buffers, pH 7.3, in 500-mL Erlenmeyer flasks. Labeled precursors (expt 1, 14 mg of **2a**-d_3; expt 2, 12 mg of **2b**-d_3 expt 3, 10 mg of L-tryptophan, 20 mg of D,L-mevalonic acid, and 10 mg of L-[$^{13}C^2H_3$]methionine) were each distributed evenly into two replacement cultures, and these were incubated with shaking for 2 days. Alkaloids were then extracted from the alkalinized (NH_4OH) culture filtrate with 1:2 2-propanol/chloroform. The organic extract was evaporated in vacuo and the residue partitioned between 2% aqueous succinic acid and methylene chloride. The aqueous layer was washed twice with CH_2Cl_2, made alkaline (NH_4OH), and extracted 3 times with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated in vacuo. All these operations were carried out in test tubes by using Pasteur pipets for the transfers to avoid contamination with silicone grease or plasticizer. The alkaloid residue was chromatographed on an alumina column (2 g Alumina Neutral containing 20% water, 80–200 mesh, Fisher Scientific; elution with 1:40 CH_3OH–CH_2Cl_2). The fraction containing **3b** was subjected to GC–MS analysis (Hewlett-Packard 5970A, SPB-5 capillary column 0.25 mm × 15 m, flow rate 1.0 mL/min, temperature program 4 min 60 °C, then 10 °C/min to 290 °C; **3b**, t_R 24 min). Isotopic composition was calculated from the MS data as described by Biemann.[27]

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Registry No. 1, 29702-35-0; 2, 112152-11-1; 2-d_3, 112152-35-9; 3a, 2390-99-0; 3b, 548-43-6; 5, 1074-86-8; 6, 112152-12-2; 7, 112152-13-3; 8, 112152-14-4; 10, 112152-15-5; (*E*)-11, 112152-16-6; (*Z*)-11, 112152-31-5; 12, 74685-00-0; 13, 102301-81-5; 14, 68900-05-0; (*E*)-15, 112152-17-7; (*Z*)-15, 112152-32-6; 16, 112152-18-8; 17, 112152-19-9; (*E*)-18, 112152-20-2; (*Z*)-18, 112152-33-7; 19, 112152-21-3; 20, 112152-22-4; 21, 112152-23-5; 22, 112152-24-6; 23, 5417-21-0; d_3-23, 112152-36-0; 24, 112152-25-7; d_3-24, 112152-37-1; 25, 112152-26-8; 26, 112152-27-9; 27, 112152-28-0; 29, 112152-29-1; 30, 112152-30-4; 30 (desilylated), 112152-34-8; $HOCH_2COCH_3$, 116-09-6; $ClCO_2CH_2CCl_3$, 17341-93-4; $H_3CCOCH{=}PPh_3$, 1439-36-7; $LiCH_2OCH_2CH_2SiMe_3$, 112152-38-2; $(PhCH_2)_2NCH(CO_2Me)_2$, 112152-39-3; CD_3I, 865-50-9; H-Trp-OH, 73-22-3.

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A Facile Synthesis of Statine and Analogues by Reduction of β-Keto Esters Derived from Boc-Protected Amino Acids. HPLC Analyses of Their Enantiomeric Purity

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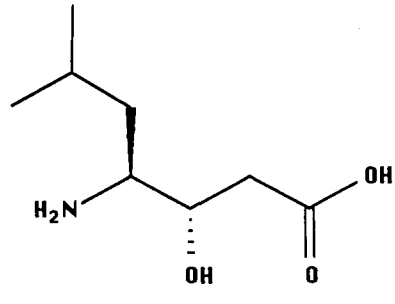
The synthesis of γ-amino-β-keto ester derivatives **3a–e** from *N*-Boc-protected L-amino acids by *N,N'*-carbonyldiimidazole activation and treatment with the magnesium enolate of hydrogen ethyl malonate is described. Racemization during activation, which depended upon the reaction conditions for imidazolide formation, was minimized for **3a–c** but was almost complete in the case of *N*-Boc-*S*-methylcysteine (**2d**). The diastereoselectivity for reducing intermediate **3** with sodium borohydride, potassium tri-*sec*-butylborohydride (K-Selectride), and other reducing agents to the (3*S*,4*S*)- and (3*R*,4*S*)-statine derivatives **4** and **5** was examined. A high selectivity for the *S,S* diastereomers, but low reactivity, was observed for the sterically demanding K-Selectride. Diastereomerically pure Boc-(*S,S*)-statine ethyl ester (**4a**) (with enantiomeric purity ee = 97%) and its analogue **4b** (*N*-Boc-AHPPA-OEt; ee = 95%) were obtained after $NaBH_4$ reduction in THF–MeOH (98:2) in 27% and 41% total yield. The enantiomeric purity of (*S,S*)/(*R,R*)-statine and analogues was determined by precolumn derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) followed by HPLC analyses on a silica gel column. The synthesis of a new histidine side-chain analogue of statone is described.

Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (1), is a constituent of the naturally occurring small peptide pepstatin[1] which is a strong and general inhibitor of aspartic proteinases, e.g., pepsin, renin, and cathepsin D.[2] The importance of the statine stereochemistry for the slow and tight-binding inhibition has been demonstrated by kinetic studies.[3,4d] Because statine has proven useful for developing new inhibitors of aspartic proteinases, numerous syntheses of 1 and its N-protected ester derivatives have been published.[4] Most of these methods proceed via condensation of an N-protected (*S*)-amino aldehyde with a metalated ethyl acetate to form the 3*S*,4*S* and 3*R*,4*S*



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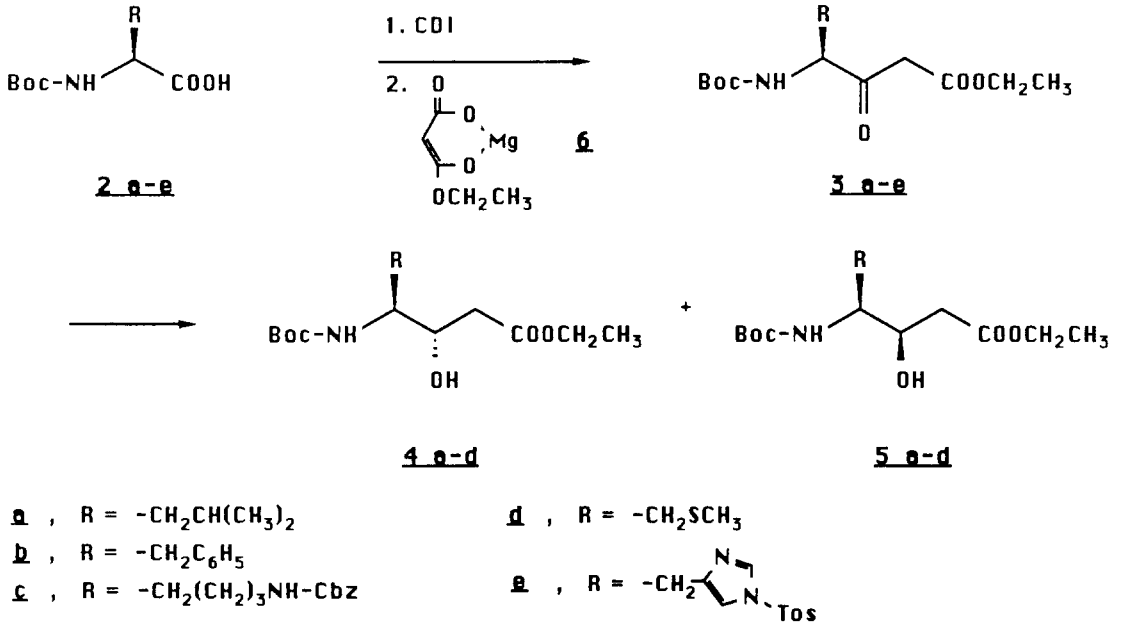
Scheme I



diastereoisomer mixture of Boc-statine ester.

We report here a convenient high yield, high optical purity two-step synthetic route to Boc-(*S*,*S*)-statine ethyl ester (**4a**) and its analogue *N*-Boc-(3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid ethyl ester (Boc-AHPPA-OEt, **4b**)[5] based on the efficient formation and subsequent reduction of the β-keto esters **3**. This approach is a modification[6,7] of one first reported by Steulmann and Klostermeyer.[4c] The stereochemical course of this reaction pathway has not been examined previously, and for this purpose we have developed a new indirect HPLC method to determine the enantiomeric purity of statine derivatives. New histidine and cysteine side-chain analogues also were prepared by this method.

Results and Discussion

The β-keto ester compounds **3a–c,e** were obtained in good yield and high purity from the corresponding N-protected L-amino acids **2** (Scheme I) by use of a slightly modified procedure described recently for the reaction with achiral amino acids.[8] Activation of the amino acid carboxyl with *N*,*N*′-carbonyldiimidazole (CDI from Aldrich Chemical Co.), followed by treatment of the resulting imidazolide with the nucleophilic magnesium enolate **6**, and spontaneous decarboxylation during acidic workup provided compounds **3** which were easily purified by recrystallization or by flash chromatography on silica gel (Table I, method A). However, stereochemical analyses of the Boc-statines formed by $NaBH_4$ reduction[9] established that considerable racemization had occurred, the extent of which depended upon the nature of the amino acid side chain. For example, the optical purity of **4a** (from **3a** prepared by method A) was 92% and only 58% for **4b**. Higher specific rotations were not obtained when the imidazolides derived from **2a–c** were reacted with the neutral magnesium salt of the malonic half ester[10] instead of the dianion. Because *N*,*N*′-carbonyldiimidazole has been reported to cause racemization of protected amino acids under certain conditions during peptide synthesis,[11] we attempted to suppress the racemization at what becomes C-4 in the β-keto esters by using lower initial reaction temperatures and shorter reaction times. Thus, reaction of CDI with **2** in THF at 0 °C for a shorter total reaction time for the activation of the amino acid (1 h at 0 °C, then 3 h at room temperature) gave higher optical rotations for **3a–c**, although slightly lower yields of products were obtained. Analogues **4a,b** were obtained with high enantiomeric purity (ee ≥95–97%, Table II, entry 3 and 5) following reduction of **3a,b**, demonstrating that under these conditions only a small amount of racemization (1.5–2.5%) had occurred. Similarly, little racemization occurred when the aliphatic side chain amino acid N^{α}-Boc-N^{ϵ}-(Cbz)lysine (**2c**) was converted to **3c**. Very similar rotations were obtained for **3c** obtained from two different procedures (Table I). On the other hand, the cysteine-derived β-keto ester **3d** was almost completely racemized when prepared from L-*S*-methylcysteine by this method, presumably due to a catalytic effect of the imidazole base

Table I. Formation of β-Keto Ethyl Esters 3 from N-Protected Amino Acids 2a–e

compd	method[a]	mmol	$[\alpha]^{22}_D$, deg (c 2, MeOH)	yield, %
3a	A	5.0	−51.3[b]	83
	B	5.0	−54.8[b]	72
b	A	5.0	−35.5	88
	B	5.0	−56.3	82
c	A	5.0	−24.7	81
	B	2.5	+25.5[c]	70
d	B	2.5	−0.2	78
e	A	5.0	−20.6[d]	82

[a] A, 25 °C, 12-h activation; B, 0 °C; 1 h → room temperature, 3-h activation. For details, see Experimental Section. [b] Lit.[4c] $[\alpha]^{22}_D$ −49.5° (1, MeOH). [c] Obtained from N^{α}-Boc-N^{ϵ}-(Cbz)-D-lysine. [d] c = 1.5 in methanol.

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Table II. Reduction of the β-Keto Ester 3 to the *N*-Boc-statine Diastereomers 4/5. Diastereoselectivity as a Function of Reaction Conditions and Enantiomeric Purity of the Final Products 4

entry	compd	reducing agent	conditions[a]	diastereomer ratio 4:5	yield 4/5, %	enantiomeric ratio[b] (*S,S*)-4[c]:(*R,R*)-4
1	**3a**	$NaBH_4$	THF, 3 h	31:69	92	97:3[d]
2			THF–EtOH (98:2), 3 h	28:72	80[e]	96:4[d]
3			THF–MeOH (98:2), 2 h	39:61	97	98.5:1.5
4		K-*sec*-Bu_3BH_4	THF[f]	90:10[g]	15–20[h]	
5	**3b**	$NaBH_4$	THF, 5 h	37:63	98	97.5:2.5
6			EtOH, 0.5 h	17:83	87[e]	
7			THF–MeOH (98:2), 2 h	53:47	97	95.5:4.5[i]
8		K-*sec*-Bu_3BH_4	THF	96:4[g]	24	97:3
9	**3c**	$NaBH_4$	THF–MeOH (98:2), 6 h	35:65[g,j]	94	
10	**3d**	$NaBH_4$	THF, 1 h	42:58	37[e]	55:45[k]

[a] For details, see Experimental Section. [b] Determined by HPLC after derivatization with GITC (see text). [c] Compound (*S,S*)-**4a** (entry 3) was a colorless oil with $[\alpha]^{22}_D$ −37.9° (1, MeOH) (lit.[4e] $[\alpha]^{22}_D$ −37.9° (0.84, MeOH). (*S,S*)-**4b** (entry 5) was recrystallized from ether–*n*-hexane: mp 89–90°C; $[\alpha]^{22}_D$ −34.6° (1, MeOH) (lit.[5] mp 88–89 °C; $[\alpha]^{24}_D$ −35.9 (1, MeOH)). ^1H NMR and MS data of compounds **4** and **5** were consistent with their chemical structure, TLC on silica gel (solvent B) showed single spots in each case. [d] Obtained from **3a** with $[\alpha]^{22}_D$ −51.3° (2, MeOH). [e] Not optimized. [f] Reaction scale 0.15 mmol. [g] Determined by HPLC on a reversed phase Bondapak C 18 column with acetonitrile–water as eluant. [h] Estimated by TLC. [i] Obtained from **3b** with $[\alpha]^{22}_D$ −53.1° (2, methanol). [j] Diastereomers **4c**/**5c** are not separable on silica gel on a preparative scale.[22] [k] Ratio of (3*S*,4*R*)/(3*R*,4*S*)-**4d**.

during amino acid activation. Carboxyl-activated N,S-protected cysteine derivatives are known to racemize readily in the presence of base.[12]

Reduction of the β-keto esters **3a–d** with $NaBH_4$ at −78 °C gave excellent yields of the *N*-Boc-statine derivatives as a mixture of the 3*S*,4*S* and 3*R*,4*S* diastereomers **4a–d** and **5a–d**, respectively (Scheme I, Table II). These diastereomers, except **4c**/**5c**,[22] were easily separated by silica gel column chromatography.[4e,5] In each case when tetrahydrofuran or aliphatic alcohols were used as solvents, the *R,S* configurated isomer formed predominantly. A similar stereochemical course has been reported very recently[13] for the reduction of two ester analogues of **3a**. The diastereoselectivity in THF is further directed toward the *R,S* isomer when the complexing lanthanide salt, $CeCl_3$,[14] is present (for example, the ratio **4b**/**5b** was 23:77). However, when a mixed solvent system THF–MeOH (98:2)[15] was used, the yield of (*S,S*)-**4** was increased significantly, compared to the ratio of diastereomers **4**/**5** obtained when the reduction was run separately in these solvents. Lower reaction temperature (−78 °C versus 0 °C) did not improve the stereochemical outcome but did decrease the amount of minor unidentified side products.

When the bulky reducing agent, potassium tri-*sec*-butylborohydride (K-Selectride), was used in an attempt to increase the yield of the desired **4**, the diastereoselectivity dramatically changed, affording predominantly or almost exclusively the *S,S* isomers **4a,b**. Unfortunately, the conversion of **3** to **4** was poor, and although the reaction conditions (solvent, temperature, inverse reaction, etc.[16]) were extensively varied, the best yields for **4b** did not exceed 25%. Most of the unreacted **3b** was reisolated after column chromatography, and no methylation of **3b** was observed when methyl iodide was added to the reaction mixture containing **3b** and K-Selectride.[17] Thus, high steric hindrance appears to be the main reason for the low reactivity of the β-keto esters with this reducing agent[18] although enolization cannot be excluded by these experiments. Low total yields of the diastereomer mixture (20–25%) and a high reductive selectivity for the keto group at low temperatures were also observed in the reaction of **4a,b** with K(OPr-*i*)$_3$BH and the bulky aluminum hydride (Li(OBu-*t*)$_3$AlH; however, here formation of the *R,S* stereoisomers was favored to a similar extent [(*S,S*)/(*R,S*) about 35:65)] in each case.

Table III. Separation of Enantiomeric Statine Derivatives 4a,b,d as Their Diastereomeric Thiourea Derivatives Formed with GITC[21]

compd	t_1[a] [min]	t_2 [min]	α[b]	R_S[c]
4a	11.6 (*R,R*)	14.7	1.35	2.6
4b[d]	14.3 (*R,R*)	16.7	1.23	1.4
4d	17.2 (*R,S*)	19.3	1.15	0.93

[a] Retention time; the configuration of the statine compound that is first eluted as its thiourea derivative is given in parentheses. Chromatographic conditions: RSil silica gel 5 μm (Alltech), column 4.6 × 250 mm; eluant *n*-hexane–2-propanol (93:7); flow 1.5 mL/min; UV detection at 254 nm. [b] Separation factor $\alpha = (t_2 - t_0)/(t_1 - t_0)$; t_0 = void volume. [c] Peak resolution $R_S = 2(t_2 - t_1)/w_1 + w_2$. [d] Eluant: *n*-Hexane–2-propanol (91:9); flow rate 1.0 mL/min.

The enantiomeric purity of **4a,b,d** (see Table II) was established by formation and HPLC analyses of diastereoisomeric derivatives. In separate operations, samples of racemic **4a,b**, prepared from the corresponding D,L-amino acids, and enantiomerically enriched samples were N-deprotected followed by reaction of the free amino group with commercially available, optically active isothiocyanates. When (*R*)- and (*S*)-α-methylbenzyl isothiocyanate (AMBI)[19] were used, base-line separations of the resulting pairs of thiourea diastereomers were achieved by HPLC on a silica gel column by using *n*-hexane–2-propanol as eluant. However, this method did not give a 1:1 ratio of diastereomers when racemic **4a,b** was used. Furthermore, substantially different values of ee were obtained for identical enantiomerically enriched samples after reaction with (*R*)- and (*S*)-AMBI. We therefore concluded

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(17) Reaction conditions: To 0.25 mmol of **3b** in 2.5 mL of THF was added 2.4 equiv of K-Selectride at −78 °C. After 1 h, 3.5 equiv of MeI was added and the mixture was warmed up to 0 °C and stirred for 10 min (a white precipitate appeared), before being quenched with 1 N HCl. After usual workup no additional reaction products were detectable by TLC.
(18) According to ^1H NMR spectral data, the keto tautomers of compounds **3** are strongly favored in chloroform solutions with ratios for the keto/enol form being ≥10:1. A similar result was found for **3b** in THF at room temperature.
(19) This reagent has been briefly described for the HPLC determination of the enantiomeric purity of a side-chain analogue of **4a**: see Boger, J.; et al. *J. Med. Chem.* **1985**, *28*, 1779.

that this is not an appropriate reagent[20] for the determination of ee of statine derivatives. On the other hand, neglectably small kinetic effects (diastereomer ratio 1.002 to 1.005 for racemic **4a,b**) were observed when enantiomerically pure 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)[21,25] was used to derivatize the statine analogues (Table III). The high accuracy of this method was verified by analyzing samples of **4b** with known optical purity. The GITC method allows also the determination of the enantiomeric composition of statine 1 by forming the ethyl ester derivative[24] prior to the reaction with GITC.

As a result of this study, the Boc-protected (3*S*,4*S*)-statine ester **4a** and its analogue **4b** are easily synthesized from the N-protected amino acids by the two-step procedure depicted in Scheme I in 27% and 41% overall yields, respectively. The products showed high enantiomeric purity, as confirmed by a simple derivatization and HPLC method, comparable to that of compounds obtained via aldol addition to L-α-amino aldehydes.[4,5] Although the described synthesis does not circumvent the disadvantage of diastereoisomer separation by column chromatography, it can be regarded as a convenient alternative for the preparation of new statine derivatives (e.g., **4c–e**).

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker WP-200 in $CDCl_3$ with tetramethylsilane as internal standard (chemical shifts in ppm). Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN. For column chromatography, Merck grade 60 silica, 230–400 mesh, was used. TLC was performed on 0.25-mm-thickness silica gel glass plates (Merck silica gel 60 F-254). The following solvent systems were used: A, *n*-hexane–ethyl acetate (1:1); B, *n*-hexane–ethyl acetate (7:3). HPLC analyses were carried out on a Waters system consisting of two Model 510 HPLC pumps in combination with an Automated Gradient Controler, a UV Detector Model 441, and a Data Module Model 730.

β-Keto Ester Formation. General Procedure, Method A. To a solution of 5 mmol of the Boc-protected L-amino acid in 20 mL of THF was added *N,N'*-carbonyldiimidazole (0.97 g, 6 mmol). The resulting mixture was stirred for 12 h at room temperature followed by cooling to 0 °C. Distilled hydrogen ethyl malonate (0.99 g, 7.5 mmol; prepared from the potassium salt by treatment of an aqueous solution with excess of concentrated hydrochloric acid at 0 °C and extraction of the free acid with ether) dissolved in 15 mL of THF was reacted with isopropyl magnesium chloride (15 mL of a 2 M solution in THF) at 0 °C for 30 min and then at room temperature for 45 min and at 40–50 °C for 30 min under nitrogen. This solution of the magnesium enolate was added dropwise with stirring via cannulae to the cooled imidazolide solution. The resulting mixture was, after warming to room temperature, stirred for 4 h and then poured into ice-cold 1 N hydrochloric acid. The aqueous phase was extracted twice with ethyl acetate, and the combined organic extracts were washed with saturated NaCl, dried over $MgSO_4$, and evaporated to give the crude β-keto esters as oils. These were purified by column chromatography on silica gel using *n*-hexane/ethyl acetate as eluant.

Method B. To a solution of 2.5–5.0 mmol of the Boc-protected amino acid in 8–15 mL of THF, cooled to 0 °C, was added *N,N'*-carbonyldiimidazole (120 mmol %). The reaction mixture was stirred for 1 h at 0 °C and for 3 h at room temperature and then cooled to −20 °C before the magnesium enolate solution in THF (prepared from 150 mmol % hydrogen ethyl malonate and 3.75–7.5 mL (300 mmol %) of a 2 M solution of isopropyl magnesium chloride in THF; see description in Method A) was added dropwise. The reaction mixture was stirred for 45 min at −20 °C and for 3 h after warming to room temperature. Quenching and workup of the reaction followed the procedure described in method A.

(4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-6-methyl-3-oxoheptanoic acid ethyl ester (3a): mp 36–38 °C (lit.[4c] mp 32 °C); R_f(B) 0.38; ^{1}H NMR ($CDCl_3$)[23] δ 0.9–1.0 (m, 6 H), 1.28 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.45 (s, 9 H), 1.25–1.8 (m, 3 H), 3.58 (m, AB, *J* = 16 Hz, 2 H, $COCH_2$), 4.21 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 4.36 (m, 1 H, CH), 4.91 (m, 1 H, NH). Anal. Calcd ($C_{15}H_{27}NO_5$): C, 59.78; H, 9.03; N, 4.65. Found: C, 60.01; H, 9.25; N, 4.54.

(4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-3-oxo-5-phenylpentanoic acid ethyl ester (3b): mp 54–56 °C (ether–*n*-hexane); R_f(B) 0.34; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.41 (s, 9 H), 2.9–3.2 (m, 2 H, CH_2Ar), 3.47 (m, AB, *J* = 15 Hz, 2 H, $COCH_2$), 4.16 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 4.55 (m, 1 H, CH), 4.95–5.05 (m, 1 H, NH), 7.1–7.35 (m, 5 H, Ar H). Anal. Calcd ($C_{18}H_{25}NO_5$): C, 64.46; H, 7.51; N, 4.17. Found: C, 64.46; H, 7.64; N, 4.11.

(4*S*)-8-[(Benzyloxycarbonyl)amino]-4-[(*tert*-butyloxycarbonyl)amino]-3-oxooctanoic acid ethyl ester (3c): mp 64–66 °C; R_f(A) 0.34; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.41 (s, 9 H), 1.3–1.95 (m, 6 H), 3.15 (m, 2 H, $NHCH_2$), 3.50 (m, AB, *J*, 16 Hz, 2 H, $COCH_2$), 4.14 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 4.27 (m, 1 H, CH), 4.85–5.0 (m, 1 H, NH), 5.08 (s, 2 H, CH_2Ar), 5.2–5.3 (m, 1 H, NH), 7.35 (s, 5 H, Ar H). Anal. Calcd ($C_{23}H_{34}N_2O_7$): C, 61.32; H, 7.61; N, 6.21. Found: C, 61.12; H, 7.52; N, 6.19.

(4*R*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-(methylthio)-3-oxopentanoic acid ethyl ester (3d): mp 57–58 °C (ether–*n*-hexane); R_f(A) 0.48; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.46 (s, 9 H), 2.14 (s, 3 H, SCH_3), 2.8–3.05 (m, 2 H, CH_2), 4.63 (m, AB, *J* = 16 Hz, 2 H, $COCH_2$), 4.38 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 4.50 (m, 1 H, CH), 5.39 (m, 1 H, NH). Anal. Calcd ($C_{13}H_{23}NO_5S$): C, 51.13; H, 7.59; N, 4.58. Found: C, 51.55; H, 7.47; N, 4.88.

(4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-[1-[(4-methylphenylsulfonyl)]imidazol-4-yl]-3-oxopentanoic acid ethyl ester (3e): mp 93–96 °C (ether–*n*-hexane); R_f(A) 0.25; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7 Hz, 3 H, CH_2CH_3), 1.42 (s, 9 H), 2.44 (s, 3 H, CH_3), 2.95–3.1 (m, 2 H, CH_2Ar), 3.57 (s, 2 H, $COCH_2$), 4.16 (q, *J* = 7 Hz, 2 H, CH_2CH_3), 4.54 (m, 1 H, CH), 5.75 (m, 1 H, NH), 7.08 (s br, 1 H, imid. H), 7.3–7.4 (m, 2 H, Ar H), 7.5–7.85 (m, 2 H, Ar H), 7.90 (s br, 1 H, imid. H). Anal. Calcd ($C_{22}H_{29}N_3O_7S$): C, 55.10; H, 6.10; N, 8.76. Found: C, 55.00; H, 6.36; N, 8.67.

Reduction of β-Keto Esters 3 by $NaBH_4$. To a stirred solution of **3** (1.50 mmol) in 15 mL of dried THF or THF–MeOH (98:2) at −78 °C was added sodium borohydride (76 mg, 2.01 mmol) in one portion. The reaction mixture was further stirred for 1.5–2 h. The reaction was quenched by pouring the mixture into ice-cold 1 N hydrochloric acid covered with ethyl ether. The aqueous phase was extracted with ether, and the combined organic layers were dried over $MgSO_4$ and evaporated. After column chromatography on silica gel with *n*-hexane–ethyl acetate mixtures, the 3-hydroxy esters **4** were obtained in diastereomerically pure form or as a mixture of diastereomers in the case of **4c/5c**.

Reduction of 3b by K-Selectride. To a solution of **3b** (335 mg, 1.0 mmol) in dried THF (10 mL) was added K-Selectride (2.4 mL of a 1 M solution in THF) at −78 °C under a nitrogen at-

(20) Both (*R*)- and (*S*)-AMBI (Transworld Chem., Inc., Chevy Chase, MD) contained 0.5–0.65% of the opposite enantiomer as determined by HPLC using pure (+)- and (−)-ephedrine, see: Gal, J.; Sedman, A. J. *J. Chromatogr.* **1984**, *314*, 275.

(21) (a) Kinoshita, T.; Kasahara, Y.; Nimura, N. *J. Chromatogr.* **1981**, *210*, 77. (b) Gal, J. *J. Chromatogr.* **1984**, *307*, 220. The reagent (ee >99.9%, determined after derivatization with (+)- and (−)-ephedrine) was purchased from Polysciences, Inc., Warrington, PA.

(22) Salituro, F. G.; Agarwal, N.; Hofmann, T.; Rich, D. H. *J. Med. Chem.* **1987**, *30*, 286–295.

(23) This and the following NMR data include only assignments for the predominant keto tautomeric form.[18]

(24) *N*-Boc-statine-OEt (**4a**) can be prepared under mild and racemization-free conditions in 65–70% yields (0.15 mmol scale) from *N*-Boc-statine-OH by reaction of the cesium salt of the carboxylic acid with ethyl iodide, according to a reported procedure: Wang, S.-S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kuleska, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.

(25) Jones, M.; Sueiras-Diaz, J.; Szelke, M. In *Peptides Structure and Function. Proceedings of Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chem. Co.; Rockford, IL, 1985; pp 759–762.

mosphere. The reaction mixture was stirred for 2.5 h and then poured into ice-cold 0.5 N hydrochloric acid. The aqueous phase was extracted with ethyl acetate, the organics dried over $MgSO_4$ and evaporated to give a colorless oil. Chromatography on silica gel with *n*-hexane–ethyl acetate (80:20) gave 240 mg (71%) of unreacted **3b** and 80 mg (24%) of pure (*S,S*)-**4b** as white crystals, $[a]^{22}_D$ – 34.1° (1, MeOH), after recrystallization from ethyl acetate–*n*-hexane.

Reduction reactions with potassium triisopropoxyborohydride (3 mmol %, 3 h at –23 °C) and lithium tri-*tert*-butoxyaluminiohydride (5 mmol %, 4 h at –78 °C) in THF and workup were performed in a similar manner.

Derivatization with 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Isothiocyanate (GITC). About 1 mg of N-Boc-protected **4a,b,d** was dissolved in 0.3 mL of CH_2Cl_2, and 0.15 mL of trifluoroacetic acid was added at 0 °C. After being stirred for 30 min, the mixture was evaporated, taken up in CH_2Cl_2 (2 mL), and evaporated. The residue in 1 mL of CH_2Cl_2 was treated twice with 0.5 mL of saturated $NaHCO_3$ solution by mixing the two phases for 30 s (Vortex Mixer) followed by aspirating the aqueous layer. The organic solution of the free base was shortly dried over Na_2SO_4, decanted into a vial, and concentrated to a volume of 0.2–0.3 mL by a stream of dry nitrogen, and 3 mg of GITC was added. After 1 h at room temperature, aliquots of the mixture were injected for HPLC analysis.

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Registry No. **2a**, 13139-15-6; **2b**, 13734-34-4; **2c**, 2389-45-9; **2d**, 16947-80-1; **2e**, 35899-43-5; **3a**, 58521-44-1; **3b**, 112271-08-6; **3c**, 98045-07-9; **3d**, 112271-09-7; **3e**, 112271-10-0; **4a**, 67010-43-9; **4b**, 72155-46-5; **4c**, 98045-10-4; (±)-**4d**, 112271-11-1; **5a**, 67010-44-0; **5b**, 72155-47-6; **5c**, 98063-00-4; (±)-**5d**, 112271-12-2; $HOOCCH_2COOEt$, 1071-46-1.

Notes

A Short and Efficient Synthesis of (3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-hydroxypentanoic Acid Ethyl Ester

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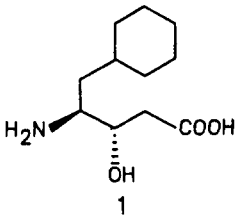
(3*S*,4*S*)-4-Amino-3-hydroxy-6-methylheptanoic acid (statine) is a key element of the naturally occurring aspartic protease inhibitor pepstatin and has been incorporated into the natural substrate for human renin, yielding potent inhibitors of this enzyme.[1] ACHPA ((3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (1)), an analogue of statine in which the isobutyl group has been replaced by a cyclohexymethyl group, has been found to give exceedingly potent renin inhibitors. In some cases, these inhibitors have been 50-fold more potent than the corresponding statine-containing analogues.[2] We required large amounts of a protected derivative of enantiomerically pure **1** for use in various aspects of our program to design potent, orally active inhibitors of renin.



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Previous methods for synthesizing these types of two-carbon homologated derivatives from naturally occurring α-amino acids proceed through organometallic additions to the reductively derived α-amino aldehydes.[2] The low yields, difficulties in scale-up, and ease of racemization of the aldehydes[3] led us to investigate an alternative method of synthesis of a protected version (**6**) of ACHPA. This methodology is shown in Scheme I.

L-Phenylalanine (**2**) was reduced to the hexahydro acid **3** with PtO_2 (98%) and protected as the *N*-α-*t*-Boc derivative by using Schotten–Baumen conditions to afford **4** (100%). The two-carbon homologation sequence was readily accomplished in one pot by sequential formation of the imidazolide derivative, addition of a mixture of malonic acid monoethyl ester[4]/$MgCl_2$(anhydrous)/Et_3N, and standard workup.[5] This gave the β-keto ester **5** as a thick oil (62%). Direct standard hydride reduction of **5** (e.g. $NaBH_4$; $LiAl(O\text{-}t\text{-}Bu)_3H$; $Zn(BH_4)_2$, etc.) gave predominantly the undesired 3*R*,4*S* isomer **7**, and although catalytic hydrogenation (Raney Ni/H_2/EtOH/65 °C) using the previously reported conditions[5b] gave approximately a 1:1 mixture of **6** and **7**, it also caused significant racemization (ca. 15%) of the C-4 center and produced the desired 3*S*,4*S* isomer **6** in only 15% isolated chemical yield. However, the use of $NaCNBH_3$/THF in the presence of glacial acetic acid afforded a 1:1 mixture of **6** and **7** with minimal racemization at C-4. The undesired isomer **7** crystallizes more easily and much of its is removed in this way. The thereby enriched **6** is purified by silica gel chromatography to give the optically pure material (40%). The enantiomeric purity of **6** was determined to be 92.4–92.7% ee by HPLC analysis of the 2,3,4,6-tetrahydro-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivative.[6]

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(5) For examples using various methods and types of magnesium enolates of malonic acid derivatives, see: (a) Morishima, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1973**, *36*, 115. (b) Descamps, M.; Verstraeten, W.; Mandataire, C. D. European Patent 0 165 226, December 18, 1985.

(6) See accompanying paper describing a related topic by Daniel H. Rich and Jurgen Maibaum.

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