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RESEARCH ARTICLE

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A practical diastereoselective synthesis of (−)-bestatin

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Diastereoselective addition of nitromethane to Boc-*D*-Phe-H in the presence of sodium hydride in diethyl ether/hexane containing 15-crown-5 and subsequent *N*,*O*-protection with 2,2-dimethoxypropane gave *trans*-oxazolidine in a diastereomeric ratio of >16:1. The oxazolidine was easily separated by column chromatography, which after Nef reaction was coupled to H-Leu-OtBu. The 8-step synthesis afforded (−)-bestatin in an overall yield of 24.7% after deprotection and ion exchange.

KEYWORDS

Bestatin, Nitroaldol, Nef reaction, (*2S, 3R*)-3-amino-2-hydroxy-4-phenylbutanoic acid, diastereoselective

1 | INTRODUCTION

Bestatin (**1;** Figure 1), a competitive inhibitor of aminopeptidase B, is currently used as a drug, either alone or in combination with other antibiotics or anticancer drugs, for the treatment of cancer and bacterial infections.[1,2] Bestatin has also shown potential for the treatment of HIV as well as an anti-inflammatory drug.[3,4] Due to the therapeutic importance of bestatin, several strategies for its synthesis have been developed.[5] These syntheses have utilized chiral auxiliaries, sugars, chiral synthons, or asymmetric catalysis to influence the chirality. Our goal was to develop a synthesis, which was robust, scalable, and utilized no expensive chiral catalysts or auxiliaries.

Retrosynthetic analysis of bestatin showed that it contains a (*2S, 3R*)-3-amino-2-hydroxy-4-phenylbutanoic acid (**2;** AHPBA) moiety and *L*-leucine. AHPBA can be conveniently synthesized from *D*-phenylalanine aldehyde (**3;** *D*-Phe-H), which already contains a chiral carbon with required configuration (Figure 1).

Thus, we envisioned that a suitably protected *D*-Phe-H can be utilized as the starting material to synthesize bestatin. A literature survey revealed that the *N*-protected *D*-Phe-H has been exploited for the synthesis of bestatin.[6-12] Furthermore, the diastereomeric ratios of the nucleophilic addition products varied from 1:1 to 9.5:1 depending on the nucleophile and other contributing factors as shown in Figure 2. Generally, the formation of *syn* isomer was favored due to stabilization of the chelation-controlled Cram cyclic transition state. However, when diprotected phenylalaninal, *N,N*-$(Bzl)_2$-Phe-H was used for nucleophilic addition reaction or when the reaction was influenced by a chiral catalyst (**9**), the *anti*-isomer predominated, suggesting that the reaction occurred *via* a non-chelated intermediate in conformance with Felkin-Anh model.[13-15]

In our approach, we utilized nitromethane as a masked functionality for the creation of a carboxylic acid *via* Nef reaction to generate the protected AHPBA moiety, the core peptidomimetic component of **1**.[16] In this publication, we would like to report that by choosing an appropriate base and solvents mixture, desired stereochemical outcome of nucleophilic addition of nitromethane to Boc-*D*-Phe-H can be obtained even in the absence of a chelating agent. These efforts led to an efficient diastereoselective synthesis of bestatin in overall good yield.

2 | MATERIALS AND METHODS

Except amino acid derivatives, all other reagents and solvents were procured from Sigma-Aldrich (Milwaukee, WI) and were used without further purification unless otherwise stated. Analytical HPLC was performed on a C18, reversed-phase column (YMC, 150 × 4.6 mM, 5 μ) using Shimadzu LC10 equipped with dual pumps and a diode array detector. Buffer A consisted of 0.05% TFA in water, and buffer B was 0.05% TFA in acetonitrile. Flow rate was 1.0 mL/min. Three methods were used: Method A: a linear gradient from 30% buffer B to 80% buffer B in 25 minutes, method B: a linear gradient from 60% buffer B to 100% buffer B in 20 minutes, and method C: a linear gradient from 15% buffer B to 75% buffer B in 30 minutes. The mass spectra were obtained using in-house MALDI-MS (Voyager DE™ Pro Biospectrometry™ Workstation, Applied Biosystems, CA) and Waters Acquity H-Class Xevo G2 TOF UPLC-MS System (Waters, MA) equipped with ESI probe. The ^{1}H and ^{13}C NMR spectra were obtained using a 600-MHz Varian Unity600 NMR spectrometer in $CDCl_3$ or DMSO-d_6 as solvent (Emory University, Atlanta, GA). Elemental

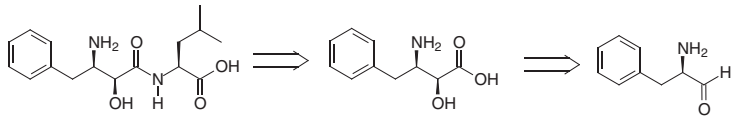


FIGURE 1 Retrosynthesis of bestatin

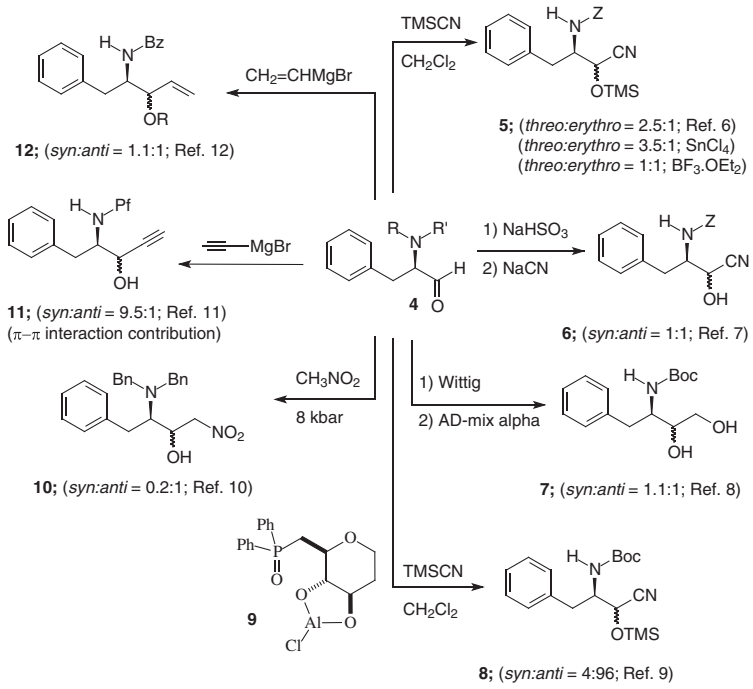


FIGURE 2 Diastereomeric ratios of nucleophilic addition reaction to *N*-protected *D*-Phe-H

analysis was performed by Atlantic Microlab Inc. (Norcorss, GA). Diastereomeric excess was measured using HPLC and NMR spectrometer. Optical rotation measurements on purified samples were recorded using Autopol IV automatic polarimeter (Rudolph Research Analytical, NJ).

2.1.1 | *tert*-Butyl *N*-((*2R*, *3R*)-3-hydroxy-4-nitro-1-phenylbutan-2-yl)-carbamate (14)/*tert*-butyl N-((*2R*, *3S*)-3-hydroxy-4-nitro-1-phenylbutan-2-yl)-carbamate (15)

To a suspension of NaH (36.1 g, 904 mmol; 60% dispersion in mineral oil) in hexanes (350 mL) at 0°C was added nitromethane (44.5 mL, 822 mmol) followed by addition of diethyl ether (1450 mL), Boc-*D*-Phe-H[17,18] (102.5 g, 411 mmol), and 15-crown-5 (80 mL, 411 mmol) in 30-minute intervals of each. The reaction mixture was stirred at 0°C for 1 hour and allowed to proceed at ambient temperature overnight. Reaction was monitored by HPLC. After 22 hours, reaction mixture was cooled to 0°C and acidified with 1 N aq. HCl (1050 mL) to pH 4. The resulting mixture was diluted with ethyl acetate (2000 mL), and the 2 layers were separated. The organic layer was washed with 0.5 N aq. HCl (2 × 750 mL), brine (1 × 750 mL), saturated aq. $NaHCO_3$ (2 × 750 mL), and brine (2 × 750 mL). The organic layer was dried over Na_2SO_4 and evaporated at reduced pressure to afford a yellowish residue, which upon trituration with hexane gave a precipitate. Filtration of the precipitate yielded a mixture of **14** and **15** as slightly off-white solid (81.7 g, 64%). A small sample of the above mixture was purified by semi-prep HPLC to isolate **14** and **15** as white solids for analytical purposes.

14: RP-HPLC, method A: *t*R 11.42 minutes. $[\alpha]_D^{24}$ = +29.0 (*c* 0.1, THF). Accurate mass analysis using ESI-MS gave peak at: *m/z* 333.2538 ($C_{15}H_{22}N_2O_5Na$) [M + Na]$^+$; calcd. *m/z* = 310.1528 ($C_{15}H_{22}N_2O_5$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32 (2H, *t*, *J* = 7.4 Hz), 7.27 to 7.22 (3H, *m*), 4.84 (1H, *d*, *J* = 8.1 Hz), 4.48 to 4.44 (2H, *m*), 4.31 (1H, *ddd*, *J* = 2.2 Hz, 2.9 Hz, 6.1 Hz), 3.84 (1H, *q*, *J* = 7.7 Hz), 3.40 (1H, *bs*), 2.97 to 2.93 (2H, *m*), 1.42 (9H, *s*) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ 155.93, 137.19, 129.20, 128.70, 126.81, 80.28, 79.20, 68.43, 53.89, 38.13, 28.22 ppm.

15: RP-HPLC, method A: *t*R 10.84 minutes. $[\alpha]_D^{27}$ = +6.00 (*c* 0.1, THF). Accurate mass analysis using ESI-MS gave peak at: *m/z* 349.2300 ($C_{15}H_{22}N_2O_5K$) [M + K]$^+$; calcd. *m/z* = 310.1528 ($C_{15}H_{22}N_2O_5$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32 (2H, *t*, *J* = 7.6 Hz), 7.26 to 7.25 (1H, *m*), 7.21 (2H, *d*, *J* = 7.6 Hz), 4.58 (1H, *d*, *J* = 7.0 Hz), 4.50 (1H, *d*, *J* = 12.9 Hz), 4.43 to 4.41 (1H, *m*), 4.31 (1H, *bs*), 3.90 (1H, *bs*), 3.01 to 2.98 (1H, *m*), 2.88 to 2.85 (1H, *m*), 1.37 (9H, *s*) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ 156.15, 136.44, 129.29, 128.82, 127.02, 80.62, 78.48, 71.12, 54.23, 36.19, 28.18 ppm.

2.1.2 | *tert*-Butyl (*4R*, *5R*)-4-benzyl-2,2-dimethyl-5-nitromethyl-1,3-oxazolidine-3-carboxylate (16)

To a solution of the mixture of **14** and **15** (81.5 g, 263 mmol) in dry acetone (1200 mL) and 2,2-dimethoxypropane (1200 mL) at 0°C was added boron trifluoride etherate (3 mL) dropwise. The reaction was stirred for 1 hour at 0°C and overnight at ambient temperature. The HPLC showed formation of 71.6% product as a single peak (*t*R = 19.49 minutes, method A). The reaction was quenched with triethyl amine (3.5 mL). The volatiles were removed under reduced

pressure, and the resulting viscous residue was diluted with diethyl ether (2500 mL) and partitioned with saturated aq. $NaHCO_3$ (1000 mL). The organic layer was separated and washed with saturated aq. $NaHCO_3$ (1 × 300 mL), brine (1 × 300 mL), 0.25 N HCl (2 × 300 mL), and brine (4 × 300 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to afford the crude product (86.0 g, 93%), which was purified by silica gel column chromatography using a gradient of ethyl acetate (0%–6%) in hexane to afford **16** (46.28 g, 54%) as a white solid, $[\alpha]_D^{23}$ = +12.8 (*c* 0.5, $CHCl_3$) and its *cis* isomer (2.79 g, 3.26%), $[\alpha]_D^{23}$ = +22 (*c* 0.5, $CHCl_3$).

Accurate mass analysis using ESI-MS gave peak at: *m/z* 373.2966 ($C_{18}H_{26}N_2O_5Na$) $[M + Na]^+$; calcd. *m/z* = 350.1841 ($C_{18}H_{26}N_2O_5$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.35 (2H, *t*, *J* = 7.5 Hz), 7.29 to 7.24 (3H, *m*), 4.65 (1H, *dt*, *J* = 4.0, 4.4 Hz), 4.34 to 4.31 (1H, *m*), 4.01 to 3.96 (2H, *m*), 3.43 to 3.41 (1H, *m*), 2.81 to 2.78 (1H, *m*), 1.64 (3H, *s*), 1.57 (9H, *s*), 1.50 (3H, *s*) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ 151.67, 136.82, 129.30, 128.93, 127.14, 95.60, 80.80, 78.01, 75.73, 61.41, 39.05, 28.48, 27.26 ppm.

2.1.3 | (*4R*, *5S*)-4-benzyl-3-[(*tert*-butoxy)carbonyl]-2,2-dimethyl-1,3-oxazolidine-5-carboxylic acid (17)

To a solution of **16** (30 g, 85.7 mmol) in methanol (750 mL) precooled to 0°C was added a solution of KOH (14.4 g, 257 mmol) in methanol (500 mL). Five minutes later, a solution of $KMnO_4$ (41 g, 257 mmol) and Na_2HPO_4 (36 g, 257 mmol) in water (250 mL) was added, and the resulting mixture was stirred vigorously. After 2 hours, the reaction was quenched by adding a slurry prepared from Na_2SO_3 (54 g, 343 mmol), NaCl (65 g, 1111 mmol), and 1 N HCl (900 mL, 900 mmol). The resulting mixture was diluted with ethyl acetate (3000 mL), stirred for 5 minutes, and filtered to remove the brown precipitate that formed. The 2 layers were separated, and the organic layer was washed with 0.2 N aq. HCl (2 × 300 mL) and brine (3 × 300 mL). It was dried over Na_2SO_4 and evaporated under reduced pressure to afford **17** (28.8 g, 100%) as an off-white solid, which was 94.45% pure by RP-HPLC, method A: *t*R 14.29 minutes. $[\alpha]_D^{24}$ = 30.0 (*c* 0.1, THF). Accurate mass analysis using ESI-MS gave peak at: *m/z* 336.2961($C_{18}H_{26}NO_5$) $[M + H]^+$; calcd. *m/z* = 335.1732($C_{18}H_{25}NO_5$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.31 (2H, *t*, *J* = 7.4 Hz), 7.29 to 7.22 (3H, *m*), 5.93 to 5.91 (2H, *b*), 4.51 (1H, *d*, *J* = 8.8 Hz), 4.39 (1H, *d*, *J* = 2.6 Hz), 3.23 (1H, *dd*, *J* = 2.6 Hz, 13.6 Hz), 3.01 to 2.98 (1H, *b*), 1.62 (3H, *s*), 1.54 (9H, *s*), 1.41 (3H, *s*) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ 174.67, 151.59, 136.97, 129.80, 128.60, 126.83, 96.46, 80.70, 76.47, 61.13, 38.75, 28.49, 27.38 ppm.

2.1.4 | *tert*-Butyl-(*4R*, *5S*)-4-benzyl-5-(((*2S*)-1-(*tert*-butoxy)-4-methyl-1-oxopentan-2-yl)-carbamoyl)-2,2-dimethyl-1,3-oxazolidine-3-carboxylate (18)

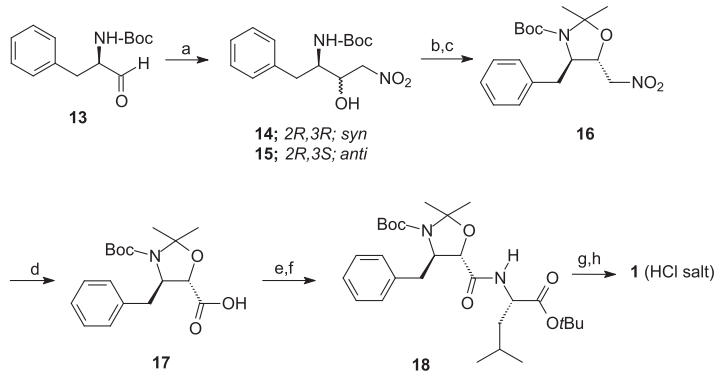
To a solution of **17** (27.88 g, 83.22 mmol) in dry THF (560 mL) was added *N*-methylmorpholine (9.1 mL) at 0°C. After vigorous stirring for 10 minutes at the same temperature, the mixture was cooled to −12 ± 2°C, and *iso*butyl chloroformate (10.8 mL, 83.22 mmol) was added dropwise. After 30 minutes, a suspension of H-Leu-O*t*Bu.HCl (22.35 g, 100 mmol) in dimethyl formamide (56 mL) and *N*-methylmorpholine (9.3 mL) was added. After 90 minutes at the same temperature, the reaction was quenched with 1 N HCl (200 mL). The reaction mixture was partitioned with ethyl acetate (2500 mL), followed by washing with 0.25 N aq. HCl (3 × 200 mL), saturated aq. $NaHCO_3$ (3 × 200 mL), and brine (3 × 200 mL). The organic layer was dried over Na_2SO_4 and evaporated to afford a viscous residue (44.9 g, 89.2%), which after flash silica gel column chromatography in hexane/ethyl acetate (0%–6%) afforded a crystalline white solid (34.9 g, 69.4% over 2 steps). RP-HPLC, method B: *t*R 12.39 minutes. $[\alpha]_D^{26}$ = −8.4 (*c* 0.5, $CHCl_3$). Accurate mass analysis using ESI-MS gave peak at: *m/z* 505.4783 ($C_{28}H_{45}N_2O_6$) $[M + H]^+$; calcd. *m/z* = 504.3199 ($C_{28}H_{44}N_2O_6$). ^1H NMR (600 MHz, $CDCl_3$): δ 7.27 (2H, *t*, *J* = 7.7 Hz), 7.24 to 7.19 (3H, *m*), 6.77 (1H, *d*, *J* = 8.1 Hz), 4.45 (2H, *dd*, *J* = 8.1 Hz, 14.0 Hz), 4.29 (1H, *d*, *J* = 4.8 Hz), 3.15 (2H, *d*, *J* = 10.6 Hz), 1.66 to 1.61 (3H, *m*), 1.60 (3H, *s*), 1.52 (9H, *s*), 1.45 (9H, *s*), 1.25 (3H, *bs*), 0.95 (6H, *d*, *J* = 5.5 Hz) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ 171.55, 170.37, 151.64, 136.93, 130.23, 128.48, 128.37, 126.63, 96.01, 81.94, 80.34, 77.32, 60.57, 51.26, 42.13, 28.47, 27.99, 25.09, 22.55, 22.30 ppm.

2.1.5 | (*2S*)-2-((*2S*, *3R*)-3-amino-2-hydroxy-4-phenylbutanamido)-4-methylpentaoic acid hydrochloride (1)

Compound **18** (10.0 g, 19.84 mmol) was dissolved in a mixture of trifluoroacetic acid (190 mL) and water (10 mL) precooled to 0°C. After 2 hours, an additional amount of water (10 mL) was added. After stirring for additional 30 minutes, the volatiles were removed under reduced pressure. The residue was precipitated with acetonitrile/diisopropyl ether to afford a white solid (8.0 g). The solid was dissolved in 20% acetonitrile/water and passed through Dowex Cl^- form ion-exchange resin and lyophilized to yield **1** (6.4 g, 99.7%) as a white powder. RP-HPLC, method C: *t*R 7.32 minutes. $[\alpha]_D^{25}$ = −14.2 (*c* 1, 1.0 N HCl). Accurate mass analysis using ESI-MS gave peak at: *m/z* 309.2910 ($C_{16}H_{25}N_2O_4$) $[M + H]^+$; calcd. *m/z* = 308.3733 ($C_{16}H_{24}N_2O_4$). ^1H NMR (600 MHz, DMSO-d_6): δ 12.69 (1H, *bs*), 8.21 (1H, *d*, *J* = 7.7 Hz), 8.12 (3H, *bs*), 7.33 (4H, *bs*), 7.25 (1H, *bs*), 6.82 (1H, *bs*), 4.21 to 4.19 (1H, *m*), 4.00 (1H, *s*), 3.51 (1H, *s*), 2.98 to 2.93 (2H, *m*), 1.69 to 1.64 (2H, *m*), 1.63 to 1.51 (1H, *m*), 0.88 (3H, *d*, *J* = 6.6 Hz), 0.86 (3H, *d*, *J* = 6.6 Hz) ppm. ^{13}C NMR (600 MHz, DMSO-d_6): δ 173.65, 171.02, 136.57, 129.5, 128.60, 126.87, 68.22, 54.42, 50.54, 34.48, 34.33, 22.75, 21.66. Anal. Calcd for $C_{16}H_{24}N_2O_4$.HCl. 0.25H_2O: C, 54.93; H, 6.87; N, 8.01; Cl, 10.16. Found: C, 54.89; H, 7.03; N, 7.99; Cl, 10.08 ppm.

3 | RESULTS

As shown in Scheme 1, Boc-*D*-Phe-OH was converted into Boc-*D*-Phe-H (**13**) *via* Weinreb amide by reacting with *N*,*O*-dimethylhydroxylamine followed by reduction with lithium aluminum hydride in 76% yield (over 2 steps) according to the reported procedures.[17,18] The aldehyde (**13**) was reacted with nitromethane in the presence of NaH in diethyl ether/hexane containing 15-crown-5 to afford a mixture of nitroaldols (**14**/**15**).[19,20] The mixture was not separated because it had the tendency to precipitate on the silica gel column due to poor solubility in ethyl acetate/hexane solvent mixture, but carried to the next step without purification. However, in our initial studies, we isolated small amounts of nitroaldols, **14** and **15**, by



SCHEME 1 Reagents and conditions: a) nitromethane (2 equiv.), NaH (2.2 equiv.), 15-crown-5 (1.0 equiv.), Et_2O/hexane (8:2), then 0.25 N aq. HCl; 64%; b) 2,2-dimethoxypropane, $BF_3.OEt_2$, then Et_3N; c) silica gel column chromatography, 54%; d) $KMnO_4$ (3 equiv.), KOH (3 equiv.), Na_2HPO_4 (3 equiv.), MeOH/H_2O (5:1), quant.; e) *i*BuOCOCl/NMM, 30 minutes, then H-Leu-O*t*Bu.HCl/NMM, DMF, 90 minutes; f) silica gel column chromatography, 69.4% over 2 steps; g) TFA/H_2O (9:1), 99%; h) Cl^- ion-exchange resin, then lyophilization, 99%

RP-HPLC and carried each through the entire synthesis separately in order to confirm the retention times and determine stereochemistry. Bestatin derived from *anti*-isomer **15** showed optical rotation of $[\alpha]_D^{26}$ + 3.8 (*c* 1, 1 N HCl; *cf. vide supra*).

The mixture of nitroaldols (**14/15**) was treated with 2,2-dimethoxypropane in the presence of boron trifluoride etherate to afford a mixture of dimethyl oxazolidines in 72% yield.[21] The two isomers, ie, *trans* and *cis* were formed resulting from *syn* and *anti* nitroaldols, respectively, and were easily separated by silica gel column chromatography (R_f values: *trans*-isomer **16:** 0.42, *cis*-isomer 0.51, solvent: 20% ethyl acetate/Hexane, visualization: ninhydrin). The isolated yield of the desired *trans*-isomer-**16** was 54%. Its *cis*-isomer was also isolated but in 3.26% yield only.

The *trans*-isomer **16** was subjected to Nef reaction using a modified procedure.[22] The optimized conditions required 3 equivalents each of potassium permanganate, potassium hydroxide, and disodium hydrogen phosphate in methanol/water (5:1) for 2 hours. Upon completion of reaction, 0.25 N aq. HCl was added to quench the reaction, and the precipitated manganese dioxide was filtered. Upon work up, fully protected AHPBA (**17**) was obtained in >95% yield. No purification was required as the reaction was very clean.

The fully protected acid thus obtained was reacted with H-Leu-O*t*Bu.HCl using mixed anhydride method generated from the reaction of **17** with *iso*butyl chloroformate and *N*-methylmorpholine (NMM). The coupling reaction was also tried using EDAC/HOBt as activator. NMM was used as a base to neutralize the bound HCl. Both reactions afforded quantitative yield of the coupled product (**18**).[23]

Cleavage of the fully protected bestatin (**16**) was performed with 90% TFA/H_2O, which afforded bestatin (**1**) in quantitative yield as the TFA salt. The TFA salt of **1** was dissolved in 20% acetonitrile/water and passed through a short Dowex ion-exchange column to afford the HCl salt of bestatin (**1**) in an overall yield of 24.7% after lyophilization. Its purity was 99.7% as determined by RP-HPLC, and its optical rotation was $[\alpha]_D^{25}$ −14.2 (*c* = 1, 1.0 N HCl); lit.[24] $[\alpha]_D^{25}$ −14.3 (*c* = 0.5, 1.0 N HCl).

4 | DISCUSSION

As shown in Table 1, the nitroaldol reaction of Boc-*D*-Phe-H with nitromethane was tried using different bases, including 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene, tetramethyl guanidine (TMG) in toluene, sodium hydride (NaH) in THF, and NaH in diethyl ether/hexane/15-crown-5. The isolated yields of nitroaldols **14/15** varied from 57% to 73% and the ratios of **14** to **15** ranged from 3.54 to 5.76 as determined by RP-HPLC. We also tried LDA in THF as a base, but the yield was poor (39%).[25] Because NaH in diethyl ether/hexane/15-crown-5 gave the best ratio of the desired nitroaldol (**14**), we were able to increase the scale of reaction to 100 g using these conditions. The optimized reaction conditions utilized 2.2 equiv. of NaH and 2 equiv. of nitromethane in diethyl ether/hexane (5:1) containing 1 equiv. of 15-crown-5 at 0°C overnight. Under these experimental conditions, an inseparable mixture of **14/15** was obtained in 68% yield as an off-white solid. The other impurities were either volatile or easily washed off by precipitation of product with ethyl acetate/hexane solvent mixture.

Based on the observation of *syn* to *anti* ratios in Figure 2, addition of a nucleophile to *N*-protected *D*-Phe-H preferentially occurs *via* a chelation-controlled transition state.[26] However, in the present study, there was no chelating metal present. Perhaps, hydrogen-bonding

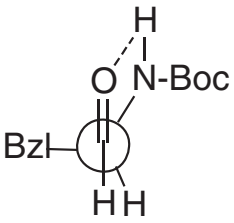
TABLE 1 Reaction conditions and products of nitroaldol reaction[a]

Entry	Solvent	Base (Equiv.)	Temp/Time	14:15	Yield[c]
1	Toluene	DBU (2)	0°C, 1 hour; rt, 12 hours	4.11:1	71%
2	Toluene	TMG (2)	0°C, 1 hour; rt, 2 hours	4.12:1	57%
3	THF	NaH (2)	0°C, 1 hour; rt, 3 hours	3.54:1	73%
4	Et_2O/Hexane[b]	NaH (2.2)	0°C, 1 hour; rt, 22 hours	5.76:1	64%
5	THF	LDA (1.05)	−72-0°C, 30 minutes	n.d.	39%

[a]Reaction performed with 2 equiv. of nitromethane.

[b]Reaction contained 1 equiv. of 15-crown-5 ether.

[c]Isolated yield.



19

FIGURE 3 Plausible hydrogen-bonded transition state

between the Boc-NH- and C=O of the aldehyde group, as depicted by **19** (Figure 3) stabilizes transition state, much in the way a chelation-controlled transition state would operate, albeit of weaker nature. The fact that bases DBU (pK_a 12.0), TMG (pK_a 13.6), and nitromethane (pK_a ~10 in water; 17.2 in DMSO) are not sufficiently strong bases to deprotonate the carbamate (pK_a ~20.8), nor Na^+ is a chelator, lends some credence to the stabilization afforded in transition state due to H-bonding.[27] The enhanced ratio of *syn* to *anti* upon use of crown ether in a relatively nonpolar solvent mixture further supports the statements above (dielectric constants: THF 7.52, Et_2O 4.267, toluene 2.38, hexane 1.89).[28]

The practicality of this synthetic strategy was observed in *N,O*-acetal formation with 2,2-dimethoxpropane in the presence of boron trifluoride etherate leading to oxazolidine **16**. It was found that the *syn*-diastereoisomer **14** reacted approximately 3 times faster than the *anti*-isomer **15** as monitored by RP-HPLC. Thus, the reaction mixture was further enriched in the desired stereoisomer (*dr* > 16:1), which was isolated in good yield after silica gel column chromatography. Our research corroborates the earlier observation of enhancement in diastereoisomeric ratio upon cyclization of an allylic acetate (**12**) to oxazoline from 1.1:1 (*syn/anti*) to >14:1 (*trans/cis*) by Goerge et al.[12]

The reaction conditions of Nef reaction were modified due to heterogeneity of the reaction mixture. A mixture of methanol water (5:1) was used instead of *t*BuOH.[22] Furthermore, the ratio of MeOH to water was critical. Use of excess water led to isolation of very little or no product.

The reaction of fully protected acid (**17**) with H-Leu-O*t*Bu.HCl occurred in quantitative yield using either mixed anhydride method or EDAC/HOBt as activator. Acidolytic cleavage of all the protecting groups followed by counter-ion exchange afforded the final product as HCl salt.

Thus, we have developed a convenient stereoselective synthesis of a fully protected AHPBA (**17**) intermediate by utilizing nitroaldol and Nef reactions, which after coupling with H-Leu-O*t*Bu.HCl and subsequent deprotection of the acid-labile groups in a single step, afforded (−)-bestatin. The 8-step synthesis starting from Boc-*D*-Phe-OH is robust, scalable, and required only 2 silica gel column chromatography purifications.

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