Synthesis of Boc-(2S, 3R)-3-Amino-2-Hydroxy-4-Phenylbutanoic Acid, a Moiety of Bestatin

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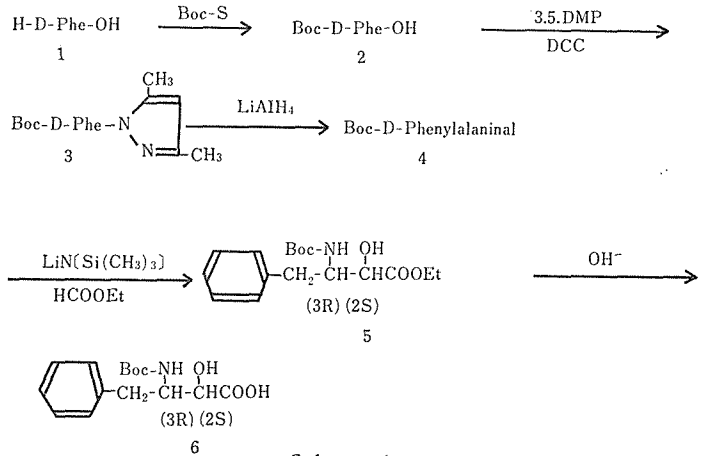
Bestatin,[1-2] (2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoyl-leucine, is a low molecular weight inhibitor of aminopeptidase B and leucine aminopeptidase, in which (2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoic acid is the novel amino acid abbreviated as (2S, 3R)-AHPA.

Owing to the properties of enhancing delayed type hypersensitivity and of being effective against experimented murine tumors, bestatin is now widely clinically tested as an immunotherapeutic agent against cancer.[3-5]

The synthesis of bestatin including AHPA has been reported by UMEZAWA et al.[6-8] However, an efficient method for the stereospecific synthesis of optically pure (2S, 3R)-AHPA has not yet been developed.

In the present paper, we wish to report the attempt to synthesize Boc-(2S, 3R)-AHPA (6) stereospecifically. The synthesis of **6** is outlined in Scheme I.



Scheme 1

Received April 30, 1981

The abbreviations follow IUPAC-IUB tentative rules as described in "The American Society of Biological Chemists Inc., Rockville Pike, Bethesda Md." Additional abbreviations used are as follows : AHPA, 3-amino-2-hydroxy-4-phenylbutanoic acid ; Boc, *tert*-butyloxycarbonyl ; Boc-S, 2-(*tert*-butyloxycarbonylthio)-4,6-dimethylpyrimidine ; DMP, dimethylpyrazole ; DCC, dicyclohexylcarbodiimide ; Statin, 4-amino-3-hydroxy-6-methylheptanoic acid.

Boc-D-Phe-OH (2) was prepared by using 2-(*t*-butyloxycarbonylthio)-4,6-dimethylpyrimidine (93%). Quite recently a method for the preparation of the corresponding Boc-amino aldehyde from Boc-amino acid methyl ester was reported[9-10] by using diisobutylaluminum hydride which was prepared from triisobutylaluminum.[11] The reagent used there, however, are not easily commercially available, so we obtained the amino aldehyde (4) via 3,5-dimethylpyrazolide (3). Namely, **2** was coupled with 3,5-dimethylpyrazole using dicyclohexylcarbodiimide to give a crystalline product (3) in 96% yield. **3** was reduced with lithium aluminum hydride in tetrahydrofurane. However, **4** could not be obtained by the usual reduction condition.[7] Then, we investigated the optimum reduction condition by using lithium aluminum hydride and a better and faster preparation was achieved by using such reduction conditions ; 1.5 mole eq. of lithium aluminum hydride at -18°～-19° for 15 min (85% yield).

The compound **5** has two asymmetric carbons, in which as far as we use D-phenylalanine as a starting material, the configuration at C_3 is R-form and in order to obtain the compound having S-form at C_2, we used hexamethyldisilazyllithium[12-14] as a coupling reagent.

In 1975, KLOSTERMEYER et al[15] first introduced this reagent to the stereochemical synthesis of Boc-(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid [abbreviated as Boc-(3S, 4S)-Statin] which is a moiety of microbial inhibitor, "pepstatin" and reported that the reaction product contains 94% of the (3S, 4S)-diastereomer. In 1978, however, RICH et al[9] have reported the similar experiments according to a modification of the procedure of KLOSTERMEYER[15] and obtained Boc-Statin ethyl ester in 80% yield as a mixture of diastereomers [60% (3S, 4S) ; 40% (3R, 4S)] and also suggested in their paper that the Boc-Statin obtained by KLOSTERMEYER contains only 77% of the (3S, 4S) diastereomer.

In applying this reagent for synthesizing **5**, there are some differences between AHPA and Statin from the viewpoint of the properties of condencing pairs (the former is Boc-D-phenylalaninal + LiCOOEt ; the latter is Boc-L-leucinal + $LiCH_2$-COOEt), and needed to modify the procedure of KLOSTERMEYER.

In fact, performing the condensation reaction according to the method of KLOSTERMEYER at -78° for 5 min gave no product except the compound from lack of formyl group. The yield of **5** was improved to 46% by adjusting the reaction temperature at -20° and prolonging the reaction time to 2 hr at -20° following for 37 hr at room temperature. **5** was obtained as an oil having levo-rotatory optical rotation, $[\alpha]_D^{21}$ -40.0° (c=0.1, MeOH). The compound **5** was, however, not found in the literature. Then, **5** was saponified to Boc-(2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoic acid (6) and the dicyclohexylammonium salt of **6** was obtained as a crystal, mp. 154-156°, $[\alpha]_D^{21}$ +44.0 (c=0.1, AcOH) [Lit.[7] mp. 158-159°, $[\alpha]_D^{25}$ +

51.9° (c=0.89, AcOH)].

From the results obtained above, although the reaction between Boc-D-phenylalaninal (4) and LiCOOEt could not proceed stereospecifically, it may at least be concluded that Boc-AHPA-OEt (5) and Boc-AHPA (6) were synthesized stereoselectively.

EXPERIMENTAL SECTION

All melting points are uncorrected. IR spectra were measured by a Hitachi EPI-G_2 infrared spectrometer, PMR spectra by a Hitachi R-24 in deuterochloroform. Chemical shifts were expressed in ppm from TMS ($\delta=0$). Optical rotation was measured by an Applied Electric Lab. Ltd. Model MP-1T. Thin layer chromatography was performed on silica gel G plates using the mixture of $CHCl_3$: MeOH : AcOH (95 : 5 : 3) [Rf (1)] and 20% ethyl acetate in benzene [Rf (2)] as eluent.

Boc-D-Phe-OH (2)

H-D-Phe-OH (16.91 g, 0.1 mole) and triethylamine (21.4 ml, 0.15 mole) were dissolved in H_2O (55 ml) and to the mixture Boc-S[16] (27.1 g, 0.11 mole) in dioxane (55 ml) was added. The mixture was stirred for 24 hr at room temperature. After the reaction was complete, H_2O (55 ml) was added and washed three times with ethyl acetate (200 ml). The cold aqueous layer was acidified with sat. citric acid aq. (pH 3) and the product was extracted with ethyl acetate. The cold ethyl acetate solution was washed with 10% citric acid aq., sat. NaCl aq. and dried over anhyd. Na_2SO_4. After the removal of ethyl acetate, the oily substance was crystallyzed from ethanol-H_2O. 25.28g (93%) mp. 80–82°, $[\alpha]_D^{21}$ −30.0 (c=0.1, MeOH), Rf (1)= 0.59.

Boc-D-Phe-3,5-dimethylpyrazolide (3)

Boc-D-Phe-OH (2) (25.15 g, 0.095 mole) and 3,5-dimethylpyrazole (10.93g, 0.11 mole) were treated with DCC (19.56 g, 0.095 mole) in $CHCl_3$ (1.8 l) at room temperature for two days. After removal of dicyclohexylurea, the solvent was evaporated. The solid residue was dissolved in ethyl acetate and washed twice with 10% citric acid aq., twice with sat. $NaHCO_3$ aq. and sat. NaCl aq. The solvent phase was dried with anhyd. Na_2SO_4 and evaporated to give a solid residue which was recrystallyzed from EtOH-H_2O. 28.63g (88%) mp. 69–70°, $[\alpha]_D^{21}$ −85.0 (c=1, MeOH), Rf (2)=0.84.

Boc-D-Phenylalaninal (4)

To a solution of **3** (1.36g, 4 mmole) in dry tetrahydrofurane (80ml) was added a suspension of $LiAlH_4$ (0.24g, 6 mmole) in dry tetrahydrofurane (80 ml) keeping the temperature at −15∼−19° under N_2 stream. After stirring for 15 min at the same temperature, 2N-HCl was added slowly (pH 3–4) at a temperature below −20° under

a N_2 stream. After the removal of $Al(OH)_3$ by filtration, the solvent was evaporated. The residue was dissolved in ether and the residual $Al(OH)_3$ was again removed off. The etherate was washed with H_2O and dried over anhyd. Na_2SO_4. The white crystalline substance was obtained after removal of the ether. 0.85g (85%), mp. 95–104°, $[\alpha]_D^{21}$ −10.0 (c=0.1, MeOH), Rf (2)=0.96, IR ν_{max}^{KBr} cm^{-1} : 1370, 1390 (*t*-butyl group), 1510 (CONH), 1610 (benzene ring), 1700 (CHO), 3000–3100 (=C–H), 3350 (NH). PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.40 [9H, s, $C(CH_3)_3$], 3.05 (2H, m, $-CH_2-$), 5.03–5.33 (2H, m, –NH–CH–), 7.13 (5H, s, aromatic protons), 9.50 (1H, s, CHO).

Hexamethyldisilazyllithium

To a solution of metal lithium (1.84g) in dry ether (20 ml), phenyl bromide (20.0 g, 0.13 mole) in dry ether (40 ml) was added with stirring under a N_2 stream and then stirred for 2 hr at room temperature. After removal of the excess lithium, hexamethyldisilazane (21.9g, 0.14 ml) was dropped into the stirred etherate under a N_2 stream and the solution was refluxed for 1 hr. After the solvent was evaporated, the crude product was distilled *in vacuo* and white crystal (9.14g) was obtained. 43%, bp. 130°/4mmHg, mp. 68–71° (Lit.[12] 115°/1mmHg, 70–72°).

Boc-(2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoic acid ethyl ester (*5*)

To a stirred solution of hexamethyldisilazyllithium (0.17 g, 1.0 mmole) in dry tetrahydrofurane (2.5 ml), ethyl formate (0.09 ml, 1.0 mmole) was added under a N_2 stream at −20°. After 15 min, Boc-D-phenylalaninal (4) (0.26 g, 1.0 mmole) in dry tetrahydrofurane (1.0 ml) was dropped into the solution. The reaction mixture was stirred at the same temperature for 2 hr, then at room temperature for 37 hr. After the solution was acidified with 2N-HCl (pH 3–4), the solvent was removed and the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with sat. $NaHCO_3$ aq., 10% citric acid aq., H_2O and dried over $MgSO_4$. Removing the solvent gave an oil (0.15g, 46%). $[\alpha]_D^{21}$ −40.0 (c=0.1, MeOH), Rf (2)=0.65 (tailing), PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.20 (3H, t, J=8Hz, $COOCH_2CH_3$), 1.40 [9H, s, $C(CH_3)_3$], 4.05 (2H, q, J=8Hz, $COOCH_2CH_3$), 6.00 (1H, s, OH), 7.15 (5H, s, aromatic protons)

Boc-(2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoic acid (*6*)

5 (0.1 g, 0.3 mmole) was dissolved in the mixture of dioxane (5 ml) and water (5 ml). The solution was maintained at pH 10 with 1N-NaOH (thymolphthalein was used as an indicator) for 30 min, then acidified with cold 1N-HCl (pH 2–3) and allowed to stand overnight at room temperature. After the pink colored solution being concentrated, the residual oil was dissolved in ethyl acetate and dicyclohexylamine (0.073 g, 0.4 mmole) was added. The solid was obtained by diluting with ether and recrystallyzed from ethanol-*n*-hexane. White crystal, 0.12g, 81.1%, mp. 154–156°, $[\alpha]_D^{21}$ +44.0 (c=0.1, AcOH) [Lit.[7] mp. 158–159°, $[\alpha]_D^{25}$ +51.9° (c=0.89, AcOH)]

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摘　　要

Boc-(2S, 3R)-3-アミノ-2-ヒドロキシ-4-フェニルブタン酸（ベスタチンの部分構造）の合成

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本論文は，ベスタチン（アミノペプチダーゼB及びロイシンアミノペプチダーゼの活性阻害物質，並びに免疫調節物質）分子中の異常アミノ酸である（2S,3R)-3-アミノ-2-ヒドロキシ-4-フェニルブタン酸〔(2S,3R)-AHPAと略〕(6) の立体特異的合成法の試みに関する研究を扱っている。本実験の大きな隘路は，Scheme Ⅰ 中の Boc-フェニルアラニナール(4) から Boc-(2S,3R)-AHPA-OEt (5)への合成であった。この際，ヘキサメチルジシラジルリチウムを用いて反応条件を詳細に検討した結果，当初目的とした立体特異的反応は進まなかったが，反応が立体選択的に進行する条件を見いだし，表題の化合物を最終的に得ることが出来た。