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A Stereospecific Synthesis of (-)-Bestatin from L-Malic Acid

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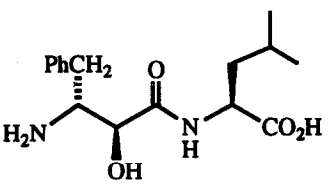
Abstract: A highly diastereospecific route to (-)-Bestatin from L-Malic Acid has been developed. This approach features a stereocontrolled alkylation of diethyl (*S*)-malate and proceeds through an oxazolidone via a Curtius Rearrangement.

(-)-Bestatin, which was isolated from *Streptomyces olivoreticuli*,[2] is a potent inhibitor of leucine aminopeptidase (K_i = 20 nM) and aminopeptidase B (K_i = 60 nM).[3,4] It has been reported that bestatin has antitumor, as well as antimicrobial properties and it is believed that these characteristics are associated with its ability to inhibit cell-surface aminopeptidases.[5] Previous approaches to the (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid (AHPBA) portion of bestatin have utilized chiral glyoxylate methodology,[6] cyanohydrin chemistry of α-amino aldehydes,[4,7] epoxide opening by azide ion,[8] iodocyclocarbamation of allylic amines[9] and hydroxamination of a substituted alkene.[10] We sought a more highly stereoselective route to AHPBA analogs which could be easily applied to a wider variety of bestatin derivatives. We report herein (**Scheme**) a stereospecific synthesis of (-)-bestatin from commercially available L-malic acid which should make bestatin analogs more readily accessible.

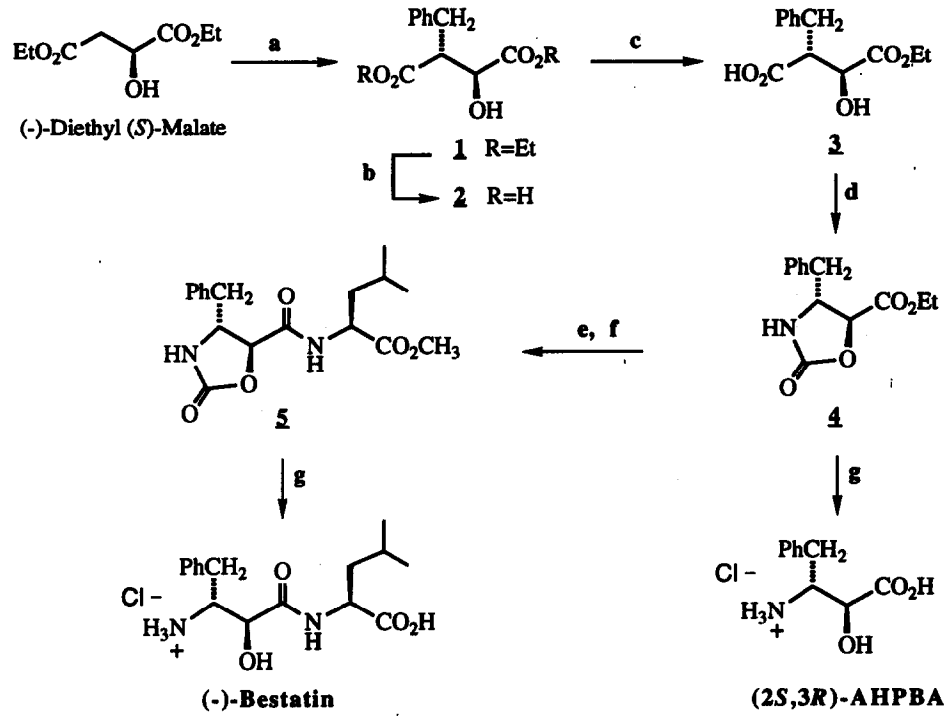


(-)-Bestatin

Several years ago, Seebach and coworkers reported that the diesters of L-malic acid could be alkylated at C-3 using two equivalents of LDA and various alkyl halides.[11] The reactions were reported to proceed in moderate yields (~50 %) with good diastereoselectivity (~9:1). We found that if lithium hexamethyldisilazide (LHMDS) was used, (-)-diethyl (*S*)-malate[12] could be alkylated with benzyl bromide with >35:1 selectivity (NMR) for the R isomer at C-3 in 70 % yield. It is not clear to us why this subtle change should so dramatically enhance the diastereoselectivity of this alkylation and we are examining the reaction in greater detail. The diastereomers of **1** were inseparable by flash chromatography and therefore carried on as a mixture.

For our route to bestatin, we required a selective saponification of the C-4 ester of **1** so that the appropriate Curtius rearrangement could be employed. Since it is the C-1 ester that is most susceptible to hydroxide attack, we utilized the procedure of Miller and coworkers[13] to obtain the required monoacid **3**. Diester **1** was fully saponified using excess 1N sodium hydroxide to give the diacid **2** as a 38:1 mixture of diastereomers (HPLC analysis). Treatment of **2** with trifluoroacetic anhydride gives an intermediate cyclic anhydride, which is

Scheme[14]



Reagents and Conditions: a) LHMDS (2 equ.) -78°C, $PhCH_2Br$, -78°C to 25°C (70 %). b) 1N NaOH, dioxane, reflux (100 %). c) (i) TFAA, 0°C, (ii) EtOH, 25°C (97 %). d) DPPA, Et_3N, toluene, 90°C (65 %). e) LiOH, THF, H_2O (100 %). f) Leu-OCH_3 HCl, NMM, EDC, HOBt, DMF (64 %). g) 1N NaOH, EtOH, reflux; acidify (100 %).

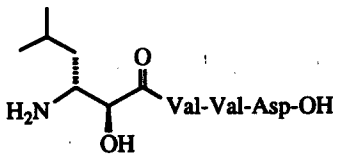
subsequently opened with an equivalent of ethanol to give the monoacid **3**. Ethanol is directed to the carbonyl carbon adjacent to the hydroxy group exclusively.

With the C-4 acid in hand, we turned our attention to the Curtius rearrangement and found that treatment of **3** with triethylamine and diphenylphosphoryl azide[15] (DPPA) (Aldrich) produces an intermediate isocyanate, which is trapped by the C-2 hydroxyl group to give oxazolidone **4**[16] in good yield. We found that the best yields were obtained when a toluene solution of acid and triethylamine was heated to 90°C prior to the addition

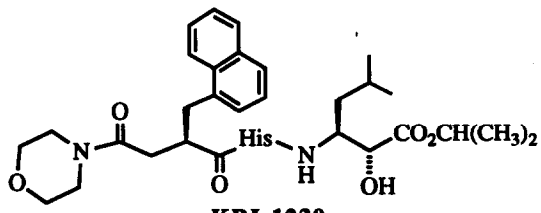
of the DPPA. Oxazolidone **4** was formed without detectable loss of diastereomeric purity and was separated from the minor diastereomer by flash chromatography. The synthesis of (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid (AHPBA) was completed by saponification using 1N sodium hydroxide. This material was identical (NMR, HPLC and mass spec) to commercially available AHPBA (Sigma, Lot 71H5825).

For the synthesis of (-)-bestatin, the ester of oxazolidone **4** could be saponified using LiOH at 25°C in quantitative yield. The acid was coupled to leucine methyl ester using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 1-hydroxybenzotriazole (HOBt) and N-methylmorpholine (NMM) in DMF to give **5**.[17] This material was converted to (-)-bestatin by saponification with 1N sodium hydroxide in refluxing ethanol. Our synthetic bestatin was identical in every respect (mp, NMR, HPLC and optical rotation)[18] to commercially available (-)-bestatin (Sigma, Lot 51H58502).

Some of the advantages to this route should be pointed out. Both optical isomers of malic acid are readily available from commercial sources. This approach also allows for a wider variety of alkyl substituents attached to the 3-amino-2-hydroxy acids since countless alkyl halides are readily available. The related aminopeptidase inhibitor, (-)-amastatin,[3,7] and the unnatural amino acid, norstatine, which is the key component in the renin inhibitor, KRI-1230,[19] should both be available using this methodology. Additionally, all four diasteromers of the 3-amino-2-hydroxy acids should be obtainable via the selection of the appropriate malic acid enantiomer and an optional Mitsunobu inversion[20] of the hydroxy group following the alkylation. We are presently investigating these and other applications of this methodology and will report our findings in due course.



(-)-Amastatin



KRI-1230

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12. (-)-Diethyl (*S*)-malate can be prepared on a 50 g scale from L-malic acid using the procedure outlined in Fischer, E.; Speier, A. *Chem. Ber.* **1895**, *28*, 3252.

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14. All compounds were characterized on the basis of their spectral properties (^{1}H-NMR, ^{13}C-NMR and high resolution mass spec.). Acceptable combustion analyses were obtained on compounds **2**, **4** and **5**.

15. A similar reaction was performed on an unsubstituted derivative to prepare (-)-isoserine: Maeda, H.; Suzuki, M.; Sugano, H.; Matsumoto, K. *Synthesis* **1988**, 401.

16. Compound **4**: clear oil, ^{1}H-NMR ($CDCl_3$, 300 MHz) ∂ 1.28 (t, 3H, J=7.1 Hz), 2.96 (dd, 1H, J=13.6 and 7.3 Hz), 3.05 (dd, 1H, J=13.6 and 5.9 Hz), 4.14 (m, 1H), 4.25 (q, 2H, J=7.1 Hz), 4.69 (d, 1H, J=4.8 Hz), 6.33 (bs, 1H) and 7.21-7.42 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz) ∂ 14.1, 41.7, 57.1, 62.3, 77.2, 127.5, 129.1, 129.3, 135.2, 157.5 and 168.6. HRMS calcd. for $C_{13}H_{15}NO_4$ $(M+H)^+$: 250.1079. Found: 250.1093. Anal. calcd.: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.54; H, 6.10; N, 5.51.

17. Compound **5**: white needles, mp 130.5-131°C, ^{1}H-NMR ($CDCl_3$, 400 MHz) ∂ 0.90 (d, 6H, J=6.1 Hz), 1.60 (m, 3H), 2.83 (dd, 1H, J=13.7 and 8.7 Hz), 3.14 (dd, 1H, J=13.7 and 4.4 Hz), 3.73 (s, 3H), 4.17 (m, 1H), 4.60 (m, 1H), 4.62 (d, 1H, J=5.1), 5.82 (bs, 1H), 6.97 (d, 1H, J=9.6) and 7.15- 7.32 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz) ∂ 21.8, 22.8, 25.0, 41.0, 41.9, 50.7, 52.5, 57.4, 78.1, 127.4, 129.0, 129.4, 135.5, 156.9, 168.6 and 172.4. HRMS calcd. for $C_{18}H_{24}N_2O_5$ $(M+Li)^+$: 355.1845. Found: 355.1889.

18. Synthetic bestatin: $[\alpha]^{25}_D$ -12.3 (c=0.35, 1N HCl). mp 225-227°C (dec.).

Sigma bestatin: $[\alpha]^{25}_D$ -12.5 (c=0.44, 1N HCl). mp 231-233°C (dec.).

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