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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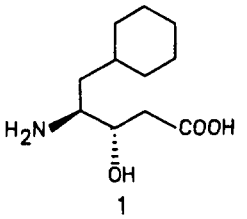
(3*S*,4*S*)-4-Amino-3-hydroxy-6-methylheptanoic acid (statine) is a key element of the naturally occurring aspartic protease inhibitor pepstatin and has been incorporated into the natural substrate for human renin, yielding potent inhibitors of this enzyme.[1] ACHPA ((3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic 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.



1

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]

(1) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature (London)* **1983**, *303*, 81.

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(3) Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236 and references cited therein.

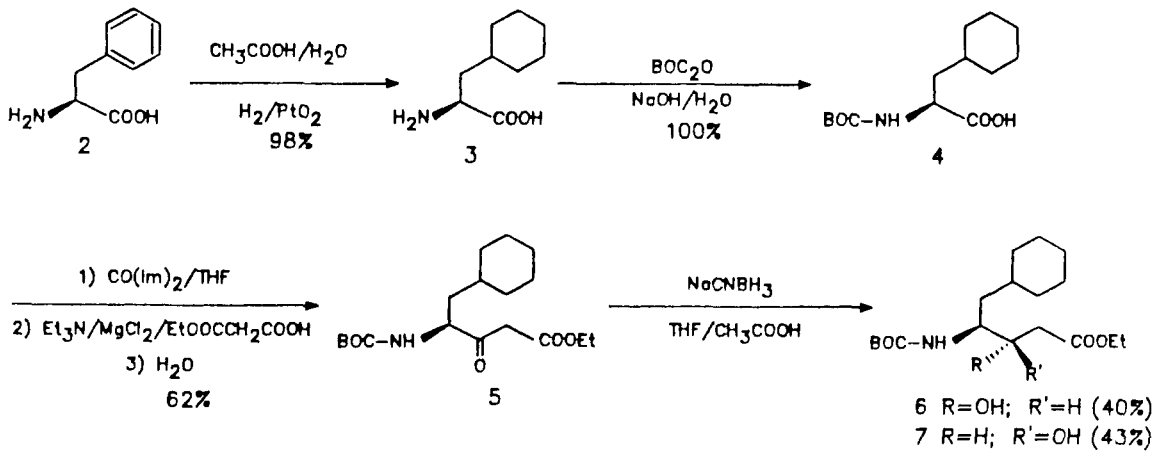
(4) Strube, R. E. *Organic Synthesis*; Wiley: New York, 1963; Collect. Vol. IV, p 417.

(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.

0022-3263/88/1953-0873$01.50/0

Scheme I



We have used this methodology to prepare **6** and **7** on scales of up to 500 g and have found no decrease in quantity or quality of the product.[7]

Experimental Section

Proton nuclear magnetic resonance (NMR) spectra were measured on a Varian XL-300 spectrometer. All shifts are reported as parts per million downfield from tetramethylsilane. Infrared spectra (IR) were measured on a Perkin-Elmer 283 spectrometer and are calibrated against the 1601-cm^{-1} band of polystyrene. Melting points were determined on a Haake-Buchler melting point apparatus and are uncorrected. Thin layer chromatographic analyses were done on E. Merck silica gel 60 (0.25 mm) plates (F-254); eluants are reported as percent by volume.

2-Amino-3-hexahydrophenylpropionic Acid (3). A solution of 50.00 g (0.303 mol) of L-phenylalanine (**2**) in 200 mL of glacial acetic acid and 140 mL of water was treated with 2.50 g of platinum oxide and the mixture of hydrogenated at 45 psig of hydrogen at 50 °C for 18 h on a Parr shaker apparatus. The reaction mixture was cooled to room temperature and the semisolid mass dissolved by adding additional acetic acid and methanol (ca. 100 mL each). The solution was filtered through a small pad of Celite and the pad was washed with additional methanol. The combined filtrates were evaporated completely in vacuo and the remaining solid triturated with anhydrous ether (2 × 500 mL) and filtered. The while solid was air-dried by suction filtration to afford 50.80 g (98%) of 2-amino-3-hexahydrophenylpropionic acid (**3**) as a white powder, mp 295–297 °C (begins to sublime at 290 °C): NMR (D_2O; 300 MHz) δ 0.80–1.00 (m, 2 H), 1.05–1.46 (m, 4 H), 1.50–1.80 (m, 7 H), 3.72 (dd, 1 H); IR ($CHCl_3$) 3500–2400 (v br), 1580 cm^{-1}.

2-[(*tert*-Butyloxycarbonyl)amino]-3-hexahydrophenylpropionic Acid (4). 2-Amino-3-hexahydrophenylpropionic acid (**3**) (45.00 g, 0.263 mol) was dissolved in a mixture of 580 mL of 0.5 N sodium hydroxide and 450 mL of dioxane. The resulting solution was cooled to 0 °C and treated dropwise with 63.11 g (66.50 mL, 0.290 mol) of di-*tert*-butyl dicarbonate over a period of ca. 30 min. The reaction mixture was allowed to warm to room temperature of its own accord (ca. 3 h) and stirred vigorously for an additional 17 h. Most of the volatiles were evaporated in vacuo and the remaining solution was cooled to 0 °C and acidified to pH 1 to 2 with 1 N $KHSO_4$. The mixture was extracted with 3 × 200 mL of methylene chloride, the combined organic solutions were dried over anhydrous sodium sulfate, and the volatiles were evaporated in vacuo. This afforded 71.30 g of **4** (100%) as a very viscous, colorless oil that was very pure by spectroscopic analysis: NMR ($CDCl_3$; 300 MHz) δ 0.75–1.30 (m, 6 H), 1.42 (s, 9 H), 1.43–1.84 (m, 7 H), 4.33 (m, 1 H), 4.82 (d, J = 7.5 Hz, 1 H); IR ($CHCl_3$) 3500–2550 (v br), 3450, 2950, 1710, 1160 cm^{-1}.

3-Oxo-(4*S*)-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentanoic Acid Ethyl Ester (5). Imidazolide Solution. A solution of carboxylic acid **4** (1060 g, 3.90 mol) in 7 L of tetrahydrofuran was stirred at room temperature while 705 g (4.35 mol) of *N,N*-carbonyldiimidazole was added in portions of 100 g. Strong gas evolution occurred but there was no increase in temperature of the reaction. The solution was then allowed to stir at room temperature for 20 h. During this period the solution became slightly yellow.

Magnesium Malonate Solution. A solution of 567 g (4.30 mol) of malonic acid monoethyl ester in 5 L of tetrahydrofuran was cooled to 0 °C in a methanol–ice bath. Magnesium chloride (225 g, 2.40 mol) was added all in one portion, followed by the dropwise addition of 480 g (660 mL, 4.73 mol) of triethylamine. The triethylamine was added over a period of ca. 20 min so that the temperature of the reaction did not exceed 10 °C. The reaction mixture became a white slurry and was stirred at 0 °C for 1 h.

The slurry from the magnesium malonate solution as prepared above was added all at once to the imidazolide solution at room temperature. The residue from the malonate solution was rinsed in with a small amount of tetrahydrofuran. The resulting slurry was stirred at room temperature for 3.5 h. The reaction mixture was concentrated in vacuo (ca. 90% of the THF removed) and the residue partitioned between 5 L of ether and 5 L of water. The layers were separated and the aqueous layer extracted with 3 × 3 L portions of ether. The combined organic layers were washed with 3 × 1 L of water and 1 × 1 L of saturated NaCl, dried over sodium sulfate and magnesium sulfate, and concentrated in vacuo to afford 1261 g of a viscous material as the crude product.

The crude material was dissolved in 3 L of methylene chloride and filtered through 3 kg of silica gel (70–230 mesh) in a large sintered glass funnel. Additional methylene chloride (25 L) was used to elute the desired ketone from the silica gel. Evaporation of the solvent afforded 822 g (62%) of desired ketone **5** was a viscous oil: $[\alpha]_D$ −38.4° (*c* 1, MeOH); NMR ($CDCl_3$; 300 MHz) δ 0.80–1.42 (m, containing 3-H t at δ 1.28, 13 H), 1.45 (s, 9 H), 1.53–1.92 (m, 2 H), 3.51 (d, J = 14.4 Hz, 1 H), 3.58 (d, J = 14.4 Hz, 1 H), 4.19 (q, 2 H), 4.39 (m, 1 H), 4.90 (d, J = 7.8 Hz, 1 H); IR ($CHCl_3$) 2950, 1710, 1485, 1370, 1145 cm^{-1}.

(3*S*)-3-Hydroxy-(4*S*)-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentanoic Acid (6) and (3*R*)-3-Hydroxy-(4*S*)-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentanoic Acid (7). A solution of 612 g (1.80 mol) of keto ester **5** in 6 L of tetrahydrofuran was cooled in an ice bath and treated with 126 g (2 mol) of sodium cyanoborohydride. The resulting solution was stirred and treated with 355 g (340 mL, 5.90 mol) of glacial acetic acid added dropwise over a period of 20 min. The ice bath was then removed and the reaction mixture stirred at room temperature for 16 h.

The mixture was concentrated under reduced pressure to ca. 20% of the original volume and added to 2 L of water. The aqueous layer was extracted with 4 × 1 L of ether and the combined ether layers were washed with 1 L of water and then with 500-mL portions of saturated sodium bicarbonate until the aqueous layer was neutral pH. The organic solution was washed with 1 L of water and 1 L of saturated NaCl, dried over sodium

(7) After submission of this manuscript, an efficient and stereospecific method for synthesizing some other statine derivatives appeared. Cf. Jouin, P.; Castro, B.; Nisato, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177.

sulfate and magnesium sulfate, and concentrated in vacuo to afford 717 g of a thick golden oil.

Hexanes (700 mL) was added to the oil and the mixture heated until a clear yellow solution was obtained. The solution was allowed to cool and the flask scratched to induce crystallization. The vessel was cooled in a refrigerator for ca. 20 h. The solid was filtered off, washed with a small amount of cold hexanes, and dried in vacuo. The resulting solid was recrystallized from 400 mL of hexanes to afford 176 g of pure 3*R*,4*S* material **7**. The combined mother liquors from above were concentrated under reduced pressure and the enriched mixture was completely separated by preparative HPLC using a Waters Prep 500 utilizing two silica gel packs and 9% acetone in hexanes as the eluant (flow rate 250 mL/min). The appropriate fractions were combined and the solvents evaporated under reduced pressure. These procedures afforded 245 g (40%) of the desired 3*S*,4*S* isomer **6** and 266 g (43%) of the 3*R*,4*S* isomer **7**.

(3*S*,4*S*)-6: mp 67-69 °C; R_f 0.35 (20% acetone in hexanes); $[\alpha]_D$ -32° (*c* 1.4, MeOH) (lit.[2] -37°); NMR ($CDCl_3$; 300 MHz) δ 0.80-1.92 (m containing 3-H t at δ 1.28 and 9-H s at δ 1.44, 13 H), 2.40-2.62 (m, 2 H), 3.26 (br s, 1 H), 3.60-3.70 (m, 1 H), 4.01 (d, J = 9.0 Hz, 1 H), 4.18 (q, 2 H), 4.69 (d, J = 9.6 Hz, 1 H); IR ($CHCl_3$) 3460 (br), 2940, 1705, 1490, 1165 cm^{-1}.

(3*R*,4*S*)-7: mp 81-83 °C; R_f 0.25 (20% acetone in hexanes); $[\alpha]_D$ -16.7° (*c* 1.6, MeOH); NMR ($CDCl_3$; 300 MHz) δ 0.70-1.92 (m, containing 3-H t at δ 1.28 and 9-H s at δ 1.45, 13 H), 2.41-2.52 (m, 2 H), 3.40 (br s, 1 H), 3.62-3.78 (m, 1 H), 4.00 (m, 1 H), 4.17 (q, 2 H), 4.54 (d, J = 10.2 Hz, 1 H); IR ($CHCl_3$) 450 (br), 2930, 1700, 1485, 1445, 1370, 1165, 1030 cm^{-1}.

Acknowledgment. We thank Drs. David Melillo and David Tschaen for helpful discussions regarding the two-carbon homologation procedure. We also express our appreciation to Drs. Daniel Rich and Jurgen Maibaum for performing the ee analysis and for sharing their results with us prior to publication.

Registry No. **2**, 63-91-2; **3**, 27527-05-5; **4**, 37736-82-6; **5**, 112151-92-5; **6**, 98105-43-2; **7**, 98105-44-3; $EtOCOCH_2COOH$, 1071-46-1.

Methyl Ester Nonequivalence in the ^1H NMR Spectra of Diastereomeric Dipeptide Esters Incorporating N-Terminal α-Phenylglycine Units

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Numerous methods for following racemization during peptide coupling reactions have been reported.[1] Techniques based on NMR spectroscopy have the advantage of simplicity and rapid applicability and are therefore especially suited for quick survey work where high sensitivity is not required. The first such test, described by Halpern and Weinstein,[2] took advantage of the *C*-methyl doublets arising from alanine units built into various protected diastereomeric dipeptides. Davies, Thomas, and Williams[3] subsequently recommended the use of *N*-benzoyl dipeptide methyl esters since different methyl ester singlets were observed in many such cases and the appropriate peaks occur in an uncluttered region of the spectrum. While not limited to a specific amino acid such as alanine, the Davies method requires the presence of a terminal *N*-benzoyl substituent and thus diastereomeric dipeptide methyl esters bearing classic urethane-type protecting groups such as benzyloxycarbonyl, *tert*-butyloxycarbonyl, or (9-fluorenylmethyl)oxycarbonyl do not exhibit nonequivalence. Visualization of the effect in such cases requires deblocking and subsequent benzoylation.

While surveying conditions which might lead to racemization during the coupling of various FMOC amino acid chlorides,[4] we initially examined a moderately sensitive model, FMOC phenylalanine chloride, with the coupling step being followed by 4-(aminomethyl)piperidine (4-AMP) deblocking and N-benzoylation. In order to magnify the susceptibility to racemization[5] we subsequently shifted to a study of analogous reactions of the FMOC derivative of α-phenylglycine chloride. Although a nonprotein amino acid, α-phenylglycine is readily available in both L and D forms and its selection proved to be especially fortunate. Initial studies involved reaction of [(9-fluorenylmethyl)-oxycarbonyl]-L- and [(9-fluorenylmethyl)oxycarbonyl]-D-α-phenylglycine chlorides with alanine methyl ester followed by in situ examination of the reaction mixtures by ^1H NMR analysis. The *C*-methyl doublets of the diastereomeric dipeptides **1** differed as expected from the work of Weinstein and co-workers.[2] However, we were pleas-

FMOC-(L or D)-Phg-Ala-OMe
1

antly surprised to find that, in addition, the methyl ester singlets were also well separated (δ 3.59, 3.66). Indeed the separation for these *urethanes* (0.07 ppm) is about the same as observed by Davies and co-workers[3] for a variety of related *N*-benzoyl derivatives (range ca. 0.04-0.1 ppm). These ester peaks were also well separated in DMSO-d_6, a result which contrasts with the previous recommendation that all traces of polar solvents be removed prior to NMR measurement in view of coalascence of the two peaks in such media.

The effect observed appears general for dipeptides bearing other C-terminal amino acid units. In some cases the diastereomeric pairs were isolated and characterized; in other cases reaction products were examined in solution (see Table I and Experimental Section). That the effect arises from the presence of the α-phenyl substituent at the N-terminal amino acid position and is unrelated to any particular N-terminal acyl function is confirmed by the observation of two widely separated methyl ester peaks in the cases of a number of other diastereomeric N-substituted dipeptide derivatives **2** (X = BOC, Z, Ts; X_{aa} = Ala or Phe) as well as a free amino dipeptide methyl ester (**2**, X = H; X_{aa} = Phe).

X-Phg-X_{aa}-OMe
2

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