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Amino Acids and Peptides. XLVII. Facile Synthesis of Flavacol, Deoxymuta-aspergillic Acid and Optically Active Deoxyaspergillic Acid from Dipeptidyl Aldehydes[1-3)]

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2(1*H*)-Pyrazinone ring-containing natural products, flavacol, deoxymuta-aspergillic acid and finally optically active deoxyaspergillic acid, were easily synthesized by a newly developed procedure for preparation of 2(1*H*)-pyrazinone derivatives from dipeptidyl aldehyde hydrochlorides. The absolute configuration of natural deoxyaspergillic acid was synthetically determined as *S*.

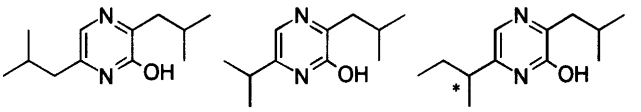
Key words 2(1*H*)-pyrazinone; optically active deoxyaspergillic acid; total synthesis; dipeptidyl aldehyde

Various 3,6-disubstituted 2(1*H*)-pyrazinones, such as flavacol **1**, deoxymuta-aspergillic acid **2** and deoxyaspergillic acid **3** (Fig. 1) have been found in secondary metabolites of *Aspergillus*[4,5)] and *Streptomyces*.[6,7)] Because of their structural relationship to aspergillic acid, which exhibits antibiotic and smooth muscle relaxation activity, they have been of interest to both organic chemists and biochemists.[8)] Therefore, extensive efforts have been directed toward the development of synthetic methods for 2(1*H*)-pyrazinone derivatives. Various synthetic procedures have been developed, but some inherent problems remain to be resolved.[9,10)]

Previously, **1** was synthesized through D,L-leucyl-D,L-leucyl anhydride according to the reported method[10)] and **2**[11,12)] was synthesized by condensation of α-oxiimino-isovaleraldehyde with α-amino-γ-methylvaleronitrile (D,L-leucyl nitrile), followed by reduction with sodium hydrosulfide.[13)] Several attempts to synthesize **3** have been made.[14-16)] However, the methods are complicated, and the produt was racemic.

Here we present a facile synthesis of **1**, **2** and optically active **3** by a newly developed and convenient method and the determination of the absolute configuration of natural deoxyaspergillic acid.

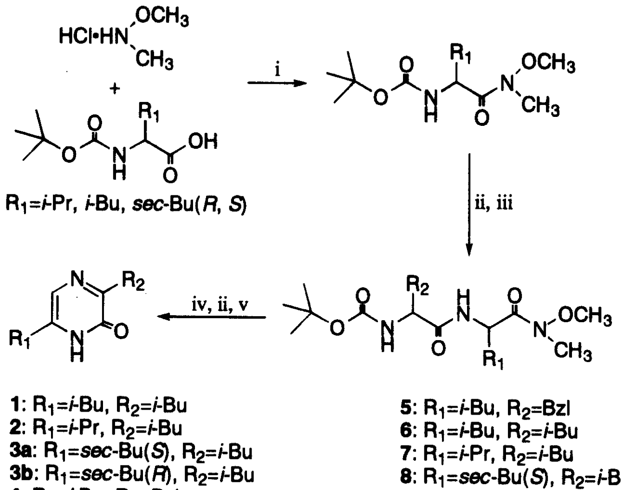
Previously, we reported a simple and convenient synthetic procedure for 2(1*H*)-pyrazinone derivatives from dipeptidyl chloromethyl ketones.[17-19)] Various kinds of dipeptidyl chloromethyl ketones were prepared and converted in good yields to the corresponding 2(1*H*)-pyrazinone derivatives by brief refluxing in MeOH. The corresponding dipeptidyl methyl ketones were also easily converted to 2(1*H*)-pyrazinone derivatives through a different cyclization mechanism from that of the chloromethyl ketones.[19,20)] The above novel method can afford 2(1*H*)-pyrazinone derivatives in which desired substituent(s) can be introduced at position(s) 3 and/or 6. However, position 5 of the 2(1*H*)-pyrazinone derivatives is substituted with a methyl group. We considered that dipeptidyl aldehydes might afford the 2(1*H*)-pyrazinone derivatives without a substituent at position 5 by a similar cyclization mechanism to that of the dipeptidyl methyl ketones. Therefore, we attempted to prepare the 2(1*H*)-pyrazinone derivatives from the dipeptidyl aldehydes. According to Chart 1, Boc–Leu-*N*,*O*-dimethylhydroxyamate (DMA) was prepared from Boc–Leu–OH and DMA using the 1,3-dicyclohexylcarbodiimide (DCC)/DMAP procedure.[21)] After removal of the Boc group, the resultant amine was coupled with Boc–Phe–OH by a mixed anhydride procedure to give Boc–Phe–Leu–DMA (**5**). This amide was treated with LiAlH$_4$ to afford Boc–Phe–Leu–H. After removal of the Boc group of this aldehyde with 5.0 N HCl–dioxane, the resultant amine hydrochloride was cyclized to 3-benzyl-6-isobutyl-2(1*H*)-pyrazinone (**4**) by stirring at room temperature in various solvents (DMF, dioxane, MeCN and MeOH) for 13 h. Among them, MeCN was the best solvent. Although the yield was not high, we did not study side reactions. The results showed that, as expected, 2(1*H*)-pyrazinone derivatives could be easily obtained from the dipeptidyl alde-



1 **2** **3**

Fig. 1. Structures of Flavacol **1**, Deoxymuta-aspergillic Acid **2** and Deoxyaspergillic Acid **3**

* indicates an asymmetric carbon atom.



1: R$_1$=*i*-Bu, R$_2$=*i*-Bu
2: R$_1$=*i*-Pr, R$_2$=*i*-Bu
3a: R$_1$=*sec*-Bu(*S*), R$_2$=*i*-Bu
3b: R$_1$=*sec*-Bu(*R*), R$_2$=*i*-Bu
4: R$_1$=*i*-Bu, R$_2$=Bzl

5: R$_1$=*i*-Bu, R$_2$=Bzl
6: R$_1$=*i*-Bu, R$_2$=*i*-Bu
7: R$_1$=*i*-Pr, R$_2$=*i*-Bu
8: R$_1$=*sec*-Bu(*S*), R$_2$=*i*-Bu
9: R$_1$=*sec*-Bu(*R*), R$_2$=*i*-Bu

Chart 1.

Reagents and conditions: (i) DCC, DMAP; (ii) HCl–dioxane; (iii) isobutyl chloroformate, Et$_3$N, Boc-AA-OH(AA: Phe, Leu); (iv) LiAlH$_4$, THF, −15 °C; (v) MeCN, 13 h, r.t.

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Table 1. mp, t_R, $[\alpha]_D$ and MS of Natural and Synthetic Deoxyaspergillic Acid

	Natural **3**	**3a**	**3b**
mp (°C)	83	80—81	80—80.5
t_R (min)$^{a)}$	15.46	15.46	15.46
$[\alpha]_D^{21\,b)}$	+11.8°	+11.3°	−11.2°
MS (M^+)	208	208	208

a) See Experimental for HPLC conditions. *b*) $c=1.0$, $CHCl_3$.

hydes.

This method was applied to the synthesis of naturally occurring 2(1*H*)-pyrazinone derivatives, because our novel method can easily afford 2(1*H*)-pyrazinone derivatives under slightly acidic conditions. It was also considered that this method might be suitable for the synthesis of optically active **3**, because **3** possesses one asymmetric carbon atom (Fig. 1) and this position is easily racemized during heating with alkali.[11)]

First of all, this method was applied to the synthesis of **1** and **2**. Boc–Leu–OH was coupled with H–Leu–DMA by a mixed anhydride procedure to afford Boc–Leu–Leu–DMA (**6**), which was converted to Boc–Leu–Leu–H by treatment with $LiAlH_4$. After removal of the Boc group, the resultant HCl·H–Leu–Leu–H in MeCN was stirred at room temperature for 13 h to afford **1**. The structure of this compound was confirmed by NMR and MS analysis and the melting point was identical with that of natural **1**. Similarly, Boc–Leu–OH was coupled with H–Val–DMA by a mixed anhydride method to give Boc–Leu–Val–DMA (**7**), which was treated with $LiAlH_4$ to give Boc–Leu–Val–H. After removal of the Boc group, the resultant HCl·H–Leu–Val–H in MeCN was stirred at room temperature for 13 h to afford **2**. The structure of this compound was confirmed by NMR and MS analysis and the melting point was identical with that of natural **2**.[12)]

Finally, this new method was applied to the synthesis of optically active **3**. According to Chart 1, Boc–Leu–Ile–DMA (**8**) and Boc–Leu–allo-Ile–DMA (**9**) were prepared. These amides were converted to **3a** and **3b** (*S* and *R* at the asymmetric center of the *sec*-butyl group, respectively). The synthetic **3a** and **3b** exhibited a single peak at the same retention time on HPLC analysis as that of natural **3**. Their physicochemical data are summarized in Table 1. From these results, the absolute configuration of the natural deoxyaspergillic acid could be confirmed as *S*. From the NMR data for **3a** and **3b**, we can deduce that deoxyaspergillic acid might exist as a pyrazinone, rather than pyrazinol, form.[19)] Thus, we were able to develop a facile and racemization-free synthetic procedure for the 2(1*H*)-pyrazinone derivatives (**1**, **2** and **3**) from dipeptidyl aldehydes and have succeeded in the first total synthesis of optically active **3**, as well as determination of the absolute configuration of natural **3** as *S*. Our convenient synthetic procedure should contribute to studies on the structure–antibacterial activity relationship of these compounds.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus without correction. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co.). ^1H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a Bruker AM400 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (δ-value). The *J* values are given in Hz. The ^{13}C signals were assigned with the aid of distortionless enhancement by polarization transfer (DEPT) experiments, and multiplicities are indicated by p (primary), s (secondary), t (tertiary) or q (quaternary). Mass spectra were measured with a Hitachi M-2000 mass spectrometer using the EI technique. For HPLC analysis, the following conditions were employed; solvent: eluent A, 0.05% TFA/H_2O; eluent B, 0.05% TFA/MeCN; 80% A for 5 min, to 20% A for 20 min, 20% A for 5 min, to initial conditions for 10 min; column: Waters Nova-Pak C18 (3.9 × 150 mm), flow rate: 1 ml/min; retention time is reported as t_R. On TLC (Kieselgel G, Merck), Rf^1, Rf^2 and Rf^3 values refer to the systems of $CHCl_3$, AcOEt and EtOH (30 : 19 : 1), AcOEt and hexane (1 : 2), and $CHCl_3$, MeOH and AcOH (90 : 8 : 2), respectively.

Boc–Phe–Leu–DMA (5) A mixed anhydride [prepared from Boc–Phe–OH (2.9 g, 11.0 mmol), Et_3N (1.7 ml, 12.1 mmol) and isobutyl chloroformate (1.44 ml, 11 mmol) as usual] in THF (80 ml) was added to an ice-cold solution of H–Leu–DMA·HCl [prepared from Boc–Leu–DMA[21)] (3.0 g, 11.0 mmol) and 5.0 N HCl–dioxane (13.2 ml, 66.0 mmol) as usual] in DMF (80 ml) containing Et_3N (1.7 ml, 12.1 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water and dried over Na_2SO_4, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt, yield 3.6 g (77.6%), mp 141—145 °C, $[\alpha]_D^{25}$ −20.1° ($c=1.0$, MeOH), Rf^2 0.22. *Anal.* Calcd for $C_{22}H_{35}N_3O_5$: C, 62.7; H, 8.37; N, 9.97. Found: C, 62.8; H, 8.43; N, 9.85.

3-Benzyl-6-isobutyl-2(1*H*)-pyrazinone (4) Lithium aluminium hydride (89.8 mg, 2.37 mmol) was added to a stirred solution of **5** (0.5 g, 1.18 mmol) in tetrahydrofuran (THF) (20 ml). Reduction was complete within 30 min. The product was hydrolyzed with a solution of potassium hydrogen sulfate in water. The resultant aldehyde was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water and dried over Na_2SO_4, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. The Boc group was removed by treatment with 5.0 N HCl–dioxane (1.4 ml, 7.1 mmol) and the resultant amine was dissolved in MeCN (150 ml). This solution was stirred at room temperature for 13 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water and dried over Na_2SO_4, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and purified by silica-gel column chromatography (eluent, $CHCl_3$: AcOEt : EtOH = 30 : 19 : 1), yield 59.8 mg (20.9%), mp 105—107 °C, Rf^1 0.52. ^1H-NMR ($CDCl_3$ 400 MHz) δ: ppm 13.1 (1H, br, NH), 7.39—7.17 (6H, m, 3-$CH_2\underline{Ph}$+5-H), 4.09 (2H, s, 3-$C\underline{H}_2Ph$), 2.37 (2H, d, $J=7.3$ Hz, 6-$C\underline{H}_2CH(CH_3)_2$), 2.06 (1H, m, $J=6.8$ Hz, 6-$CH_2C\underline{H}(CH_3)_2$), 0.98 (6H, d, $J=6.6$ Hz, 6-$CH_2CH(C\underline{H}_3)_2$). ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: ppm 158.2 (q, C-2), 155.5 (q, C-3), 138.5 (q, C-1′), 137.9 (q, C-6), 129.4 (t, C-2′, 6′), 128.3 (t, C-3′, 5′), 126.4 (t, C-4′), 123.4 (t, C-5), 39.6 (s, 6-$\underline{C}H_2CH(CH_3)_2$), 39.4 (s, 3-$\underline{C}H_2Ph$), 28.1 (t, 6-$CH_2\underline{C}H(CH_3)_2$), 22.2 (p, 6-$CH_2CH(\underline{C}H_3)_2$). *Anal.* Calcd for $C_{15}H_{18}N_2O \cdot 0.1H_2O$: C, 73.8; H, 7.51; N, 11.5. Found: C, 73.7; H, 7.43; N, 11.4.

Boc–Leu–Leu–DMA (6) A mixed anhydride [prepared from Boc–Leu–OH (0.72 g, 3.13 mmol), Et_3N (0.48 ml, 3.45 mmol) and isobutyl chloroformate (0.41 ml, 3.13 mmol) as usual] in THF (60 ml) was added to an ice-cold solution of H–Leu–DMA·HCl [prepared from Boc–Leu–DMA[21)] (0.86 g, 3.13 mmol) and 5.4 N HCl–dioxane (3.5 ml, 18.8 mmol) as usual] in DMF (60 ml) containing Et_3N (0.48 ml, 3.45 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $NaHCO_3$ and water and dried over Na_2SO_4, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.84 g (69.3%), mp 173—174 °C, $[\alpha]_D^{25}$ −56.8° ($c=1.0$, MeOH), Rf^1 0.56, Rf^2 0.29. *Anal.* Calcd for $C_{19}H_{37}N_3O_5$: C, 58.9; H, 9.62; N, 10.8. Found: C, 58.7; H, 9.79; N, 10.9.

3-Isobutyl-6-isobutyl-2(1*H*)-pyrazinone (Flavacol) (1) Lithium aluminium hydride (47.0 mg, 1.3 mmol) was added to a stirred solution of **6** (0.25 g, 0.65 mmol) in THF (15 ml). Reduction was complete within 30 min. The product was hydrolyzed with a solution of potassium hydrogen sulfate in water. The resultant aldehyde was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. The Boc group was removed by treatment with 7.2 N HCl–dioxane (0.9 ml, 6.5 mmol) and the resultant amine was dissolved in MeCN (75 ml). This solution was stirred at room temperature for 13 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and purified by flash silica-gel column chromatography (eluent, $\mathrm{CHCl_3}$: AcOEt : EtOH = 30 : 19 : 1), yield 14.5 mg (10.7%), mp 145—146 °C (natural, 144 °C), Rf^1 0.62, Rf^2 0.46, t_R 16.06 min (natural, 16.06 min). ^{1}H-NMR ($\mathrm{CDCl_3}$ 400 MHz) δ: ppm 12.9 (1H, br s, NH), 7.18 (1H, s, 5-H), 2.65 (2H, d, J = 7.1 Hz, 3-$\mathrm{C\underline{H}_2}$–$\mathrm{CH(CH_3)_2}$), 2.39 (2H, d, J = 7.3 Hz, 6-$\mathrm{C\underline{H}_2CH(CH_3)_2}$), 2.21 (1H, nonet, 3-$\mathrm{CH_2C\underline{H}(CH_3)_2}$), 2.09 (1H, nonet, 6-$\mathrm{CH_2C\underline{H}(CH_3)_2}$), 0.98 (6H, d, J = 6.6 Hz, 3-$\mathrm{CH_2CH(C\underline{H}_3)_2}$), 0.97 (6H, d, J = 6.7 Hz, 6-$\mathrm{CH_2CH(C\underline{H}_3)_2}$). ^{13}C-NMR ($\mathrm{CDCl_3}$ 100 MHz) δ: ppm 158.3 (q, C-2), 156.4 (q, C-3), 137.7 (q, C-6), 123.0 (t, C-5), 41.5 (s, 3-$\mathrm{\underline{C}H_2CH(CH_3)_2}$), 39.5 (s, 6-$\mathrm{\underline{C}H_2CH(CH_3)_2}$), 28.2 (t, 3-$\mathrm{CH_2\underline{C}H(CH_3)_2}$), 27.0 (t, 6-$\mathrm{CH_2\underline{C}H(CH_3)_2}$), 22.7 (p, 3-$\mathrm{CH_2CH(\underline{C}H_3)_2}$), 22.2 (p, 6-$\mathrm{CH_2CH(\underline{C}H_3)_2}$). MS m/z: 208 $\mathrm{M^+}$.

Boc–Leu–Val–DMA (7) A mixed anhydride [prepared from Boc–Leu–OH (0.89 g, 3.84 mmol), $\mathrm{Et_3N}$ (0.59 ml, 4.23 mmol) and isobutyl chloroformate (0.5 ml, 4.23 mmol) as usual] in THF (60 ml) was added to an ice-cold solution of H–Val–DMA·HCl [prepared from Boc–Val–DMA[21)] (1.0 g, 3.84 mmol) and 5.4 N HCl–dioxane (4.27 ml, 23.1 mmol) as usual] in DMF (60 ml) containing $\mathrm{Et_3N}$ (0.59 ml, 4.23 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.81 g (57.1%), mp 155—157 °C, $[\alpha]_\mathrm{D}^{25}$ −65.6° (c = 1.0, MeOH), Rf^1 0.77, Rf^3 0.3. *Anal.* Calcd for $\mathrm{C_{18}H_{35}N_3O_5}$: C, 57.9; H, 9.45; N, 11.3. Found: C, 57.6; H, 9.67; N, 11.2.

3-Isobutyl-6-isopropyl-2(1*H*)-pyrazinone (Deoxymuta-aspergillic Acid) (2) Lithium aluminium hydride (50.8 mg, 1.34 mmol) was added to a stirred solution of Boc–Leu–Val–DMA (**7**) (0.25 g, 0.67 mmol) in THF (10 ml). Reduction was complete within 30 min. The product was hydrolyzed with a solution of potassium hydrogen sulfate in water, and the resultant aldehyde was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. The Boc group was removed by treatment with 7.2 N HCl–dioxane (0.93 ml, 6.7 mmol) and the resultant amine was dissolved in MeCN (75 ml). This solution was stirred at room temperature for 13 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from acetone, yield 16.0 mg (11.8%), mp 104—105 °C (lit.[9)] 111 °C), Rf^2 0.44, t_R 11.11 min. ^{1}H-NMR ($\mathrm{CDCl_3}$ 400 MHz) δ: ppm 13.0 (1H, br s, NH), 7.22 (1H, s, 5-H), 2.85 (1H, m, 6-$\mathrm{C\underline{H}(CH_3)_2}$), 2.66 (2H, d, J = 7.1 Hz, 3-$\mathrm{C\underline{H}_2CH(CH_3)_2}$), 2.21 (1H, m, 3-$\mathrm{CH_2C\underline{H}(CH_3)_2}$), 1.34 (6H, d, J = 7.0 Hz, 6-$\mathrm{CH(C\underline{H}_3)_2}$), 0.97 (6H, d, J = 6.7 Hz, 3-$\mathrm{CH_2CH(C\underline{H}_3)_2}$). ^{13}C-NMR ($\mathrm{CDCl_3}$ 100 MHz) δ: ppm 158.4 (q, C-2), 157.1 (q, C-3), 143.6 (q, C-6), 120.2 (t, C-5), 41.5 (s, 3-$\mathrm{\underline{C}H_2CH(CH_3)_2}$), 30.1 (t, 6-$\mathrm{\underline{C}H(CH_3)_2}$), 27.0 (t, 3-$\mathrm{CH_2\underline{C}H(CH_3)_2}$), 22.7 (p, 3-$\mathrm{CH_2CH(\underline{C}H_3)_2}$), 21.1 (p, 6-$\mathrm{CH(\underline{C}H_3)_2}$). MS m/z: 194 $\mathrm{M^+}$.

Boc–Leu–Ile–DMA (8) A mixed anhydride [prepared from Boc–Leu–OH (0.8 g, 3.4 mmol), $\mathrm{Et_3N}$ (0.52 ml, 3.7 mmol) and isobutyl chloroformate (0.45 ml, 3.7 mmol) as usual] in THF (50 ml) was added to an ice-cold solution of H–Ile–DMA·HCl [prepared from Boc–Ile–DMA[21)] (0.92 g, 3.4 mmol) and 6.5 N HCl–dioxane (2.6 ml, 17.0 mmol) as usual] in DMF (50 ml) containing $\mathrm{Et_3N}$ (0.52 ml, 3.7 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.8 g (61.3%), mp 132—134 °C, $[\alpha]_\mathrm{D}^{25}$ −56.2° (c = 1.0, MeOH), Rf^3 0.55. *Anal.* Calcd for $\mathrm{C_{19}H_{37}N_3O_5}$: C, 58.9; H, 9.62; N, 10.8. Found: C, 58.9; H, 9.78; N, 10.9.

3-Isobutyl-6-(*S*)-*sec*-butyl-2(1*H*)-pyrazinone (3a) Lithium aluminium hydride (94.0 mg, 2.6 mmol) was added to a stirred solution of **8** (0.5 g, 1.3 mmol) in THF (30 ml). Reduction was complete within 30 min. The product was hydrolyzed with a solution of potassium hydrogen sulfate in water. Then, the resultant aldehyde was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. The Boc group was removed by treatment with 5.4 N HCl–dioxane (2.4 ml, 13.0 mmol) and the resultant amine was dissolved in MeCN (150 ml). This solution was stirred at room temperature for 13 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from acetone, yield 72.2 mg (26.7%), mp 80—81 °C, $[\alpha]_\mathrm{D}^{25}$ +11.3° (c = 1.0, $\mathrm{CHCl_3}$), Rf^2 0.5, Rf^3 0.63, t_R 15.46 min. ^{1}H-NMR ($\mathrm{CDCl_3}$ 400 MHz) δ: ppm 12.8 (1H, br s, NH), 7.19 (1H, s, 5-H), 2.65 (2H, d, J = 6.8 Hz, 3-$\mathrm{C\underline{H}_2CH(CH_3)_2}$), 2.56 (1H, m, 6-$\mathrm{C\underline{H}(CH_3)CH_2CH_3}$), 2.21 (1H, m, 3-$\mathrm{CH_2C\underline{H}(CH_3)_2}$), 1.82—1.6 (2H, m, 6-$\mathrm{CH(CH_3)C\underline{H}_2CH_3}$), 1.33 (3H, d, J = 7.0 Hz, 6-$\mathrm{CH(C\underline{H}_3)CH_2CH_3}$), 0.97 (6H, d, J = 6.7 Hz, 3-$\mathrm{CH_2CH(C\underline{H}_3)_2}$), 0.91 (3H, d, J = 7.4 Hz, 6-$\mathrm{CH(CH_3)CH_2C\underline{H}_3}$). ^{13}C-NMR ($\mathrm{CDCl_3}$ 100 MHz) δ: ppm 158.4 (q, C-2), 157.0 (q, C-3), 142.6 (q, C-6), 121.2 (t, C-5), 41.6 (s, 3-$\mathrm{\underline{C}H_2}$-$\mathrm{CH(CH_3)_2}$), 37.3 (t, 6-$\mathrm{\underline{C}H(CH_3)CH_2CH_3}$), 28.5 (s, 6-$\mathrm{CH(CH_3)\underline{C}H_2}$-$\mathrm{CH_3}$), 27.0 (t, 3-$\mathrm{CH_2\underline{C}H(CH_3)_2}$), 22.7 (p, 3-$\mathrm{CH_2CH(\underline{C}H_3)_2}$), 18.7 (p, 6-$\mathrm{CH(\underline{C}H_3)CH_2CH_3}$), 11.8 (p, 6-$\mathrm{CH(CH_3)CH_2\underline{C}H_3}$). MS m/z: 208 $\mathrm{M^+}$. *Anal.* Calcd for $\mathrm{C_{12}H_{20}N_2O}$: C, 69.2; H, 9.68; N, 13.5. Found: C, 69.0; H, 9.88; N, 13.3.

Boc–Leu–allo-Ile–DMA (9) A mixed anhydride [prepared from Boc–Leu–OH (1.15 g, 5.0 mmol), $\mathrm{Et_3N}$ (0.84 ml, 6.0 mmol) and isobutyl chloroformate (0.66 ml, 5.0 mmol) as usual] in THF (80 ml) was added to an ice-cold solution of H–allo-Ile–DMA·HCl [prepared from Boc–allo-Ile–DMA (0.92 g, 3.4 mmol) and 5.4 N HCl–dioxane (5.6 ml, 30.0 mmol) as usual] in DMF (80 ml) containing $\mathrm{Et_3N}$ (0.84 ml, 6.0 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from EtOH, yield 0.94 g (48.5%), mp 135—137 °C, $[\alpha]_\mathrm{D}^{25}$ −47.3° (c = 1.0, MeOH), Rf^1 0.78. *Anal.* Calcd for $\mathrm{C_{19}H_{37}N_3O_5}$: C, 58.9; H, 9.62; N, 10.8. Found: C, 58.8; H, 9.89; N, 10.7.

3-Isobutyl-6-(*R*)-*sec*-butyl-2(1*H*)-pyrazinone (3b) Lithium aluminium hydride (47.0 mg, 1.3 mmol) was added to a stirred solution of **9** (0.25 g, 0.65 mmol) in THF (15 ml). Reduction was complete within 30 min. The product was hydrolyzed with a solution of potassium hydrogen sulfate in water. The resultant aldehyde was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration. The Boc group was removed by treatment with 5.4 N HCl–dioxane (1.2 ml, 6.5 mmol) and the resultant amine was dissolved in MeCN (150 ml). This solution was stirred at room temperature for 13 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% $\mathrm{NaHCO_3}$ and water and dried over $\mathrm{Na_2SO_4}$, then the solvent was evaporated. Petroleum ether was added to the residue to afford crystals, which were collected by filtration and recrystallized from acetone, yield 55.7 mg (41.2%), mp 80—80.5 °C, $[\alpha]_\mathrm{D}^{25}$ −11.2° (c = 1.0, $\mathrm{CHCl_3}$), Rf^2 0.5, Rf^3 0.63, t_R 15.46 min. ^{1}H-NMR ($\mathrm{CDCl_3}$ 400 MHz) δ: ppm 12.8 (1H, br s, NH), 7.19 (1H, s, 5-H), 2.65 (2H, d, J = 6.8 Hz, 3-$\mathrm{C\underline{H}_2CH(CH_3)_2}$), 2.56 (1H, m, 6-$\mathrm{C\underline{H}(CH_3)CH_2}$-$\mathrm{CH_3}$), 2.21 (1H, m, 3-$\mathrm{CH_2C\underline{H}(CH_3)_2}$), 1.82—1.6 (2H, m, 6-$\mathrm{CH(CH_3)C\underline{H}_2CH_3}$), 1.33 (3H, d, J = 7.0 Hz, 6-$\mathrm{CH(C\underline{H}_3)CH_2CH_3}$), 0.97 (6H, d, J = 6.7 Hz, 3-$\mathrm{CH_2CH(C\underline{H}_3)_2}$), 0.91 (3H, d, J = 7.4 Hz, 6-$CH(CH_3)CH_2C\underline{H}_3$). ^{13}C-NMR ($CDCl_3$ 100 MHz) δ: ppm 158.3 (q, C-2), 157.1 (q, C-3), 142.6 (q, C-6), 121.2 (t, C-5), 41.6 (s, 3-$\underline{C}H_2CH(CH_3)_2$), 37.3 (t, 6-$\underline{C}H(CH_3)CH_2CH_3$), 28.4 (s, 6-$CH(CH_3)$-$\underline{C}H_2CH_3$), 26.9 (t, 3-$CH_2\underline{C}H(CH_3)_2$), 22.6 (p, 3-$CH_2CH(\underline{C}H_3)_2$), 18.6 (p, 6-$CH(\underline{C}H_3)CH_2CH_3$), 11.7 (p, 6-$CH(CH_3)CH_2\underline{C}H_3$). MS *m*/*z*: 208 M^+. *Anal.* Calcd for $C_{12}H_{20}N_2O$: C, 69.2; H, 9.68; N, 13.5. Found: C, 68.9; H, 9.97; N, 13.2.

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References and Notes

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