Total Synthesis of Anti-microtubule Diketopiperazine Derivatives: Phenylahistin and Aurantiamine

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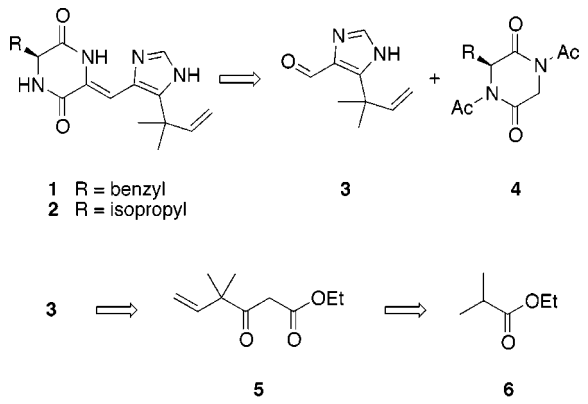
Introduction

The closely related phenylahistin[1] **1** and aurantiamine[2] **2** have been isolated from *Aspergillus ustus* NSC-F038 and *Penicillium aurantiogriseum*. These diketopiperazine derivatives consist of an L-phenylalanine or L-valine residue, respectively, and a unique isoprenylated dehydrohistidine residue with a quaternary carbon at the 5-position of the imidazole ring. The former is a member of a new class of colchicine-like microtubule binding agents that exhibit cytotoxic activities against a wide variety of tumor cell lines.[1,3,4] The latter was isolated as a blue fluorescence compound for chemotaxinomic indication of growth in some toxigenic varieties of *P. aurantiogriseum*;[2] however, its biological function is unknown. The total syntheses of these natural products are useful to understand their precise biological functions and to develop more potent antitumor agents based on the diketopiperazine structures. Herein, we report the total syntheses of both diketopiperazine derivatives starting from ethyl isobutyrate **6**.

Results and Discussion

Previously, our efforts were focused on the preliminary modification of phenylahistin such as reduction of olefin structures, methylation of nitrogen atoms, and synthesis of derivatives with the substituent at the 5-position of the imidazole ring either removed or changed to a methyl group, to determine the structural features responsible for the biological activity.[5] From the biological evaluation of these derivatives, we found that a uniplanar pseudotricyclic structure, formed by the hydrogen bonding between the diketopiperazine and the imidazole ring, and the substituent at the 5-position of the imidazole ring were important for the anti-microtubule activity of the phenylahistin derivatives. This biologically important template of phenylahistin suggested two attractive sites for further modification to develop more potent antitumor agents, i.e., the side chain of the phenylalanine residue and the 5-position of the imidazole ring. Accordingly, our next goal was to establish a suitable synthetic route for preparing phenylahistin, which can lead to the development of more potent derivatives for anti-microtubule activity and also help clarify the mechanisms of action of these diketopiperazine derivatives.

Scheme 1



In the total synthesis, the key steps of our approach were focused on the formation of the isoprenylated imidazole ring and its condensation with diketopiperazine derivatives. Fortunately, a method for the preparation of the 5-position-substituted imidazole-4-carboxylate from chlorinated β-ketoesters was reported by Durant et al.,[6] and condensation of imidazolecarboxaldehyde with a diketopiperazine analogue was also reported by Bond et al. for the synthesis of viridamine,[7] another closely related diketopiperazine analogue from *Penicillium viridicatum*. Therefore, to synthesize **1** and **2**, the proposed retrosynthetic route is shown in Scheme 1.

In the synthesis of β-ketoester **5** as a precursor for the imidazole derivative, we could not follow the Claisen condensation method of Vig et al.[8] as reported for the reaction between **9** and ethyl acetate (60% yield) due to the predominant production of ethyl acetoacetate. To effectively synthesize **5**, we followed a new synthetic procedure as shown in Scheme 2. Ethyl isobutyrate **6** was converted to **7** by aldol condensation using acetaldehyde in the presence of LDA. The hydroxyl group of **7** was tosylated in the presence of pyridine to yield **8**, which was subsequently converted to an olefin **9** by using DBU as a base and solvent. Saponification of the obtained olefin ester **9** with 4 N sodium hydroxide afforded 2,2-dimethyl-3-butenoic acid **10**. Compound **10** was obtained at a 92% yield from **6** through four steps with no intermediate purification (final yield of 74% with each

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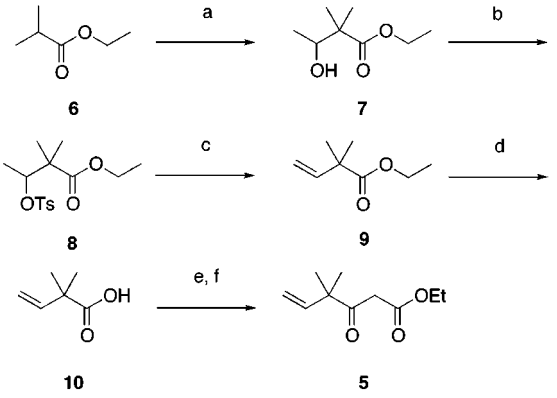
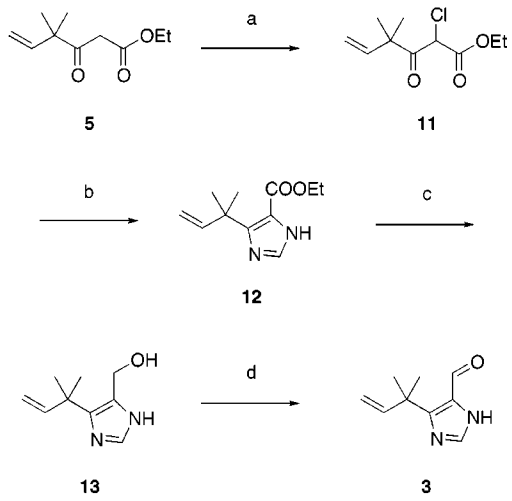
Scheme 2[a]



[a] Reagents and conditions: (a) LDA, CH_3CHO, THF, −70 °C, 88%; (b) Tos-Cl, pyridine, rt, 88%; (c) DBU, reflux (140 °C), 96%; (d) 4 N NaOH, EtOH, rt, 99%; (e) $SOCl_2$, reflux; (f) $EtOCOCH_2$-COOH, BuLi, THF, −70 to −10 °C, 85% (two steps).

Scheme 3[a]



[a] Reagents and conditions: (a) SO_2Cl_2, $CHCl_3$, reflux, 77%; (b) formamide, H_2O, reflux (145 °C), 48%; (c) DIBALH, toluene, −30 °C, 50%; (d) MnO_2, acetone, rt, 95%.

step purification). Next, in the preparation of β-ketoester, we adopted an efficient one-step synthesis introduced by Wierenga et al.,[9] in which acid chloride of **10** was reacted with dilithio dianion of monoethylmalonate in THF to give **5** without any difficulty in obtaining a good yield (85%).

In the synthesis of 5-substituted imidazole-4-carboxylate, we followed the synthetic protocol of Durant et al.[6] The synthetic pathway is outlined in Scheme 3. Briefly, β-ketoester **5** was chlorinated with sulfuryl chloride to yield **11**, which was later refluxed with formamide in the presence of water to give **12**. After purification using silica gel chromatography, **12** was reduced with DIBALH to give the alcohol **13**. In the conversion of alcohol to aldehyde, MnO_2 was employed for the oxidation to prepare aldehyde **3** in high yield.

Initially, condensation of aldehyde **3** with the diacetyldiketopiperazine derivative **4** was carried out in the presence of LDA and HMPA, since this procedure afforded the phenylahistin analogues, although at less than 10% yield.[5] However, in the present situation the reaction

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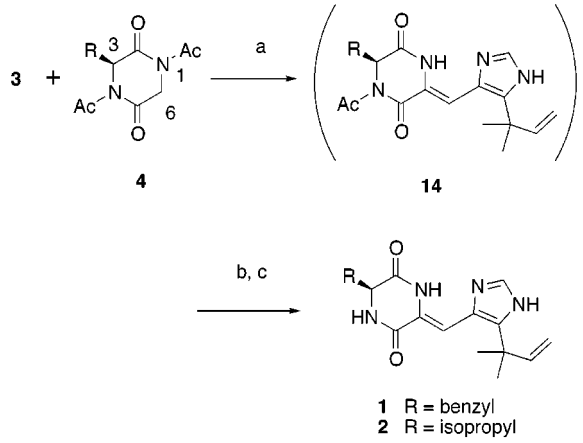
Scheme 4[a]



[a] Reagents and conditions: (a) ^{t}BuOK, n-BuOH, DMF, rt; or Cs_2CO_3, DMF, rt; (b) 28% NH_4OH, rt; (c) chiral HPLC.

with LDA gave only a trace amount of the product. This low reactivity of aldehyde **3** was probably due to the steric hindrance of the geminal dimethyl groups on the 5-position of the imidazole ring. Hence, we performed the above reaction with several bases, and when ^{t}BuOK or Cs_2CO_3 was adopted (Scheme 4), the production of intermediate **14** was predominantly detected in the reaction mixture by HPLC analysis. Deacetylation was carried out by adding excess amounts of aqueous NH_4OH to the reaction mixture and then neutralizing with AcOH, followed by direct application of the reaction mixture to RP-HPLC for the purification. After lyophilization of the desired fractions, **1** was obtained as a white powder with a yield of 5% (in case of ^{t}BuOK) and 21% (in case of Cs_2CO_3) respectively, indicating that Cs_2CO_3 was the preferred base for this condensation, even though the yield was not high. Aurantiamine **2** was also obtained from diacetyl-cyclo(L-Val-Gly) using Cs_2CO_3 as a base with a yield of 35%, which was comparatively higher than that of phenylahistin. Since the benzyl group of the Phe residue in phenylahistin was located out of and over the diketopiperazine ring, in a conformation which was reported to be the most energetically favorable for a diketopiperazine with an aromatic amino acid residue,[10] this conformation may prevent the attack of enolate anions to sterically hindered aldehydes, resulting in the lower yield of phenylahistin in comparison with that of aurantiamine.

To check the optical purity of the synthesized compounds, **1** and **2** were subjected to chiral chromatography.[11] Severe racemization was observed during the final condensation step due to the base treatment (Scheme 4, step a). In the case of phenylahistin, almost complete racemization was observed, whereas, in the case of aurantiamine, 31% of the other enantiomer was obtained. This observation also suggested that enolate ions at the C6 site, derived from diacetyl-cyclo(L-Phe-Gly), were less reactive than that from diacetyl-cyclo(L-Val-Gly), as a result of which the α-carbon (C6) of the Phe residue becomes racemized. The target compounds with the L-amino acid residue were separated by chiral HPLC,[11] resulting in the total synthesis of (−)-phenylahistin and

(10) Liwo, A.; Ciarkowski, J. *Tetrahedron Lett.* **1985**, *26*, 1873.

(11) **1** and **2** were isolated by HPLC using chiral column (CHIRALCEL OD, 10 × 250 mm) eluted with a mixture of hexane and ethanol (5:1 and 8:1, respectively) at a flow rate of 3 mL/mim.

(−)-aurantiamine. Total yields of these compounds as derived from **6** were 1 and 3%, respectively (14% yield from **6** to aldehyde **3**). Since these findings suggest that in this type of condensation it is difficult to prevent the racemization of amino acid residues located on the opposite side of the diketopiperazine ring, alternative synthetic routes such as the preparation of isoprenylated dehydrohistidine residue, followed by amide bond formation leading to the diketopiperazine ring, will be necessary to improve this method or solve this problem.

Synthetic phenylahistin **1** and aurantiamine **2** were characterized using spectroscopic techniques. The physical properties of and the characterizations performed on the synthetic materials were found to be identical to those for the natural products. Since the (−)-enantiomer of the native phenylahistin was more potent than the (+)-enantiomer, we focused on the biological activity concerning only the (−)-enantiomer. The cytotoxic effect on P388 cell proliferation was performed for the synthetic compounds (**1**, **2**) and native (−)-phenylahistin. The activity of **1** was almost similar to that of native (−)-phenylahistin, while in case of aurantiamine, **2** was 40 times less potent than (−)-phenylahistin. All details regarding the biological activities will be published elsewhere. Further derivatization of the phenylahistin structure using the above synthetic methodology will contribute to the better understanding on the structure−activity relationship of phenylahistin and to develop more potent antitumor agents based on the diketopiperazine structure. Studies in this regard are in progress.

In conclusion, phenylahistin **1**, a member of a new class of colchicine-like microtubule binding agents with a diketopiperazine structure, and its closely related derivative aurantiamine **2** have been synthesized, which will pave the way for the development of potent antitumor drugs.

Experimental Section

General Information. All solvents were reagent grade and dried prior to use. Column chromatography was performed using 70−230 mesh silica gel. Melting points (mp) are uncorrected. ^{1}H NMR spectra were recorded at 270 and 300 MHz, and ^{13}C NMR spectra were recorded at 67.5 and 75 MHz. Optical rotations were measured using the sodium D line ($\lambda = 589$ nm). High-resolution mass spectra were analyzed by using the electron impact (EI) or fast atom bombardment (FAB) method. Infrared (IR) spectra were obtained using KBr pellets or $CHCl_3$ solution.

Ethyl 2,2-Dimethyl-3-hydroxybutanoate (7). To a solution of ethyl isobutyrate (42 g, 0.362 mol) in THF (300 mL) was added 200 mL of 2.0 M solution of LDA in heptane/THF/ethylbenzene at −70 °C under argon atmosphere, and the mixture was stirred at the same temperature for 30 min. To this solution was then added a solution of acetaldehyde (24.4 mL, 0.436 mol) in THF (40 mL) at −70 °C. After stirring for 30 min at the same temperature, saturated NH_4Cl (100 mL) was added, and the solution was allowed to warm to room temperature. The reaction mixture was extracted with EtOAc two times, washed with 2 N HCl, 5% $NaHCO_3$, and saturated NaCl three times, respectively, dried over $MgSO_4$, and concentrated in vacuo. Distillation of the residual oil at 120 °C (64 mmHg) gave 51.2 g (88%) of **7** as an oil: IR ($CHCl_3$) 1700 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17 (q, $J = 7$ Hz, 2 H), 3.86 (q, $J = 7$ Hz, 1H), 2.72 (br s, 1H), 1.27 (t, $J = 7$ Hz, 3H), 1.18 (s, 3H), 1,17 (s, 3H), 1.15 (d, $J = 7$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 72.5, 60.6, 47.0, 22.4, 19.8, 17.7, 14.1; HRMS (FAB) m/z 161.1167 ($M^+ + 1$) (calcd for $C_8H_{17}O_3$: 161.1178).

Ethyl 2,2-Dimethyl-3-(4-toluenesulfonyloxy)butanoate (8). To a solution of **7** (45.5 g, 0.281 mol) in dry pyridine (92 mL) was added 4-toluenesulfonyl chloride (59.6 g, 0.312 mol) at 4 °C, and the mixture was stirred at room temperature for 48 h under argon atmosphere. Then 1 N HCl (100 mL) was added to the reaction mixture at 4 °C, and the mixture was extracted twice with EtOAc (200 mL). The combined organic layers were washed with 1 N HCl until the aqueous phase became less than pH 3, and then with saturated NaCl three times, dried over Na_2SO_4, and concentrated in vacuo. The residual pale orange oil was purified by column chromatography on silica (4.5 × 30 cm) using hexane−EtOAc (100:1 to 6:1) as an eluant to give colorless crystals of **8** (89.7 g, 88%): mp 38−39 °C; IR ($CHCl_3$) 1730 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, $J = 8$ Hz, 2H), 7.32 (d, $J = 8$ Hz, 2H), 4.96 (q, $J = 6$ Hz, 1H), 4.05 (m, 2H), 2.45 (s, 3H), 1.23 (d, $J = 6$ Hz, 3H), 1.22 (t, $J = 7$ Hz, 3H), 1.13 (s,3H), 1.12 (s,3H); ^{13}CNMR (75 MHz,$CDCl_3$) δ 174.9, 144.5, 134.6, 129.7, 127.7, 83.0, 60.9, 46.9, 21.7, 21.6, 19.6, 16.5, 14.0; HRMS (EI) m/z 314.1178 (M^+) (calcd for $C_{15}H_{22}O_5S$: 314.1188). Anal. Calcd for $C_{15}H_{22}O_5S$: C, 57.30; H, 7.05. Found: C, 57.20, H, 7.03.

Ethyl 2,2-Dimethyl-3-butenoate (9). A mixture of **8** (41.5 g, 0.132 mol) and DBU (30 mL, 0.2 mol) was refluxed at 140 °C for 3 h. After being cooled to room temperature, the reaction mixture was poured into ice-chilled ether, the organic layer was washed with 1 N HCl until the aqueous phase became less than pH 3, then 5% $NaHCO_3$, and saturated NaCl three times, respectively, and dried over Na_2SO_4, and ether was removed in vacuo. Distillation of the residual oil afforded **9** as an oil; bp 142 °C; yield 18.0 g (96%): IR ($CHCl_3$) 1730 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.03 (dd, $J = 11$, 18 Hz, 1H), 5.09 (dd, $J = 1$, 18 Hz, 1H), 5.06 (dd, $J = 1$, 11 Hz, 1H), 4.12 (q, $J = 7$ Hz, 2H), 1.30 (s, 6H), 1.25 (t, $J = 7$ Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 176.3, 142.7, 112.7, 60.7, 44.8, 24.6, 14.1. HRMS (EI) m/z 142.0997 (M^+) (calcd for $C_8H_{14}O_2$: 142.0994).

2,2-Dimethyl-3-butenoic Acid (10). To a solution of **9** (16.8 g, 0.118 mol) in EtOH (50 mL) was added 4 N NaOH (44 mL, 0.176 mol), and the mixture was stirred at room temperature for 16 h. After the solvent was removed in vacuo, the residue was acidified to pH 2 by addition of 1 N HCl and then extracted twice with EtOAc, washed with saturated NaCl three times, dried over Na_2SO_4, and concentrated in vacuo to give an oil of **10** (13.4 g, 99%): IR ($CHCl_3$) 1700 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.05 (dd, $J = 11$, 17 Hz, 1H), 5.15 (dd, $J = 1$, 17 Hz, 1H), 5.11 (dd, $J = 1$, 11 Hz, 1H), 1.33 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 182.6, 141.9, 113.4, 44.7, 24.4. HRMS (EI) m/z 114.0695 (M^+) (calcd for $C_6H_{10}O_2$: 114.0681). Anal. Calcd for $C_6H_{10}O_2$: C, 63.14; H, 8.83. Found: C, 62.81, H, 8.53.

Ethyl 3-Oxo-4,4-dimethyl-5-hexenoate (5). To 5.88 g (51.5 mmol) of **10** in a 50 mL round-bottom flask fitted with a reflux condenser was added 5.64 mL (77.3 mmol) of thionyl chloride and one drop of DMF. The reaction mixture was stirred for 10 min at room temperature and then refluxed at 80 °C for 2 h. After cooling, the residual thionyl chloride was removed by evaporator under mild reduced pressure, and the residual crude acid chloride was applied to the following reaction. To 100 mL of THF under argon atmosphere with stirring, monoethyl malonate (11.9 g, 90.2 mmol) and several milligrams of 2,2′-bipyridyl as an indicator were added. After cooling to −70 °C, n-BuLi (97 mL of 1.6 M solution in hexane, 0.155 mol) was added slowly while allowing the temperature to rise to −10 °C. After the pink indicator was visible at −10 °C (pink indicator gradually decayed during stirring at −10 °C), the heterogeneous solution was recooled to −65 °C and the solution of the above prepared acid chloride in THF (15 mL) was added dropwise over 10 min. The reaction mixture was stirred for 1 h at −65 °C and then allowed to rise to 0 °C for 1 h. The reaction solution was poured into 100 mL of ether and 1 N HCl and stirred for 2 h. The layers were separated, and the organic phase was washed with saturated $NaHCO_3$ and saturated NaCl three times, dried over Na_2SO_4, and concentrated in vacuo. The residual oil was purified by column chromatography on silica (3.2 × 25 cm) using hexane−EtOAc (200:1 to 50:1) as an eluant to give an oil of **5** (8.1 g, 85%): IR ($CHCl_3$) 1790, 1740 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.89 (dd, $J = 10$, 17 Hz, 1H), 5.21 (dd, $J = 1$, 10 Hz, 1H), 5.20 (dd, $J = 1$, 17 Hz, 1H), 4.17 (q, $J = 7$ Hz, 2H), 3.51 (s, 2H); 1.27 (t, $J = 7$ Hz, 3H), 1.26 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 205.1, 167.4, 141.3, 115.4, 61.2, 51.4, 44.6, 23.3(2C), 14.2. HRMS (EI) m/z 184.1107 (M^+) (calcd for $C_{10}H_{16}O_3$: 184.1099).

Ethyl 2-Chloro-3-oxo-4,4-dimethyl-5-hexenoate (11). A 10.25 g (55.6 mmol) amount of **5** in 40 mL of $CHCl_3$ was treated with 4.7 mL (58.4 mmol) of sulfuryl chloride at 4 °C. The reaction mixture was stirred for 30 min at room temperature and then refluxed for 2 h. On cooling, the clear solution was washed with water, 5% $NaHCO_3$, water, and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo. The residual oil was purified by column chromatography on silica (3.2 × 20 cm) using hexane−EtOAc (400:1 to 75:1) as an eluant to give an oil of **11** (9.39 g, 77%): IR ($CHCl_3$) 1725, 1760 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.96 (dd, J = 11, 17 Hz, 1H), 5.294 (d, J = 11 Hz, 1H), 5.288 (d, J = 17 Hz, 1H), 5.19 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 1.35 (s, 3H), 1.33 (s, 3H), 1.29 (t, J = 7 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 201.1, 165.1, 140.5, 116.5, 63.0, 55.2, 51.6, 23.7, 23.4, 13.9. HRMS (EI) m/z 218.0698 (M^+) (calcd for $C_{10}H_{15}O_3Cl$: 218.0710).

Ethyl 5-(1,1-Dimethyl-2-propenyl)imidazole-4-carboxylate (12). A mixture of 7.83 g (35.81 mmol) of **11**, 14.2 mL (0.358 mol) of formamide, and 1.29 mL (71.6 mmol) of water was refluxed at 145 °C for 4 h. On cooling, to the reaction mixture was added 100 mL of $CHCl_3$, and it was washed with 10% Na_2CO_3 and saturated NaCl three times, dried over Na_2SO_4, and concentrated in vacuo. The residual oil was purified by column chromatography on silica (3.2 × 20 cm) using $CHCl_3$−MeOH (50:1) as an eluant to give 4.29 g (48%) of a white solid **12**: mp 88−90 °C; IR ($CHCl_3$) 1705 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.82 (br, 1H), 7.54 (s, 1H), 6.28 (dd, J = 10, 18 Hz, 1H), 5.08 (dd, J = 1, 18 Hz, 1H), 5.05 (dd, J = 1, 10 Hz, 1H), 4.33 (q, J = 7 Hz, 2H), 1.57 (s, 6H), 1.34 (t, J = 7 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 161.3, 149.1, 145.2, 133.3, 122.6, 111.8, 60.6, 38.5, 26.6 (2C), 14.4. HRMS (EI) m/z 208.1221 (M^+) (calcd for $C_{11}H_{16}N_2O_2$: 208.1212). Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74, N, 13.45. Found: C, 63.38, H, 7.82, N, 13.28.

(5-(1,1-Dimethyl-2-propenyl)imidazol-4-yl)methanol (13). To a solution of **12** (3.0 g, 14.4 mmol) in toluene (60 mL) was added 47.5 mL of 1.0 M DIBALH dropwise in toluene at −50 °C under argon atmosphere, and the mixture was stirred, allowing the temperature rise up to −20 °C for 1 h. This solution was recooled to −30 °C, and then 30 mL of a mixture of MeOH and AcOH (2:1) was added slowly. After the mixture was stirred for 30 min at 0 °C, 50 mL of water, 50 mL of EtOAc, and 10% Na_2CO_3 up to pH 10 were added, and the resulting precipitate was removed by filtration. To the filtrate was added NaCl up to saturation, and the organic layer was washed with 10% Na_2CO_3 (once) and saturated NaCl (three times), dried over Na_2SO_4, and concentrated in vacuo. The residual solid was recrystallized from ether to obtain 1.18 g (50%) of white crystals **13**: mp 171−173 °C; IR (KBr) 3460, 2980, 1460, 1015 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 7.49 (s, 1H), 6.11 (dd, J = 11, 17 Hz, 1H), 5.04 (dd, J = 1, 17 Hz, 1H), 5.02 (dd, J = 1, 11 Hz, 1H), 4.58 (s, 2H), 1.44 (s, 6H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 147.6, 138.5, 134.0, 131.4, 111.4, 57.2, 39.0, 28.4 (2C). HRMS (EI) m/z 166.1105 (M^+) (calcd for $C_9H_{14}N_2O$: 116.1106). Anal. Calcd for $C_9H_{14}N_2O$: C, 65.03; H, 8.49, N, 16.85. Found: C, 65.20, H,8.67, N, 16.56.

5-(1,1-Dimethyl-2-propenyl)imidazole-4-carboxaldehyde (3). To a solution of **13** (0.3 g, 1.80 mmol) in acetone (70 mL) was added 1.6 g of MnO_2, and the mixture was stirred at room temperature for 1 h. After filtration to remove MnO_2, the solvent was removed by evaporation, and the residual white powder was purified by column chromatography on silica (1.6 × 20 cm) using $CHCl_3$−MeOH (200:1 to 25:1) as an eluant to give 280 mg (95%) of white crystalline **12**: mp 109−111 °C; IR ($CHCl_3$) 1650, 1340 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.01 (br, 1H), 7.80 (s, 1H), 6.22 (dd, J = 11, 18 Hz, 1H), 5.20 (dd, J = 1, 18 Hz, 1H), 5.16 (dd, J = 1, 11 Hz, 1H), 1.56 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 181.5, 156.8, 146.2, 137.4, 128.6, 112.3, 39.8, 28.5 (2C). HRMS (EI) m/z 164.0944 (M^+) (calcd for $C_9H_{12}N_2O$: 164.0950). Anal. Calcd for $C_9H_{12}N_2O$: C, 65.83; H, 7.37, N, 17.06, Found: C, 65.65, H, 7.40, N, 16.73.

(−)-Phenylahistin (1). To a mixture solution of **3** (100 mg, 0.61 mmol) and diacetyl-cyclo(l-Phe-Gly)[5] (351 mg, 1.28 mmol) in dry DMF (5 mL) was added Cs_2CO_3 (198 mg, 0.61 mmol), and the mixture was stirred at room temperature for 16 h. To the reaction mixture was added 5 mL of 28% aqueous NH_4OH. After being stirred at room temperature for 12 h, the reaction mixture was neutralized with AcOH at 4 °C, and the resultant solution was purified by preparative reverse phase (RP) HPLC with a column (μBondasphere 5C_{18} 100 A, 19 × 150 mm) employing a linear gradient from 24 to 33% CH_3CN in 0.1% aqueous TFA over 18 min at a flow rate of 12 mL/min to give 60 mg (21%) of **1** (TFA salt) as a white fluffy powder. However, since almost complete racemization was observed in purified **1**, the (−)-form of **1** was isolated by HPLC using a chiral column (CHIRALCEL OD, 10 × 250 mm) eluted with a mixture of hexane and ethanol (5:1) at a flow rate of 3.0 mL/min. 7% yield (15 mg) from **3**: white solid, mp 229−231 °C; $[\alpha]^{23}_D$ −268° (c = 0.1, MeOH); IR ($CHCl_3$) 1670, 1640, 1430 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.0 (br s, 1H, NH), 8.96 (br s, 1H, NH), 7.55 (s, 1H), 7.22−7.38 (m, 5H), 6.88 (s, 1H), 6.03 (dd, J = 11, 17 Hz, 1H), 5.69 (br s, 1H, NH), 5.21 (dd, J = 1, 11 Hz, 1H), 5.16 (dd, J = 1, 17 Hz, 1H), 4.33 (ddd, J = 2, 3, 10 Hz, 1H), 3.47 (dd, J = 3, 14 Hz, 1H), 2.93 (dd, J = 10, 14 Hz, 1H), 1.51 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 159.9, 144.6, 136.6, 135.5, 132.3 (2C), 129.5, 129.1, 127.4, 123.9, 113.5, 105.3, 57.2, 41.3, 37.6, 27.9 (2C). HRMS (EI) m/z 350.1736 (M^+) (calcd for $C_{20}H_{22}N_4O_2$: 350.1743). Anal. Calcd for $C_{20}H_{22}N_4O_2 \cdot CF_3COOH \cdot H_2O$: C, 54.77; H, 5.22, N, 11.61. Found: C, 54.62, H, 5.00, N, 11.23.

Aurantiamine (2). This compound was prepared according to the same procedure described for **1** starting from aldehyde **3** (100 mg) and diacetyl-cyclo (L-Val-Gly) in the presence of Cs_2CO_3. After the reaction, the resultant solution was purified using RP-HPLC with the same conditions described for the purification of **1** except for a column (YMC-Pac ODS-AM 120 A, 20 × 250 mm) and a linear gradient from 21 to 24% CH_3CN over 12 min to give 89 mg (35%) of **2** (TFA salt) as a white fluffy powder. Since purified **2** contained 31% of a racemized compound with D-valine (38% ee), further purification by HPLC with a chiral column was performed using the same procedure described for the purification of **1** with a mixture of hexane and ethanol (8:1) as an eluant. 20% (36 mg) yield from **3**; white solid, mp 237−238 °C; $[\alpha]^{24}_D$ −111° (c = 0.1, MeOH); IR ($CHCl_3$) 1670, 1640, 1430 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 12.0 (br s, 1H, NH), 9.07 (br s, 1H, NH), 7.55 (s, 1H), 6.93 (s, 1H), 6.04 (dd, J = 11, 17 Hz, 1H), 6.00(br s, 1H, NH), 5.21 (d, J = 11 Hz, 1H), 5.17 (d, J = 17 Hz, 1H), 4.05 (dd, J = 2, 3 Hz, 1H), 2.47 (dhept, J = 3, 7 Hz, 1H), 1.52 (s, 6H), 1.06 (d, J = 7 Hz, 3H), 0.96 (d, J = 7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.0, 160.7, 144.6, 136.5. 132.3 (2C), 123.8, 113.5, 105.1, 61.2, 37.6, 33.0, 28.0 (2C), 18.7, 15.9. HRMS (EI) m/z 302.1749 (M^+) (calcd for $C_{16}H_{22}N_4O_2$: 302.1743). Anal. Calcd for $C_{16}H_{22}N_4O_2 \cdot CF_3COOH \cdot 0.5MeOH$: C, 51.39; H, 5.83, N, 12.96. Found: C, 51.72, H, 6.02, N, 13.25.

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Supporting Information Available: Copies of both ^1H and ^{13}C NMR spectra of compounds **1**−**3**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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