Regio- and Stereo-specific Synthesis of *threo*-3-Amino-2-hydroxy-Acids, Novel Amino-acids contained in Aminopeptidase Inhibitors of Microbial Origin

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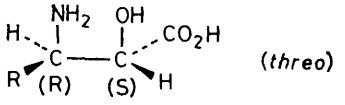
threo-3-Amino-2-hydroxy-4-phenylbutanoic acid, a novel amino-acid contained in bestatin, an inhibitor of aminopeptidase B and leucine aminopeptidase, and *threo*-3-amino-2-hydroxy-5-methylhexanoic acid, a novel amino-acid contained in amastatin, an inhibitor of aminopeptidase A and leucine aminopeptidase, have been synthesized from *cis*- and *trans*-2-olefinic acids *via* regiospecific ring-opening of *cis*-2,3-epoxy-acids by ammonia. Direct hydroxyamination of the 2-olefinic acids, however, gave 2-amino-3-hydroxy-acids as the major products.

RECENTLY, bestatin,[1] (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl-*S*-leucine,[2] which inhibits aminopeptidase B and leucine aminopeptidase but not aminopeptidase A, and amastatin,[3] (2*S*,3*R*)-3-amino-2-hydroxy-5-methyl-hexanoyl-*S*-valyl-*S*-valyl-*S*-aspartic acid,[4] which inhibits aminopeptidase A and leucine aminopeptidase but not aminopeptidase B, have been isolated

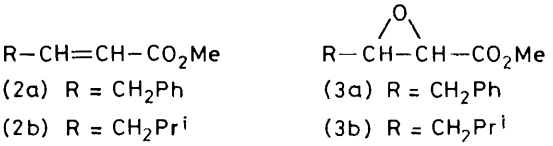


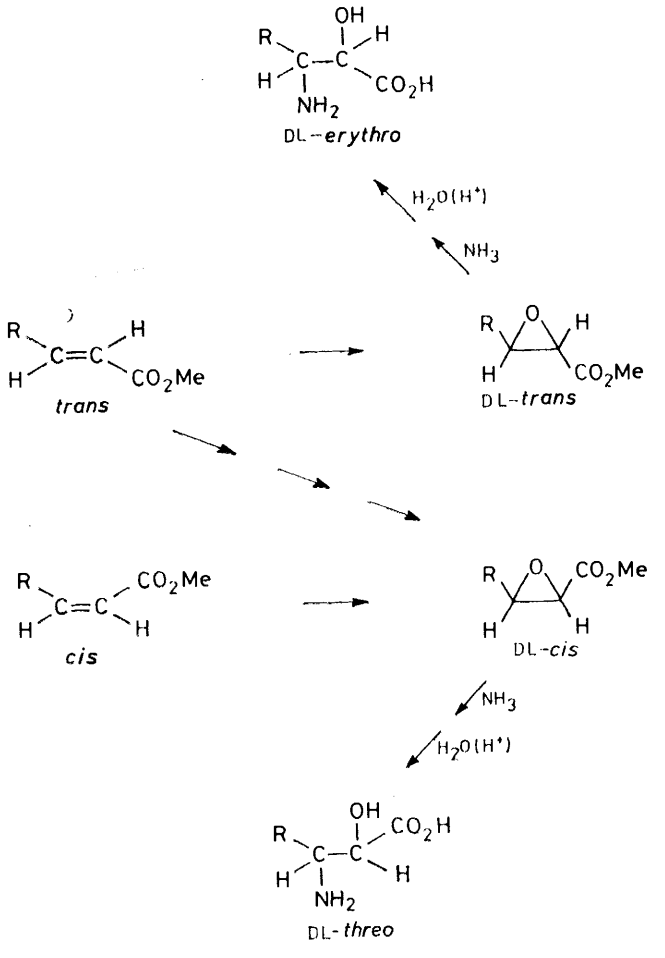
(*threo*)

(1)

a; R = CH_2Ph (2*S*,3*R*)-AHPA
b; R = CH_2Pr^i (2*S*,3*R*)-AHMA

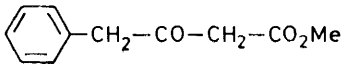
from the cultured broths of soil streptomyces. Neither of them inhibits carboxypeptidases and endopeptidases. They both have novel (2*S*,3*R*)-3-amino-2-hydroxy-acid structures and these unusual amino-acid residues are essential for their bioactivities. Syntheses of (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoic acid (1a) [abbreviated as (2*S*,3*R*)-AHPA] and (2*S*,3*R*)-3-amino-2-hydroxy-5-methylhexanoic acid (1b) [abbreviated as (2*S*,3*R*)-AHMA] have been already achieved starting from D-phenylalanine [2,5] or D-leucine,[4,5] respectively. Regio- and stereo-specific hydroxyamination of 2-olefinic acids appears to be the other feasible route to the synthesis of these amino-acids. If the direct hydroxyamination [6] proceeds in a regiospecifically favourable way, DL-*threo*-AHPA and -AHMA, the DL-forms of natural ones, should, since the hydroxyamination is a *cis*-addition, be available from the readily obtainable corresponding *trans*-2-olefinic acids. Thus, methyl *E*-4-phenylbut-2-enoate, *trans*-(2a), was prepared from phenylacetaldehyde and methoxycarbonylmethylene-triphenylphosphorane. Hydroxyamination of *trans*-(2a) with osmium tetraoxide and chloramine-T,[6] however, afforded the undesired α-amino-β-hydroxy-derivative as the major product. The ratio of the yield of the *threo*-AHPA derivative to the α-amino-β-hydroxy-derivative was found to be 2 : 5 after separation.





Therefore, hydroxyamination of 4-phenylbut-2-enoate *via* ring-opening of 2,3-epoxy-4-phenylbutanoate by ammonia was examined. To test the regiospecificity of

the hydroxyamination by ring-opening of the epoxide, methyl *trans*-2,3-epoxy-4-phenylbutanoate, *trans*-(3a), which is readily available by pertrifluoroacetic acid oxidation of *trans*-(2a), was treated with ammonia, although it was expected to give *erythro*-AHPA even if the amination proceeded in a regiospecifically favourable way. At room temperature for six days, the oxiran ring was opened exclusively in the expected way and gave DL-*erythro*-AHPA after the concomitant acid hydrolysis.



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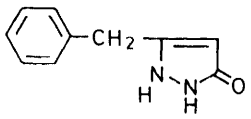

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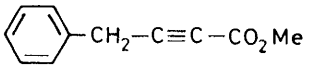
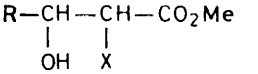


(6)

It was also confirmed that DL-*threo*-AHPA was formed from *cis*-(3a) by the regiospecific ammonolysis. Compound *cis*-(3a) was derived from *cis*-(2a) by peracid oxidation in a similar manner described above. The latter was synthesized by three steps: (i) condensation of the β-ketoester (4) with hydrazine hydrate to give the pyrazolone (5); (ii) treatment of the latter with thallium nitrate in methanol solution to give yne-ester (6); (iii) hydrogenation of (6) with Lindlar catalyst to give *cis*-(2a).

To avoid the troublesome synthesis of *cis*-(2a), the synthesis of *cis*-(3a) from *trans*-(2a) was attempted. The *threo*-glycol (7a) was obtained from *trans*-(2a) by the Sharpless procedure.[7] The monotosylate (8a) was prepared by treatment of (7a) with tosyl chloride in pyridine. Finally, treatment of (8a) with sodium hydride in benzene furnished *cis*-(3a). Thus, the regio- and stereospecific synthesis of DL-*threo*-AHPA, the DL-form of natural AHPA, from *E*- and *Z*-4-phenylbut-2-enoic acids *via* *cis*-2,3-epoxy-4-phenybutanoate was established.



(7a) R = CH_2Ph, X = OH

(7b) R = CH_2Pr^i, X = OH

(8a) R = CH_2Ph, X = OTs

(8b) R = CH_2Pr^i, X = OTs

In a similar manner, DL-*threo*-AHMA, the DL-form of natural AHMA, was synthesized by regiospecific amination of *cis*-(3a), which was derived from *trans*-(2b) *via* *threo*-(7b).

EXPERIMENTAL

N.m.r. spectra were recorded on JEOL, JNM-PMX 60 spectrometer in deuteriochloroform unless otherwise stated and are reported as chemical shifts downfield from tetramethylsilane. I.r. spectra were obtained on JASCO IR-G spectrometer. Kieselgel 60 (Merck) was used for column chromatography and Kieselgel 60 F_{254} plates for thin layer chromatography. Anhydrous sodium sulphate was used as a drying agent for solutions in organic solvents. The OsO_4 catalyst solution was prepared as follows: OsO_4 (1 g) was dissolved in reagent grade t-butyl alcohol (199 ml) and 90+% t-butyl hydroperoxide (1 ml). Each ml of this stock solution contained 5 mg (2.0 mmol) of OsO_4.

Preparation of the trans-α,β-*Unsaturated Esters* (2).—A solution of an appropriate aldehyde (50 mmol) and methoxycarbonylmethylenetriphenylphosphorane (50.9 mmol) in benzene (50 ml) was stirred at room temperature for 18 h. The solution was evaporated under reduced pressure and the residue was washed with n-hexane (2 × 50 ml). After evaporation of the solvent, the residual oil was chromatographed on silica gel (100 g) using benzene as eluant.

(i) *Methyl* E-4-*Phenylbut*-2-*enoate*, trans-(2a). From phenylacetaldehyde was obtained *trans*-(2a) as an oil (92%), the n.m.r. spectrum of which indicated the absence of *cis*-(2a); $\nu_{max.}$ (film) 1 720, 1 658, 1 605, and 1 500 cm^{-1}; δ 3.46 (2 H, d × d, J = 7 and 2 Hz, 4-H) and 5.78 (1 H, d × t J = 16 and 2 Hz, 2-H) (Found: C, 74.7; H, 7.15%; *m/e* 176. $C_{11}H_{12}O_2$ requires C, 74.97; H, 6.86%; M^+ 176).

(ii) *Methyl* E-5-*methylhex*-2-*enoate*, trans-(2b). From 3-methylbutyl aldehyde was obtained *trans*-(2b) as an oil (90%), b.p. 80—82 °C/26 mmHg; $\nu_{max.}$ (film) 1 730, 1 663, 1 470, and 1 440 cm^{-1}; δ 5.75 (1 H, d × t J = 16 and 0.5 Hz, 2-H) (Found: C, 67.9; H, 9.8%; *m/e* 142. $C_8H_{14}O_2$ requires C, 67.57; H, 9.93%; M^+ 142).

Preparation of 3-*Benzyl*-3-*pyrazolin*-5-*one* (5).—A solution of the β-ketoester (4) (4 g, 21 mmol) and 90% hydrazine hydrate (1.1 g, 24 mmol), in ethanol (10 ml) was stirrred at room temperature for 3 h. The alcohol was removed under reduced pressure. The resulting crystals were filtered off, followed by washing with small amount of cold ethanol to give the title compound (2.86 g, 78%); this was dried for 1—2 h over anhydrous calcium chloride and then used without further purification, m.p. 192—193 °C (from ethanol); $\nu_{max.}$ (Nujol) 3 140, 3 050, 3 025, 1 640, 1 610, and 1 600 cm^{-1}; δ [$(CD_3)_2SO$] 3.80 (2 H, s, 6-H), 5.23 (1 H, s, 4-H), and 10.43 (2 H, s, $w_{1/2}$ = 16 Hz 1-H, 2-H) (Found: C, 69.25; H, 5.6; N, 15.75%; *m/e* 174. $C_{10}H_{10}N_2O$ requires C, 68.95; H, 5.79; N, 16.08%; M^+ 174).

Preparation of Methyl 4-*Phenylbut*-2-*ynoate* (6).—A solution of thallium(III) nitrate (2.457 g, 63 mmol) in methanol (10 ml) was added to a suspension of the 5-pyrazolone (5) (522 mg, 30 mmol) in methanol (50 ml). The reaction mixture was stirred for 20 min at room temperature followed by an additional 20 min at reflux; it was then reduced to approximately half its volume by evaporation on a rotary evaporator. The cooled reaction mixture was filtered to remove precipitated thallium(I) nitrate. The filter cake was washed with chloroform (40 ml) and water (40 ml) was added to the filtrate. The chloroform layer was separated and two additional extractions with chloroform (20 ml) were carried out. The combined chloroform layer was washed once with 5% aqueous sodium carbonate (10 ml), twice with water (20 ml), and then dried. The chloroform was filtered through a short column of Florisil (100—200 mesh; 30 g). The chloroform was removed on a rotary evaporator to afford the pure yne-ester (6) (444 mg, 85%) as a slightly yellow liquid, $\nu_{max.}$ (film) 2 225, 1 715, 1 600, and 1 495 cm^{-1}; δ 3.73 (5 H, s, OCH_3, 4-H) (Found: C, 76.15; H, 5.65%; *m/e* 174. $C_{11}H_{10}O_2$ requires C, 75.84; H, 5.79%; M^+ 174).

Hydrogenation of the Yne-ester (6).—A solution of the yne-ester (6) (340 mg, 19.5 mmol) in ethanol (5 ml) with 1 drop of quinoline was hydrogenated on 5% Pd–$BaSO_4$ (50 mg) with a hydrogen atmosphere at room temperature for 30 min. The catalyst and solvent were removed, and the residue was chromatographed on silica gel (5 g). The fractions eluted with benzene afforded methyl *Z*-4-phenylbut-2-enoate, *cis*-(2a) (316 mg, 92%) as a liquid, whose n.m.r. spectrum indicated the absence of *trans*-(2a), $\nu_{max.}$ (film) 1 725, 1 645, 1 600, and 1 495 cm^{-1}; δ 4.03 (2 H, d × d, J = 7 and 2 Hz, 4-H) and 5.83 (1 H, d × t, J = 12 and 2 Hz, 2-H) (Found: C, 75.2; H, 6.75%, *m/e* 176. $C_{11}H_{12}O_2$ requires C, 74.97; H, 6.86% M^+ 176).

Preparation of Glycidic Esters (3) *by Epoxidation of* α,β-*Unsaturated Esters* (2) *with Pertrifluoroacetic Acid*.—A solution of pertrifluoroacetic acid was prepared from 90% hydrogen peroxide (50 mmol), trifluoroacetic anhydride (55 mmol), and methylene chloride (15 ml). This reagent was added during a 10 min period to a well stirred, boiling mixture of *cis*- or *trans*-(2a) (10 mmol), disodium hydrogen phosphate (120 mmol), and methylene chloride (50 ml). After the exothermic reaction had subsided, the solution was refluxed overnight. The resulting mixture was stirred with water until all the inorganic salts had dissolved. The organic layer was separated and the aqueous layer was extracted with methylene chloride (25 ml). The combined methylene chloride extracts were washed with 10% aqueous sodium hydrogen carbonate (10 ml) and dried. Most of the solvent was evaporated under reduced pressure and the residual liquid was chromatographed on silica gel (50 mg) and eluted with benzene–n-hexane (4 : 1).

(i) *Methyl* trans-2,3-*epoxy*-4-*phenylbutanoate*, trans-(3a). From *trans*-(2a) was obtained *trans*-(3a) as an oil (78%), b.p. 88—90 °C/0.07 mmHg; $\nu_{max.}$ (film) 1 750, 1 600, 1 495, and 1 440 cm^{-1}; δ 3.26 (1 H, d, J = 2 Hz, 2-H) (Found: C, 68.6; H, 6.55%, *m/e* 192. $C_{11}H_{12}O_3$ requires C, 68.73; H, 6.29%; M^+ 192).

(ii) *Methyl* cis-2,3-*epoxy*-4-*phenylbutanoate*, cis-(3a). From *cis*-(2a) was obtained *cis*-(3a) as an oil (72%), b.p. 85—87 °C/0.07 mmHg; $\nu_{max.}$ (film) 1 750, 1 600, 1 495, and 1 440 cm^{-1}; δ 3.55 (1 H, d, J = 4 Hz, 2-H) (Found: C, 68.7; H, 6.45%; *m/e* 192. $C_{11}H_{12}O_3$ requires C, 68.73; H, 6.29%; M^+ 192).

cis-*Hydroxylation of the* trans-α,β-*Unsaturated Esters* (2).—A solution of the appropriate *trans*-ester (10 mmol), $Et_4NOAc{\cdot}4H_2O$ (2.5 mmol), and 70% t-butyl hydroperoxide (*ca.* 17 mmol) in acetone (15 ml) was chilled by stirring in an ice-bath; OsO_4 stock solution (1 ml) was then added in one portion. After 1 h the ice-bath was removed and the content was allowed to stand overnight at room temperature. The resulting golden solution was diluted with ether (20 ml) and chilled by stirring in an ice-bath. Thereafter a freshly prepared 20% $NaHSO_3$ solution (2 ml) was added, the ice cooling was stopped and the contents of the flask was stirred for 1 h at room temperature. The aqueous layer was saturated with NaCl and the two phases were partitioned. The organic layer was washed with brine. The combined brine layers were extracted twice with 10 ml portions of ether. All organic layers were combined and dried and evaporated.

(i) *Methyl* threo-2,3-*dihydroxy*-4-*phenylbutanoate* (7a). From *trans*-(2a) was obtained *threo*-(7a) as a solid (78%), m.p. 78—80 °C (from ether–n-hexane); $\nu_{max.}$ (Nujol) 3 375, 3 200, 1 725, and 1 610 cm^{-1}; δ 2.91 (2 H, d, J = 7 Hz, 4-H) and 3.93—4.50 br (3 H, 2-H, 3-H, OH × 1) (Found: C, 62.95; H, 6.8%, *m/e* 210. $C_{11}H_{14}O_4$ requires C, 62.84; H, 6.71%; M^+ 210).

(ii) *Methyl* threo-2,3-*dihydroxy*-5-*methylhexanoate* (7b). From *trans*-(2b) was obtained *threo*-(7b) as a solid (75%), m.p. 80—81 °C (from ether–n-hexane); $\nu_{max.}$ (Nujol) 3 400, 3 250, 1 730, and 1 290 cm^{-1}; δ 0.97 (6 H, d, J = 6 Hz, CH_3 × 2) and 3.93—4.27 br (3 H, 2-H, 3-H, OH × 1) (Found: C, 54.9; H, 8.9%, *m/e* 176. $C_8H_{16}O_4$ requires C, 54.33; H, 9.15%; M^+ 176).

Preparation of the threo-*Monotosylates* (8).—A solution of the *threo*-glycol (6a) or (7b) (23.8 mmol) and tosyl chloride (30 mmol) in pyridine (3 ml) was stirred at room temperature for 20 h. The solution was poured onto ice and the product was extracted with ethyl acetate. The organic layer was washed with dilute hydrochloric acid, water, and brine, and then evaporated after drying. The residual oil was chromatographed on silica gel (20 g). The fractions eluted with benzene–ethyl acetate (4 : 1) afforded the desired monotosylate.

(i) *Methyl* threo-3-*hydroxy*-2-*tosyloxy*-4-*phenylbutanoate* (8a). Compound *threo*-(8a) was obtained from *threo*-(7a) as an oil (82%); $\nu_{max.}$ (film) 3 600, 1 765, 1 600, 1 380, and 1 180 cm^{-1}; δ 2.80 (2 H, d, J = 7 Hz, 4-H), 3.90—4.50 (1 H, m, 3-H) and 4.89 (1 H, d, J = 3 Hz, 2-H) (Found: C, 59.7; H, 5.4%, *m/e* 364. $C_{18}H_{20}O_6S$ requires C, 59.33; H, 5.53%; M^+ 264).

(ii) *Methyl* threo-3-*hydroxy*-2-*tosyloxy*-5-*methylhexanoate*, (8b). Compound *threo*-(8b) was obtained from *threo*-(7b) as an oil (86%); $\nu_{max.}$ (film) 3 520, 1 765, 1 595, 1 370, and 1 195 cm^{-1}; δ 0.83 (3 H, d, J = 7 Hz, CH_3), 0.89 (3 H, d, J = 7 Hz, CH_3), and 4.77 (1 H, d, J = 3 Hz, 2-H) (Found: C, 54.85; H, 6.6%; *m/e* 330. $C_{15}H_{22}O_6S$ requires C, 54.54; H, 6.71%; M^+ 330).

Preparation of the cis-*Glycidic Esters* (3) *from the* threo-*Monotosylates* (8).—A solution of the monotosylate (8a) or (8b) (1.1 mmol) and sodium hydride (1.3 mmol) in dry benzene (8 ml) was heated under reflux for 18 h. Work-up as usual gave an oil which was chromatographed on silica gel (5 g) with bezene as eluant.

(i) *Methyl* cis-2,3-*epoxy*-4-*phenylbutanoate*, cis-(3a). From monotosylate-(8a) was obtained *cis*-(3a) as an oil (90%), whose i.r., n.m.r. and g.c. retention time were identical with those of the authentic *cis*-(3a) derived from the *cis*-(2a).

(ii) *Methyl* cis-2,3-*epoxy*-5-*methyl hexanoate*, cis-(3b). From monotosylate-(8b) was obtained *cis*-(3b) as an oil (78%), b.p. 57—58 °C/0.3 mmHg; $\nu_{max.}$ (film) 1 760, 1 475, 1 445, and 1 210 cm^{-1}; δ 0.95 (3 H, d, J = 6 Hz, CH_3), 1.00 (3 H, d, J = 6 Hz, CH_3), and 3.53 (1 H, d, J = 5 Hz, 2-H) (Found: C, 61.0; H, 9.1%; *m/e* 158. $C_8H_{14}O_3$ requires C, 60.74; H, 8.92%; M^+ 158).

Ammonolysis of the Glycidic Esters (3).—A solution of an appropriate glycidic ester (3.6 mmol) in 29% NH_4OH (6 ml) and methanol (10 ml) was kept at room temperature for 6 days. After evaporation of the solvent, the residue was dissolved in 2N-HCl (5 ml) and refluxed for 1 h. The reaction mixture was adsorbed on a Dowex 50W (H-Type 30 ml) column and eluted with 2N-NH_4OH to afford a solid, the t.l.c. character of which was identical with that of the corresponding 3-amino-2-hydroxy-acid (development: n-butanol–acetic acid–water = 4 : 1 : 1 and ethyl acetate–pyridine–acetic acid–water = 5 : 5 : 1 : 3), high-performance liquid chromatography [column: Aminex A-5 (BioRAD), development: 0.2M-citrate buffer pH 6.89]; the n.m.r. (CD_3CO_2D) characteristics were also identical.

(i) Compound *trans*-(3a) yielded DL-*erythro*-AHPA (72%),

m.p. 228—230 °C (decomp.) (Found: C, 61.75; H, 6.6; N, 7.2. $C_{10}H_{13}NO_3$ requires C, 61.52; H, 6.71; N, 7.18%).

(ii) Compound *cis*-(3a) yielded DL-*threo*-AHPA (70%), m.p. 247—249 °C (decomp.) (Found: C, 61.8; H, 6.7; N, 7.05. $C_{10}H_{13}NO_3$ requires C, 61.52; H, 6.71; N, 7.18%).

(iii) Compound *cis*-(3b) yielded DL-*threo*-AHMA (68%), m.p. 243—245 °C (decomp.) (Found: C, 52.45; H, 9.3; N, 8.55. $C_7H_{15}NO_3$ requires C, 52.15; H, 9.38; N, 8.69%).

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