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Chemoenzymatic formal total synthesis of (−)-bestatin

Brent D. Feske and Jon D. Stewart*

Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

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Abstract—A highly stereoselective, enzymatic reduction of an α-chloro-β-keto ester provided the key intermediate for a total synthesis of the α-hydroxy-β-amino acid moiety of (−)-bestatin. The reduction product was cyclized to a glycidic ester that was opened in a Ritter reaction with benzonitrile, affording a *trans*-oxazoline, which was hydrolyzed under acidic conditions to the target molecule.

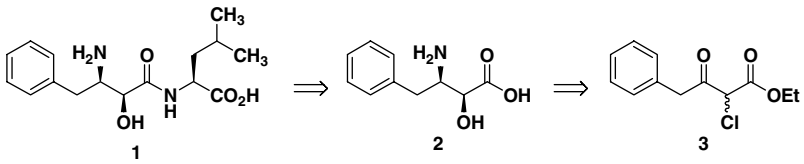
1. Introduction

Bestatin **1** was isolated in 1976 by Umezawa et al. on the basis of its ability to inhibit aminopeptidase B (Scheme 1).[1] Presently, **1** is used as an oral medication for the treatment of cancer and bacterial infection in Japan. This drug is often used in conjunction with other antibiotics and anticancer agents because of its ability to elicit T cell proliferation, thereby enhancing the immune response.[2] Shortly after its discovery, Nakamura et al. used X-ray crystallography to confirm the absolute configuration of bestatin[3] and Suda et al. published the first total synthesis, starting from D-phenylalanine.[4] Bestatin's biological activity and intriguing structure have engaged the interest of several groups, and a number of asymmetric syntheses have been disclosed.[5–15] Much of the synthetic interest centers on the *syn*-α-hydroxy-β-amino acid moiety, which recurs in a number of other biologically active molecules such as Taxol® side-chain,[16] microginin,[17] amistatin,[18] phebestin,[19] and probestin.[20,21]

Since the initial report, most of the published routes to bestatin have relied on chiral pool starting materials to supply the initial asymmetric center(s). We have pursued a complementary strategy, using enzyme-mediated reductions of prochiral ketones to generate homochiral intermediates for further synthetic transformations. Our recent efforts have focused on carbonyl reductases from bakers' yeast. After analyzing *Saccharomyces cerevisiae* genome,[22] we identified, cloned, and expressed a library of yeast reductases.[23] In many cases, the individual enzymes afforded much higher stereoselectivities when compared to whole bakers' yeast cells, which have traditionally been employed in preparative reactions. Herein, we report on how access to a diastereo- and enantioselective enzyme reduction facilitates a concise synthesis of (−)-bestatin, which does not rely on a chiral pool starting material.

2. Results and discussion

(2*S*,3*R*)-α-Hydroxy-β-amino acid **2** is the key target for our synthesis since Suda et al. have shown that it can be converted to bestatin by *N*-protection, coupling with L-leucine and subsequent deprotection.[4] We have recently developed a synthetic approach to homochiral



Scheme 1.

* Corresponding author. Tel.: +1 352 846 0743; fax: +1 352 846 2095; e-mail: jds2@chem.ufl.edu

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α-hydroxy-β-amino acids, which relies on a Ritter reaction of glycidic esters[24] and we hoped that such an approach might also be applicable to bestatin. We planned to prepare the necessary epoxide intermediate by reducing α-chloro-β-keto ester **3** with subsequent ring closure under mildly basic conditions.[25] The high acidity of the α-proton in **3** allows a stereoselective reduction to proceed as a dynamic kinetic resolution that could potentially yield a single chlorohydrin diastereomer in 100% molar yield, thereby avoiding the usual 50% yield penalty exacted by standard kinetic resolutions.

β-Keto ester **4**, prepared from Meldrum's acid by an acylation/decarboxylation strategy[26,27] was chlorinated with sulfuryl chloride to afford **3** (Scheme 2).[28] A sub-stoichiometric ratio of SO_2Cl_2 was employed to avoid the formation of the α,α-dichlorinated adduct, which proved very difficult to separate from the desired product. Our previous screening studies revealed that **3** was a substrate for three of the yeast reductases in our collection: short-chain dehydrogenases YGL039w and YGL157w produced the (2*S*,3*S*)-chlorohydrin in 41% and >98% ee, respectively, while aldose reductase YDR368w afforded the (2*R*,3*S*)-diastereomer in >98% ee and >98% de.[29] Since the latter chlorohydrin was required for the synthesis of (−)-bestatin, we scaled up the reduction of **3** by using whole cells of an engineered *Escherichia coli* strain that overexpressed the YDR368w dehydrogenase as a fusion protein with glutathione *S*-transferase. The reduction was carried out in a 1 L fermenter and a glucose solution was added along with **3** to allow intracellular recycling of the essential NADPH cofactor. As **3** was quite toxic toward the *E. coli* cells, it was added portionwise and a non-polar polymeric resin (XAD-4) was present during the reaction to maintain a low aqueous substrate concentration.[30] We also observed that a small, variable fraction (ca. 5%) of added **3** suffered reductive dechlorination during the early stages of the bioconversion.[31] Despite this minor complication, chlorohydrin **5** routinely accumulated to a final concentration of >1 g/L in the fermentation broth, from which it could be isolated in 82% yield as a single stereoisomer.

As precedented by the work of Azerad and co-workers,[25] ring closure of chlorohydrin **5** proceeded smoothly in the presence of excess K_2CO_3 to afford *cis*-glycidate **6** whose relative stereochemistry was evident from the $J_{2,3}$ value of 4.5 Hz in the ^1H NMR. The epoxide was opened by benzonitrile in the presence of a stoichiometric quantity of $BF_3{\cdot}OEt_2$ to yield a single product. The *trans*-stereochemistry of **7** was assigned on the basis of its $J_{2,3}$ value of 6.3 Hz. We were gratified to find that this Ritter reaction proceeded with no C_3 epimerization, in contrast to our earlier synthesis of Taxol® side-chain, where a 5:1 mixture of *trans*- and *cis*-oxazolines were observed.[24] Herein, C_3 is secondary, rather than benzylic, and it is this that likely promotes a tighter association of the nitrogen nucleophile and avoids the formation of a free carbocation intermediate. Acidic hydrolysis of **7** proceeded uneventfully to afford **2**, which was isolated as its hydrochloride salt. The spectral data and specific rotation of our synthetic material, $[\alpha]_D = +23$ (*c* 1.3, 1 M HCl) were identical to those of an authentic sample of the natural product measured in our laboratory. Since **2** has been converted to bestatin,[4] this completed our formal total synthesis of the target.

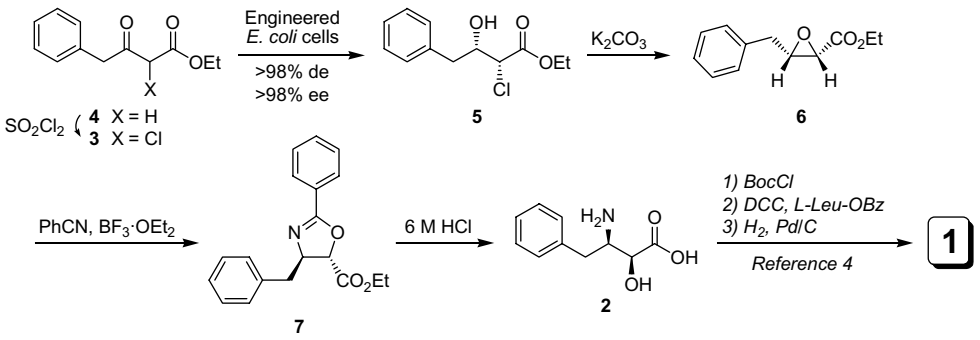
3. Conclusion

Our successful synthesis of the key bestatin subunit further underscores the synthetic utility of employing individual bakers' yeast reductases in organic synthesis, rather than native yeast cells. When commercial yeast cells were used to reduce **3**, a mixture of all four possible stereoisomeric chlorohydrins was obtained with the desired (2*R*,3*S*)-diastereomer represented ca. 5% of the total product.[29] In contrast, the approach used here (employing an engineered *E. coli* strain) for the same reduction afforded only a single product in >98% de and >98% ee. This ability to carry out highly stereoselective reductions at scales useful for preparative organic synthesis, combined with the Ritter reaction, provides a useful and potentially general route to homochiral α-hydroxy-β-amino acids.

4. Experimental

4.1. General

LB medium contained 1% bacto-tryptone, 0.5% bacto-yeast extract, and 1% NaCl and the strain used in this study can be obtained from the authors. Optical rotations were measured from $CHCl_3$ solutions at rt



Scheme 2.

unless otherwise indicated. GC analyses were carried out with DB-17 (0.25 mm × 25 m) or Chirasil-Dex CB (0.25 mm × 25 m) columns for normal and chiral separations, respectively. GC samples from the bioconversion were prepared by vortex mixing 0.50 mL portions of the reaction mixture and EtOAc for 10 s followed by brief centrifugation to remove insoluble cell debris. The conversion of **7** to **2** was monitored by reversed-phase HPLC (4.6 × 250 mm C_{18} column) using a water–CH_3CN solvent system (both solvents containing 0.1% trifluoracetic acid). Glucose concentrations during the bioconversion were assayed by a commercial kit using the Trinder reagent. Non-aqueous reactions were carried out in dried glassware under an argon atmosphere. β-Keto ester **4** was prepared using the method described by Chowdhury et al.[27]

4.1.1. Ethyl 2-chloro-3-oxo-4-phenylbutyrate 3. Sulfuryl chloride (5.7 mL, 60 mmol, 0.60 equiv) was added over a 15 min period to a solution of β-keto ester **4** (15.5 g, 75 mmol) in $CHCl_3$ (170 mL) at room temperature. After stirring for an additional 3 h (or until no further reaction occurred according to TLC), water (100 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 50 mL). The combined organics were dried over $MgSO_4$, concentrated in vacuo, and the residue purified by flash chromatography on silica gel (hexanes:toluene, 1:1) to yield 13.2 g of **3** as a pink oil in addition to unreacted starting material (3.4 g) (92% yield of **3** based on recovered SM). ^{1}H NMR: ($CDCl_3$) δ 7.32 (m, 5H), 4.87 (s, 1H), 4.22 (q, 2H, $J = 6.2$ Hz), 4.01 (d, 2H, $J = 4.5$ Hz), 1.27 (t, 3H, $J = 6.2$ Hz). ^{13}C NMR: ($CDCl_3$) δ 196.5, 165.1, 132.7, 129.9, 129.0, 127.7, 63.4, 60.6, 45.9, 14.1. IR (film): ν (cm^{-1}): 2987, 1764, 1692, 1269, 1185.

4.1.2. Ethyl (2*R*,3*S*)-2-chloro-3-hydroxy-4-phenylbutyrate 5. A 45 mL portion of LB medium supplemented with 30 μg/mL kanamycin was inoculated with a single colony of *E. coli* BL21(DE3)(pIK4) and the culture shaken overnight at 37 °C. The preculture was diluted 1:100 into 4 L of the same medium in a New Brunswick M19 fermenter. The culture was grown for 2 h at 37 °C with a stir rate of 800 rpm and an air flow of 0.5 vessel volumes per min (vvm) until reaching an $OD_{600} = 0.6$. After cooling to 28 °C over 15 min, reductase overexpression was induced by adding isopropylthio-β-D-galactoside to a final concentration of 100 μM and the culture was maintained under the same conditions for an additional 6 h. Cells were collected by centrifugation (6000*g* for 10 min at 4 °C), then half of the biomass (25 g wet weight) was resuspended in 1 L of 10 mM KP_i (pH 5.6) containing 4 g/L glucose. The bioconversion was carried out in a Braun Biostat B fermenter with the temperature, pH, and dissolved oxygen maintained at 30 °C, 5.6 (3 M NaOH titrant) and 75% saturation (fixed air flow of 0.25 vvm and variable stirring rate), respectively. Solid XAD-4 resin (0.5 g) was added, followed by portions of neat **3** (0.2 mL) approximately every hour over a total of 12 h to provide a final added substrate concentration of 5 mM. A glucose stock solution was also added after 3 and 6 h to keep the glucose concentration at approximately 4 g/L. The progress of the reaction was monitored by GC and the consumption of both **3** and glucose slowed significantly after 7 h. After 24 h, the reaction product was gently extracted with CH_2Cl_2 (2 × 300 mL) to avoid forming an emulsion.[29] The combined organics were dried over $MgSO_4$, concentrated in vacuo, and the residue subjected to silica gel chromatography (cyclohexane:Et_2O, 85:15) to afford 1.1 g of **5** as a colorless oil (82% yield). $[\alpha]_D = +24$ (*c* 0.7, $CHCl_3$). ^{1}H NMR: (C_6D_6) δ 7.06 (m, 5H), 4.24 (ddd, 1H, $J_1 = 3.3$ Hz, $J_2 = 6.6$ Hz, $J_3 = 7.1$ Hz), 4.05 (d, 1H $J = 3.3$ Hz), 3.78 (q, 2H, $J = 7.2$ Hz), 2.82 (dd, 1H, $J_1 = 7.1$ Hz, $J_2 = 13.8$ Hz), 2.72 (dd, 1H, $J_1 = 6.6$ Hz, $J_2 = 13.8$ Hz), 2.4 (br s, 1H), 0.79 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR: ($CDCl_3$) δ 169.1, 137.1, 129.8, 129.2, 127.4, 73.4, 63.0, 61.2, 40.4, 14.4. IR (film): ν (cm^{-1}): 3483, 1740, 1300, 1183, 1025. Anal. Calcd for $C_{12}H_{15}ClO_3$: C, 59.39; H, 6.23. Found: C, 59.75; H, 6.72.

4.1.3. Ethyl (2*S*,3*S*)-*cis*-4-phenyl-2,3-oxiranebutanoate 6. K_2CO_3 (1.7 g, 12.4 mmol, 3 equiv) and water (390 μL) were added to a solution of chlorohydrin **5** (1.0 g, 4.1 mmol) in DMF (19 mL). After stirring for 5 h at rt, the mixture was diluted with water (50 mL) and the aqueous layer extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with water to remove residual DMF (6 × 5 mL), dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography on silica gel (cyclohexane:Et_2O, 85:15) to afford 0.84 g of **6** as a colorless oil (99% yield). $[\alpha]_D = +37$, (*c* 3.0, $CHCl_3$). Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.89; H, 6.84. Found: C, 70.10; H, 6.91. Spectral data matched those reported for the racemic material.[32]

4.1.4. (4*S*,5*R*)-4,5-Dihydro-2-phenyl-4-carboethoxy-5-benzyl-1,3-oxazole 7. $BF_3{\cdot}OEt_2$ (62 μL, 0.49 mmol, 1 equiv) was added over 10 min to a solution of glycidic ester **6** (100 mg, 0.49 mmol) in benzonitrile (900 μL) cooled to 0 °C. The reaction was allowed to warm to rt over a 3 h period. Saturated $NaHCO_3$ (2 mL) was added and the mixture was stirred for an additional 2 h before being diluted with water (10 mL). After extracting with CH_2Cl_2 (3 × 15 mL), the combined organics were dried over $MgSO_4$, concentrated in vacuo, and purified by silica gel chromatography (cyclohexane:Et_2O, 9:1) to yield 0.12 g of **7** as a colorless oil (78% yield). $[\alpha]_D = -57$, (*c* 2.0, $CHCl_3$). ^{1}H NMR: (C_6D_6) δ 8.37 (m, 2H), 7.28 (m, 8H), 4.90 (ddd, 1H, $J_1 = 6.3$ Hz, $J_2 = 6.3$ Hz, $J_3 = 6.9$ Hz), 4.78 (d, 1H, $J = 6.3$ Hz), 3.93 (m, 2H), 3.18 (dd, 1H, $J_1 = 6.3$ Hz, $J_2 = 13.8$ Hz), 2.92 (dd, 1H, $J_1 = 6.9$ Hz, $J_2 = 13.8$ Hz), 0.91 (t, 3H, $J = 7.2$ Hz). ^{13}C NMR: (C_6D_6) δ 170.2, 163.2, 131.8 (other aromatic signals obscured by solvent) 80.3, 73.7, 61.2, 42.1, 14.0. IR (film): ν (cm^{-1}): 3029, 2982, 1752, 1655, 1206, 1027, 695. Anal. Calcd for $C_{19}H_{19}O_3N$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.63; H, 6.14; N, 4.56.

4.1.5. (2*S*,3*R*)-2-Hydroxy-3-amino-4-phenylbutyric acid 2 hydrochloride. To a solution of oxazoline **7** (323 mg, 1.04 mmol) in a mixture of EtOH (8 mL) and water (6 mL) was added concentrated HCl (15 mL). The mixture was held at reflux for 8 h before being concentrated under reduced pressure. After resuspending the residue

in water (3 mL) and EtOH (1.5 mL), Dowex 50WX8-100 resin (5 g) was added and gently swirled by hand for 5 min. The resin was collected by filtration and washed successively with water (15 mL) and EtOH (15 mL). The desired product was eluted by washing with 40 mL of 2 M NH_4OH. The filtrate was lyophilized to afford 0.16 g of a white powder that was dissolved in 6 M HCl (4 mL) before being lyophilized to afford **2** as its hydrochloride salt (83% yield) mp 201–203 °C, $[\alpha]_D = +23$ (*c* 1.3, 1 M HCl). A sample of authentic material (obtained from Sigma–Aldrich) gave mp 202–204 °C and $[\alpha]_D = +23$, (*c* 1.0, 1 M HCl). All spectral data (obtained on the same instruments under the same conditions) were identical for both the synthetic and authentic material.

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References

1. Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1976**, *29*, 97–99.
2. Abe, F.; Ino, K.; Bierman, P. J.; Talmadge, J. E.; Sawada, H.; Ogino, T.; Ishizuka, M.; Aoyagi, T. *Int. Congress Ser.* **2001**, *1218*, 155–159.
3. Nakamura, H.; Suda, H.; Takita, T.; Aoyagi, T.; Umezawa, H.; Itaka, Y. *J. Antibiot.* **1976**, *29*, 102–103.
4. Suda, H.; Takita, T.; Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 600–601.
5. Nishizawa, R.; Saino, T.; Suzuki, M.; Fujii, T.; Shirai, T.; Aoyagi, T.; Umezawa, H. *J. Antibiot.* **1983**, *36*, 695–699.
6. Pearson, W. H.; Hines, J. V. *J. Org. Chem.* **1989**, *54*, 4235–4237.
7. Norman, B. H.; Morris, M. L. *Tetrahedron Lett.* **1992**, 6803–6806.
8. Palomo, C.; Aizpurua, J. M.; Cuevas, C. *J. Chem. Soc., Chem. Commun.* **1994**, 1957–1958.
9. Koseki, K.; Ebata, T.; Matsushita, H. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 534–536.
10. Bergmeier, S. C.; Stanchina, D. M. *J. Org. Chem.* **1999**, *64*, 2852–2859.
11. Seki, M.; Nakao, K. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 1304–1307.
12. Semple, J. E.; Owens, T. D.; Nguyen, K.; Levy, O. E. *Org. Lett.* **2000**, *2*, 2769–2772.
13. Lee, B. W.; Lee, J. H.; Jang, K. C.; Kang, J. E.; Kim, J. H.; Park, K. M.; Park, K. H. *Tetrahedron Lett.* **2003**, 5905–5907.
14. Kudyba, I.; Raczko, J.; Jurczak, J. *Tetrahedron Lett.* **2003**, 8685–8687.
15. Wasserman, H. H.; Petersen, A. K.; Xia, M. D. *Tetrahedron* **2003**, *59*, 6771–6784.
16. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
17. Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, 501–504.
18. Aoyagi, T.; Tobe, H.; Kojima, F.; Hamada, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 636–638.
19. Nagai, M.; Kojima, F.; Naganawa, H.; Hamada, M.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1997**, *50*, 82–84.
20. Aoyagi, T.; Yoshida, S.; Nakamura, Y.; Shigihara, Y.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1990**, *43*, 143–148.
21. Yoshida, S.; Nakamura, Y.; Naganawa, H.; Aoyagi, T.; Takeuchi, T. *J. Antibiot.* **1990**, *43*, 149–153.
22. Stewart, J. D.; Rodriguez, S.; Kayser, M. M. Enzyme Technologies for Pharmaceutical and Biotechnological Applications. In *Cloning, Structure and Activity of Ketone Reductases from Baker's Yeast*; Kirst, H. A., Yeh, W.-K., Zmijewski, M. J., Eds.; Marcel Dekker: New York, 2001, pp 175–208.
23. Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827–12832.
24. Feske, B. D.; Kaluzna, I. A.; Stewart, J. D. *J. Org. Chem.*, submitted for publication.
25. Cabon, O.; Buisson, D.; Larchevêque, M.; Azerad, R. *Tetrahedron: Asymmetry* **1995**, *6*, 2211–2218.
26. Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**, *43*, 2087–2088.
27. Chowdhury, S. F.; Guerrero, H.; Brun, R.; Ruiz-Perez, L. M.; Pacanowska, D. G.; Gilbert, I. H. *J. Enzym. Inhib. Med. Chem.* **2002**, *17*, 293–302.
28. Matsuki, K.; Sobukawa, M.; Kawai, A.; Inoue, H.; Takeda, M. *Chem. Pharm. Bull.* **1993**, *41*, 643–648.
29. Kaluzna, I. A.; Feske, B. D.; Wittayanan, W.; Ghiviriga, I.; Stewart, J. D. *J. Org. Chem.* **2005**, *70*, 342–345.
30. Anderson, B. A.; Hansen, M. M.; Harkness, A. R.; Henry, C. L.; Vicenzi, J. T.; Zmijewski, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 12358–12359.
31. Jörg, G.; Bertau, M. *ChemBioChem* **2004**, *4*, 87–92.
32. Hoffman, R. V.; Johnson, M. C.; Okonya, J. F. *J. Org. Chem.* **1997**, *62*, 2458–2465.