 Hz), 2.51 (m, 1H), 2.04 (m, 4H), 1.82 (m, 1H), 1.3-1.7 (m, 20H), 0.94 (m, 18H). ^{13}C NMR ($CDCl_3/CD_3OD$): δ 177.58, 171.70, 171.61, 171.54, 156.43, 78.36, 73.90, 58.66, 52.86, 48.02, 47.67, 40.74, 39.34, 37.57, 31.07, 30.05, 28.63, 27.49, 25.61, 23.96, 22.78, 22.48, 22.23, 21.41, 21.23, 20.54, 18.28, 17.47. FAB-MS: Calculated for $[C_{30}H_{56}N_4O_8Na]^+$: 623.3996. Found: 623.3999.

Ac-Val-Lys(N_ε-Boc)-(2*R*, 3*S*, 4*S*)-2-isobutyl-statine-Ala-OMe (10a). ^{1}H NMR ($CDCl_3/CD_3OD$): 4.47 (m, 1H), 4.20 (m, 1H), 4.07 (m, 2H), 3.69 (s, 3H), 3.53 (d, 1H, 9.2 Hz), 3.01 (t, 2H, *J* = 6.5 Hz), 2.39 (m, 1H), 2.01 (m, 4H), 1.82 (m, 1H), 1.1-1.7 (m, 23H), 0.94 (d, 3H, *J* = 3.5 Hz), 0.91 (d, 3H, *J* = 3.5 Hz), 0.86 (m, 12H). FAB-MS: Calculated for $[C_{34}H_{63}N_5O_9Na]^+$: 708.4523. Found: 708.4527.

Ac-Val-Lys-(2*R*, *3S*, 4*S*)-2-isobutyl-statine-Ala-OMe (11a). ESI-MS: $[M+H]^+$ = 586.6.

Fmoc-Lys(N_ε-Boc)-(2*S*, 3*S*, 4*S*)-2-isobutyl-statine benzyl ester (6b). ^{1}H NMR ($CDCl_3$): δ 7.74 (d, 2H, *J* = 7.5 Hz), 7.57 (d, 2H, *J* = 7.2 Hz), 7.26-7.40 (m, 9H), 6.51 (d, 1H, *J* = 8.5 Hz), 5.70 (d, 1H, *J* = 6.1 Hz), 5.16 (d, 2H, *J* = 11.8 Hz), 5.04 (d, 2H, *J* = 11.8 Hz), 4.81 (br s, 1H), 4.35 (d, 2H, *J* = 6.4 Hz), 4.18 (t, 1H, *J* = 7.2 Hz), 3.87 (br m, 1H), 3.71 (d, 1H, *J* = 9.0 Hz), 2.99 (br m, 1H), 2.97 (br m, 1H), 1.77 (br m, 1H), 1.3-1.9 (m, 12 H), 1.43 (s, 9H), 0.85 (m, 12 H). ^{13}C NMR ($CDCl_3$): δ 174.2, 171.6, 171.1, 156.1, 143.7, 143.5, 141.1, 135.6, 128.5, 128.3, 128.1, 127.6, 126.9, 124.9, 119.8, 79.1, 73.6, 66.9, 66.4, 60.3, 54.8, 50.5, 47.9, 46.9, 41.2, 39.7, 38.6, 32.3, 29.3, 28.3, 26.1, 24.5, 23.6, 22.5, 22.3, 21.2, 20.9. FAB-MS: Calculated for $[C_{45}H_{61}N_3O_8Na]^+$: 794.4356. Found: 794.4344.

Fmoc-Val-Lys(N_ε-Boc)-(2S, 3S, 4S)-2-isobutyl-statine benzyl ester (7b). ^{1}H NMR ($CDCl_3$): δ 7.74 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 2H, *J* = 5.5 Hz), 7.3-7.4 (m, 9H), 6.89 (br m, 2H), 5.89 (d, 1H, *J* = 6.5 Hz), 5.17 (d, 1H, *J* = 12.2 Hz), 5.01 (d, 1H, *J* = 12.2 Hz), 4.83 (br m, 1H), 4.58 (m, 1H), 4.47 (m, 1H), 4.33 (m, 1H), 4.19 (m, 1H), 4.04 (br m, 1H), 3.93 (m, 1H), 3.64 (m, 1H), 2.93 (m, 2H), 2.55 (br t, 1H, *J* = 8.1 Hz), 1.99 (m, 1H), 1.73 (m, 1H), 1.15-1.70 (m, 22H), 0.83 (m, 18H). ^{13}C NMR ($CDCl_3$): δ 174.1, 171.1, 171.0, 156.3, 156.0, 143.8, 143.5, 141.23, 141.18, 135.7, 128.5, 128.4, 128.2, 127.7, 127.1, 127.0, 125.1, 124.9, 119.91, 119.87, 78.9, 73.9, 66.6, 66.4, 60.2, 53.14, 50.22, 47.79, 47.20, 41.32, 39.97, 38.61, 32.37, 31.48, 29.45, 28.37, 26.11, 24.57, 23.63, 22.69, 22.52, 22.31, 21.08, 18.41, 17.29. FAB-MS: Calculated for $[C_{50}H_{70}N_4O_9]^+$: 871.5221. Found: 871.5254.

Ac-Val-Lys(N_ε-Boc)-(2*S*, 3*S*, 4*S*)-2-isobutyl-statine benzyl ester (8b). ^{1}H NMR ($CDCl_3$/CD_3OD): δ 7.83 (d, 1H, *J* = 7.5 Hz), 7.59 (d, 1H, *J* = 8.1 Hz), 7.34 (m, 5H), 5.23 (d, 1H, *J* = 12.2 Hz), 4.99 (d, 1H, *J* = 12.2 Hz), 4.27 (m, 1H), 4.17 (m, 1H), 3.93 (dd, 1H, *J* = 1.7, 9.4 Hz), 3.66 (dd, 1H, *J* = 6.8, 8.3 Hz), 2.93 (m, 2H), 2.57 (m, 1H, *J* = 8.1 Hz), 2.03 (s, 3H), 1.99 (m, 1H), 1.76 (m, 1H), 1.15-1.70 (m, 22H), 0.83 (m, 18H). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 174.35, 171.68, 171.59, 171.54, 171.32, 156.36, 135.30, 128.00, 127.99, 127.75, 78.63, 72.85, 66.07, 58.71, 53.17, 53.08, 49.64, 47.53, 39.48, 38.54, 31.08, 30.26, 28.73, 27.76, 25.70, 24.06, 23.08, 22.35, 22.07, 21.75, 21.40, 20.29, 18.41, 17.24. FAB-MS: Calculated for $[C_{37}H_{62}N_4O_8Na]^+$: 713.4465. Found: 713.4451.

Ac-Val-Lys(N_ε-Boc)-(2*S*, 3*S*, 4*S*)-2-isobutyl-statine (9b). ^{1}H NMR (CDCl3/CD3OD): __4.22 (m, 1H), 4.07 (d, 1H, J = 7.2 Hz), 3.86 (m, 1H), 2.96 (m, 2H), 2.40 (m, 1H), 1.95 (m, 4H), 1.75 (m, 1H), 1.15-1.70 (m, 20H), 0.83 (m, 18H).__13C NMR: _ 176.43, 171.71, 171.63, 171.57, 156.46, 78.62, 72.78, 58.72, 53.01, 49.85, 47.26, 40.86, 38.02, 30.95, 30.14, 28.73, 27.64, 25.61, 24.01, 23.03, 22.31, 21.97, 21.65, 21.61, 20.69, 18.42, 17.59.__FAB-MS: Calculated for [C30H56N4O8Na]+: 623.3996. Found: 623.4012.

Ac-Val-Lys(N_-Boc)-(2S, 3S, 4S)-2-isobutyl-statine-Ala-OMe (**10b**). 1H NMR (CDCl3/CD3OD): _ 8.43 (d, 1H, J = 7.7 Hz), 8.04 (m, 2H), 7.22 (d, 1H, J = 8.1 Hz), 5.59 (br s, 1H), 4.54 (d, 1H, J = 12.2 Hz), 4.13 (m, 1H), 4.00 (m, 1H), 3.78 (s, 3H), 3.43 (br m, 1H, J = 1.7, 9.4 Hz), 3.38 (br m, 1H), 3.08 (m, 2H), 2.25 (m, 1H), 2.06 (s, 3H), 2.01 (m, 1H), 1.20-1.75 (m, 24H), 0.83 (m, 18H). FAB-MS: Calculated for [C34H63N5O9Na]+: 708.4523. Found: 708.4548.

Ac-Val-Lys-(2S, 3S, 4S)-2-isobutyl-statine-Ala-OMe (**11b**). ESI-MS: [M+H]+ = 586.6.