Syntheses of Antifungal Aureobasidin A Analogs with Alkyl Chains for Structure-activity Relationship

TORU KUROME,* TETSUYA INOUE, KAZUTOH TAKESAKO and IKUNOSHIN KATO

Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., 3-4-1 Seta, Otsu, Shiga 520-21, Japan

KAORU INAMI and TETSUO SHIBA

Peptide Institute, Protein Research Foundation, 4-1-2 Ina, Minoh, Osaka 562, Japan

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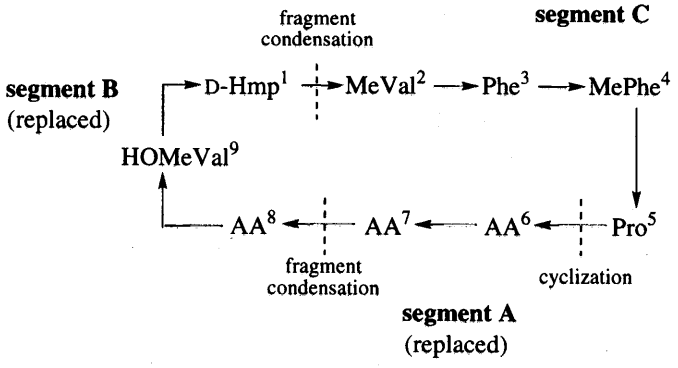
The syntheses of aureobasidin A (AbA) derivatives with alkyl chains and their *in vitro* structure-biological activity relationships are discussed. The analogs replaced at positions 6, 7, or 8 of AbA with either L-glutamic acid, δ-hydroxy-L-norvaline, or δ-hydroxy-*N*-methyl-L-norvaline are prepared. The γ-carboxyl or δ-hydroxyl group of these new amino acids was coupled with acids, alcohols, or amines with alkyl chains. While the analogs having L-glutamic acid residue at positions 6 or 8 showed weak activity, esterification of the γ-carboxyl group with benzyl or shorter alkyl (C_4 or C_6) alcohols, significantly enhanced the activities. Introduction of longer alkyl (C_{14}) chain to the same amino acids residues at positions 6, 7, or 8 resulted in total loss of antifungal activity. Among the lipophilic analogs in [L-Glu^6] derivatives, the C_6 alcohol ester showed the strongest antifungal activity against *Candida* spp. so far tested. None of the derivatives showed activity against *Cryptococcus neoformans*.

Aureobasidin A (AbA, Fig. 1), an antibiotic produced by *Aureobasidium pullulans* R106, exhibits a strong antifungal activity *in vitro* as well as *in vivo* against many pathogenic fungi including *Candida albicans*, with low toxicity.[1,2] It seems to be a promising antifungal agent. AbA is a cyclic depsipeptide composed of one hydroxy acid and eight amino acids, four of which are *N*-methylated.[3,4] Antifungal lipopeptides like echinocandins and pneumocandins have a long alkyl chains on *N*-acyl side chains. Presence of such alkyl side chain is requisite for their high activity.[5,6] On the other hand, in more than 20 congeners of natural aureobasidin[7~9],

Fig. 1. Strategy for synthesis of analogs.



Aureobasidin A (**1**) : AA^6; *a*Ile, AA^7; MeVal, AA^8; Leu

Scheme 3. Replacement at Leu[8] in aureobasidin A.

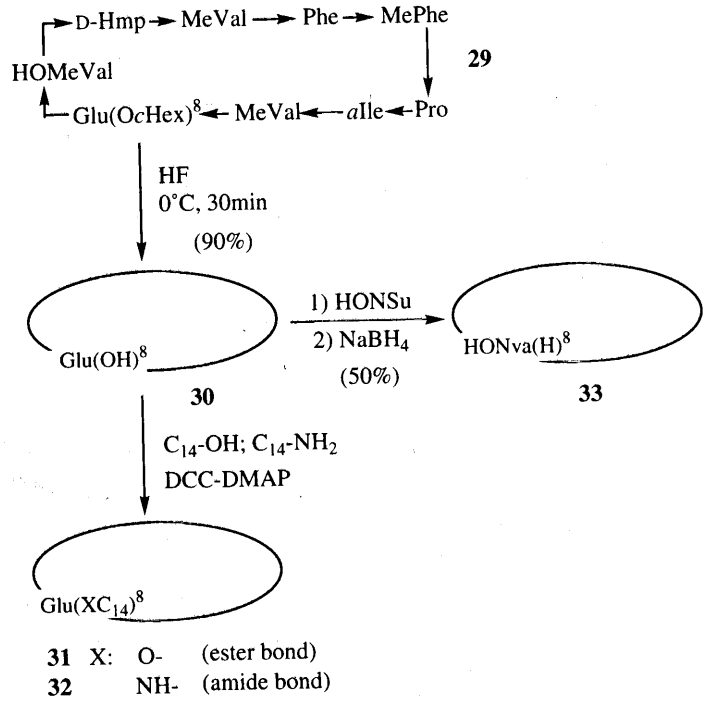


Table 1. MICs for analogs with alkyl chain in the replaced amino acids at positions 6, 7, or 8.

		MIC (μg/ml)					
		C.a.		*C.k.*	*C.g.*	*C.n.*	*S.c.*
Compound	(no.)	0136	0171	0301	1062	0354	9763
Aureobasidin A (AbA)	(**1**)	0.025	0.025	0.4~0.8	0.1~0.2	0.8~1.6	0.1~0.4
[L-Glu[6]]-AbA	(**10**)	12.5	12.5	25	>25	>25	>25
[L-Glu[8]]-	(**30**)	>25	>25	>25	>25	>25	>25
[L-HONva[6]]-	(**12**)	6.3	3.2	6.3	>25	>25	>25
[L-HOMeNva[7]]-	(**21**)	0.8	0.8	1.6	1.6	12.5	6.3
[L-HONva[8]]-	(**33**)	3.1	3.1	1.6	12.5	>25	>25
[L-Glu(OBzl)[6]]-	(**9**)	0.2	0.1	1.6	0.4	>25	>25
[L-Glu(OC_4)[6]]-	(**11a**)	0.4	0.4	1.6	3.2	>25	6.3
[L-Glu(OC_6)[6]]-	(**11b**)	0.4	0.2	3.2	1.6	>25	1.6
[L-Glu(OC_8)[6]]-	(**11c**)	0.8	0.8	6.3	1.6	>25	3.1
[L-Glu(OC_{10})[6]]-	(**11d**)	1.6	1.6	>25	>25	>25	12.5
[L-Glu(OC_{14})[6]]-	(**11e**)	>25	>25	>25	>25	>25	>25
[L-Glu(O*cis*Δ^3C_6)[6]]-	(**11f**)	0.2	0.1	0.8	0.4	>25	1.6
[L-Glu(O*trans*Δ^3C_6)[6]]-	(**11g**)	0.2	0.1	1.6	0.8	>25	0.8
[L-HONva(C_5CO)[6]]-	(**13**)	0.2	0.1	0.8	0.4	>25	0.8
[L-HOMeNva(Bzl)[7]]-	(**20**)	0.8	0.8	3.1	>25	>25	>25
[L-HOMeNva($C_{13}CO$)[7]]-	(**22**)	>25	>25	>25	>25	>25	>25
[L-Glu(O*c*Hex)[8]]-	(**29**)	0.2	0.013	3.1	1.6	>25	12.5
[L-Glu(OC_{14})[8]]-	(**31**)	>25	>25	>25	>25	>25	>25
[L-Glu(NHC_{14})[8]]-	(**32**)	>25	>25	>25	>25	>25	>25

Candida albicans (*C.a.*) TIMM 0136 and TIMM 0171, *C. kefyr* (*C.k.*) TIMM 0301, *C. glabrata* (*C.g.*) TIMM 1062, *Cryptococcus neoformans* (*C.n.*) TIMM 0354, *Saccharomyces cerevisiae* (*S.c.*) TIMM 9763.

glutamination of L-Glu in preference to a peptide cyclization. The benzyl group of **9** was removed by treatment with H_2/Pd-black to give [L-Glu^6]-AbA (**10**), which was used to prepare alkyl derivatives [L-Glu(OR)^6]-AbA (**11a** to **11e**). Reduction of an active ester of γ-carboxyl group of Glu in the peptide (**10**) with sodium borohydride ($NaBH_4$)[12] gave its δ-hydroxyl analog [L-HONva^6]-AbA (**12**) (71%). The hydroxyl group of **12** was esterified with hexanoic acid (C_6) by DCC-DMAP method to give **13**.

We tried a replacement of L-MeVal^7 with L-MeGlu, for introduction of alkyl group at the residue at position 7. However, *N*-methylation of Boc-L-Glu(OBzl)-OH (**2**) by the method of BENOITON *et al.* using NaH and CH_3I[13] failed, probably due to a lactamization between the activated Boc-NH and the γ-carboxyl group of L-Glu. Instead, the carboxyl ester of **2** was first reduced to give Boc-L-HONva(Bzl)-OH (**14**). Boc-L-HOMeNva(Bzl)-OPac (**17**) was prepared from **14** as described (See Experimental). The dipeptide segment A Boc-L-*a*Ile-L-HOMeNva(Bzl)-OH was then used for preparation of [L-HOMeNva(Bzl)^7]-AbA (**20**) by the same procedure as those used in preparation of **9**. The Bzl group of **20** was removed by a treatment with H_2/Pd-black to give [L-HOMeNva^7]-AbA (**21**) (Scheme 2). Acylation of **21** with myristic acid (C_{14}) by DCC-DMAP method yielded [L-HOMeNva($C_{13}CO$)^7]-AbA (**22**) in 57% yield.

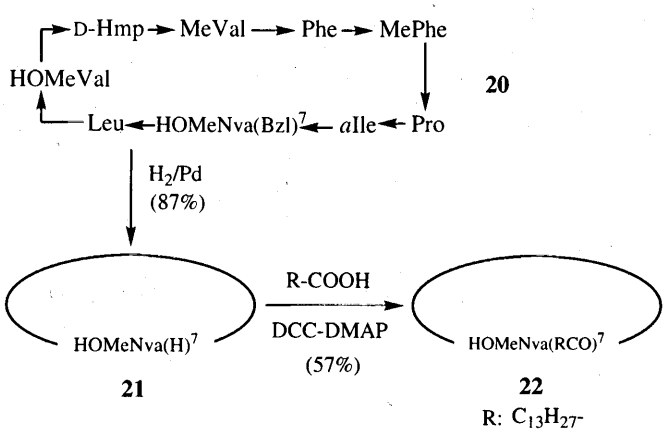
For synthesis of alkyl derivatives at the position 8 residue of AbA, we replaced L-Leu^8 with L-Glu(O*c*Hex). In this derivative, the *c*Hex group was chosen as a protection of γ-carboxyl group of L-Glu, since the protection must be stable enough under condition for a selective deprotection of Bzl group as a protection of α-carboxyl group of L-HOMeVal^9 (See Experimental). The depsipeptide Boc-L-Glu(O*c*Hex)-L-HOMeVal-D-Hmp-OH (**26L**) was used as the segment B to obtain [L-Glu(O*c*Hex)^8]-AbA (**29**). The treatment of **29** with hydrogen fluoride (HF)[14] to remove the *c*Hex group yielded [L-Glu^8]-AbA (**30**) in a good yield of 90% (Scheme 3). The γ-carboxyl group of **30** was also coupled with myristyl alcohol or myristylamine to give alkyl derivatives (**31**) and (**32**) respectively. Reduction of the γ-carboxyl group of **30** gave [L-HONva^8]-AbA (**33**) by the similar method described in the synthesis of **12**.

Antifungal Activity

Replacement of neutral amino acids at positions 6 and 8 with acidic amino acid L-Glu, caused loss of antifungal activities in **10** and **30** (Table 1). While the analog (**12**) replaced at position 6 with hydroxyamino acids showed a low activity, acylation of the hydroxy group with *n*-hexanoic acid caused a higher activity. Likewise, esterification of the carboxyl group in **10** and **30** with shorter alcohol or benzyl alcohol enhanced the activities. Acylation or esterification with long alkyl chain such as myristic acid (C_{14}) or myristyl alcohol resulted in total loss of the activity. The position for introduction of alkyl chains made no marked difference in the effect on the antifungal activities.

Among ester derivatives (**11a**$\sim$**e**) with alkyl chain at γ-carboxyl group of Glu^6, as well as *a*Ile^6 analog, the compound (**11b**) with hexyl ester group exhibited the best antifungal activity. Double bond in the alkyl chains gave little effects for antifungal activity as shown in [Glu(*cis*Δ^3C_6)^6]-AbA (**11f**) and [Glu(*trans*Δ^3C_6)^6]-AbA (**11g**). Among the alkyl derivatives, [Glu(O*c*Hex)^8]-AbA (**29**) exhibited a characteristic activity against *C. albicans* TIMM0171 (MIC$<$0.0125 μg/ml). Consequently, a cyclic alkyl group, rather than a linear one, caused a change of the spectrum of antifungal activity

Scheme 2. Replacement at MeVal^7 in aureobasidin A.



alkyl chain has never been found. An introduction of an alkyl chain to an amino acid residue in AbA may enhance a hydrophobic interaction of the agent with fungal cell surface molecules, resulting in increased biological activity.

We have developed a total chemical synthesis of AbA.[10] This synthetic strategy is applicable for preparation of other aureobasidin analogs and derivatives. We chose three amino acid residues, 6, 7, and 8, respectively L-*allo*isoleucine (L-*a*Ile), *N*-methyl-L-valine (L-MeVal), and L-leucine (L-Leu) in AbA for introduction of alkyl chain. These positions are exchangeable with aliphatic amino acids such as L-Val[6], L-Leu[6], L-Val[7], L-MeLeu[7], L-MeIle[7], L-*a*Ile[8] and L-norvaline (L-Nva)[8] without loss of antifungal activity[7~9,11]. Additionally, heptapeptide H-L-Leu[8]-L-HOMeVal[9]-D-Hmp[1]-L-MeVla[2]-L-Phe[3]-L-MePhe[4]-L-Pro[5]-OPac (**6**), which was obtained as an intermediate in the synthesis of AbA[10], is useful as a common peptide for preparation of analogs replaced at positions 6 and 7 of the natural compounds.

In this paper, we will describe the chemistry leading to lipopeptide derivatives of AbA which have a lipophilic moiety at amino acids of positions 6, 7, or 8, and their antifungal activities.

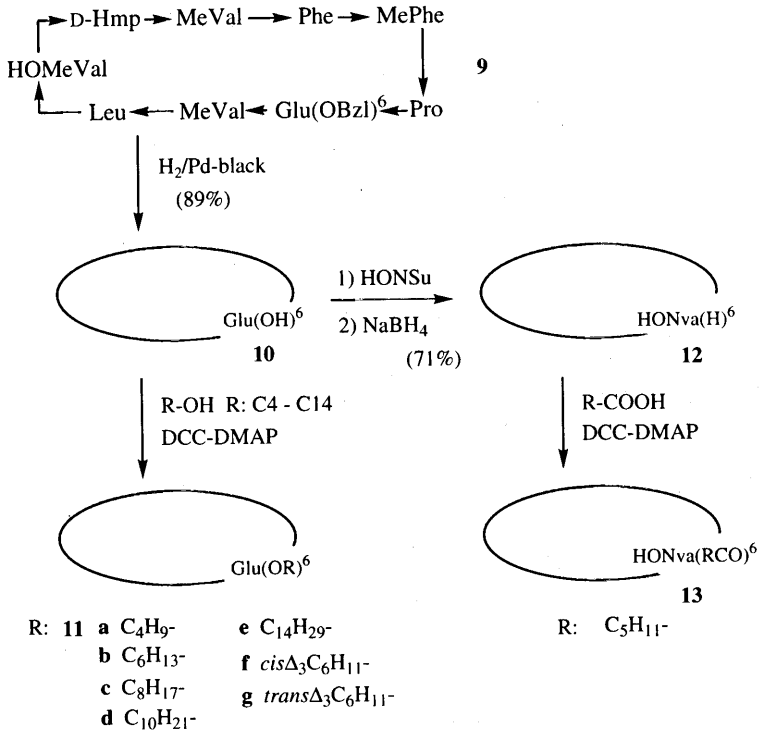
Results and Discussion

Chemistry

The alkyl derivatives of AbA were prepared by replacing one of the amino acid residues, L-*a*Ile[6], L-MeVal[7] or L-Leu[8] with L-Glu, δ-hydroxy-L-norvaline (L-HONva), or δ-hydroxy-*N*-methyl-L-norvaline (L-HOMeNva), and then an appropriate alkyl chain was introduced either to the carboxyl or hydroxyl group *via* an ester or amide bond by dicyclohexylcarbodiimide-dimethylaminopyridine (DCC-DMAP) method. For preparation of new cyclic depsipeptides [L-Glu(OBzl)[6]]-AbA (**9**), [L-HOMeNva(Bzl)[7]]-AbA (**20**) and [L-Glu(O*c*Hex)[8]]-AbA (**27**), we prepared linear nonapeptide AA[6]-AA[7]-AA[8]-L-HOMeVal[9]-D-Hmp[1]-L-MeVla[2]-L-Phe[3]-L-MePhe[4]-L-Pro[5] as shown in Fig. 1 by Boc strategy from three segments A, B and C. Cyclizations of the obtained nonapeptides were carried out between L-AA[6] and L-Pro[5] in yields of 5% to 34%.

[L-Glu(OBzl)[6]]-AbA (**9**) (Scheme 1), was synthesized by coupling of the segment A *i.e.*, Boc-Glu(OBzl)-MeVal-OH (**5**) with the heptapeptide H-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**6**) prepared from segment B and C, as an intermediate in the synthesis of AbA, followed by cyclization of the linear nonapeptide obtained **9** in 5% yield. This low yield compared with that of cyclization to AbA may be caused by a pyro-

Scheme 1. Replacement at *a*Ile[6] in aureobasidin A.



of aureobasidins.

Compounds **11e**, **22**, **31**, and **32**, with longer alkyl group such as myristyl or myristoyl resulted in the complete loss of antifungal activities. In contrast, in echinocandins the introduction of longer alkyl group like linoleoyl, enhanced activity. In aureobasidins, a balances of the steric sizes in the cyclic skeleton and side chains seemed to be very important for the antifungal activity. All analogs tested were completely inactive against *C. neoformans*. The loss of activities against *C. neoformans* in all analogs including one even with subtle structural change may suggest a different mode of action of the antibiotics between *C. neoformans* and *C. albicans*.

Experimental

General

The following spectroscopic and analytical instruments were used: ^{1}H NMR, JEOL JNM-GSX-270 (270 MHz, ref. TMS), JNM-A500 (500 MHz, ref. TMS); FAB-MS (Fast Atom Bombardment Mass Spectrometry), JEOL JMS-DX302; PD-MS (Plasma Desorption Mass Spectrometry), Applied Bio-Systems Inc. BIO-ION 20. Merck Kieselgel 60 F_{254} (silica gel, Merck) was used for TLC. Merck Kieselgel 60 (silica gel; 0.04~0.063 mm, Merck) was used for flash chromatography.

Synthesis of [L-Glu(OBzl)^6]-AbA (**9**)

Boc-L-Glu(OBzl)-L-MeVal-OPac (**4**):

Boc-L-Glu(OBzl)-OH (**2**) (1.8 g, 5.25 mmol) and HCl·H-L-MeVal-OPac (**3**) (1.0 g, 3.50 mmol) were dissolved in CH_2Cl_2 (10 ml). To the mixture, PyBroP (2.50 g, 5.25 mmol) and DIEA (2.44 ml, 14.0 mmol) were added. The mixture was stirred at 0°C for 2 hours and then at room temperature for 12 hours. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc, washed successively with satd. aq. $NaHCO_3$, satd. aq. NaCl, 10% aq. citric acid and satd. aq. NaCl, and then dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by silica gel chromatography (toluene/EtOAc (5:1)) to give **4** as colorless oil (1.98 g, 99% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.85 (d, 2H, J=7.6 Hz), 7.61 (t, 1H J=7.4 Hz), 7.47 (t, 2H, J=7.6 Hz), 5.40 (br s, 1H), 4.79 (m, 1H), 3.16 (s, 3H), 2.49 (m, 2H), 1.87 (m, 1H), 1.43 (s, 9H), 1.09 (d, 3H, J=6.6 Hz), 0.89 (d, 3H, J=6.6 Hz).

Boc-L-Glu(OBzl)-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**7**):

The compound (**4**) (2.54 g, 4.47 mmol) was dissolved in 90% aq. AcOH (224 ml). To the solution, zinc dust (14.6 g, 220 mmol) was added at 0°C. The mixture was ultrasonically stirred at 0°C for 1.5 hours. After removal of insoluble materials, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc, washed successively with 10% aq. citric acid and satd. aq. NaCl, and then dried over $MgSO_4$. After concentration under reduced pressure, the residue was crystallized from hexane to give Boc-L-Glu(OBzl)-L-MeVal-OH (**5**) as colorless oil (2.0 g, 96% yield).

The compound (**5**) (39.9 mg, 85.9 μmol) and HCl·H-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**6**) (60.0 mg, 57.3 μmol) obtained as described in the previous paper[10], were dissolved in dimethylformamide (DMF) (250 μl). To the solution, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HOOBt) (11.3 mg, 68.8 μmol) and water-soluble carbodiimide (WSCD) (11.2 μl, 63.1 μmol) were added at 0°C. The mixture was stirred at 0°C for 9.5 hours. After addition of EtOAc it was then washed, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by silica gel TLC (benzene/acetone 3:1) to give **7** as colorless powder (76.0 mg, 92% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.88 (d, 2H, J=7.1 Hz), 7.62 (t, 1H J=7.4 Hz), 7.49 (t, 2H, J=7.5 Hz), 6.70 (m, 1H), 5.60 (t, 1H, J=7.2 Hz), 5.46 (m, 1H), 1.41 (s, 9H), FAB-MS m/z 1442 (M+H).

HCl·H-L-Glu(OBzl)-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (**8**):

The Pac group in **7** was removed as described for the synthesis of **5** using **7** (70.0 mg, 48.5 μmol) and zinc dust (634 mg, 9.70 mmol). Boc-L-Glu(OBzl)-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH was obtained as colorless oil (37.7 mg, 74% yield).

To Boc-L-Glu(OBzl)-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (32.7 mg, 24.7 μmol) was added TFA (951 μl, 12.4 mmol), and the mixture was allowed to stand at 0°C for 30 minutes. After concentration under reduced pressure, the residue was dissolved in ethyl ether. To the solution, 5.5 N HCl/dioxane solution (6.8 μl, 37.1 μmol) was added at 0°C and the resulting mixture was allowed to stand for 30 minutes at 0°C to give **8** as colorless crystals (30.8 mg, 98% yield). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.23~7.12 (m, total 10H), 5.24 (t, 1H, J=6.1 Hz), 5.12 (s, 1H), 5.10 (s, 1H), 4.89 (m, 1H), 4.74 (m, 1H), 4.65 (d, 1H, J=11.2 Hz), 4.50 (d, 1H, J=11.2 Hz), 4.40 (m, 1H), 4.13 (m, 1H), FAB-MS m/z 1224 (M+H).

[L-Glu(OBzl)^6]-AbA (**9**):

The compound (**8**) (20.0 mg, 15.7 μmol) and DIEA (5.5 μl, 31.4 μmol) were dissolved together in CH_2Cl_2 (8.0 ml). The solution was added dropwise to a solution of PyBroP (36.6 mg, 78.5 μmol) and DIEA (13.7 μl, 78.5 μmol) in CH_2Cl_2 (8.0 ml) within 3.5 hours at 0°C. The mixture was stirred at 0°C for 2 hours. After concentration under reduced pressure, the residue was dissolved in EtOAc and the mixture was washed, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified with silica gel TLC ($CHCl_3$/MeOH 19:1) to give **9** as colorless powder (1.05 mg, 5% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.40 (m, 1H), 5.81 (m, 1H), 5.72 (m, 1H), 5.16 (s, 2H), 5.07 (s, 1H), 4.95 (m, 2H), 4.71 (s, 1H), 4.10 (m, 1H), 3.88 (m, 1H), FAB-MS *m/z* 1206 (M+H), 1228 (M+Na).

[L-Glu^6]-AbA (**10**):

To a solution of **9** (6.0 mg, 4.91 μmol) in MeOH (15 ml) was added palladium black (15 mg). Hydrogen gas was bubbled into the mixture at room temperature for 2 hours. After filtration of the catalyst, the filtrate was concentrated under reduced pressure to give **10** as colorless powder (5.0 mg, 89% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.47 (m, 1H), 5.67 (s, 1H), 5.41 (m, 1H), 5.18 (m, 1H), 4.89 (m, 1H), 4.79 (m, 1H), 4.68 (d, 1H, J=10.7 Hz), 4.48 (d, 1H, J=9.8 Hz), 1.46 (s, 3H), 1.17 (s, 3H), FAB-MS *m/z* 1117 (M+H), 1139 (M+Na).

[L-Glu(OC$_4$)^6]-AbA (**11a**):

[L-Glu^6]-AbA (**10**) (2.0 mg, 1.79 μmol), 1-butanol (0.82 μl, 8.84 μmol) and DMAP (0.11 mg, 0.89 μmol) were dissolved together in CH_2Cl_2 (40 μl). To the mixture, DCC (0.74 mg, 3.58 μmol) was added at 0°C. The mixture was stirred at 0°C for 4 hours and purified by silica gel TLC ($CHCl_3$/MeOH 19:1) to give **11a** as colorless powder (2.0 mg, 95% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.40 (d, 1H J=7.3 Hz), 4.94 (m, 3H), 4.66 (d, 1H, J=11.7 Hz), 4.11 (m, 2H), FAB-MS *m/z* 1173 (M+H), 1195 (M+Na).

The following compounds (**11b**~**g**) were obtained by the same procedure. The structures of these were determined by ^{1}H NMR and FAB-MS.

[L-Glu(OC$_6$)^6]-AbA (**11b**):

1-Hexanol (1.13 μl, 8.84 μmol) was used to produce **11b** (colorless powder, 94% yield).

[L-Glu(OC$_8$)^6]-AbA (**11c**):

1-Octanol (1.5 μl, 8.84 μmol) was used to give **11c** (colorless powder, 54% yield).

[L-Glu(OC$_{10}$)^6]-AbA (**11d**):

1-Decanol (1.71 μl, 8.84 μmol) was used to give **11d** (colorless powder, 99% yield).

[L-Glu(OC$_{14}$)^6]-AbA (**11e**):

1-Tetradecanol (0.82 μl, 8.84 μmol) was used to give **11e** (colorless powder, 95% yield).

[L-Glu(O*cis*Δ^3C$_6$)^6]-AbA (**11f**):

cis-3-Hexen-1-ol (0.80 μl, 6.70 μmol) was used to give **11f** (colorless powder, 85% yield).

[L-Glu(O*trans*Δ^3C$_6$)^6]-AbA (**11g**):

trans-3-Hexen-1-ol (0.80 μl, 6.70 μmol) was used to give **11g** (colorless powder, 80% yield).

[L-HONva^6]-AbA (**12**):

To a solution of **10** (4.0 mg, 3.58 μmol) and *N*-hydroxysuccinimde (HONSu) (0.83 mg, 7.16 μmol) in DMF (50 μl) was added WSCD·HCl (1.40 mg, 7.16 μmol) at 0°C. The mixture was stirred at 0°C for 5 hours and dissolved in EtOAc. The mixture was washed, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was dissolved in THF (50 μl). To the solution, sodium borohydride ($NaBH_4$) (0.34 mg, 8.95 μmol) was added at 0°C. The mixture was stirred at 0°C for 3.5 hours, added a 10% aq. citric acid cooled on ice, and extracted with EtOAc. The extract was washed with satd. aq. NaCl and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified with silica gel TLC ($CHCl_3$/MeOH 19:1) to give **12** as colorless powder (2.80 mg, 71% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.48 (d, 1H J=7.1 Hz), 4.65 (m, 1H), 4.14 (m, 1H), 3.75 (m, 2H), FAB-MS *m/z* 1103 (M+H), 1125 (M+Na).

[L-HONva(C$_5$CO)^6]-AbA (**13**):

The compound (**13**) was synthesized as described for the synthesis of **11a** using **12** (2.0 mg, 1.81 μmol) and hexanoic acid (1.14 μl, 9.05 μmol). The desired product (**13**) was obtained as colorless powder (2.18 mg, 100% yield). The structure of **13** was determined by ^{1}H NMR and FAB-MS.

Synthesis of [L-HOMeNva(Bzl)^7]-AbA (**20**)

Boc-L-HONva-OH (**14**):

To a suspension of lithium aluminum hydride ($LiAlH_4$) (1.23 g, 32.5 mmol) in ethyl ether (18 ml) was added dropwise a solution of **2** (6.0 g, 17.8 mmol) in ethyl ether (18 ml) at 0°C within 30 minutes. The mixture was stirred at room temperature for 30 minutes and refluxed for 3.5 hours. To the resulting mixture, 10% aq. citric acid was added within 30 minutes, and the insoluble material was

filtered. The organic layer of the filtrate was washed with satd. aq. NaCl, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified with silica gel column chromatography ($CHCl_3$/MeOH/AcOH 95:5:3) to give **14** as colorless oil (2.56 mg, 62% yield). ^{1}H NMR (270 MHz, DMSO-d_6) δ 4.42 (m, 1H), 3.84 (m, 1H), 1.38 (s, 9H).

Boc-L-HOMeNva(Bzl)-OPac (**17**):

The compound (**17**) was prepared from **14** by the procedure as follows:

Protection of OH group in **14**; To the solution of **14** (500 mg, 2.14 mmol) in DMF (5.0 ml), NaH (124 mg, 5.14 mmol) was added, and the mixture was stirred at 0°C for 1 hour. To the mixture, benzyl bromide (330 μl, 2.78 mmol) was added, and the mixture was stirred at 0°C for 4.5 hours. Boc-L-HONva(Bzl)-OH (**15**) was obtained as colorless solid (341 mg, 49% yield). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.37~7.26 (m, 5H), 4.42 (s, 2H), 1.36 (s, 9H).

N-Methylation of **15**; The compound (**15**) (77.3 mg, 0.24 mmol) and CH_3I (119 μl, 1.91 mmol) was dissolved in THF (5 ml). To the mixture, NaH (17.2 mg, 0.72 mmol) was added at 0°C. The mixture was stirred at room temperature for 4 hours. Boc-L-HOMeNva(Bzl)-OH (**16**) was obtained as colorless oil (45.1 mg, 56% yield). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.38~7.25 (m, 5H), 4.45 (s, 2H), 2.70 (s, 3H), 1.40 (s, 9H).

Protection of carboxyl group in **16**; To the solution of **16** (96.1 mg, 0.29 mmol) in EtOAc (1.0 ml), phenacyl bromide (62.5 g, 0.31 mmol) and Et_3N (43.6 μl, 0.31 mmol) at 0°C. The mixture was stirred at room temperature for 4.5 hours. The compound (**17**) was obtained as colorless solid (84.1 mg, 65% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, 2H, J=7.6 Hz), 7.61 (m, 1H), 7.49 (m, 2H), 7.28 (s, 2H), 5.39 (d, 1H, J=15.3 Hz), 4.52 (s, 2H), 3.53 (t, 2H, J=6.3 Hz), 2.88 (s, 3H), 2.16 (m, 1H), 1.95 (m, 1H), 1.70 (s, 2H), 1.45 (s, 9H).

Boc-L-*a*Ile-L-HOMeNva(Bzl)-OPac (**18**):

The Boc group in **17** was removed with 4.0 N HCl/dioxane solution to give HCl·H-L-*a*Ile-L-HOMeNva(Bzl)-OPac. The compound (**18**) was synthesized as described for the synthesis of **4** using Boc-L-*a*Ile-OH (48.6 mg, 0.21 mmol) and HCl·H-L-HOMeNva(Bzl)-OPac (55.0 mg, 0.14 mmol). (66.8 mg, 84% yield).

Boc-L-*a*Ile-L-HOMeNva(Bzl)-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**19**):

The Pac group in **18** was removed with Zn/AcOH as described in the synthesis of **7**. Then, Boc-L-*a*Ile-L-HOMeNva(Bzl)-OH (25.3 mg, 54.4 μmol) was coupled with **6** (38.0 mg, 36.3 μmol) as described for the synthesis of **7**. (colorless oil 26.2 mg, 50% yield).

[L-HOMeNva(Bzl)^7]-AbA (**20**):

After deprotections of Pac and Boc group in **19**, HCl·H-L-*a*Ile-L-HOMeNva(Bzl)-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (15.0 mg, 11.8 μmol) was used for the cyclization to **20** as described for the synthesis of **9**. The compound (**20**) was obtained as colorless solid. (4.1 mg, 34.0% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.43 (m, 1H), 5.73 (s, 1H), 5.67 (t, 1H, J=7.5 Hz), 5.23 (m, 1H), 5.13 (m, 1H), 4.46 (s, 2H), FAB-MS m/z 1207 (M+H), 1229 (M+Na).

[L-HOMeNva^7]-AbA (**21**):

The compound (**21**) was synthesized as described for the synthesis of **10** using **21** (3.0 mg, 2.46 μmol). (colorless powder 2.4 mg, 87% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.45 (m, 1H), 5.71 (s, 1H), 5.65 (m, 1H), 5.23 (m, 1H), FAB-MS m/z 1116 (M+H), 1138 (M+Na).

[L-HOMeNva(Cl_3CO)^7]-AbA (**22**):

The compound (**22**) was synthesized as described for the synthesis of **11a** using **21** (2.0 mg, 1.77 μmol) and myristic acid (0.61 mg, 2.65 μmol). (colorless powder 1.22 mg, 57% yield). The structure of **22** was determined by ^{1}H NMR and FAB-MS.

Synthesis of [L-Glu(O*c*Hex)^8]-AbA (**29**):

Boc-L-Glu(O*c*Hex)-DL-HOMeVal-OBzl (**23**):

The compound (**23**) was synthesized as described for the synthesis of **4** using Boc-L-Glu(O*c*Hex)-OH (2.71 g, 8.22 mmol) and HCl·H-DL-HOMeVal-OBzl (1.5 g, 5.48 mmol). (colorless oil 2.16 g, 72% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 4.77 (m, 2H), 3.12 (s, 3H), 2.89 (m, 1H), 2.28 (m, 1H), 1.16 (s, 3H).

Boc-L-Glu(O*c*Hex)-DL-HOMeVal-D-Hmp-OPac (**25**):

Boc-L-Glu(O*c*Hex)-DL-HOMeVal-OH (**24**) was synthesized as described for the synthesis of 10 using **23** (1.00 g, 2.20 mmol). (colorless oil 830 mg, 105% yield).

The compound (**24**) (420 mg, 0.92 mmol), H-D-Hmp-OPac (275 mg, 1.10 mmol) and 4-pyrrolidinopyridine (40.8 mg, 0.28 mmol) were dissolved together in THF (2 ml). To the mixture, DCC (226 mg, 1.10 mmol) was added at 0°C. The mixture was stirred at room tem-

perature for 18 hours. After concentration under reduced pressure, the residue was dissolved in EtOAc, washed, dried over $MgSO_4$, purified by silica gel chromatography (toluene/EtOAc 5 : 1) to give **25** as colorless oil. (465 mg, 74.5% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.90 (d, 2H, J=7.1 Hz), 7.62 (t, 1H J=7.5 Hz), 7.49 (t, 2H, J=7.6 Hz), 5.32 (s, 1H), 3.31 (s, 3H), 2.38 (m, 2H), 2.12 (m, 2H), 1.56 (m, 2H), 1.42 (s, total 12H), 1.19 (s, 3H), 1.06 (d, 3H, J=6.8 Hz), 0.96 (t, 3H, J=7.8 Hz).

Boc-L-Glu(O*c*Hex)-L-HOMeVal-D-Hmp-OH (**26L**):

The compound (**26L**) was synthesized as described for the synthesis of **5** using **25** (470 mg, 0.68 mmol) and zinc dust (8.90 g, 136 mmol). Boc-L-Glu(O*c*Hex)-DL-HOMeVal-D-Hmp-OH (**26DL**) was separated from each other by HPLC (column: YMC SH-363-5, 30 × 250 mm, eluted with 60% MeCN/H_2O). (colorless oil, 113 mg, 30% calculated from the L-L-D isomer in **25**).

Boc-L-Glu(O*c*Hex)-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**27**):

The compound (**27**) was synthesized as described for the synthesis of **4** using **26L** (67.0 mg, 114 μmol) and HCl·H-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (119 mg, 171 μmol). (colorless oil 101 mg, 72% yield).

Boc-L-*a*Ile-L-MeVal-L-Glu(O*c*Hex)-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**28**):

The Boc group in **27** was removed with TFA as described in the synthesis of **8**. The deprotected peptide (65.0 mg, 56 μmol) was coupled with Boc-L-*a*Ile-L-MeVal-OH (28.9 mg, 83.9 μmol) as described for the synthesis of **7**. (colorless oil 61.8 mg, 76% yield).

[L-Glu(O*c*Hex)^8]-AbA (**29**):

After deprotections of Pac and Boc group in **28**, HCl·H-L-*a*Ile-L-MeVal-L-Glu(O*c*Hex)-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (35.0 mg, 27.6 μmol) was cyclized as the same method described for the synthesis of **2** using PyBroP (64.3 mg, 138 μmol), DIEA (33.7 μl, 193 μmol) and CH_2Cl_2 (27.6 ml). (colorless solid 9.6 mg, 29% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.51 (d, 1H, J=5.9 Hz), 5.32 (d, 1H, J=11.0 Hz), 5.22 (d, 1H, J=11.2 Hz), FAB-MS m/z 1198 (M+H), 1220 (M+Na).

[L-Glu^8]-AbA (**30**):

To the compound (**30**) (6.5 mg, 5.35 μmol) was added HF (5 ml) under cooling with dry ice-MeOH bath. The mixture was stirred at 0°C for 30 minutes. After removal of HF under reduced pressure, the residue was dissolved in dioxane and lyophilized. The obtained powder was purified by TLC ($CHCl_3$/MeOH 19 : 1) to give **30** as colorless solid (5.50 mg, 90% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.51 (d, 1H, J=5.6 Hz), 5.75 (s, 1H), 5.27 (d, 1H, J=11.0 Hz), 5.00 (m, 1H), 4.64 (d, 1H, J=11.0 Hz), 4.44 (m, 1H), FAB-MS m/z 1117 (M+H), 1139 (M+Na).

[L-Glu(OC$_{14}$)^8]-AbA (**31**):

The compound (**31**) was synthesized as described for the synthesis of **11a** using [L-Glu^8]-AbA (2.0 mg, 1.76 μmol) and myristyl alcohol (0.57 mg, 2.65 μmol). (colorless powder 1.70 mg, 73% yield). The structure of **31** was determined by ^{1}H NMR and FAB-MS.

[L-Glu(NHC$_{14}$)^8]-AbA (**32**):

The compound (**32**) was synthesized as described for the synthesis of **11a** using [L-Glu^8]-AbA (2.0 mg, 1.76 μmol), myristylamine (1.20 mg, 5.30 μmol), HOBt (0.07 mg, 0.53 μmol) and DCC (0.73 mg, 3.52 μmol). (colorless powder 2.06 mg, 88% yield). The structure of **32** was determined by ^{1}H NMR and FAB-MS.

[L-HONva^8]-AbA (**33**):

The compound (**33**) was synthesized as described for the synthesis of **12** using **30** (2.0 mg, 1.76 μmol), HONSu (0.41 mg, 3.52 μmol), WSCD·HCl (0.68 mg, 3.52 μmol) and $NaBH_4$ (0.17 mg, 4.40 μmol). (colorless solid 0.96 mg, 50% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.34~5.12 (m, 3H), 5.05~4.77 (m, 3H), 4.66~4.14 (m, 3H), FAB-MS m/z 1103 (M+H), 1125 (M+Na).

In Vitro Antifungal Activity

The minimum growth-inhibitory concentrations (MICs) for fungi were determined by the agar dilution method using Sabouraud-dextrose (SD) agar plate. A loopful of a suspension of a fungi containing approximately 5×10^7 cells/ml was streaked on the surface of the agar plates containing graded concentrations of compounds. After incubation at 30°C for 3 days, MIC was defined as the lowest compound concentration at which no fungal growth could be detected.

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