4.48 (s, 1 H), 6.32 (d, 2 H, J = 7.5 Hz), 6.83 (t, 2 H, J = 7.5 Hz), 7.05 (t, 1 H, J = 7.5 Hz), 7.42 (t, 1 H, J = 7.6 Hz), 7.48 (d, 1 H, J = 3.4 Hz), 7.61 (t, 2 H, J = 7.5 Hz), 7.78 (t, 1 H, J = 7.5 Hz), and 7.95 (d, 2 H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 41.8, 44.8, 60.7, 63.3, 127.7, 127.8, 128.5, 129.4, 129.5, 130.1, 132.9, 134.2, 134.4, 137.0, 139.3, and 147.5. Anal. Calcd for $C_{24}H_{23}NO_4S_2$: C, 63.55; H, 5.11; N, 3.09. Found: C, 63.52; H, 5.16; N, 3.06.

Preparation of 1-Phenyl-2,3-bis(phenylsulfonyl)-1,3-butadiene (15). To a stirred solution containing (*Z*)-1-phenyl-2-(phenylsulfonyl)-3-(phenylthio)-2-buten-1-ol (**13**)[17] in 20 mL of dichloromethane at −78 °C was added 1.84 g of MCPBA (65%). The mixture was stirred for 15 min at −78 °C and then allowed to warm to room temperature, after which stirring was continued for an additional 5 h. The reaction mixture was poured into a separatory funnel containing a 10% aqueous sodium sulfite solution. The aqueous layer was extracted twice with dichloromethane, and the combined organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a white solid, which was recrystallized from methanol–ether to give (*Z*)-1-phenyl-2,3-bis(phenylsulfonyl)-2-buten-1-ol (**14**) as a white crystalline solid in 92% yield, mp 191–192 °C, which exhibits the following spectroscopic characteristics: IR (KBr) 3480, 3060, 1440, 1290, 1140, 1070, 930, 760, 730, 690, 570, and 630 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.04 (s, 3 H), 4.07 (d, 1 H, J = 9.6 Hz), and 7.20–8.00 (m, 15 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.7, 70.4, 124.9, 127.1, 127.3, 127.5, 128.1, 128.9, 129.2, 133.6, 134.1, 139.4, 140.8, 141.2, 151.5, and 151.9. Anal. Calcd for $C_{22}H_{20}S_2O_5$: C, 61.66; H, 4.70. Found: C, 61.59; H, 4.73.

To a solution containing 0.2 g of 1-phenyl-2,3-bis(phenylsulfonyl)-2-buten-1-ol (**14**) and 0.1 mL of triethylamine in 5 mL of dichloromethane at 0 °C was added 0.04 mL of mesyl chloride. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 4 h, after which it was poured into cold water. The aqueous layer was extracted with methylene chloride. The organic portion was washed consecutively with a 10% hydrochloric acid solution, a saturated sodium bicarbonate solution, and brine, then dried, and concentrated under reduced pressure to give a 95% yield of 1-phenyl-2,3-bis(phenylsulfonyl)-1,3-butadiene (**15**). Crystallization of the oil from chloroform–hexane gave a white solid, mp 104–105 °C, which exhibits the following spectroscopic properties: IR (KBr) 3060, 1630, 1590, 1580, 1440, 1310, 1160, 1140, 1080, 970, 740, 680, and 520 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (s, 1 H), 6.74 (s, 1 H), and 7.20–8.10 (m, 16 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.0, 128.1, 128.3, 128.4, 128.5, 129.8, 130.5, 131.6, 132.2, 133.1, 133.1, 134.1, 137.5, 138.5, 143.5, and 144.3. Anal. Calcd for $C_{22}H_{18}S_2O_4$: C, 64.37; H, 4.42. Found: C, 64.28; H, 4.45.

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Synthesis of β-Amino-α-hydroxy Acids via Aldol Condensation of a Chiral Glycolate Enolate. Synthesis of (-)-Bestatin

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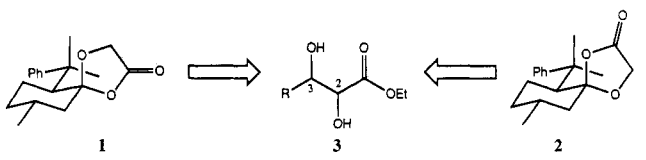
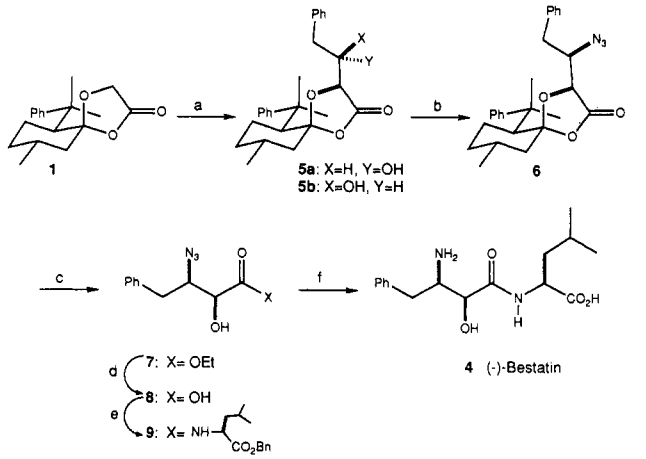
We have recently reported that the enolates of the spirocyclic 1,3-dioxolan-4-ones **1** and **2** undergo aldol condensations with aldehydes to afford, after acidic ethanolysis, α,β-dihydroxy esters **3** (Scheme I).[1] These dioxolanones are derived from 8-phenylmenthone and glycolic acid.[2] A key feature of this method is that any one of the four possible stereoisomers of **3** may be selectively prepared. Hence, the absolute stereochemistry at C-2 is completely controlled by selecting the appropriate dioxolanone **1** or **2**, and moderate to good relative stereocontrol at C-3 is governed by the choice of enolate counterion: Li^+ or Mg^{2+} gives the anti aldol, Zr^{4+} gives the syn aldol. The aldol adducts **3** are potentially useful intermediates for the synthesis of interesting biological substances. Herein, we wish to report an application of this chemistry to the synthesis of (-)-bestatin **4**.

Scheme I



Scheme II[a]



[a] [a] (i) LiHMDS, THF, −78 °C; (ii) phenyl acetaldehyde (56%, 5.7:1, **5a:5b**); [b] Ph_3P, DEAD, DPPA, THF, 0 °C to room temperature, 48 h (79%); [c] EtOH, HCl, reflux, 12 h (59%, 71% based on reacted starting material); [d] (i) LiOH, THF, H_2O, 0 °C, 1 h; (ii) HCl(aq) (91%); [e] L-leucine benzyl ester ·TsOH, HOBT, DCC, THF, 0 °C to room temperature, 16 h (68%); [f] H_2, Pd/C, MeOH, 48 h (86%).

Compounds such as bestatin[3] and amastatin[4] are examples of biologically important compounds with novel β-amino-α-hydroxy acid subunits. Bestatin is a potent aminopeptidase B inhibitor isolated from *Streptomyces*.[3,5] Clinical studies have shown its usefulness in the treatment of cancer through its ability to enhance the cytotoxic activity of known antitumor agents.[6] Since its discovery there has been continual interest in examining bestatin and analogues for biological activity.[7] Previous syntheses of the key subunit (2*S*,3*R*)-3-amino-2-hydroxy-4-phenyl-

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butanoic acid (AHPA) and of bestatin itself feature the iodocyclocarbamation of chiral allylamines,[8] the aldol condensation of α-aminoacetophenone with glyoxylic acid,[9] the stereospecific hydroxyamination of an alkene,[10] the chain extension of D-phenylalanine,[11] and the opening of an epoxy alcohol with azide ion.[12]

Our chiral glycolate enolate methodology should allow easy access to bestatin, AHPA, and related analogues. Inversion of the C-3 hydroxyl of an α,β-dihydroxy acid derivative with a nitrogen nucleophile would afford an optically active β-amino-α-hydroxy acid derivative. Inversion with azide ion offers the additional advantage of providing a useful protected version of AHPA (i.e., 8) for peptide coupling.

Results and Discussion

Dioxolanone 1 was prepared according to the previously published procedure.[2] Treatment of 1 with lithium hexamethyldisilazide followed by phenyl acetaldehyde gave the aldol products **5a** and **5b** in a 5.7:1 ratio. These diastereomers were readily separated by flash column chromatography, and no other stereoisomers were detected. The anti aldol adduct **5a** was then converted to azide **6** via a Mitsunobu inversion using diphenylphosphoryl azide (DPPA).[13] Use of phthalimide instead of DPPA in this reaction resulted in 50% elimination.

All attempts to hydrolyze **6** directly to the acid 8 under acidic or basic conditions resulted in either no reaction or a mixture of acid 8 and 2-oxo-4-phenylbutanoic acid. However, acidic ethanolysis of **6** afforded the ester **7**, which was converted to the acid 8 with lithium hydroxide.[14] No epimerization at C-2 was detected when exactly 2.0 equiv of lithium hydroxide were used. The use of a slight excess of lithium hydroxide (2.2 equiv) led to some degree (≤5%) of epimerization.

Using a standard peptide coupling procedure,[9] acid 8 was converted into the protected peptide **9**. Catalytic hydrogenation of **9** gave the free zwitterionic form of (−)-bestatin, whose physical properties precisely matched those reported in the literature. The HCl salt of the synthetic material was identical with authentic bestatin hydrochloride salt in all respects.

We have demonstrated a short, stereocontrolled route to bestatin, where the correct absolute and relative stereochemistry was produced in one step via an aldol condensation of the enolate derived from 1. In addition, azido acid 8 was shown to be a useful protected version of AHPA and may allow easy access to other peptide derivatives. Reduction of the azide to the amine in the final deprotection step is a convenient way to obtain the zwitterionic form of bestatin without resorting to tedious isolation techniques.

Experimental Section

General. Reagents and starting materials were obtained from commercial suppliers and were used without further purification, with the exception of phenylacetaldehyde, which was distilled prior to use. Tetrahydrofuran was distilled from sodium/benzophenone ketyl, immediately prior to use. Methylene chloride, hexamethyldisilazane, and triethylamine were distilled from calcium hydride immediately before use. All reactions were conducted under an atmosphere of dry nitrogen. Flash chromatography refers to liquid chromatography according to the method of Still.[15] Silica gel (230–400 mesh) was purchased from EM Reagents. Melting points were obtained on a Laboratory Devices Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory at Eagle Harbor, MI, Galbraith Laboratories Inc. at Knoxville, TN, or the Microanalytical Laboratory operated by the Department of Chemistry, University of Michigan, Ann Arbor, MI. High-resolution mass spectroscopy (HRMS) was carried out on a VG-Analytical 70-250 high-resolution mass spectrometer. Mass spectra were obtained on a Finnigan 4500 GC/MS-EICI system. FT-IR spectra were obtained using a Nicolet 5-DX. ^{1}H NMR spectra were obtained using a Bruker WM-300 or WM-360 spectrometer, with tetramethylsilane as an internal standard unless otherwise stated. Multiplicities are reported as s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet. ^{13}C NMR were obtained by using a Bruker WM-300 (75 MHz) or WM-360 (90 MHz) spectrometer and are reported relative to deuterated chloroform unless otherwise stated.

(3*S*,5*R*,6*S*,9*R*)-3-[(1*S*)-1-Hydroxy-2-phenylethyl]-9-methyl-6-(1-methyl-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-2-one (5a). Hexamethyldisilazane (1.20 g, 7.9 mmol) was cooled to 0 °C, and *n*-BuLi (5.1 mL of a 1.57 M solution in hexane, 7.9 mmol) was added dropwise. The mixture was stirred at 0 °C for 5 min and then cooled to −78 °C. Dioxolanone 1 (1.52 g, 5.3 mmol) in THF (8 mL) was added over 15 min. The reaction mixture was stirred for an additional 10 min and then phenyl acetaldehyde (1.2 g, 10.8 mmol) in THF (4 mL) was added dropwise. After 15 min, saturated aqueous ammonium chloride (0.6 mL) was added dropwise and the mixture was poured into ether (100 mL) and saturated aqueous ammonium chloride (50 mL). The aqueous layer was extracted with ether (3 × 75 mL), and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to yield 2.93 g of crude yellow oil. Purification by flash column chromatography (10% ethyl acetate/hexane) yielded 1.02 g of **5a** (47%) and 0.18 g of **5b** (8%), anti/syn 5.7:1, as clear oils. For **5a**: R_f 0.27 (10% ethyl acetate/hexane); $[\alpha]^{25}_D$ +7.76° (*c* 0.9, EtOH); IR ($CHCl_3$) 3596, 3022, 3011, 2955, 1780, 1601, 1495, 1455, 1275, 1228, 1135 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5–7.1 (m, 10 H), 3.97 (m, 1 H), 3.74 (d, 1 H, *J* = 6.3 Hz), 3.0 (dd, 1 H, *J* = 13.8, 4.4 Hz), 2.9 (dd, 1 H, *J* = 13.8, 7.9 Hz), 2.4 (d, 1 H, *J* = 4 Hz, OH), 2.12 (dd, 1 H, *J* = 11.7, 4.6 Hz), 1.9–1.6 (m, 5 H), 1.44 (t, 1 H, *J* = 12.8 Hz), 1.39 (s, 3 H), 1.35 (s, 3 H), 1.28 (m, 1 H), 0.88 (d, 3 H, *J* = 6.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 149.6, 136.9, 129.7, 128.5, 128.0, 126.7, 125.9, 125.7, 115.2, 75.8, 72.7, 53.5, 49.5, 40.05, 39.1, 34.6, 29.9, 28.7, 27.7, 25.4, 21.5; MS (70 eV) *m/e* (%) 408 (M^+, 0.6), 390 (0.8), 288 (11.0), 272 (3.6), 247 (9.2), 231 (3.6), 219 (6.7), 178 (4.1), 170 (5.6), 119 (100.0), 91 (30.0), 69 (9.7); HRMS calcd for $C_{26}H_{32}O_4$ (M^+) 408.2300, found 408.2298. Anal. Calcd for $C_{26}H_{32}O_4$: C, 76.44; H, 7.89. Found: C, 76.55; H, 7.98. For **5b**: R_f 0.35 (10% ethyl acetate/hexane); $[\alpha]^{25}_D$ −10.2° (*c* 1.0, EtOH); IR (neat) 3524, 3059, 3027, 2950, 2925, 1780, 1600, 1495, 1454, 1276, 1212, 1160 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.4–7.2 (m, 10 H), 4.17 (m, 1 H), 4.0 (d, 1 H, *J* = 8 Hz), 3.12 (dd, 1 H, *J* = 14, 3.5 Hz), 3.05 (d, 1 H, *J* = 3 Hz, OH), 2.9 (dd, 1 H, *J* = 14, 8 Hz), 2.2 (dd, 1 H, *J* = 12.5, 4 Hz), 1.9–1.5 (m, 5 H), 1.44 (s, 3 H), 1.42 (s, 3 H), 1.3 (t, 1 H, *J* = 10 Hz), 0.9 (d, 3 H, *J* = 6 Hz), 0.85 (m, 1 H); ^{13}C NMR (75 MHZ, $CDCl_3$) δ 172.6, 150.4, 136.7, 129.8, 128.2, 127.9, 126.6, 125.60, 125.55, 115.0, 73.8, 70.8, 51.7, 44.8, 40.7, 39.2, 34.2, 30.2, 29.4, 25.4, 25.1, 21.2; MS (70 eV) *m/e* (%) 408 (M^+, 0.24), 390 (0.1), 346 (0.1), 320 (0.1), 290 (2.46), 272 (0.26), 261 (0.2), 231 (0.4), 215 (0.7), 199 (1.0), 178 (4.8), 170 (1.8), 119 (100), 91 (37.9), 55 (11.7), 41 (18.9); HRMS calcd for $C_{26}H_{32}O_4$ (M^+) 408.2300, found 408.2300. Anal. Calcd for $C_{26}H_{32}O_4$: C, 76.44; H, 7.89. Found: C, 76.37; H, 7.79.

(3*S*,5*R*,6*S*,9*R*)-3-[(1*R*)-1-Azido-2-phenylethyl]-9-methyl-6-(1-methyl-1-phenylethyl)-1,4-dioxaspiro[4.5]decan-2-one (6). The anti aldol product **5a** (0.82 g, 2.0 mmol) and triphenylphosphine (0.59 g, 2.3 mmol) in THF (40 mL) were cooled

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to 0 °C. Diethyl azodicarboxylate (0.39 g, 2.2 mmol) was added dropwise followed by diphenylphosphoryl azide (0.61 g, 2.2 mmol) in THF (4 mL). The resulting mixture was stirred at room temperature for 48 h in the dark. The mixture was concentrated in vacuo and purified by flash column chromatography (5% ethyl acetate/hexane) without further workup to yield 0.69 g of **6** (79%) as a clear oil: R_f 0.5 (10% ethyl acetate/hexane); $[\alpha]^{25}_D$ -6.77° (*c*, 1.31, EtOH); IR (neat) 2955, 2926, 2113, 1794, 1272, 1257, 1138 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.4–7.1 (m, 10 H), 3.8 (ddd, 1 H, *J* = 8.4, 7.1, 2.9 Hz), 3.7 (d, 1 H, *J* = 2.9 Hz), 3.04 (dd, 1 H, *J* = 13.5, 7.1 Hz), 2.97 (dd, 1 H, *J* = 13.5, 8.4 Hz), 2.16 (dd, 1 H, *J* = 7.1, 6.4 Hz), 2.03 (b d, 1 H, *J* = 7.8 Hz), 1.9–1.7 (m, 4 H), 1.5 (t, 1 H, *J* = 12.8 Hz), 1.3 (s, 3 H), 1.28 (s, 3 H), 0.92 (d, 3 H, *J* = 6.6 Hz), 0.9 (m, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.8, 149.5, 135.8, 129.4, 128.9, 128.0, 127.3, 125.9, 125.7, 115.4, 74.9, 62.7, 53.8, 49.1, 36.6, 34.7, 34.6, 29.9, 28.7, 27.6, 25.5, 21.5; MS (70 eV) *m/e* (%) 451 ($(M + NH_4)^+$, 0.3), 434 (MH^+, 1.5), 406 (1.7), 391 (0.47), 378 (0.1), 363 (0.2), 344 (0.3), 330 (2.3), 288 (19.2), 161 (5.5), 119 (100), 91 (7.6); HRMS calcd for $C_{26}H_{32}N_3O_3$ (MH^+) 434.2444, found 434.2431. Anal. Calcd for $C_{26}H_{31}N_3O_3$: C, 71.86; H, 7.19; N, 9.67. Found: C, 70.55; H, 7.22; N, 9.51.

Ethyl (2*S*,3*R*)-3-Azido-2-hydroxy-4-phenylbutanoate (7). Azidodioxolanone **6** (0.519 g, 1.19 mmol) was dissolved in HCl saturated ethanol (45 mL). The resulting solution was heated to reflux for 10 h. After being cooled to room temperature, the reaction mixture was concentrated in vacuo, diluted with methylene chloride (20 mL), and concentrated in vacuo again to yield 0.504 g of a pale yellow oil. Purification by flash column chromatography (7% ethyl acetate/hexane) yielded 0.175 g (59%) of **7** and 88.3 mg (17%) of recovered **6**: R_f 0.12 (10% ethyl acetate/hexane); $[\alpha]^{25}_D$ -7.66° (*c*, 1.11, EtOH); IR (neat) 3489, 2982, 2111, 1736, 1495, 1454, 1257, 1114 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.4–7.25 (m, 5 H), 4.38–4.22 (m, 2 H), 4.11 (dd, 1 H, *J* = 5.5, 2.2 Hz), 3.78 (td, 1 H, *J* = 7.5, 2.2 Hz), 3.16 (d, 2 H, *J* = 7.5 Hz), 3.12 (d, 1 H, *J* = 5.5 Hz, OH), 1.28 (t, 3 H, *J* = 1.7 Hz); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 172.5, 136.6, 129.3, 128.7, 126.9, 71.4, 64.2, 62.2, 36.1, 13.9; MS (70 eV) *m/e* (%) 250 (MH^+, 5.4), 222 (33.3), 207 (13.1), 189 (9.3), 176 (5.8), 160 (6.1), 148 (20.1), 130 (28.1), 120 (100.0), 103 (16.4), 91 (83.4); HRMS calcd for $C_{12}H_{16}N_3O_3$ (MH^+) 250.1192, found 250.1184. Anal. Calcd for $C_{12}H_{15}N_3O_3$: C, 57.96; H, 6.07; N, 16.86. Found: C, 57.96; H, 6.29; N, 17.07.

(2*S*,3*R*)-3-Azido-2-hydroxy-4-phenylbutanoic Acid (8). Ester **7** (120 mg, 0.48 mmol) was dissolved in THF/H_2O (9.6 mL/3:1) and cooled to 0 °C. Lithium hydroxide (40 mg, 0.96 mmol) was added, and the mixture was stirred for 1 h at 0 °C. The reaction mixture was then acidified with 2 N HCl and extracted with ether (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to yield 97.2 mg of **8** (91%): $[\alpha]^{25}_D$ + 8.63° (*c*, 1.13, EtOH); IR (neat) 3403, 3029, 2923, 2115, 1729, 1603 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.5 (b s, 2 H, COOH, OH), 7.4–7.3 (m, 5 H), 4.2 (d, 1 H, *J* = 2 Hz), 3.9 (td, 1 H, *J* = 8.2, 2 Hz), 3.2 (d, 2 H, *J* = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 176.8, 136.6, 129.4, 128.9, 127.3, 71.1, 64.0, 36.2; MS (70 eV) *m/e* (%) 239 ($(M + NH_4)^+$, 100), 232 (0.7), 222 (0.5), 208 (0.5), 196 (24.8), 176 (0.5), 168 (0.3), 150 (8.1), 136 (25.0), 120 (67.8), 108 (7.1), 91 (4.1); HRMS calcd for $C_{10}H_{11}N_3O_3NH_4$ ($(M + NH_4)^+$) 239.1144, found 239.1149.

Benzyl *N*-[(2*S*,3*R*)-3-Azido-2-hydroxy-4-phenylbutanoyl]-L-leucinate (9). Benzyl L-leucinate p-toluenesulfonic acid salt (0.147 g, 0.37 mmol), 3-hydroxybenzotriazole (55.7 mg, 0.41 mmol), and acid **8** (74.9 mg, 0.34 mmol) were all combined in THF (1.4 mL) and cooled to 0 °C. Dicyclohexylcarbodiimide (85.8 mg, 0.42 mmol) and triethylamine (38 mg, 0.37 mmol) were then added, and the mixture was stirred at room temperature for 12 h. The resulting mixture was diluted with THF (1 mL) and filtered. The filtrate was diluted with Et_2O (20 mL) and extracted with 0.5 N HCl (1 × 10 mL), 5% aqueous $NaHCO_3$ (1 × 10 mL), and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield 202 mg of an oil. Purification by flash column chromatography (40% ethyl acetate/hexane) yielded 97.2 mg (68%) of **9** (R_f 0.47) as an oil, which crystallized: mp 97–98 °C; $[\alpha]^{25}_D$ -30.6° (*c*, 2.22, $CHCl_3$); IR ($CHCl_3$) 3411, 3020, 3011, 2962, 2115, 1735, 1676, 1522, 1455, 1222, 1154 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.4–7.2 (m, 10 H), 7.15 (d, 1 H, *J* = 9 Hz), 5.18 (d, 1 H, *J* = 12 Hz), 5.14 (d, 1 H, *J* = 12 Hz), 4.7 (m, 1 H), 4.1–4.0 (m, 2 H), 3.6 (d, 1 H, *J* = 7 Hz), 3.0 (d, 2 H, *J* = 7.5 Hz), 1.8–1.6 (m, 3 H), 0.9 (d, 6 H, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 171.3, 136.7, 135.2, 129.3, 128.8, 128.6, 128.4, 128.2, 127.1, 72.6, 67.3, 64.7, 50.8, 41.3, 36.8, 24.9, 22.9, 21.7; MS (70 eV) *m/e* (%) 425 (MH^+, 18.8), 399 (9.1), 382 (3.2), 366 (3.0), 295 (9.0), 278 (15.6), 222 (3.4), 210 (4.7), 188 (3.8), 120 (100), 91 (41.5); HRMS calcd for $C_{23}H_{29}N_4O_4$ (MH^+) 425.2189, found 425.2179. Anal. Calcd for $C_{23}H_{28}N_4O_4$: C, 65.07; H, 6.88 N, 13.19. Found: C, 65.29; H, 6.75; N, 13.14.

(-)-Bestatin (4). Catalytic hydrogenation of **9** (153 mg, 0.36 mmol) over 5% Pd on carbon (220 mg) was carried out at atmospheric pressure in methanol (7.2 ml) for 48 h. The mixture was filtered through Celite and concentrated in vacuo to yield 95 mg of **4** (86%) as the free zwitterion, which was recrystallized from methanol/ethyl acetate, mp 231–236 °C dec [lit.[3] mp 233–236 °C dec]. Crystallization of this solid from 1 N HCl formed the HCl salt of **4**, which matched the authentic HCl salt obtained from Sigma (lot no. 76 F-58201): $[\alpha]^{25}D$ -14.3° (*c* 0.5, 1 N HCl); authentic sample $[\alpha]^{25}_D$ -13.0° (*c* 0.5, 1 N HCl); IR (KBr) 3397, 2961, 1713, 1662, 1539, 1256, 1184, 1158 cm^{-1}; ^{1}H NMR (300 MHz, acetic acid-d_4) δ 10.89 (s, 3 H, OH, COOH, HCl), 7.4–7.2 (m, 5 H), 4.7–4.5 (m, 2 H), 4.05 (s, 1 H), 3.25 (dd, 1 H, *J* = 15.6 Hz), 3.08 (dd, 1 H, *J* = 15, 8 Hz), 1.9–1.7 (m, 3 H), 0.94 (m, 6 H); ^{13}C NMR (90 MHz, acetic acid-d_4) δ 177.5, 173.9, 136.6, 130.9, 130.2, 128.7, 70.6, 57.3, 52.6, 41.1, 35.8, 26.1, 23.4, 22.2.

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Registry No. **1**, 104196-76-1; **4**, 58970-76-6; **4**·HCl, 65391-42-6; **5a**, 121445-49-6; **5b**, 121521-81-1; **6**, 121445-50-9; **7**, 121445-51-0; **8**, 121445-52-1; **9**, 121445-53-2; H-Leu-OBn·TsOH, 1738-77-8; $PhCH_2CHO$, 122-78-1.

Biotransformation of Some Keto Esters through the Consecutive Reuse of Immobilized *Nicotiana tabacum* Cells

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A number of biotransformations have recently been reported employing freely suspended or immobilized plant cell cultures. Most of these reports, however, were confined to the biotransformation of secondary metabolites produced by plant cell cultures;[1] there have been few examples on the biotransformation of synthetically important foreign substrates.[2,3]

For some years now we have been investigating the possibility of using immobilized biocatalysts such as bakers' yeast entrapped with calcium alginate or carrageenan in organic synthesis.[4,5] Very recently we reported the first

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