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SYNTHESIS OF ALTERNARIOLIDE ANALOGS FOR PHOTOAFFINITY-LABELING†

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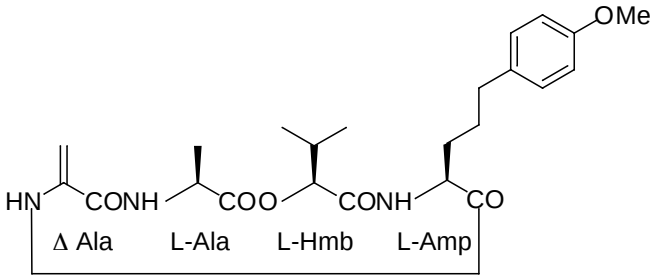
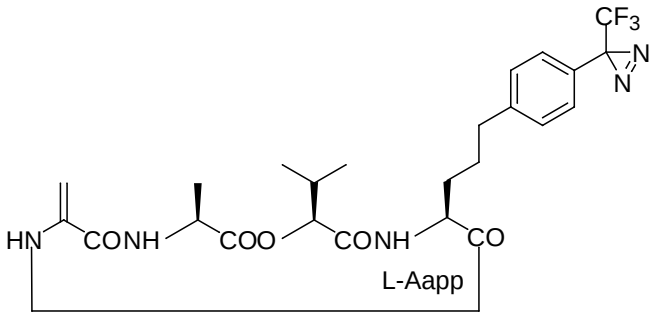
Abstract - Two photoaffinity-labeling analogs of alternariolide were synthesized. These peptides contain the 3-nitrophenyl-3-trifluoromethyldiazirine moiety for easy detection after irradiation experiment.

Alternariolide (AM-toxin I, **1**) is a host-specific phytotoxin produced by *Alternaria mali*.[1] The toxin causes necrotic brown spots on certain apple leaves that significantly damages the fruit. The host-specificity should arise from the existence of a high-affinity protein to the toxin at the infection site of the plants for the fungal pathogen.[2] To isolate the high-affinity protein, we have synthesized many labeling analogs of alternariolide (**1**).[3]



Alternariolide (**1**)



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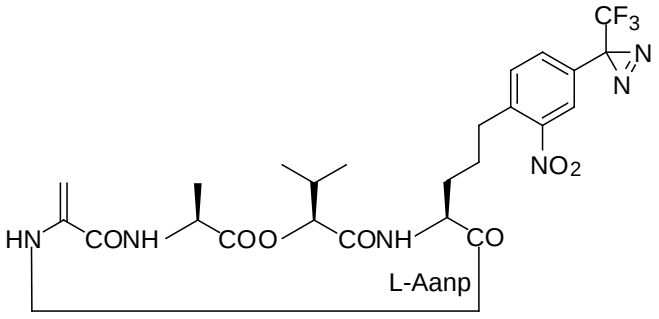
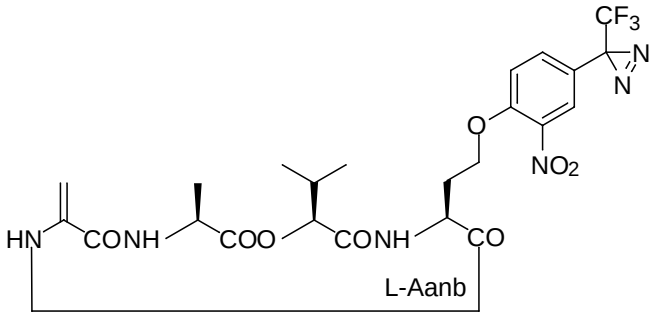


3



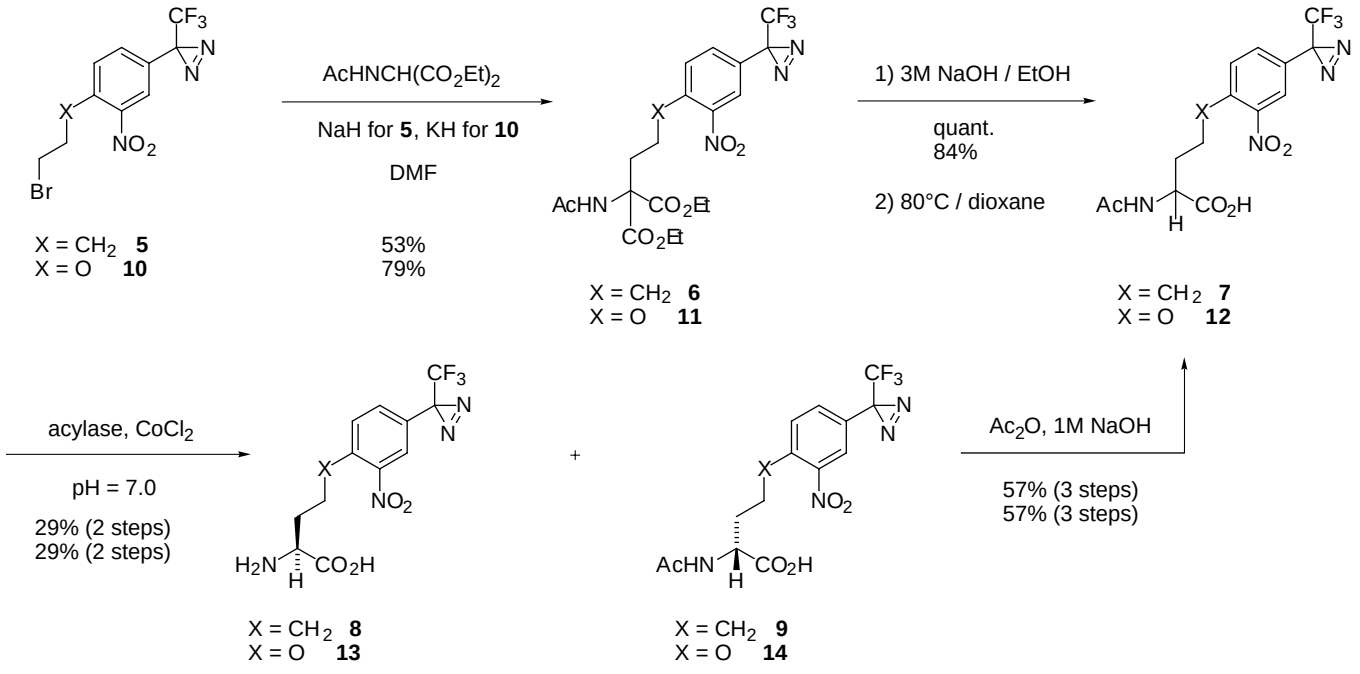
4

Recently, we reported the synthesis of the 3-trifluoromethyl-3-phenyldiazirine derivative (**2**) used to label the protein.[3(b)] The synthesized analog (**2**) showed a rather weak activity compared to that of alternariolide, and after the irradiation of **2** with albumin as a model experiment, the detection of the labeled protein was troublesome. For the easy detection of the labeled protein, according to Hatanaka's report, [4]

we synthesized some nitrophenyl derivatives of the diazirine as model compounds and tested their photoaffinity-labeling abilities in MeOH during irradiation.[5] These experiments showed that the *o*-alkylnitrobenzene derivative (a model compound of **3**) produced a complex mixture due to the hydrogen abstraction from the alkyl group by the nitro group during irradiation. To prevent the side reaction, the *ortho*-alkyl group was changed to an alkoxy group. The insertion reaction of the *o*-alkoxynitrobenzene derivative (a model compound of **4**) in MeOH cleanly proceeded under irradiation. Accordingly, to compare the toxicities and labeling abilities, the syntheses of **3** and **4** were performed.

For the labeling, L-Amp (L-2-amino-5-(4-methoxyphenyl)pentanoic acid) in **1** was replaced with L-Aanp (**8**, L-2-amino-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid) or L-Aanb (**13**, L-2-amino-4-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitro-1-phenoxy]butanoic acid). These amino acids were prepared by the following steps (Scheme 1). Starting from the bromide (**5**),[5] the amino acid function was prepared by condensation with acetamidomalonate, hydrolysis under basic conditions, and decarboxylation by heating at 80°C under neutral conditions. The obtained acetamide (**7**) was hydrolyzed using acylase to give L-Aanp (**8**) with recovery of the corresponding D-acetamide (**9**). The D-isomer was racemized with Ac_2O and NaOHaq and then recycled. The phenoxy derivative (**10**)[5] was converted to the corresponding L-amino acid (**13**) by similar reactions.

Scheme 1

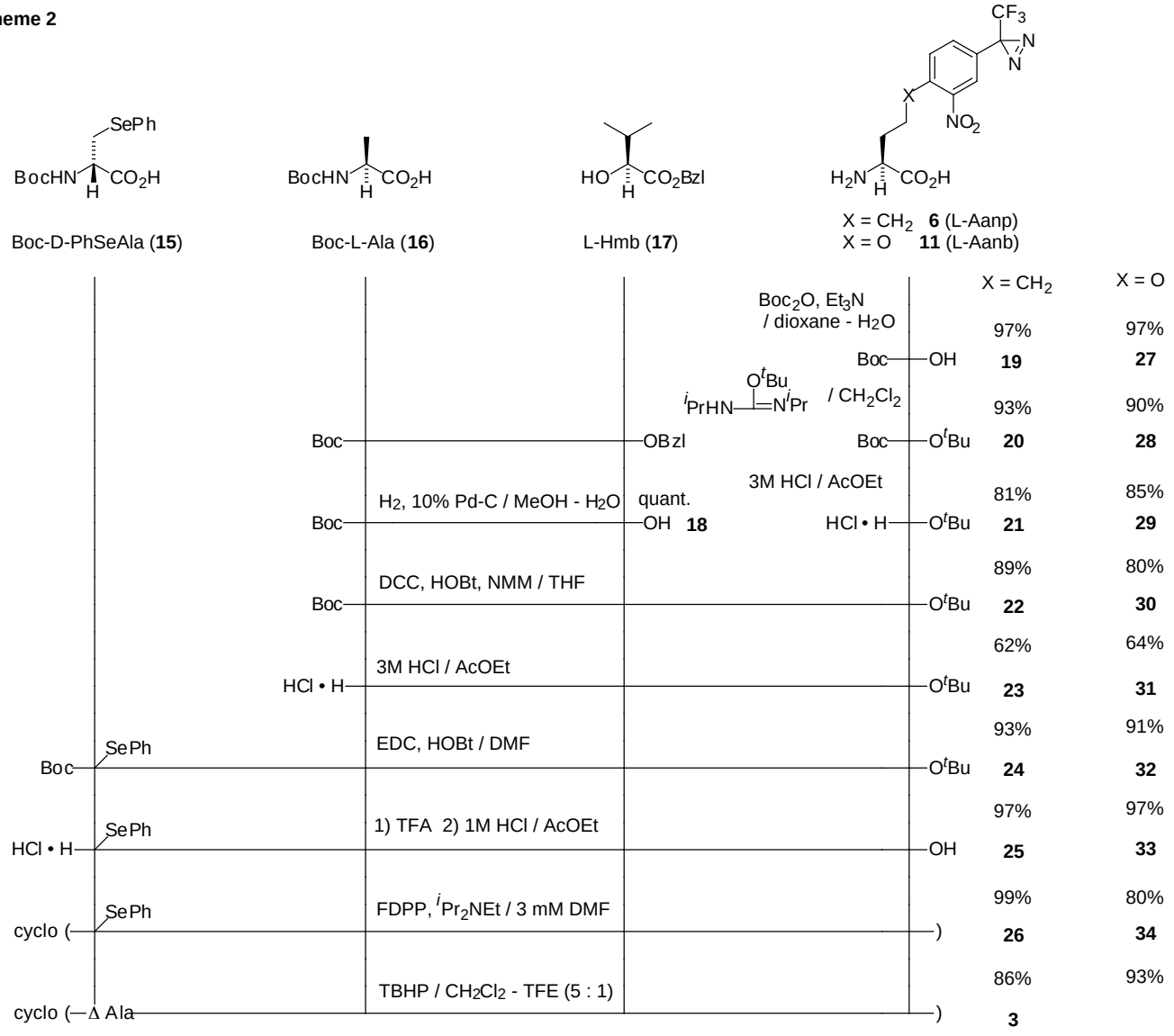


For the synthesis of the cyclic peptides, the cyclization is a crucial step. It is known that the yield from the cyclization step highly depends on the linking position, the order of the amino acid arrangement, the configuration of the amino acids, the substituent of the amides, and the protective groups which control the conformation of the linear precursor.[7] To construct the cyclic tetradepsipeptide containing dehydroalanine, incorporation of a D-amino acid is one of the approaches to solve this problem. Employment of D-β-phenylselenoalanine[6] as the dehydroalanine precursor was successful for the cyclization of some alternariolide analogs.

The amino group of L-Aanp (**6**) was protected with Boc and the carboxyl group was protected as the *t*-

butyl ester to give **20** (Scheme 2). After deprotection of the Boc group of **20** by treatment with 3M HCl / AcOEt, the resulting salt **21** was coupled with carboxylic acid (**18**) to afford **22**. The Boc group of the tridepsipeptide (**22**) was removed with 3M HCl / AcOEt and then the HCl salt was condensed with Boc-D-phenylselenoalanine (**15**) to give the linear tetradepsipeptide (**24**). For the cyclization, the protective groups on both ends of **24** were removed and the product (**25**) was treated with FDPP (pentafluorophenyl diphenylphosphinate)[8] in 3 mM DMF to yield the cyclized peptide (**26**) in excellent yield. As the last step, the phenylseleno group was removed after oxidation with TBHP to give the cyclic dehydropeptide (**3**). Another derivative (**4**) was also synthesized by a series of similar reactions.

Scheme 2



L- Hmb : L-2-Hydroxy-3-methylbutanoic acid
L- Aanp : L-2-Amino-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid
L-Aanb : L-2-Amino-4-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitro-1-phenoxy]butanoic acid
HOBt : 1-Hydroxybenzotriazole
NMM : *N*-Methylmorpholine
TFE : 2,2,2-Trifluoroethanol
FDPP : Pentafluorophenyl diphenylphosphinate

The synthetic derivatives were then exposed to the biological test (formation of necrotic spots on apple leaves of susceptible species).[1(a), 1(b)] The derivative (**3**) showed a rather weak activity while **4** showed a very weaker activity than that of **1**.

EXPERIMENTAL

Diethyl acetamido[3-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]propyl]malonate (6)

To a suspension of NaH (washed with hexane, 77 mg, 3.2 mmol) in DMF (4 mL) was added a solution of **5** (1.1 g, 3.2 mmol) and ethyl acetamidomalonate (580 mg, 2.7 mmol) in DMF (8 mL) at 0℃ and the mixture was stirred at rt for 24 h. The reaction was quenched with H_2O at 0℃ and the mixture was extracted with AcOEt (15 mL x 3). The combined extract was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (20% AcOEt - benzene) to give **6** as a pale yellow solid (690 mg, 53%) which was recrystalyzed from AcOEt - hexane to give pale yellow needles.

mp 73.5-74.5℃; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.24 (6H, t, *J* = 7.1 Hz), 1.47 (2H, m), 2.03 (3H, s), 2.43 (2H, m), 2.88 (2H, t, *J* = 7.8 Hz), 4.23 (4H, q, *J* = 7.1 Hz), 6.75 (1H, br s), 7.38 (2H, s), 7.68 (1H, s); IR (cm^{-1}, nujol) 3246, 3084, 2926, 2855, 1752, 1740, 1645, 1535, 1346, 1290, 1215, 1152, 1026, 1009.

DL-2-Acetamido-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid (7)

To a solution of **6** (13.6 g, 28 mmol) in EtOH (136 mL) was added 3M NaOH aq (136 mL) and the mixture was stirred at rt overnight. The mixture was evaporated *in vacuo* to the half volume and then washed with Et_2O (30 mL x 3). The residue was acidified to pH 2 with 1M HCl aq and the mixture was extracted with AcOEt (40 mL x 3), washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was precipitated from AcOEt - hexane to give dicarboxylic acid (quant.) as a pale yellow amorphous powder.

mp 121-122℃ (decomp); ^{1}H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.39 (2H, m), 1.85 (3H, s), 2.11 (2H, m), 2.79 (2H, t, *J* = 7.4 Hz), 7.61 (1H, d, *J* = 9.1 Hz), 7.64 (1H, d, *J* = 9.1 Hz), 7.77 (1H, s), 7.87 (1H, s), 13.16 (2H, br s); IR (cm^{-1}, nujol) 3385, 3312, 2920, 2855, 2575, 1904, 1726, 1620, 1562, 1535, 1348, 1296, 1260, 1235, 1192, 1157.

A solution of the dicarboxylic acid (11.4 g, 26 mmol) in 1,4-dioxane (350 mL) was stirred for 1 h at 80℃. After evaporation, the residue was dissolved in 1M NaOH aq (60 mL) and the mixture was washed with Et_2O (20 mL x 3). The aqueous layer was acidified to pH 2 with 1M HCl aq and the mixture was extracted with AcOEt (30 mL x 3), washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give crude carboxylic acid (**7**) as a pale yellow solid which was used to the next reaction without further purification.

L-2-Amino-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid (L-Aanp, 8)

The racemic acetamide (**7**, 10.2 g, 26 mmol) was dissolved into the least amount of 2M NaOH aq and the pH of the solution was adjusted to 6.7 with both 6M and 1M HCl aq. To the solution were added $CoCl_2$ aq (2.5 x 10^{-4} M, 12.6 mL) and acylase solution (*Aspergillus* genus, A suspension of the enzyme (8.2 g) in H_2O (10 mL) was centrifuged at 3500 rpm for 10 min and the supernatant was used.) and the volume of the mixture was adjusted to 440 mL. To the mixture was added phosphate buffer (pH = 4.7, 200 mL) and the solution was stand at 35 - 38 ℃ for 48 h and then 0℃ overnight. A yellow solid was precipitated

which was washed with H_2O and dried *in vacuo* to give L-Aanp (**8**) (2.6 g, 29% for 2 steps). mp 138℃ (decomp); $[\alpha]_D$ +18.4° (*c* 0.93, DMF : 1M HCl = 1 : 1); ^{1}H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.64 (4H, m), 2.81 (2H, t, *J* = 6.9 Hz), 3.08 (1H, t, *J* = 5.7 Hz), 7.45 (2H, t, br s), 7.63 (1H, dd, *J* = 0.9, 8.4 Hz), 7.66 (1H, d, *J* = 8.4 Hz), 7.80 (1H, d, *J* = 0.9 Hz); IR (cm^{-1}, nujol) 3115, 2924, 2855, 2726, 1605, 1578, 1539, 1505, 1344, 1182, 1155.

To the mother liquor was added 1M HCl aq and the pH of the solution was adjusted to 2. The mixture was extracted with AcOEt (600 mL x 3) and the combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved into 1M NaOH (22 mL, 22 mmol) and to the solution was added Ac_2O (3.5 mL, 37 mmol). The mixture was stirred for 1 h (during the reaction time, when the solution became turbid, the least amount of THF was added till the solution became clear). The solution was acidified to pH 2 with 1M HCl aq and to the mixture was added solid NaCl. The mixture was extracted with AcOEt (25 mL x 3) and the extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel to give **7** (5.8 g, 57% for 3 steps) which was recycled.

Diethyl acetamido[2-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitro-1-phenoxy]ethyl]malonate (11)

To a suspension of KH (35 wt% in mineral oil, 5.8 g, 50 mmol) in DMF (60 mL) were added a solution of ethyl acetamidomalonate (12.0 g, 55 mmol) in DMF (60 mL) and then a solution of bromide (**10**, 16.2 g, 46 mmol) in DMF (60 mL) at -42℃ and the mixture was stirred at rt for 24 h. The reaction was quenched with H_2O at 0℃ and the mixture was extracted with AcOEt (200 mL x 3). The combined extract was washed with H_2O, brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (20% AcOEt - benzene) to give **11** as a pale yellow oil (11.6 g, 79%).
^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 1.26 (6H, t, *J* = 7.1 Hz), 2.04 (3H, s), 2.92 (2H, t, *J* = 5.8 Hz), 4.18 (2H, t, *J* = 5.8 Hz), 4.27 (4H, m), 6.92 (1H, br s), 7.11 (1H, d, *J* = 8.8 Hz), 7.41 (1H, dd, *J* = 2.4, 8.8 Hz), 7.64 (1H, d, *J* = 2.4 Hz); IR (cm^{-1}, neat) 3403, 2988, 2942, 2907, 1744, 1676, 1626, 1541, 1507, 1470, 1370, 1271, 1233, 1194, 1159, 1094, 997, 862, 760, 681.

DL-2-Acetamido-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid (12)

The same procedure as **7**.

dicarboxylic acid: a yellow amorphous powder (precipitated from MeOH-AcOEt-pet. ether); mp 117.0-117.5℃ (decomp); ^{1}H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.88 (3H, s), 2.60 (2H, t, *J* = 6.1 Hz), 4.16 (2H, t, *J* = 6.1 Hz), 7.43 (1H, d, *J* = 9.0 Hz), 7.63 (1H, dd, *J* = 2.4, 9.0 Hz), 7.82 (1H, d, *J* = 2.4 Hz), 7.95 (1H, s); IR (cm^{-1}, nujol) 3393, 3360, 2926, 2855, 2558, 1738, 1628, 1539, 1514, 1348, 1271, 1229, 1190, 1155, 999.

The carboxylic acid (**12**, a yellow solid) was used for the next reaction without purification.

L-2-Amino-5-[4-(1-azi-2,2,2-trifluoroethyl)-2-nitrophenyl]pentanoic acid (L-Aanb, 13)

The racemic acetamide (**12**, 11.0 g, 28 mmol) was dissolved into the least amount of 2M NaOH aq and the pH of the solution was adjusted to 7.4 with both 6M and 1M HCl aq. To the solution were added $CoCl_2$

aq (2.5×10^{-4} M, 12.6 mL) and acylase solution (*Aspergillus* genus, A suspension of the enzyme (8.8 g) in H_2O (10 mL) was centrifuged at 3500 rpm for 10 min and the supernatant was used.) and the volume of the mixture was adjusted to 350 mL. To the mixture was added phosphate buffer (pH = 6.6, 300 mL) and the solution was stand at 35 - 38 ℃ for 48 h and then 0℃ overnight. A yellow solid was precipitated which was washed with H_2O and dried *in vacuo* to give L-Aanb (**13**) (2.8 g, 29% for 2 steps).
mp 135℃ (decomp); $[\alpha]_D$ +18.2° (*c* 0.67, DMF : 1M HCl = 1 : 1); ^{1}H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.99 (1H, m), 2.22 (1H, m), 3.35 (1H, t, *J* = 6.6 Hz), 4.31 (1H, m), 4.40 (1H, m), 7.47 (1H, d, *J* = 9.0 Hz), 7.66 (1H, dd, *J* = 2.4, 9.0 Hz), 7.70 (2H, br s), 7.85 (1H, d, *J* = 2.4 Hz); IR (cm^{-1}, nujol) 2924, 2855, 1593, 1539, 1346, 1312, 1273, 1227, 1209, 1154.
To the mother liquor was added 1M HCl aq and the pH of the solution was adjusted to 2. The mixture was extracted with AcOEt (600 mL x 3) and the combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved into 1M NaOH aq (24 mL, 24 mmol) and to the solution was added Ac_2O (3.8 mL, 40 mmol). The mixture was stirred for 1 h (during the reaction time, when the solution became turbid, the least amount of THF was added till the solution became clear). The solution was acidified to pH 2 with 1M HCl aq and to the mixture was added solid NaCl. The mixture was extracted with AcOEt (30 mL x 3) and the combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel to give **12** (6.3 g, 57% for 3 steps) which was recycled.

Boc-L-Ala-L-Hmb-OH (18)

A round-bottomed flask containing 10% Pd-C (3.7 g, 3.5 mmol) was filled with argon. To the flask were added a small amount of H_2O and MeOH, and then a solution of the ester (Boc-L-Ala-L-Hmb-OBzl, 13.1 g, 35 mmol). After substitution of argon with H_2, the mixture was stirred for 12 h at rt. The mixture was filtered through Celite pad and the solution was evaporated. The residue was dissolved in sat $NaHCO_3$ aq and the mixture was washed with Et_2O (100 mL x 2). The aqueous layer was acidified to pH 2 with 1M HCl aq and the mixture was extracted with AcOEt (100 mL x 3). The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give **18** (9.7 g, quant.) as a colorless oil.
$[\alpha]_D$ -50.8° (*c* 1.7, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.02 (3H, d, *J* = 6.8 Hz), 1.04 (3H, d, *J* = 6.8 Hz), 1.44 (*ca.* 9H, s), 1.46 (*ca.* 3H, s), 2.31 (1H, m), 4.22 (*ca.* 1/4H, br s), 4.40 (*ca.* 3/4H, m), 4.96 (1H, m), 5.07 (1H, d, *J* = 6.6 Hz), 6.20 (1H, br s); IR (cm^{-1}, neat) 3349, 2976, 1719, 1520, 1456, 1395, 1370, 1167, 1073, 1026.

Boc-L-Aanp (19)

L-Aanp (**6**) (2.6 g, 7.4 mmol) was dissolved in H_2O (26 mL) by addition of Et_3N (1.1 mL, 7.9 mmol) and to the solution was added a solution of Boc_2O (1.9 g, 8.7 mmol) in dioxane (26 mL). After stirring for 12 h at rt, the solution was adjusted to pH 2-3 by addition of 1M HCl aq and the mixture was extracted with AcOEt (50 mL x 3). The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel (1% MeOH - $CHCl_3$) to give **19** as a yellow oil (3.2 g, 97%).

$[\alpha]_D$ +7.3° (*c* 0.36, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.44 (9H, s), 1.76 (3H, m), 1.97 (1H, m), 2.93 (2H, br t, *J* = 4.5 Hz), 4.36 (1H, br s), 5.02 (1H, br d, *J* = 7.2 Hz), 7.39 (1H, d, *J* = 8.4 Hz), 7.43 (1H, d, *J* = 8.4 Hz), 7.70 (1H, s); IR (cm^{-1}, neat) 3320, 3098, 2980, 2936, 2876, 2625, 2575, 1717, 1537, 1456, 1399, 1368, 1348, 1289, 1235, 1192, 1161, 1060, 760.

Boc-L-Aanp-O*t*Bu (20)

To a solution of **19** (3.1 g, 6.9 mmol) in CH_2Cl_2 (100 mL) was added *O-tert*-butyl-*N*,*N'*-diisopropyl-isourea (2.1 g, 10.5 mmol) and the solution was refluxed for 12 h. When the reaction was not complete, after cooling to rt, to the mixture was added the isourea and the solution was refluxed for several hours. After evaporation of the solvent, to the residue was added 15% AcOEt - hexane and the insoluble material was filtered off. After twice filtration, the solution was evaporated and the residue was chromatographend on silica gel (10% AcOEt - hexane) to give **20** a yellow oil (3.3 g, 93%).

$[\alpha]_D$ +5.0° (*c* 0.85, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.44 (9H, s), 1.45 (9H, s), 1.68 (3H, m), 1.86 (1H, m), 2.91 (2H, t, *J* = 7.4 Hz), 4.21 (1H, m), 5.05 (1H, brd, *J* = 7.8 Hz), 7.38 (1H, dd, *J* = 1.2, 8.1 Hz), 7.43 (1H, d, *J* = 8.1 Hz), 7.69 (1H, d, *J* = 1.2 Hz); IR (cm^{-1}, neat) 3436, 3378, 2980, 2936, 2874, 1711, 1624, 1537, 1505, 1458, 1393, 1368, 1235, 1157, 1059, 760.

HCl•H-L-Aanp-O*t*Bu (21)

A solution of 3M HCl (gas) in AcOEt (33 mL, 99 mmol) was added to **20** (3.3 g, 6.5 mmol) and the mixture was stirred for 1 h at rt. After evaporation, to the residue was added Et_2O and the insoluble material was filtered off. After twice filtration, to the solution was added sat. $NaHCO_3$ aq (30 mL) and the mixture was extracted with AcOEt (20 mL x 3). The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel (100% AcOEt). To the product was added 1M HCl (gas) in AcOEt (20 mL, 60 mmol) and the mixture was evaporated to afford salt (**21**) which was crystallyzed from AcOEt - Et_2O to give yellow needles (2.3 g, 81%).

mp 136.8-137.3 ℃; $[\alpha]_D$ +7.6° (*c* 0.44, MeOH); ^{1}H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.40 (9H, s), 1.57 (1H, m), 1.79 (3H, m), 2.85 (2H, m), 3.88 (1H, t, *J* = 5.4 Hz), 7.66 (1H, dd, *J* = 1. 2, 8.1 Hz), 7.69 (1H, d, *J* = 8.1 Hz), 7.82 (1H, d, *J* = 1.2 Hz), 8.48 (3H, br s); IR (cm^{-1}, nujol) 2926, 2855, 1750, 1579, 1530, 1360, 1298, 1235, 1163.

Boc-L-Ala-L-Hmb-L-Aanp-O*t*Bu (22)

To a solution of **21** (2.3 g, 5.3 mmol) in THF (35 mL) were added HOBt (790 mg, 5.9 mmol) and NMM (0.58 mL, 5.3 mmol). After cooling to 0℃, to the solution was added a solution of DCC (1.2 g, 5.8 mmol) in THF (25 mL) and the mixture was stirred for 12 h at rt. After filtration of the insoluble material, the solution was evaporated. When the insoluble urea was appeared, the mixture was again filtered. The solution was successively washed with sat $NaHCO_3$ aq (20 mL x 3), 1M HCl aq (20 mL x 3), and brine, which was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$ - AcOEt - benzene (5:15:80)) to give **22** (3.2 g, 89%) as a yellow solid which was

reprecipitated from AcOEt - pet. ether.
mp 115.5-116.0 ℃; $[\alpha]_D$ -17.8° (*c* 0.44, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.94 (3H, d, *J* = 6.9 Hz), 0.97 (3H, d, *J* = 6.9 Hz), 1.43 (9H, s), 1.44 (9H, s), 1.48 (3H, d, *J* = 7.2 Hz), 1.74 (3H, m), 1.95 (1H, m), 2.31 (1H, m), 2.91 (2H, t, *J* = 7.8 Hz), 4.36 (1H, m), 4.47 (1H, m), 4.95 (1H, br d, *J* = 7.8 Hz), 5.07 (1H, d, *J* = 3.6 Hz), 6.96 (1H, br d, *J* = 7.2 Hz), 7.38 (1H, dd, *J* = 1.2, 8.1 Hz), 7.42 (1H, d, *J* = 8.1 Hz); IR (cm^{-1}, nujol) 3341, 3312, 2924, 2855, 1732, 1682, 1657, 1537, 1352, 1289, 1252, 1229, 1188, 1154, 1115, 1073, 997.

HCl•H-L-Ala-L-Hmb-L-Aanp-O*t*Bu (23)

A solution of 3M HCl (gas) in AcOEt (22 mL, 66 mmol) was added to **22** (3.0 g, 4.4 mmol) and the mixture was stirred for 1 h at rt. After evaporation, to the mixture was added sat $NaHCO_3$ aq and the solution was extracted with AcOEt (30 mL x 3). The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (AcOEt). To the product was added a solution of 3M HCl (gas) in AcOEt (4.4 mL, 13 mmol) and the solvent was evaporated to give **23** (1.7 g, 62%) as a yellow solid which was reprecipitated from Et_2O - pet. ether.
mp 90.0-90.5 ℃; $[\alpha]_D$ -12.6° (*c* 0.90, MeOH); ^1H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.90 (3H, d, *J* = 6.9 Hz), 0.95 (3H, d, *J* = 6.9 Hz), 1.35 (9H, s), 1.43 (3H, d, *J* = 7.2 Hz), 1.64 (4H, m), 2.14 (1H, m), 2.82 (2H, t, *J* = 6.6 Hz), 4.13 (2H, q, *J* = 7.2 Hz), 4.81 (1H, d, *J* = 4.5 Hz), 7.66 (2H, s), 7.80 (1H, s), 8.42 (1H, d, *J* = 7.2 Hz), 8.54 (3H, br s); IR (cm^{-1}, nujol) 3295, 2924, 2855, 2726, 1742, 1663, 1626, 1537, 1346, 1298, 1235, 1192, 1154, 1119, 993.

Boc-D-PhSeAla-L-Ala-L-Hmb-L-Aanp-O*t*Bu (24)

To a solution of **23** (1.5 g, 2.4 mmol) in DMF (35 mL) were added **15** (830 mg, 2.4 mmol) and HOBt (360 mg, 2.7 mmol) and the solution was cooled to -5℃. To the solution was added EDC (0.44 mL, 2.4 mmol) and the mixture was degassed under reduced pressure and filled with argon. After stirring for 12 h at rt, the reaction was quenched by addition of 1M HClaq (30 ml) and the mixture was extracted with AcOEt (30 mL x 3). The combined extract was successively washed with 1M HCl aq (30 mL x 3), sat $NaHCO_3$ aq (30 mL x 3), and brine, which was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (AcOEt - benzene - hexane (30:35:35)) to give **24** (2.0 g, 93%) as a yellow solid which was reprecipitated from AcOEt - pet. ether.
mp 129.5-130.0 ℃; $[\alpha]_D$ -4.2° (*c* 0.85, AcOEt); ^1H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.86 (3H, d, *J* = 6.9 Hz), 0.90 (3H, d, *J* = 6.9 Hz), 1.26 (3H, d, *J* = 7.2 Hz), 1.34 (9H, s), 1.35 (9H, s), 1.61 (3H, m), 1.68 (1H, m), 2.07 (1H, m), 2.81 (2H, t, *J* = 6.8 Hz), 3.05 (1H, dd, *J* = 9.3, 12.3 Hz), 3.20 (1H, dd, *J* = 5.4, 12.3 Hz), 4.12 (1H, m), 4.23 (2H, m), 4.70 (1H, d, *J* = 5.1 Hz), 6. 88 (1H, d, *J* = 8.4 Hz),7.27 (3H, m), 7.47 (2H, m), 7.63 (2H, s), 7.79 (1H, s), 8.14 (1H, d, *J* = 7.5 Hz), 8.41 (1H, d, *J* = 6.9 Hz); IR (cm^{-1}, nujol) 3333, 2924, 2855, 1730, 1678, 1659, 1528, 1348, 1311, 1244, 1188, 1157.

HCl•H-D-PhSeAla-L-Ala-L-Hmb-L-Aanp-OH (25)

A solution of **24** (454 mg, 0.5 mmol) in TFA (4.5 mL) was degassed under reduced pressure and filled

with argon and the mixture was stirred for 2 h at rt. After evaporation of TFA, to the residue was added a solution of 1M HCl (gas) in AcOEt (1.0 mL, 1 mmol) and the solution was evaporated. The resulted solid was dissolved in AcOEt and then reprecipitated by addition of Et_2O to give **25** (382 mg, 97%) as a yellow amorphous powder.

mp 115-116 ℃ (decomp); $[\alpha]_D$ -40.5° (*c* 0.83, DMF); ^{1}H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.87 (3H, d, *J* = 6.9 Hz), 0.91 (3H, d, *J* = 6.9 Hz), 1.17 (3H, d, *J* = 6.9 Hz), 1.61 (3H, s), 1.73 (1H, m), 2.09 (1H, m), 2.81 (2H, t, *J* = 7.1 Hz), 3.38 (2H, m), 4.09 (2H, m), 4.18 (1H, m), 4.71 (1H, d, *J* = 5.1 Hz), 7.31 (3H, m), 7.53 (2H, m), 7.63 (2H, s), 7.79 (1H, s), 8.20 (1H, d, *J* = 7.5 Hz), 8.45 (3H, br s), 8.98 (1H, d, *J* = 6.9 Hz), 12.6 (1H, br s); IR (cm^{-1}, nujol) 3192, 2924, 2855, 2726, 1742, 1671, 1537, 1346, 1235, 1194, 1155, 1047, 739.

Cyclo(D-PhSeAla-L-Ala-L-Hmb-L-Aanp) (26)

To a solution of **25** (962 mg, 1.2 mmol) in DMF (411 mL) were added ^{i}Pr$_2$NEt (0.86 mL, 4.9 mmol) and FDPP (947 mg, 2.5 mmol) and the mixture was degassed under reduced pressure and filled with argon. After stirring for 12 h at rt, the solvent was evaporated *in vacuo* and the residue was extracted with 5% TFE - $CHCl_3$ (15 mL x 3). The combined extract was successively washed with 1M HCl aq (15 mL x 3), sat $NaHCO_3$ aq (15 mL x 3), and brine, and evaporated *in vacuo*. The residue was dissolved in 5% TFE-$CHCl_3$ and then to the mixture was added Et_2O to produce precipitation. The precipitate was collected and the mother liquor was evaporated to the volume of 1/5. To the solution was added Et_2O to produce precipitation. The combined precipitate was reprecipitated from $CHCl_3$ - TFE - Et_2O to give **26** (892 mg, 99%) as a yellow amorphous powder.

mp 130 ℃ (decomp); $[\alpha]_D$ -101.4° (*c* 0.24, DMF); ^{1}H NMR (300 MHz, DMF-d_7, DMF-C<u>H</u>O = 8.01 ppm) δ 0.91 (3H, d, *J* = 6.9 Hz), 0.92 (3H, d, *J* = 6.9 Hz), 1.41 (3H, d, *J* = 7.2 Hz), 1.67 (3H, m), 1.84 (1H, m), 2.10 (1H, m), 2.95 (2H, m), 3.04 (1H, dd, *J* = 5.4, 12.3 Hz), 3.31 (1H, dd, *J* = 9.0, 12.3 Hz), 4.32 (1H, m), 4.53 (2H, m), 4.79 (1H, d, *J* = 7.5 Hz), 7.29 (3H, m), 7.55 (2H, m), 7.67 (1H, dd, *J* = 2.1, 8.1 Hz), 7.76 (1H, d, *J* = 8.1 Hz), 7.87 (1H, d, *J* = 2.1 Hz), 7.99 (1H, d, *J* = 8.7 Hz), 8.42 (2H, m); IR (cm^{-1}, nujol) 3293, 2924, 2855, 1740, 1651, 1532, 1346, 1256, 1235, 1194, 1148, 1049, 750, 689.

Cyclo(ΔAla-L-Ala-L-Hmb-L-Aanp) (3)

To a solution of **26** (931 mg, 1.3 mmol) in CH_2Cl_2 (25 mL) and TFE (5 mL) was added a solution of 4.29 M TBHP in CH_2Cl_2 (3.0 mL, 13 mmol) and the mixture was stirred for 8 h at rt. To the solution was added sat $NaHCO_3$ aq (35 mL) and the mixture was extracted with $CHCl_3$ (20 mL x 3). The combined extract was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The resulting solid was reprecipitated from $CHCl_3$ - Et_2O - TFE to give **3** (627 mg, 86%) as a yellow amorphous powder.

mp 160 ℃ (decomp); $[\alpha]_D$ -99.2° (*c* 0.29, DMF); ^{1}H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.86 (3H, d, *J* = 6.9 Hz), 0.87 (3H, d, *J* = 6.9 Hz), 1.34 (3H, d, *J* = 7.2 Hz), 1.49 (1H, m), 1.60 (2H, m), 1.90 (2H, m), 2.87 (2H, m), 4.30 (2H, m), 4.66 (1H, d, *J* = 5.7 Hz), 5.27 (*ca.* 1H, br s), 5.40 (*ca.* 1H, br s), 7.62 (1H, d, *J* = 8.7 Hz), 7.66 (1H, d, *J* = 8.7 Hz), 7.79 (1H, s), 7.79 (1H, s), 8.01 (1H, br

d, *J* = 7.2 Hz), 8.08 (1H, d, *J* = 9.6 Hz), 9.07 (1H, br s); IR (cm^{-1}, nujol) 3331, 3297, 3260, 2924, 2855, 1738, 1709, 1665, 1632, 1537, 1343, 1254, 1235, 1202, 1152, 1055, 992, 681.

Boc-L-Aanb (27)

The same procedure as **19**.

a yellow amorphous powder (reprecipitated from $CHCl_3$ - hexane), mp 45.0 - 46.0 ℃; $[\alpha]_D$ -14.4° (*c* 0.60, MeOH); ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 1.44 (9H, s), 2.44 (2H, m), 4.29 (2H, m), 4.51 (1H, m), 5.68 (1H, m), 7.14 (1H, d, *J* = 9.0 Hz), 7.43 (1H, dd, *J* = 2.1, 9.0 Hz), 7.72 (1H, d, *J* = 2.1 Hz); IR (cm^{-1}, neat) 3410, 2926, 2855, 1719, 1626, 1541, 1510, 1273, 1233, 1192, 1157.

Boc-L-Aanb-O*t*Bu (28)

The same procedure as **20**.

a yellow oil, $[\alpha]_D$ +3.0° (*c* 0.45, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 1.42 (9H, s), 1.46 (9H, s), 2.16 (1H, m), 2.39 (1H, m), 4.23 (2H, t, *J* = 6.7 Hz), 4.35 (1H, m), 5.38 (1H, br d, *J* = 6.6 Hz), 7.11 (1H, d, *J* = 8.8 Hz), 7.42 (1H, dd, *J* = 2.4, 8.8 Hz), 7.71 (1H, d, *J* = 2.4 Hz); IR (cm^{-1}, neat) 3422, 2980, 2936, 1711, 1626, 1543, 1507, 1393, 1354, 1237, 1233, 1159, 1090, 1059, 1030, 993, 846, 683.

HCl•H-L-Aanb-O*t*Bu (29)

The same procedure as **21**.

yellow needles (recrystallized from MeOH - AcOEt), mp 136-137 ℃; $[\alpha]_D$ -7.7° (*c* 0.69, MeOH); ^1H NMR (400 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 1.40 (9H, s), 2.26 (2H, m), 3.95 (1H, t, *J* = 6.7 Hz), 4.36 (2H, m), 7.49 (1H, d, *J* = 9.0 Hz), 7.70 (1H, dd, *J* = 2.4, 9.0 Hz), 7.88 (1H, d, *J* = 2.4 Hz), 8.53 (3H, br s); IR (cm^{-1}, nujol) 2926, 2855, 1740, 1518, 1501, 1346, 1306, 1275, 1263, 1248, 1231, 1159, 1123, 866, 841.

Boc-L-Ala-L-Hmb-L-Aanb-O*t*Bu (30)

The same procedure as **22**.

a yellow amorphous powder (reprecipitated from AcOEt - pet. ether), mp 108 - 110 ℃ (decomp); $[\alpha]_D$ -40.6° (*c* 0.24, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.97 (3H, d, *J* = 6.8 Hz), 0.98 (3H, d, *J* = 6.8 Hz), 1.40 (3H, d, *J* = 7.1 Hz), 1.40 (9H, s), 1.44 (9H, s), 2.32 (2H, m), 2.45 (1H, m), 4.03 (1H, m), 4.19 (2H, t, *J* = 6.3 Hz), 4.48 (1H, m), 4.87 (1H, br d, *J* = 6.8 Hz), 4.96 (1H, d, *J* = 3.7 Hz), 7.14 (1H, d, *J* = 9.0 Hz), 7.35 (1H, br d, *J* = 7.8 Hz), 7.43 (1H, dd, *J* = 2.2, 9.0 Hz), 7.72 (1H, d, *J* = 2.2 Hz); IR (cm^{-1}, nujol) 3362, 3337, 2924, 2855, 1726, 1686, 1661, 1626, 1537, 1352, 1327, 1279, 1231, 1190, 1159.

HCl•H-L-Ala-L-Hmb-L-Aanb-O*t*Bu (31)

The same procedure as **23**.

a yellow amorphous powder (reprecipitated from Et_2O - pet. ether), mp 102-104 ℃ (decomp); $[\alpha]_D$ -12.6°

(*c* 0.90, MeOH); ^{1}H NMR (400 MHz, DMSO-d$_6$, DMSO = 2.49 ppm) δ 0.91 (3H, d, *J* = 6.7 Hz), 0.94 (3H, d, *J* = 6.7 Hz), 1.35 (9H, s), 1.39 (3H, d, *J* = 7.3 Hz), 2.05 - 2.20 (3H, m), 4.10 (1H, q, *J* = 7.3 Hz), 4.22 (2H, m), 4.33 (1H, m), 4.78 (1H, d, *J* = 4.9 Hz), 7.46 (1H, d, *J* = 9.0 Hz), 7.67 (1H, dd, *J* = 2.4, 9.0 Hz), 7.85 (1H, d, *J* = 2.4 Hz), 8.53 (3H, br s), 8.58 (1H, d, *J* = 7.6 Hz); IR (cm^{-1}, nujol) 3397, 3218, 2922, 2855, 2724, 1746, 1672, 1626, 1539, 1273, 1233, 1194, 1157, 993, 845.

Boc-D-PhSeAla-L-Ala-L-Hmb-L-Aanb-O^{t}Bu (32)

The same procedure as **24**.

a yellow amorphous powder (reprecipitated from AcOEt - pet. ether), mp 112-113 ℃; [α]$_D$ -13.0° (*c* 0.27, AcOEt); ^{1}H NMR (400 MHz, DMSO-d$_6$, DMSO = 2.49 ppm) δ 0.85 (3H, d, *J* = 6.8 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 1.19 (3H, d, *J* = 6.6 Hz), 1.34 (9H, s), 1.35 (9H, s), 2.03 (2H, m), 2.21 (1H, m), 3.04 (1H, dd, *J* = 9.3, 12.4 Hz), 3.19 (1H, dd, *J* = 5.4, 12.4 Hz), 4.16 (2H, m), 4.22 (2H, m), 4.31 (1H, m), 4.64 (1H, d, *J* = 5.1 Hz), 6.94 (1H, d, *J* = 8.8 Hz), 7.27 (3H, m), 7.43 (1H, d, *J* = 9.0 Hz), 7.46 (2H, m), 7.63 (1H, dd, *J* = 2.4, 9.0 Hz), 7.84 (1H, d, *J* = 2.4 Hz), 8.24 (1H, d, *J* = 7.8 Hz), 8.43 (1H, d, *J* = 6.5 Hz); IR (cm^{-1}, nujol) 3328, 2924, 2855, 1725, 1674, 1657, 1626, 1535, 1520, 1350, 1331, 1279, 1250, 1231, 1196, 1152, 1022, 739.

HCl•H-D-PhSeAla-L-Ala-L-Hmb-L-Aanb-OH (33)

The same procedure as **25**.

a yellow amorphous powder (reprecipitated from CHCl$_3$ - pet. ether), mp 101-103 ℃ (decomp); [α]$_D$ -38.5° (*c* 0.88, CHCl$_3$); ^{1}H NMR (400 MHz, DMSO-d$_6$, DMSO = 2.49 ppm) δ 0.87 (3H, d, *J* = 6.7 Hz), 0.89 (3H, d, *J* = 6.7 Hz), 1.11 (3H, d, *J* = 7.3 Hz), 2.04 (2H, m), 2.23 (1H, m), 3.35 (2H, m), 4.07 (2H, m), 4.15 (1H, m), 4.26 (1H, m), 4.39 (1H, m), 4.66 (1H, d, *J* = 5.6 Hz), 7.31 (3H, m), 7.43 (1H, d, *J* = 9.0 Hz), 7.53 (2H, m), 7.63 (1H, dd, *J* = 2.2, 9.0 Hz), 7.84 (1H, d, *J* = 2.2 Hz), 8.32 (1H, d, *J* = 8.1 Hz), 8.43 (3H, br s), 8.96 (1H, d, *J* = 6.8 Hz), 12.70 (1H, br s); IR (cm^{-1}, nujol) 3387, 3198, 3057, 2926, 2855, 1738, 1669, 1626, 1537, 1352, 1271, 1233, 1194, 1155, 1046, 997, 762, 741, 691.

Cyclo(D-PhSeAla-L-Ala-L-Hmb-L-Aanb) (34)

The same procedure as **26**.

a yellow amorphous powder (reprecipitated from CHCl$_3$ - TFE - Et$_2$O), mp 133-135 ℃ (decomp); [α]$_D$ -114.7° (*c* 0.28, DMF); ^{1}H NMR (400 MHz, DMF-d$_7$, DMF-C<u>H</u>O = 8.01 ppm) δ 0.88 (3H, d, *J* = 6.8 Hz), 1.41 (3H, d, *J* = 7.3 Hz), 2.04 (2H, m), 2.33 (1H, m), 3.02 (1H, dd, *J* = 5.1, 12.4 Hz), 3.29 (1H, dd, *J* = 9.5, 12.4 Hz), 4.34 (2H, m), 4.55 (3H, m), 4.77 (1H, d, *J* = 7.3 Hz), 7.29 (3H, m), 7.55 (3H, m), 7.69 (1H, dd, *J* = 2.4, 8.8 Hz), 7.91 (1H, d, *J* = 2.4 Hz), 8.12 (1H, d, *J* = 8.5 Hz), 8.45 (1H, d, J = 9.0 Hz), 8.49 (1H, d, *J* = 8.3 Hz); IR (cm^{-1}, nujol) 3289, 3052, 2930, 2855, 1744, 1645, 1537, 1350, 1256, 1233, 1192, 1150, 1090, 1046, 990, 818, 735, 685.

Cyclo(ΔAla-L-Ala-L-Hmb-L-Aanb) (4)

The same procedure as **3**.

a yellow amorphous powder (reprecipitated from $CHCl_3$ - Et_2O), mp 121-123 ℃ (decomp); $[\alpha]_D$ -110.2° (*c* 0.22, DMF); ^1H NMR (300 MHz, DMSO-d_6, DMSO = 2.49 ppm) δ 0.81 (3H, d, *J* = 6.5 Hz), 0.83 (3H, d, *J* = 6.5 Hz), 1.35 (3H, d, *J* = 7.2 Hz), 1.90 (2H, m), 2.36 (1H, m), 4.18 (1H, q, *J* = 7.1 Hz), 4.30 (2H, m), 4.50 (1H, m), 4.63 (1H, d, *J* = 5.7 Hz), 5.30 (*ca.* 1H, br s), 5.42 (*ca.* 1H, br s), 7.43 (1H, d, *J* = 9.0 Hz), 7.66 (1H, dd, *J* = 1.8, 9.0 Hz), 7.84 (1H, d, *J* = 1.8 Hz), 8.08 (1H, d, *J* = 9.3 Hz), 8.18 (1H, br d, *J* = 5.1 Hz), 9.21 (1H, br s); IR (cm^{-1}, nujol) 3241, 3054, 2922, 2855, 1753, 1694, 1651, 1539, 1505, 1350, 1271, 1235, 1190, 1155, 1038.

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† This paper is dedicated to Professor Sho Ito on the occasion of his 77th birthday.

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