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SYNTHESIS OF THE AMIDE ANALOG OF ALTERNARIOLIDE (AM-TOXIN I), A HOST SPECIFIC PHYTOTOXIN FOR APPLE LEAVES†

Mitsuru Sakai, Toshikatsu Okuno, Kimiko Hashimoto*[2], and Haruhisa Shirahama*[1]

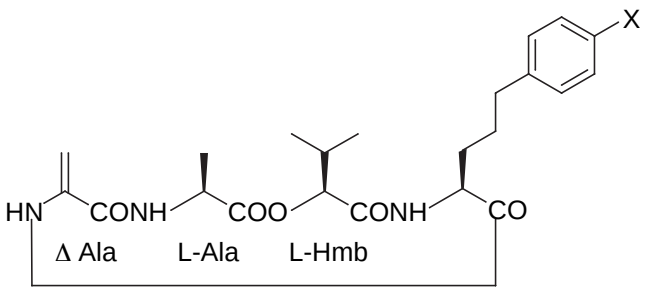
School of Science, Kwansei Gakuin University, Uegahara, Nishinomiya 662-8501, Japan

Department of Biochemistry and Biotechnology, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan

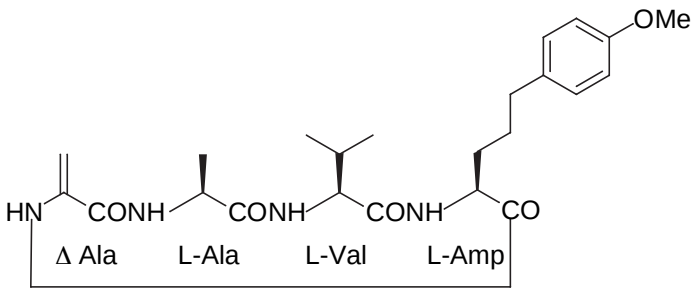
e-mail (H. S.): shiraham@sci.hokudai.ac.jp; (K. H.) kimikoh@postman.riken.go.jp

Abstract - The amide analog of alternariolide (AM-toxin I) was synthesized with high efficiency. The analog showed comparable biological activity to that of alternariolide.

Alternariolide (AM-toxin I, **1**) produced by *Alternaria mali* has been found to be responsible for the necrotic brown spots on certain apple leaves, which is the first example of a host-specific phytotoxin.[1] The fungal pathogen, *Alternaria mali*, produces alternariolide (**1**) and its congeners, AM-toxin II (**2**) and III (**3**), in the infection processes to the host plants. These compounds have been considered to function as host recognition factors.[2]



Alternariolide (AM-toxin I, **1**) : X = OMe
AM-toxin II (**2**) : X = H
AM-toxin III (**3**) : X = OH



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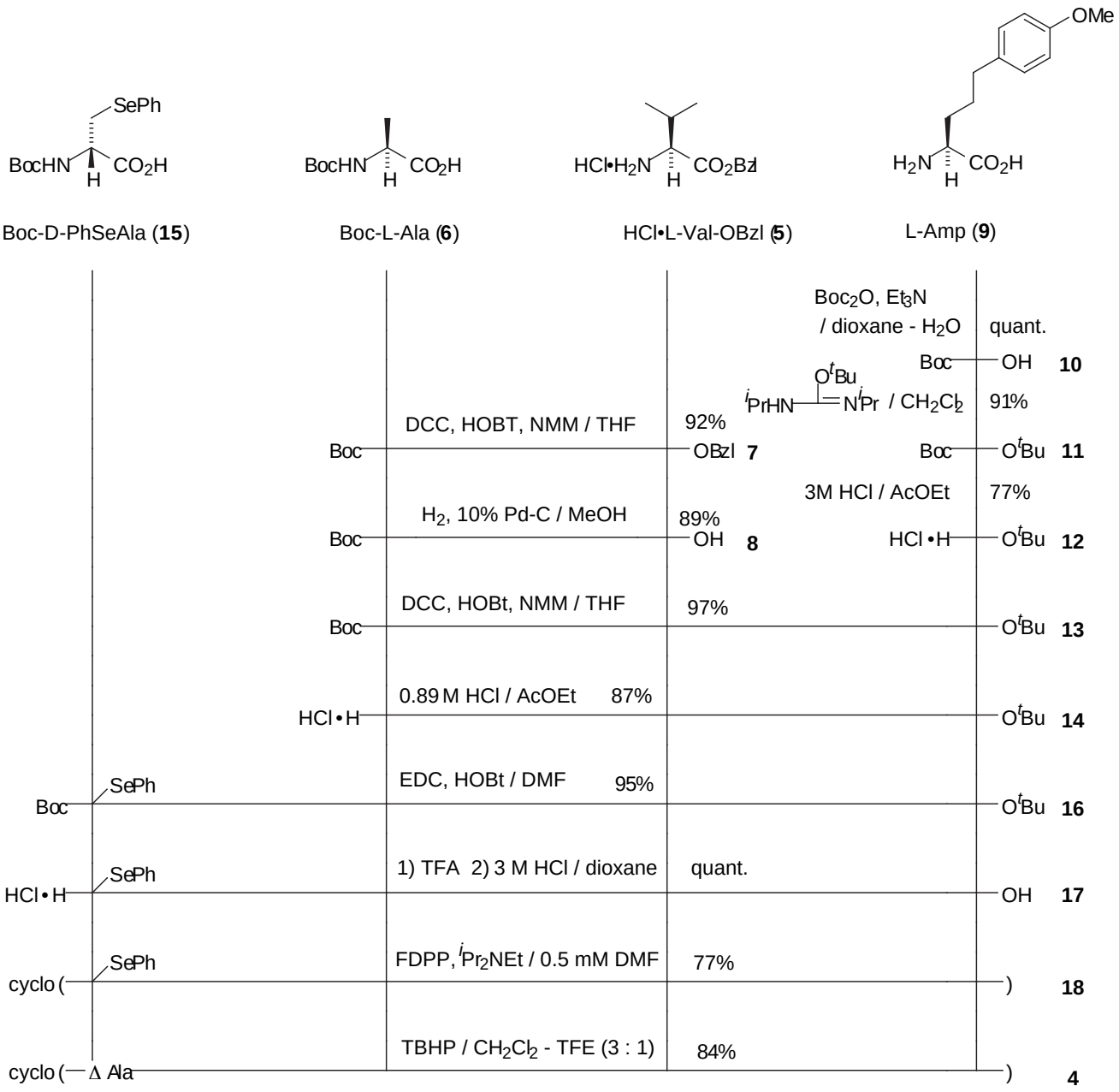
† Dedicated to Professor Yuichi Kanaoka in celebration of his 75th birthday.

Scheme 1



L-Amp : L-2-Amino-5-(4-methoxyphenyl)pentanoic acid
HOBt : 1-Hydroxybenzotriazole
NMM : *N*-Methylmorpholine
TFE : 2,2,2-Trifluoroethanol
FDPP : Pentafluorophenyl diphenylphosphinate

Accordingly, these phytotoxins should be key compounds in revealing the mechanism of the host-specificity. Recently, we developed a new synthetic method for **1** with high efficiency.[1g] To examine the applicability of the method for other derivatives,[3] we planned the synthesis of the amide analog (**4**).[4] One of the structural properties of alternariolide (**1**) is the relatively rigid cyclic structure composed of three amide bonds, one ester bond, and one

dehydroamino acid residue. Changing the ester bond to an amide bond should make its conformation more rigid because of the planarity of the amide bond. Accordingly, the cyclization step is estimated to be harder than that of **1**. Izumiya has previously synthesized the same peptide analog through a similar intermediate.[4a] During the synthesis, he encountered a difficulty in the cyclization step. The intermediate for the peptide analog resists the cyclization compared to the ester analog.

Additionally, the conformation of the alternariolide derivative might be an important factor in the biological activity, because the analogs composed of the reduced form (both L- and D-forms) of the dehydroalanine show weak activities.[5] HCl•L-Val-OBzl (**5**) was synthesized from L-Val as follows; 1) protection of the amine with a Boc group, 2) esterification of the carboxylic acid with benzyl chloride, 3) deprotection of the Boc group under acidic conditions. The HCl salt (**5**) was condensed with Boc-L-Ala (**6**) using DCC, HOBT, and NMM to give peptide (**7**) in 92% yield. Hydrogenolysis of the benzyl ester of the peptide (**7**) gave carboxylic acid (**8**) in 89% yield. A non-proteinogenic amino acid, L-Amp (**9**), was synthesized according to Shimohigashi's report[6] with slight modifications. For the condensation of L-Amp (**9**) with the peptide (**8**), the protecting groups of **9** were arranged as follows; 1) protection of the amino group as the Boc carbamate giving **10**, 2) esterification of the carboxylic acid using *t*-butylisourea[7] giving *t*-butyl ester (**11**), and 3) deprotection of the Boc group keeping the *t*-butyl ester intact under acidic conditions,[8] affording the HCl salt (**12**). The thus prepared HCl salt (**12**) was combined with **8** to afford tripeptide (**13**) in 97% yield. After removal of the Boc group of **13**, the resulting HCl salt (**14**) was condensed with Boc-D-PhSeAla[9] (**15**) to give tetrapeptide (**16**). The protecting groups of both ends in **16** were removed with TFA, and the resulting TFA salt was changed to a stable HCl salt (**17**). Cyclization of **17** was performed at a concentration of 0.5 mM in DMF with FDPP[10] to give cyclic peptide (**18**) in 77% yield. In our synthesis of alternariolide (**1**), cyclization succeeded at a 4 mM concentration; however, this peptide derivative (**17**) produced its dimer as the main product at the same concentration. At the last stage, the phenylselenyl group was oxidatively eliminated from **18** to produce the peptide analog (**4**) in 84% yield. Thus our synthetic method has been found to be applicable to the more rigid amide derivative. In this synthesis, the key steps are the cyclization and the dehydroalanine formation steps. The yields for these steps in the previous synthesis by the Izumiya group are 19% and 27%, respectively.[4a, 11] On the other hand, our process has been efficiently improved, affording 77% and 84% yields, respectively. The biological activity of **4** on susceptible

apple leaves was comparable to that of alternariolide (**1**), of which the least effective dose was 0.001 μg / mL in our bioassay system.[1h] The ^{1}H NMR spectrum of **4** appears to be more complex than that of **1**,[5] which might be due to the presence of some conformers.[4a] However, the conformational change did not affect the biological activity.

EXPERIMENTAL

HCl•L-Val-OBzl (5)

To a solution of L-Val (10.0 g, 85.5 mmol) in H_2O (100 mL) were added Et_3N (13.1 mL, 94.1 mmol) and a solution of Boc_2O (22.4 g, 103 mmol) in dioxane (100 mL) and the solution was stirred at rt overnight. The solution was concentrated to about half of the volume *in vacuo* and adjusted to pH 2 with 1M HCl. The mixture was extracted with EtOAc (100 mL x 3). The combined organic phase was washed with brine and dried over anhydrous Na_2SO_4. The filtered solution was concentrated *in vacuo* to give Boc-L-Val-OH as a colorless oil (18.5 g, quant). The crude product was used for the next step without further purification. $[\alpha]_D$ -5.3° (*c* 0.94, CH_3CO_2H) [lit.,[13] 5.8° 1.208%, AcOH; mp 77-79 °C]; ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.93 (3H, d, *J* = 6.6 Hz), 1.00 (3H, d, *J* = 6.6 Hz), 1.45 (9H, s), 2.19 (1H, m), 4.04 (*ca.* 1/4H, br), 4.24 (*ca.* 3/4H, m), 5.08 (*ca.* 7/10H, d, *J* = 8.5 Hz), 6.14 (*ca.* 3/10H, br s), 8.40 (1H, br s); IR (cm^{-1}, neat) 3326, 2973, 1688, 1512, 1370, 1169, 1094, 1019, 862, 777.

The crude Boc-L-Val-OH (16.0 g, 73.6 mmol) was dissolved in DMF (160 mL). To the solution were added Et_3N (41.2 mL, 294 mmol) and BzlCl (25.4 mL, 221 mmol) at 0 °C and the mixture was stirred overnight at rt overnight. The resulting solid (Et_3N•HCl) was filtered off. After addition of H_2O to the solution, the mixture was extracted with EtOAc (150 mL x 3). The combined organic phase was successively washed with 1M HCl (150 mL x 3), H_2O (150 mL x 3), and brine and dried over anhydrous Na_2SO_4. The filtered solution was concentrated *in vacuo* and the residue was chromatographed on silica gel (20% EtOAc-hexane) to give ester (19.2 g, 85%) as a colorless oil. $[\alpha]_D$ -2.7° (*c* 1.03, $CHCl_3$) [lit.,[14] -33.3°, *c* 2, MeOH]; ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.85 (3H, d, *J* = 6.8 Hz), 0.94 (3H, d, *J* = 6.8 Hz), 1.44 (9H, s), 2.15 (1H, m), 4.27 (1H, dd, *J* = 4.6, 8.6 Hz), 5.13 (1H, d, *J* = 12.4 Hz), 5.21 (1H, d, *J* = 12.4 Hz), 7.35 (5H, s); IR (cm^{-1}, neat) 3378, 2971, 1717, 1505, 1368, 1248, 1157, 1090, 1015, 750.

Boc-L-Val-OBzl (21.5 g, 69.9 mmol) was dissolved in TFA (200 mL) and the solution was stirred for 1 h at rt. After evaporation of TFA, the residue was dissolved in a solution of 3M HCl in dioxane (46 mL, 138 mmol) and to the

mixture was added Et_2O for crystallization. The resulting colorless needles were collected to give **5** (14.4 g, 85%). $[\alpha]_D$ -4.6° (*c* 1.01, MeOH) [lit.,[15] -10.6° (*c* 2, 1N HCl), lit.,[16] 9.1° (*c* 3, pyridine)]; mp 138 °C (recryst. from Et_2O - MeOH) [lit.,[15] 142-143 °C, lit.,[16] 138-139 °C]; ^{1}H NMR (400 MHz, D_2O, HOD = 4.65 ppm) δ 0.82 (3H, d, *J* = 8.2 Hz), 0.84 (3H, d, *J* = 8.2 Hz), 2.20 (1H, m), 3.90 (1H, d, *J* = 4.6 Hz), 5.13 (1H, d, *J* = 12.0 Hz), 5.20 (1H, d, *J* = 12.0 Hz), 7.31 (5H, m); IR (cm^{-1}, KBr disk) 2971, 2863, 1742, 1586, 1503, 1223, 1105, 1042, 741, 698.

Boc-L-Ala-L-Val-OBzl (7)

To a suspension of **5** (10.0 g, 41.0 mmol) in THF (250 mL) were added *N*-methylmorpholine (4.51 mL, 41.0 mmol), Boc-L-Ala-OH (**6**, 7.76 g, 41.0 mmol), HOBT (6.10 g, 45.1 mmol), and DCC (9.31 g, 45.1 mmol) at 0 °C. The mixture was stirred for 30 min at 0 °C and at rt overnight. The resulting precipitates were filtered off and the mother liquor was concentrated to dryness. To the residue was added a small amount of EtOAc and the resulting insoluble urea was again filtered off. The solution was concentrated *in vacuo* and the residue was chromatographed on silica gel (20% EtOAc-hexane) to give **7** (14.2 g, 92%) as a colorless oil. $[\alpha]_D$ -36.5° (*c* 0.92, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.86 (3H, d, *J* = 6.8 Hz), 0.91 (3H, d, *J* = 6.8 Hz), 1.34 (3H, d, *J* = 7.1 Hz), 1.44 (9H, s), 2.20 (1H, m), 4.19 (1H, m), 4.58 (1H, dd, *J* = 4.9, 8.0 Hz), 5.03 (1H, d, *J* = 4.9 Hz), 5.13 (1H, d, *J* = 12.2 Hz), 5.20 (1H, d, *J* = 12.2 Hz), 6.70 (1H, d, *J* = 8.0 Hz), 7.35 (5H, m); IR (cm^{-1}, neat) 3322, 2973, 1738, 1667, 1522, 1368, 1250, 1171, 752, 698; EI-HR-MS 378.2153 $[M]^+$ calcd for $C_{20}H_{30}N_2O_5$ 378.2154.

Boc-L-Ala-L-Val-OH (8)

In a round-bottomed flask was placed 10% Pd/C (3.9 g). After addition of small amount of H_2O, air in the flask was replaced with argon. To the flask was added **7** (14.0 g, 37.0 mmol) in MeOH (140 mL). After replacement of the argon with H_2, the mixture was stirred at rt overnight. The mixture was carefully filtered through a pad of Celite with suction. The resulting solution was evaporated *in vacuo* to give crude carboxylic acid (**8**) (9.49 g, 89%) as a colorless amorphous powder. $[\alpha]_D$ +8.6° (*c* 1.00, $CHCl_3$); mp 155 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.94 (3H, d, *J* = 6.7 Hz), 0.97 (3H, d, *J* = 6.7 Hz), 1.35 (3H, d, *J* = 7.1 Hz), 1.44 (9H, s), 2.25 (1H, m), 4.26 (1H, m), 4.56 (1H, dd, *J* = 4.9, 9.1 Hz), 5.23 (1H, br s), 6.96 (1H, d, *J* = 9.1 Hz); IR (cm^{-1}, KBr disk) 3364, 2978, 1707, 1634, 1553, 1507, 1252,

1171, 1073, 1024; EI-HR-MS 288.1686 $[M]^+$ calcd for $C_{13}H_{24}N_2O_5$ 288.1685.

Boc-L-Amp-OH (10)

L-Amp (**9**) was prepared according to Shimohigashi's report with slight modification. To a solution of L-Amp (**9**, 5.17 g, 23.2 mmol) in H_2O (52 mL) were added Et_3N (3.56 mL, 25.5 mmol) and a solution of Boc_2O (6.06 g, 27.8 mmol) in dioxane (52 mL) and the solution was stirred at rt overnight. The solution was concentrated to about half of the volume *in vacuo* and adjusted to pH 2 with 1M HCl. The mixture was extracted with EtOAc (50 mL x 3). The combined organic phase was washed with brine and dried over anhydrous Na_2SO_4. The filtered solution was concentrated *in vacuo* to give Boc-L-Amp-OH (**10**) as a colorless oil (7.48 g, quant). The crude product was used for the next step without further purification. $[\alpha]_D$ +5.9° (*c* 0.88, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 1.42 (9H, s), 1.66 (3H, m), 1.85 (1H, m), 2.55 (2H, m), 3.77 (3H, s), 4.28 (1H, m), 5.11 (1H, br s), 5.38 (1H, br s), 6.80 (2H, d, *J* = 8.3 Hz), 7.06 (2H, d, *J* = 8.3 Hz); IR (cm^{-1}, neat) 3343, 2978, 2936, 1713, 1512, 1368, 1246, 1173, 1036, 831; EI-HR-MS 323.1751 $[M]^+$ calcd for $C_{17}H_{25}NO_5$ 323.1733.

Boc-L-Amp-O^{t}Bu (11)

To a solution of **12** (4.4 g, 13.6 mmol) in CH_2Cl_2 (30 mL) was added *O-t*-butyl-*N,N'*-diisopropylisourea (4.08 g, 20.4 mmol) at rt and the solution was heated under reflux overnight. To the mixture was added additional amount of *O-t*-butyl-*N,N'*-diisopropylisourea (3.81 g, 19.0 mmol) and the mixture was refluxed for 7 h. After cooling to rt, the resulting insoluble urea was filtered off and the mother liquor was concentrated *in vacuo*. To the residue was added a mixture of EtOAc and hexane (1:9) and the insoluble material was again removed. The solution was concentrated and the residue was chromatographed on silica gel (10% EtOAc-hexane) to afford ester (**13**) (4.69 g, 91%) as a colorless oil. $[\alpha]_D$ +11.6° (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 1.44 (18H, s), 1.68 (4H, m), 2.57 (2H, m), 3.78 (3H, s), 4.20 (1H, m), 5.00 (1H, d, *J* = 8.1 Hz), 6.82 (2H, d, *J* = 8.5 Hz), 7.07 (2H, d, *J* = 8.5 Hz); IR (cm^{-1}, neat) 2978, 2934, 1717, 1512, 1458, 1368, 1248, 1155, 1038, 845; EI-HR-MS 379.2358 $[M]^+$ calcd for $C_{21}H_{33}NO_5$ 379.2359.

HCl•L-Amp-O^{t}Bu (12)

Boc-L-Amp-O^{t}Bu (**11**, 4.74 g, 12.5 mmol) was dissolved in 0.89 M HCl / EtOAc (70 mL, 62.3 mmol) and the solution

was stirred for 5h at rt. The solution was concentrated *in vacuo* and the residue was washed with Et_2O to afford HCl salt (**12**, 3.04 g, 77%) as colorless crystals. $[\alpha]_D$ +12.3° (*c* 0.52, MeOH); mp 143 °C (recryst from MeOH-Et_2O); ^1H NMR (400 MHz, D_2O, HOD = 4.65 ppm) δ 1.32 (9H, s), 1.49 (1H, m), 1.64 (1H, m), 1.77 (2H, m), 2.52 (2H, m), 3.69 (3H, s), 3.86 (1H, t, *J* = 5.3 Hz), 6.85 (2H, d, *J* = 8.8 Hz), 7.11 (2H, d, *J* = 8.8 Hz); IR (cm^{-1}, nujol) 2924, 2855, 1732, 1510, 1244, 1157; FAB-HR-MS 280.1888 $[M+H]^+$ calcd for $C_{16}H_{26}N O_3$ 280.1913.

Boc-L-Ala-L-Val-L-Amp-O^{t}Bu (13)

To a suspension of **12** (675 mg, 2.14 mmol) in THF (15 mL) were added *N*-methylmorpholine (0.24 mL, 2.14 mmol), dipeptide (**8**, 616 mg, 2.14 mmol), and HOBt (318 mg, 2.35 mmol) and the solution was cooled to 0°C. To the solution was added DCC (485 mg, 2.35 mmol) and the mixture was stirred for 30 min at 0°C and then at rt overnight. The resulting insoluble urea was filtered off and the mother liquor was concentrated to dryness. The residue was chromatographed on silica gel (1% EtOAc-$CHCl_3$) to give tripeptide (**13**, 1.14 g, 97%) as colorless crystals. $[\alpha]_D$ -34.0° (*c* 1.01, $CHCl_3$); mp 130-131 °C (recryst. from $CHCl_3$-pet ether); ^1H NMR (400 MHz, $CDCl_3$, TMS = 0 ppm) δ 0.92 (3H, d, *J* = 6.8 Hz), 0.94 (3H, d, *J* = 6.8 Hz), 1.32 (3H, d, *J* = 7.1 Hz), 1.43 (18H, s), 1.59 (2H, m), 1.83 (2H, m), 2.15 (1H, m), 2.54 (2H, m), 3.78 (3H, s), 4.18 (1H, br s), 4.28 (1H, m), 4.48 (1H, dd, *J* = 6.1, 13.4 Hz), 5.11 (1H, br s), 6.55 (1H, br s), 6.81 (3H, m), 7.05 (2H, d, *J* = 8.5 Hz); IR (cm^{-1}, KBr disk) 3318, 2976, 1642, 1514, 1456, 1248, 1040, 845, 700, 652; EI-HR-MS 549.3416 $[M]^+$ calcd for $C_{29}H_{47}N_3O_7$ 549.3414.

HCl•L-Ala-L-Val-L-Amp-O^{t}Bu (14)

Tripeptide (**13**, 4.5 g, 8.19 mmol) was dissolved in 0.89 M HCl / EtOAc (46 mL, 40.9 mmol) and the mixture was stirred for 5h at rt. When the solution became turbid, a small amount of *i*-PrOH was added. The solution was concentrated *in vacuo* and the residue was washed with Et_2O to afford HCl salt (**14**, 3.48 g, 87%) as a colorless amorphous powder. $[\alpha]_D$ -22.0° (*c* 0.30, MeOH); mp 179-180 °C (recryst .from MeOH-Et_2O); ^1H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.86 (3H, d, *J* = 6.8 Hz), 0.89 (3H, d, *J* = 6.8 Hz), 1.29 (3H, d, *J* = 6.8 Hz), 1.35 (9H, s), 1.59 (4H, m), 1.98 (1H, m), 2.49 (2H, m), 3.70 (3H, s), 3.93 (1H, m), 4.07 (1H, m), 4.24 (1H, m), 6.82 (2H, d, *J* = 8.5 Hz), 7.06 (2H, d, *J* = 8.5 Hz), 8.24 (3H, br s), 8.31 (1H, d, *J* = 7.1 Hz), 8.46 (1H, m); IR (cm^{-1}, nujol) 3287, 2924, 2855,

1736, 1651, 1512, 1248, 1155; FAB-HR-MS 450.2984 $[M+H]^+$ calcd for $C_{24}H_{40}N_3O_5$ 450.2968.

Boc-D-PhSeAla-L-Ala-L-Val-L-Amp-O^{t}Bu (16)

To a solution of Boc-D-PhSeAla (**15**, 2.41 g, 7.00 mmol) in DMF (60 mL) were added tripeptide (**14**, 3.40 g, 7.00 mmol) and HOBT (1.04 g, 7.70 mmol). To the cooled solution at -5 °C was added EDC (1.34 mL, 7.35 mmol). The solution was degassed under reduced pressure and filled with argon. After stirring 30 min at -5 °C, the solution was stirred at rt overnight. To the solution was added cooled H_2O and the resulting precipitate was collected. The crude crystals were recrystallized from EtOAc-hexane to give tetrapeptide (**16**, 5.42 g, 95%). $[\alpha]_D$ +12.9° (*c* 0.75, DMF); mp 199 °C (recryst .from $CHCl_3$-hexane); ^{1}H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.82 (3H, d, *J* = 6.6 Hz), 0.85 (3H, d, *J* = 6.6 Hz), 1.13 (3H, d, *J* = 7.1 Hz), 1.35 (9H, s), 1.36 (9H, s), 1.58 (4H, m), 1.95 (1H, m), 2.47 (2H, m), 3.06 (1H, m), 3.22 (1H, m), 3.69 (3H, s), 4.10 (1H, m), 4.18 (2H, m), 4.31 (1H, m), 6.81 (2H, d, *J* = 8.4 Hz), 7.02 (1H, d, *J* = 9.0 Hz), 7.06 (2H, d, *J* = 8.4 Hz), 7.27 (3H, m), 7.48 (2H, m), 7.75 (1H, d, *J* = 9.5 Hz), 8.03 (1H, d, *J* = 7.8 Hz), 8.15 (1H, d, *J* = 7.3 Hz) ; IR (cm^{-1}, nujol) 3295, 2924, 2855, 1711, 1634, 1512, 1246, 1163; FAB-HR-MS 777.3357 $[M+H]^+$ calcd for $C_{38}H_{57}N_4O_8{}^{80}Se$ 777.3342.

HCl•D-PhSeAla-L-Ala-L-Val-L-Amp-OH (17)

Tetrapeptide (**16**, 3.86 g, 4.98 mmol) was dissolved in TFA (38 mL) and the solution was stirred for 2 h at rt. After evaporation, the residue was dissolved in 3M HCl / dioxane (3.5 mL, 10.5 mmol). To the solution was added Et_2O and the resulting precipitate was collected to afford HCl salt (**17**, 3.26 g, quant) as a colorless amorphous powder. $[\alpha]_D$ -35.9° (*c* 0.85, DMF); mp 134-135 °C (recryst. from MeOH-Et_2O); ^{1}H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.83 (3H, d, *J* = 6.7 Hz), 0.85 (3H, d, *J* = 6.7 Hz), 1.06 (3H, d, *J* = 6.8 Hz), 1.61 (4H, m), 1.96 (1H, m), 2.46 (2H, m), 3.34 (2H, m), 3.69 (3H, s), 4.08 (1H, m), 4.17 (2H, m), 4.33 (1H, m), 6.81 (2H, d, *J* = 8.6 Hz), 7.06 (2H, d, *J* = 8.6 Hz), 7.30 (3H, m), 7.53 (2H, m), 8.07 (1H, d, *J* = 9.2 Hz), 8.14 (1H, d, *J* = 7.6 Hz), 8.72 (1H, d, *J* = 7.2 Hz) ; IR (cm^{-1}, nujol) 3272, 2924, 2855, 1640, 1545, 1512, 1246, 1177; FAB-HR-MS 621.2211 $[M+H]^+$ calcd for $C_{29}H_{41}N_4O_6{}^{80}Se$ 621.2191.

Cyclo(D-PhSeAla-L-Ala-L-Val-L-Amp) (18)

HCl salt (**17**, 400 mg, 0.610 mmol) was dissolved in DMF (1.2 mL). To the solution were added *i*-Pr_2NEt (0.44 mL, 2.50 mmol) and FDPP (472 mg, 1.22 mmol). The solution was degassed under reduced pressure and filled with argon. After stirring for 5 h at rt, the solution was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$-TFE and the solution was successively washed with 1M HCl (10 mL x 1), sat. $NaHCO_3$ (10 mL x 1), and brine. The solution was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was washed with Et_2O and recrystallized from $CHCl_3$-TFE-Et_2O to afford cyclic peptide (**18**, 284 mg, 77%) as colorless crystals. $[\alpha]_D$ -102.8° (*c* 0.13, DMF); mp 309-310 °C (decomp); ^{1}H NMR (400 MHz, DMF-d_7, DMF-C<u>H</u>O = 8.01 ppm) δ 0.89 (6H, m), 1.34 (3H, d, *J* = 7.3 Hz), 1.65 (4H, m), 2.10 (1H, m), 2.55 (2H, m), 3.07 (1H, m), 3.28 (1H, m), 3.76 (3H, s), 3.81 (1H, d, *J* = 9.3 Hz), 4.33 (2H, m), 4.62 (1H, m), 6.85 (2H, d, *J* = 7.8 Hz), 7.12 (2H, d, *J* = 7.8 Hz), 7.29 (3H, m), 7.54 (2H, m), 7.63 (1H, d, *J* = 9.3 Hz), 7.86 (1H, d, *J* = 9.8 Hz), 8.00 (2H, m) ; IR (cm^{-1}, nujol) 3285, 2924, 2855, 1651, 1536, 1512, 1246, 1036; EI-HR-MS 602.2030 $[M]^+$ calcd for $C_{29}H_{38}N_4O_5{}^{80}Se$ 602.2007, 600.2006 $[M]^+$ calcd for $C_{29}H_{38}N_4O_5{}^{78}Se$ 602.2016.

Cyclo(ΔAla-L-Ala-L-Val-L-Amp) (4)

To a solution of cyclic peptide (**18**, 39.8 mg, 0.0662 mmol) in CH_2Cl_2 (6 mL) and TFE (2 mL) was slowly added a solution of TBHP in CH_2Cl_2 (4.03 M, 0.48 mL, 1.93 mmol) at -5 °C. After stirring for 6 h at rt, the mixture was extracted with $CHCl_3$ (10 mL x 3). The combined extracts were washed with sat. $NaHCO_3$ (10 mL x 3) and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was washed with Et_2O and chromatographed on silica gel (5% *i*-PrOH-$CHCl_3$) to give dehydropeptide (**4**, 24.7 mg, 84%) as colorless crystals. $[\alpha]_D$ -176.1° (*c* 0.055, DMF); mp 199-200 °C (decomp, recryst from $CHCl_3$-hexane)[lit.,[4a] mp 220-223 °C]; ^{1}H NMR (400 MHz, DMF-d_7, DMF-C<u>H</u>O = 8.01 ppm, -20 °C) δ 0.88 (6H, m), 1.13 (3/2H, d, *J* = 6.0 Hz), 1.38 (3/2H, d, *J* = 6.9 Hz), 1.35-2.00 (9/2H, m), 2.15 (1/2H, m), 2.49 (2H, m), 3.53 (1/2H, m), 3.73, 3.74 (total 3H, s each), 3.89 (1/2H, m), 4.37 (1/2H, m), 4.69 (1/2H, m), 4.77 (1/2H, m), 4.86 (1/2H, m), 5.03 (1/2H, d, *J* = 8.3 Hz), 5.71 (1/2H, d, *J* = 20.0 Hz), 6.24 (1/2H, d, *J* = 20.0 Hz), 6.83 (2H, m), 7.10 (2H, m), 7.34 (1/2H, d, *J* = 8.3 Hz), 7.66 (1/2H, d, *J* = 10.4 Hz), 7.81 (1/2H, m), 8.20 (1/2H, d, *J* = 8.5 Hz), 8.61 (1/2H, d, *J* = 9.3 Hz), 8.71 (1/2H, m), 8.75 (1/2H, br s), 8.84 (1/2H, br s), 10.37 (1/2H, br s) ; IR (cm^{-1}, nujol) 3281, 2924, 2855, 1686, 1642, 1512, 1246, 1036; FAB-HR-MS 445.2458 $[M+H]^+$ calcd for $C_{23}H_{33}N_4O_5$ 445.2451.

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*[1] Present address: 2-16, Minami 18 Nishi 8, Chuo-ku, Sapporo, 064-0918, Japan

*[2] To whom correspondence should be addressed. Present address: Lab. of Biochemical Resources, Plant Science Center, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan

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