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Total Synthesis of an Antifungal Cyclic Depsipeptide Aureobasidin A [1]

Toru Kurome[b], Kaoru Inami[a], Tetsuya Inoue[b], Katsushige Ikai[b], Kazutoh Takesako*[b], Ikunoshin Kato[b], and Tetsuo Shiba*[a]

[a]Peptide Institute, Protein Research Foundation, 4-1-2 Ina, Minoh, Osaka 562, Japan and

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

Abstract: The first total synthesis of antifungal cyclic depsipeptide aureobasidin A is described. The synthesis was achieved mainly using bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP) as a coupling reagent. Peptide cyclization was carried out between L-*allo*-isoleucine (L-*a*Ile[1]) and L- Pro[9] residues in the linear nonapeptide at the final step of the synthesis. Synthesized aureobasidin A was completely identical with the natural antibiotic with respect to antifungal activity and physicochemical properties. Unusual reactions due to *N*-methylamino acid, an oxazoline-mediated reaction and an *N*, *O*-acyl migration, are also described.

INTRODUCTION

Aureobasidins are novel cyclic depsipeptide antibiotics isolated from the culture medium of *Aureobasidium pullulans* R106.[2] Aureobasidin A (**1**), as a major component of aureobasidins, exhibits strong *in vitro* as well as *in vivo* fungicidal activity against many pathogenic fungi including *Candida albicans*.[3] Therefore, it shows promise as an antifungal agent. The structure of aureobasidin A was determined mainly by the heteronuclear multiple-bond correlation (HMBC) technique and chemical degradation.[4]

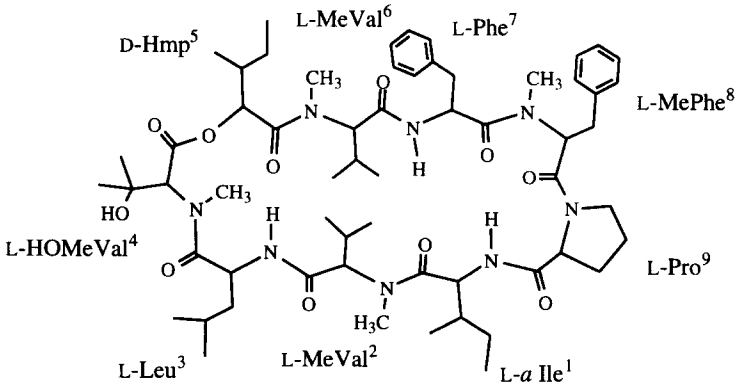


Fig. 1. Structure of aureobasidin A (**1**)

This depsipeptide is composed of one hydroxy acid, (2*R*)-hydroxy-(3*R*)-methylpentanoic acid (D-Hmp), and eight hydrophobic amino acids, four of which are *N*-methylated, *i.e.*, L-*N*-methylvaline (L-MeVal), L-*N*-methylphenylalanine (L-MePhe) and L-β-hydroxy-*N*-methylvaline (L-HOMeVal).

Only few papers have reported the total synthesis of cyclic depsipeptides containing *N*-methylamino acids. A crucial points for success of a synthesis of such cyclic depsipeptides may be in finding of efficient procedures for producing the *N*-methylamide linkage and also in the selection of a suitable position and method for the cyclization. Generally speaking, imino groups in *N*-methylamino acids are quite less reactive than primary amino groups as in usual amino acids. Furthermore, *N*-methylamino acids can easily be racemized during coupling reactions.[5] An achievement of the cyclization would depend on the conformation of the linear peptide effected by intramolecular hydrogen bonds and on the reactivities of both terminal residues involved in the cyclization reaction. An undecapeptide cyclosporin A, an immunosuppressant with antifungal activity, has been totally synthesized by Wenger *et al.* using a mixed pivalic anhydride method.[6] Its *N*-methylamino acids were condensed in yields above 80%. In general, the mixed anhydride (MA) method is a simple procedure and the peptide bond coupling proceeds promptly. On the other hand, in the coupling by the MA method, strict control of the reaction temperature is required to avoid racemization and to reduce disproportionation of MA to the corresponding symmetric anhydride of "other acid" which would cause a low yield in the reaction.

This paper is a full report on the total synthesis of aureobasidin A. In order to compare the formation reactions of two types of linkage on an ester bond and an amide bond for the final cyclization, we first tried a macrocyclization reaction at the ester linkage of a linear peptide obtained by chemical degradation of the natural aureobasidin A under different conditions, but it was not unsuccessful. We also evaluated the cyclization at the amide bond for the totally synthesized linear depsipeptide. Here we discuss unknown side reactions resulting from *N*-methylamino acids and also the control of reaction conditions to give either monomeric or dimeric cyclic peptide.

RESULTS AND DISCUSSION

Macrolactonization of the linear nonapeptide H-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-L-aIle-L-MeVal-L-Leu-L-HOMeVal-OH (2)

In a preliminary experiment to search for adequate cyclization conditions, a linear nonapeptide, H-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-L-*a*Ile-L-MeVal-L-Leu-L-HOMeVal-OH (**2**), obtained by mild alkaline hydrolysis of aureobasidin A, was tested for cyclization. We tried using several coupling reagents, including 2,4,6-trichlorobenzoyl chloride, dicyclohexylcarbodiimide (DCC), carbonyldiimidazole (CDI) and bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBroP)[7]. Some activated derivatives of the carboxyl terminus of peptide **2** were also prepared. However, none of these cyclization reactions proceeded at the ester linkage (Table 1). These results suggested that cyclization between a carboxyl group of L-HOMeVal and a hydroxyl group of D-Hmp to form an ester bond is not adequate for the total synthesis of this depsipeptide, probably because of hindrance from side chains of both terminal residues.

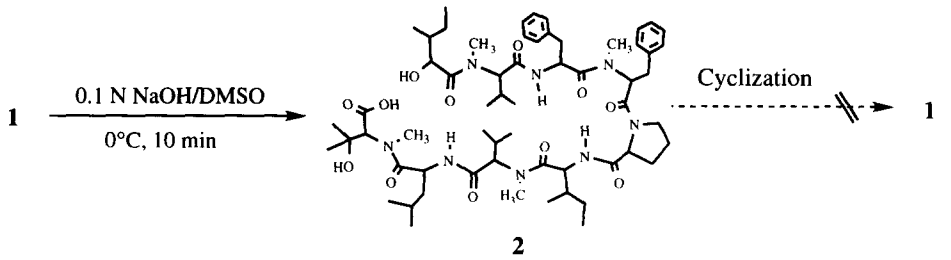


Table 1. Evaluation of Coupling Reagents for Cyclization of the Linear Peptide **2**

Reagent	Solvent	Catalyst [a]	Reaction time (hr)	Product (%)
2,4,6-trichloro-benzoyl chloride	THF	A	15	- [b]
DCC	THF	B	12	I[c] (12) II[d] (trace)
CDI	THF	B	72	no reaction
PyBroP	CH_2Cl_2	B	24	III[e] (11)

[a] A, 4-(dimethylamino)pyridine (DMAP); B, 4-pyrrolidinopyridine [b] not identified [c] I, linear peptide linked with DCC on its carboxyl group; [d] II, linear peptide linked as an amide with 4-pyrrolidinopyridine on its carboxyl group; [e] III, dehydrated linear peptide of MW 1100.

*Preparation of the linear nonapeptide H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (**20**)*

We next tried to prepare the linear peptide L-*a*Ile[1]-L-MeVal[2]-L-Leu[3]-L-HOMeVal[4]-D-Hmp[5]-L-MeVal[6]-L-Phe[7]-L-MePhe[8]-L-Pro[9] as shown in Fig. 2 in order to carry out the cyclization between L-*a*Ile[1] and L-Pro[9]. The linear depsipeptide was synthesized according to Boc strategy from three segments A, B, and C successively.

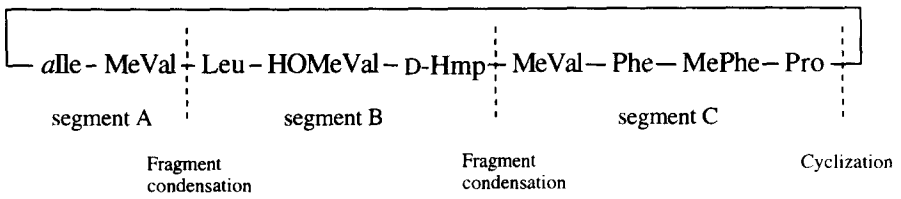


Fig. 2. Synthetic strategy

a) Synthesis of segment A (Boc-*a*Ile-MeVal) (**6**)

Several coupling reagents such as PyBroP, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Bop-Cl)[8], benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[9] and the water-soluble carbodiimide (WSCD)/1-hydroxybenzotriazole (HOBt) were used to prepare the dipeptide segment A [10] (Table 2). PyBroP gave the best yield, while Bop-Cl and other coupling reagents gave relatively poor yields.

The dipeptide thus obtained was treated with zinc/acetic acid to remove the phenacyl (Pac) group to give **6**.

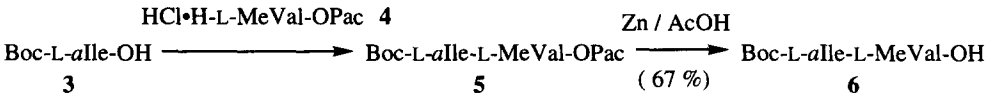
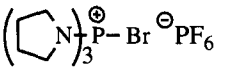


Table 2. Coupling Reaction between Boc-L-allo-Ile-OH and HCl•H-L-MeVal-OPac in Segment A

Coupling reagent	Base	Yield of **6** (%)
PyBroP	DIEA	40.9
Bop-Cl	DIEA	32.1
BOP	NMM	11.8
ClCOOBu^i	-	trace
WSCD-HOBt	-	17.3

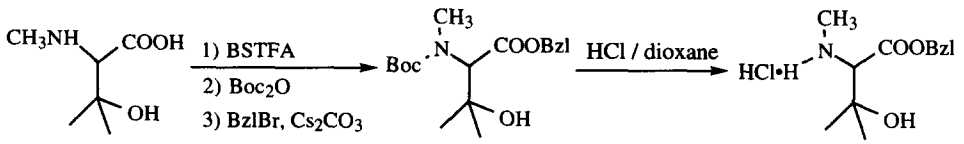
DIEA, diisopropylethylamine; NMM, *N*-methylmorpholine



b) Synthesis of segment B (Boc-L-Leu-L-HOMeVal-D-Hmp) (**12**)

DL-β-Hydroxy-*N*-methylvaline (DL-HOMeVal) (**7**) was prepared as described by Izumiya *et al.*[11] After activation and solubilization by trimethylsilylation with *N*, *O*-bis(trimethylsilyl)trifluoracetamide (BSTFA), **7** was protected with Boc for the imino group and benzyl (Bzl) for the carboxyl group successively, following deprotection of Boc group to give H-DL-HOMeVal-OBzl (**8**) in a good yield of 94% from **7** (Scheme 1).

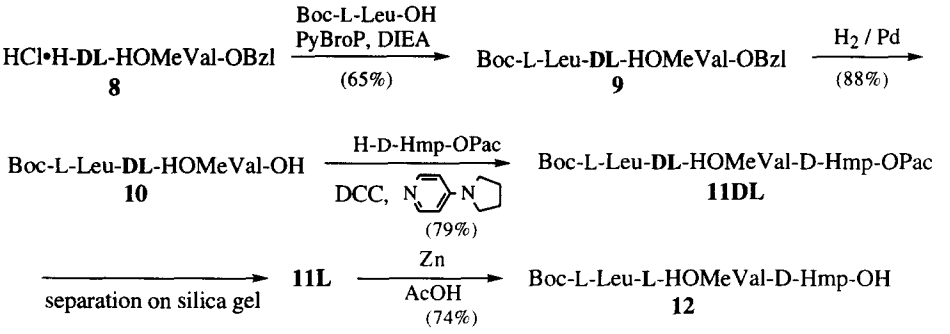
To prepare segment B, we adopted a strategy of elongation from the *N*-terminus to the *C*-terminus as shown in Scheme 2. The rationale for the choice of this strategy will be discussed later. Compound **8** was coupled with Boc-L-Leu-OH using PyBroP and DIEA to afford **9** in moderate yield (65%). This reaction proceeded without protection of the hydroxyl group of HOMeVal.[12] After deprotection of the Bzl group of **9**, the resulting *N*-protected product **10** was coupled with H-D-Hmp-OPac through an ester bond using DCC in the presence of 4-pyrrolidinopyridine as an acylation catalyst. Optimization of this coupling condition will be discussed later. The resulting diastereomers **11DL** were separated by silica gel column chromatography followed by treatment with zinc/acetic acid to obtain the L-L-D isomer **12**.[13]



H-DL-HOMeVal-OH **7**

8 (94% from **7**)

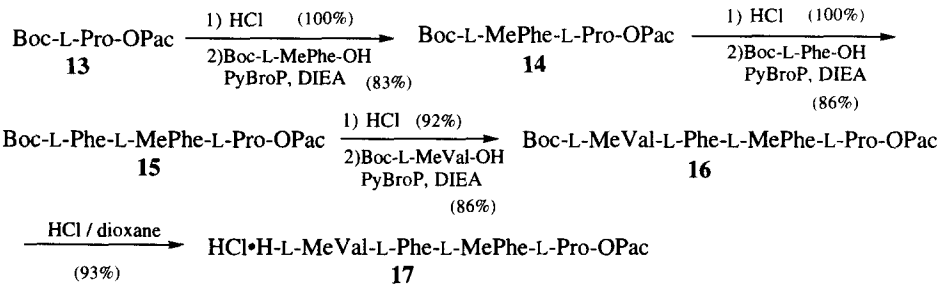
Scheme 1. Synthetic scheme of DL-β-hydroxy-*N*-methylvaline benzyl ester (**8**)



Scheme 2. Synthetic scheme of segment B

c) Synthesis of segment C (L-MeVal-L-Phe-L-MePhe-L-Pro-OPac) (**17**)

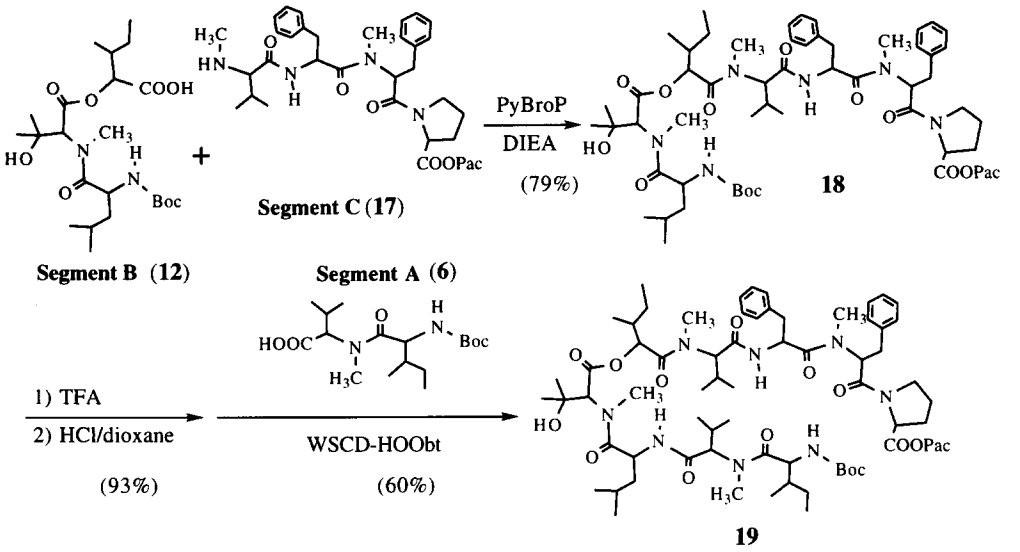
Segment C was prepared by successive condensations, starting from Boc-L-Pro-OPac as a *C*-terminus using PyBroP and DIEA in dichloromethane (CH_2Cl_2) (Scheme 3). No serious racemizations were observed at each step of the synthesis of this segment.[14]



Scheme 3. Synthetic scheme of segment C

d) Fragment condensation (Scheme 4)

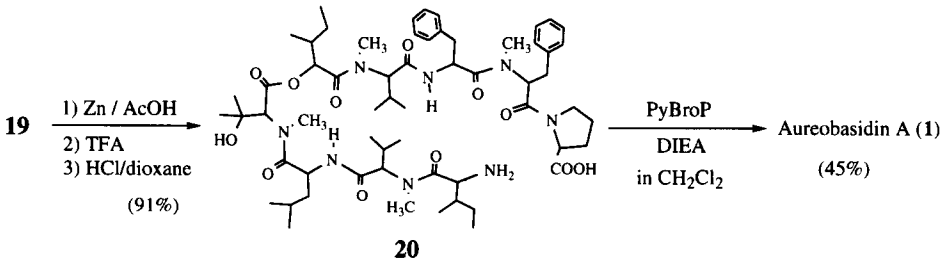
Condensation of segment B (**12**) with segment C (**17**) was carried out using PyBroP to give Boc-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**18**) in a yield of 79% without racemization of D-Hmp. After removal of the Boc group of **18**, the resulting deprotected peptide was condensed with segment A (**6**) by a method using WSCD and 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HOObt) to give the linear depsipeptide (**19**). This WSCD-HOObt method gave **19** as the main product with only slight racemization on MeVal[2], in 6:1 ratio for L/D, whereas PyBroP gave a 1:1 mixture of the diastereomers. The diastereomers were separated by silica gel column chromatography, giving the optically pure nonapeptide **19** in 60% yield.



Scheme 4. Condensation of segment A, B and C

Synthesis of aureobasidin A (Scheme 5)

Both protecting groups *i.e.*, Boc and Pac in the linear depsipeptide **19**, were removed successively, and the resulting free peptide **20** was cyclized with PyBroP in CH_2Cl_2 under high-dilution condition (10^{-3} M) to afford the desired cyclic monomeric peptide of L-Pro residue as the main product in 45% yield and the racemized D-isomer on Pro residue of the cyclic monomeric peptide in 7.6% yield, respectively (Scheme 5). No cyclic dimer was detected on cyclization with PyBroP. The main product of the cyclic monomer thus obtained was completely identical with the natural product in all respects including TLC, HPLC, ^{1}H-NMR, and antifungal activities (Table 3).



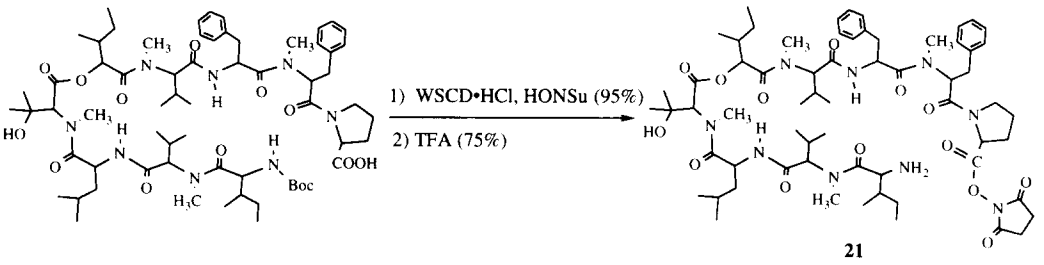
Scheme 5. Cyclization

Table 3. Minimum Inhibitory Concentrations (μg/ml) of Synthetic and Natural Aureobasidin A

	Synthetic	Natural
Candida albicans TIMM 0136	0.0125	0.0125
Candida albicans TIMM 1768	1.6	3.1
Candida kefyr TIMM 0301	0.4	0.4
Candida glabrata TIMM 1062	0.2	0.1
Cryptococcus neoformans TIMM 0354	0.8	0.8
Saccharomyces cerevisiae ATCC 9763	0.2	0.2
Aspergillus fumigatus TIMM 2766	>25	>25

*Cyclization of the linear nonapeptide H-L-alle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (**20**) by the active ester method*

As an alternative procedure, we tried cyclization of the linear depsipeptide **20** by the active ester method. The *N*-hydroxysuccinimide ester (ONSu) **21** of this depsipeptide obtained via Boc derivative of **20** gave a cyclic dimer as the major product (36%) and the monomer in only 14% yield at 10^{-3} M in CH_2Cl_2 in the cyclization (Scheme 6 and Table 4).



Scheme 6. Preparation of *N*-hydroxysuccinimide ester of the linear depsipeptide

Table 4. Cyclizations of **20** and Its *N*-Hydroxysuccinimide Ester (ONSu) **21**

Solvent	Activation	Product (%)	
		1 (Cyclic monomer)	Cyclic dimer
CH_2Cl_2	PyBroP	45	0
CH_2Cl_2	ONSu (**22**)	14	36
DMF	PyBroP	no reaction	
DMF	ONSu (**22**)	16	<4

The difference in the pattern of products of the PyBroP method from that of the *N*-hydroxysuccinimide ester (the active ester) method in CH_2Cl_2 was probably arised from the difference in the reactivities of the two coupling reagents. The coupling reaction by PyBroP proceeded rapidly under the high-dilution condition, forcing intramolecular coupling, to afford the desired cyclic monomer as the major product. In contrast, as the reaction speed in the active ester method was quite slow, intermolecular couplings occurred during the reaction in CH_2Cl_2 in addition to intramolecular couplings, resulting in dimer production even at 10^{-3} M.

On the other hand, in dimethylformamide (DMF), the active ester **21** gave the cyclic monomer as a major product in a relatively low yield of 16% together with the dimer at less than 4%, although the reaction in DMF proceeded more slowly than in CH_2Cl_2. Such different patterns in dimer formation may be due to a solvation feature of the linear peptide molecule depending on the solvent. Each solvent may cause a different conformation of the linear peptide **20**. Though the intramolecular hydrogen bonds found in the cyclic derivative of aureobasidin[15] could not be detected in the linear peptide from ^{1}H-NMR study in $CDCl_3$ or DMSO-d_6, the rotating frame nuclear Overhauser and exchange spectroscopy (ROESY) spectra showed some signals of the nuclear Overhauser effect (NOE) in both solvents and some difference of signals between them (Fig. 3). NOE signals detected by the ROESY spectrum suggested that the peptide **20** in $CDCl_3$, may have a bent conformation similar to that in CH_2Cl_2. In contrast, the peptide **20** in DMSO-d_6, may take a stretched conformation, considering the similarity to the solvent feature of DMF. Thus, the bent conformation of the peptide in CH_2Cl_2 will lead to either intramolecular or intermolecular cyclization depending on the actual peptide concentration in the cyclization as described above, resulting in a difference in ratio of the cyclic monomer and cyclic dimer. On the other hand, in DMF, the stretched conformation may decrease the chance of cyclization by the active ester method, and furthermore the solvation of the linear peptide with DMF may prevent the peptide molecules from coming closer each other, leading to less formation of the cyclic monomer as the major product in this solvent.

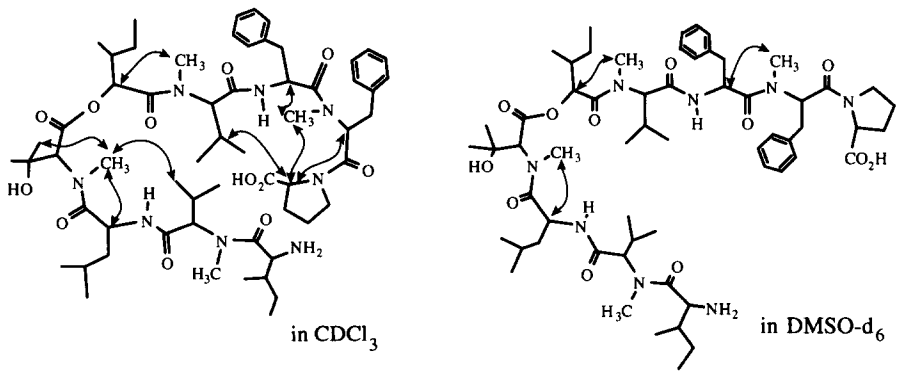


Fig. 3. NOEs detected by ROESY and plausible conformation of the linear peptide in solvents used for cyclization

Unusual reactions caused by N-methylamino acids.

a) Optimization of the coupling condition between L-HOMeVal[4] and D-Hmp[5]

Coupling between Boc-DL-HOMeVal-OH and H-D-Hmp-OPac by PyBroP and DIEA unexpectedly gave Boc-D-Hmp-OPac (**22**) (29% yield) instead of the desired depsipeptide Boc-DL-HOMeVal-D-Hmp-OPac.

The mechanism of formation of **22** is proposed in Fig. 4. When the carboxyl group of Boc-DL-HOMeVal-OH is activated by PyBroP, the carbonyl oxygen of the Boc group intramolecularly attacks the carboxyl carbon resulting in formation of an oxazolinium compound of Boc-DL-HOMeVal-OH as an intermediate.[16] Finally, the hydroxyl group of H-D-Hmp-OPac attacks the position 2 carbon of the oxazoline ring to produce Boc-D-Hmp-OPac.

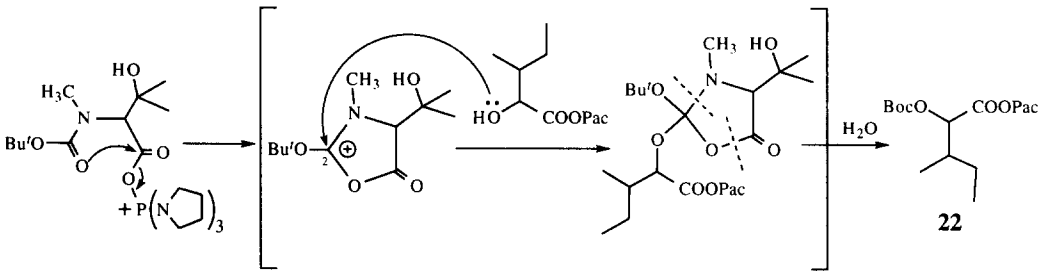


Fig. 4. Plausible mechanism of the oxazoline-mediated reaction

In order to avoid the formation of the oxazoline intermediate, DCC was used as a milder activation reagent than PyBroP. Addition of the acylation catalyst 4-pyrrolidinopyridine to the reaction mixture led to preferable coupling between the carbonyl oxygen of Boc-DL-HOMeVal-OH and the hydroxyl group of H-D-Hmp-OPac. The results are shown in Table 5. In Runs 2 and 3, the desired peptide Boc-DL-HOMeVal-D-Hmp-OPac was obtained in low yields. Since the position of the Boc group in Boc-DL-HOMeVal may cause formation of the oxazoline intermediate, as mentioned above, Boc-L-Leu-DL-HOMeVal-OH was used in place of Boc-DL-HOMeVal as an acid component in the sebsequent experiments. Yields of the coupling product in Run 4 and particularly in Run 5 were superior to those in Runs 2 and 3, showing the importance of the position of Boc as well as a mild activation reagent. Boc-L-Leu-DL-HOMeVal-D-Hmp-OPac (**11DL**) was thus prepared in a good yield of 79%. The reaction conditions in Run 5 were then used to prepare **11DL** on a large scale for elongation from the *N*- to the *C*-terminus, as shown in Scheme 2.

Table 5. Optimization of the Coupling Reaction between HOMeVal and Hmp

Run	Acid Component	Alcohol Component	Condensation Reagent	Catalyst	Solvent	Yield(%)
1	Boc-DL-HOMeVal-OH	H-D-Hmp-OPac	PyBroP		CH_2Cl_2	0
2	Boc-DL-HOMeVal-OH	H-D-Hmp-OPac	PyBroP	+	CH_2Cl_2	29
3	Boc-DL-HOMeVal-OH	H-D-Hmp-OPac	DCC	+	THF	37
4	Boc-L-Leu-DL-HOMeVal-OH	H-D-Hmp-OPac	PyBroP	+	CH_2Cl_2	43
5	Boc-L-Leu-DL-HOMeVal-OH	H-D-Hmp-OPac	DCC	+	THF	79

Catalyst : 4-pyrrolidinopyridine

b) *N*, *O*-Acyl migration between OH group in Hmp[1] and NH_2 group in Phe[3] under acidic conditions (Fig. 5).

To prepare linear peptide **20**, we first tried condensation of two fragments, *i.e.*, L-*a*Ile-L-MeVal-L-Leu-L-HOMeVal and D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro, between the carboxyl group of L-HOMeVal and the hydroxyl group of D-Hmp residues. For this condensation, tetrapeptide **17** was coupled with Boc-D-Hmp-OH followed by deprotection of the Boc group with hydrogen chloride (HCl)/dioxane solution. In this reaction, we unexpectedly obtained a tripeptide phenacyl ester (**25**) (41% yield) instead of the desired pentapeptide **23**. This indicated that deprotection by HCl caused *N*, *O*-acyl migration between the amino group of Phe and the hydroxyl group of Hmp, resulting in formation of a lactone derived from Hmp-MeVal to produce **24** as shown in Fig. 5.

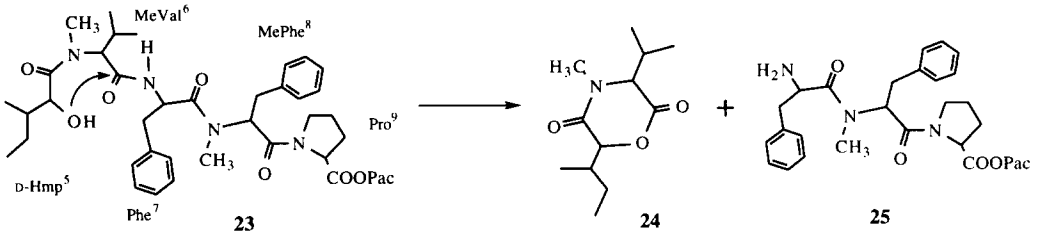


Fig. 5. Plausible mechanism of *N*, *O*-acyl migration

When trifluoroacetic acid (TFA) was used instead of HCl for deprotection, the desired peptide **23** having the free hydroxyl group was obtained in a yield of 69%, although the acyl migration could not be suppressed completely. We thus recognized that the peptide Hmp-MeVal may give the six-membered lactone more easily than normal peptide (Hmp-Val), being ascribed to an increase of the *cis* *N*-methylamide isomer.

CONCLUSION

We achieved the first total synthesis of the novel cyclic depsipeptide, aureobasidin A, which contains four *N*-methylamino acids. The synthetic route opens the way to the preparation of new members of the aureobasidin family and to the study of their structure-activity relationships. The product of the final cyclization is greatly influenced by the conformation of the linear peptide, which depends on the solvent used. When PyBroP was the coupling reagent, satisfactory results were obtained for both the yield and the stereochemical control of the coupling of *N*-methylamino acid except for the coupling between Boc-DL-HOMeVal-OH and H-D-Hmp-OPac and the fragment condensation of segment A and B. Some unusual reactions, an oxazoline-mediated reaction of Boc-DL-HOMeVal-OH activated with PyBroP and an *N*, *O*-acyl migration between the hydroxyl group in Hmp and the amino group in Phe under acidic condition, contributed to the successful strategy for the synthesis. Synthetic aureobasidin A was found to be quite identical to the natural one in all respects including biological activities as shown in Table 3. Thus, we were able to achieve the first total synthesis of the novel depsipeptide aureobasidin A.

Experimental

General remarks

Melting points are not corrected. The following spectroscopic and analytical instruments were used: optical rotation, JASCO DIP-181; ^{1}H-NMR, JEOL JNM-GSX-270 (270 MHz, ref. TMS), JNM-A500 (500 MHz, ref. TMS); FAB-MS (Fast Atom Bombardment Mass Spectrometry), PD-MS (Plasma Desorption Mass Spectrometry), JEOL JMS-DX302, Applied Bio-Systems Inc. BIO-ION 20; amino acid analysis, JEOL JCL-300. Merck Kieselgel 60 F_{254} (silica gel, Merck) was used for thin layer chromatography. Merck Kieselgel 60 (silica gel; 0.04 ~ 0.063 mm, Merck) was used for flash chromatography. The *N*-methylamino acids were analyzed by HPLC with the method used for post-column derivatization described in a previous paper.[17] The stereochemistry of the amino acids were examined by HPLC with a chiral column. Equipment — The HPLC system consisted of an Altex model 100A pump, a Shimadzu CTO-6A column oven, and a Soma S-310A UV detector. Analytical conditions — Column: Daicel Chiralpak WH; mobile phase, 1 mM $CuSO_4$; column temperature, 50°C; flow rate, 1.5 ml/minute; detection, UV absorption at 220 nm.

H-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-L-alle-L-MeVal-L-Leu-L-HOMeVal-OH (2)

To a solution of aureobasidin A (**1**) (1.0 g, 0.91 mmol) in dimethyl sulfoxide (DMSO) (91 ml) was added 1 N aq. NaOH (9.1 ml), and the mixture was stirred at 0°C for 10 min. The reaction mixture was neutralized with 1 N aq. HCl solution (9.1 ml). The solution was added to 0.1 N aq. HCl (900 ml) and the precipitate was collected, dissolved in acetonitrile (MeCN), and chromatographed on a Soken-ODS column (80 X 500 mm) with 57% MeCN, giving **2** (45 mg). ^{1}H-NMR ($CDCl_3$) δ 8.15 (m, 1H, NH), 7.75 (m, 1H, NH), 7.27-7.09 (m, 10H, ar. Phe, MePhe), 6.65 (d, 1H, J=9.5 Hz), 5.49 (br.s, 1H, OH HOMeVal), 5.39 (m, 1H, α-CH), 5.14-5.10 (m, 2H), 4.93 (m, 1H), 4.83 (br.q, 1H, α-CH), 4.63 (br.d, 1H, α-CH), 4.33 (d, 1H, J=10.3 Hz, α-CH MeVal), 4.13 (d, 1H, J=2.44 Hz, α-CH), 3.25 (s, 3H, *N*-CH_3), 3.20 (s, 3H, *N*-CH_3), 3.02 (s, 3H, *N*-CH_3), 2.47 (s, 3H, *N*-CH_3), 1.36 (s, 3H, γ-CH_3 HOMeVal), 1.28 (s, 3H, γ-CH_3 HOMeVal), 1.07-0.71 (m, CH_3), FAB-MS m/z 1119 (M+H).

Cyclization of the linear nonapeptide H-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-L-alle-L-MeVal-L-Leu-L-HOMeVal-OH (2)

Method A : Mixed anhydride method using 2,4,6-trichlorobenzoyl chloride

To a solution of **2** (10.0 mg, 8.94 μmol) in toluene (1.5 ml) were added triethylamine (12.4 μl, 84.4 μmol) and 2,4,6-trichlorobenzoyl chloride (14.0 μl, 89.4 μmol). This mixture was stirred at room temperature for 24 h. After filtration of insoluble materials, the filtrate was added dropwise to a solution of 4-(dimethylamino)pyridine (DMAP) (21.8 mg, 179 μmol) in toluene (7.0 ml) within 4 h at room temperature. The mixture was stirred for another 15 h. After concentration of the solution under reduced pressure, the residue obtained was purified by silica gel thin layer chromatography (TLC) (developed with a mixture of hexane/ethyl acetate (EtOAc) (5:1) and extracted with EtOAc). The desired cyclic compound could not be detected (TLC, ^{1}H-NMR).

Method B : DCC-pyrrolidinopyridine method

A solution of **2** (10.0 mg, 8.94 μmol) and 4-pyrrolidinopyridine (0.7 mg, 4.47 μmol) in THF (4.5 ml) was added dropwise to a solution of DCC (9.2 mg, 44.7 μmol) in THF (4.5 ml) at 0°C within 4 h. The mixture was stirred at 0°C for 12 h. After concentration of the solution under reduced pressure, the residue obtained was purified by silica gel TLC (developed with a mixture of chloroform ($CHCl_3$)/methanol (MeOH) (19:1)) to give only the peptide linked with DCC (I) (1.2 mg, 12% yield) and the peptide linked with 4-pyrrolidinopyridine (II) (trace), whereas the desired cyclic compound could not be detected (Table 1). Compound I, PD-MS 1306.3 (M), 1329.6 (M+Na). Compound II, PD-MS 1265.3 (M), 1288.7 (M+Na).

Method C : Carbonyldiimidazole method

A solution of **2** (10.0 mg, 8.94 μmol) and 4-pyrrolidinopyridine (0.7 mg, 4.47 μmol) in THF (4.5 ml) was added dropwise to a solution of carbonyldiimidazole (CDI) (7.3 mg, 44.7 μmol) in THF (4.5 ml) at 0°C within 4.5 h. The mixture was stirred at 0°C for 12 h then at room temperature for 1 h. The resulting mixture was analyzed by TLC (developed with a mixture of $CHCl_3$/MeOH/acetic acid (95:5:3)), however, only the starting materials were detected.

Method D : PyBroP method

A solution of **2** (10.0 mg, 8.94 μmol) and 4-pyrrolidinopyridine (0.7 mg, 4.47 μmol) in CH_2Cl_2 (4.5 ml) was added dropwise to a solution of PyBroP (20.9 mg, 44.7 μmol) and DIEA (7.8 μl, 44.7 μmol) in CH_2Cl_2 (4.5 ml) at 0°C within 4.5 h. The mixture was stirred at 0°C for 12 h. After concentration under reduced pressure, the residue was purified by silica gel TLC (developed with a mixture of $CHCl_3$/MeOH (19:1)) to obtain the dehydrate linear compound III as the main product (1.1 mg, 11% yield). FAB-MS m/z 1101 (M+H) (Table 1).

*HCl·H-L-MeVal-OPac (**4**)*

To a solution of Boc-L-MeVal-OH (4.94 g, 21.4 mmol) in acetone (50 ml) were added triethylamine (3.30 ml, 23.8 mmol) and phenacyl bromide (4.77 g, 24.0 mmol). This mixture was stirred at 0°C for 1 h and then at room temperature for 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc, washed successively with water, saturated aq. $NaHCO_3$ and saturated aq. NaCl, and then dried over magnesium sulfate ($MgSO_4$). The solution was concentrated under reduced pressure to dryness. To the residue obtained, a 5.5 N HCl/dioxane solution (77.8 ml, 0.428 mol) was added, and the mixture was allowed to stand at room temperature for 30 min. After concentration under reduced pressure, the mixture was crystallized from ethyl ether, and washed with cold ether to give **4** as colorless crystals (6.08 g, 99% yield). mp. 166-167°C, $[\alpha]_D^{20}$ +13.2° (*c* 0.76, MeOH), ^{1}H-NMR ($CDCl_3$) δ 10.4 (m) and 9.6 (m)(NH), 7.89 (d, 2H, J=7.1 Hz), 7.64 (t, 1H, J=7.3 Hz), 7.50 (dd, 2H, J=7.3, 7.6 Hz), 5.60 (d, 1H, J=16.2 Hz), 5.48 (d, 1H, J=16.2 Hz), 3.78 (m, 1H, α-CH), 2.94 (t, 3H, J=5.3 Hz, *N*-CH_3), 2.75 (m, 1H, β-CH), 1.27 (d, 6H, J=6.8 Hz, γ-CH_3).

*Boc-L-alle-L-MeVal-OPac (**5**)*

Boc-L-alle-OH (891 mg, 3.85 mmol) and HCl·H-L-MeVal-OPac (**4**) (1.00 g, 3.50 mmol) were dissolved in CH_2Cl_2 (7 ml). To the mixture, PyBroP (1.96 g, 4.20 mmol) and DIEA (2.13 ml, 12.3 mmol) were added. The mixture was stirred at 0°C for 3 h and then at room temperature for 17 h. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc, washed successively with water, saturated aq. $NaHCO_3$ and saturated aq. NaCl, and then dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by silica gel chromatography (silica gel: 40 g, eluted with $CHCl_3$) to give **5** as a colorless oil (662 mg, 41% yield). $[\alpha]_D^{20}$ -114.8° (*c* 0.54, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.89 (d, 2H, J=7.1 Hz), 7.60 (d, 1H, J=7.6 Hz), 7.49 (t, 2H, J=7.5 Hz), 5.25 (br.d, 1H, NH *a*lle), 5.14 (d, 1H, J=10.5 Hz, α-CH MeVal), 4.63 (dd, 1H, J=9.4, 5.2 Hz, α-CH *a*lle), 3.16 (s, 3H, *N*-CH_3MeVal), 2.30 (m, 1H, β-CH *a*lle), 1.84 (m, 1H, β-CH), 1.43 (s, 9H, Boc), 1.09 (d, 3H, J=6.6 Hz, γ-CH_3 *a*lle), 0.96 (t, 3H, J=7.3 Hz, δ-CH_3 *a*lle), 0.89 (d, 3H, J=6.8Hz, γ-CH_3), 0.87 (d, 3H, J=6.8 Hz, γ-CH_3), Anal. Calcd. for $C_{25}H_{28}N_2O_6$: C, 66.36; H, 6.24; N, 4.19. Found: C, 66.32; H, 6.28; N, 4.06.

*Boc-L-alle-L-MeVal-OH (**6**)*

Boc-L-alle-L-MeVal-OPac (**5**) (657 mg, 1.42 mmol) was dissolved in 90% aq. acetic acid (AcOH) (70 ml). Zinc dust (4.64 g, 71.0 mmol) was then added to the solution at 0°C. The mixture was ultrasonically stirred at 0°C for 7.5 h. After removal of insoluble materials by filtration, the solvent was removed under reduced pressure and the residue was dissolved in EtOAc, washed successively with 10 % aq. citric acid and saturated aq. NaCl, and then dried over $MgSO_4$. After concentration under reduced pressure, the residue was crystallized from hexane and washed with hexane to give **6** as colorless crystals (325 mg, 67% yield). mp.

130-131°C, $[\alpha]_D^{20}$ -121.5° (*c* 0.49, MeOH), ^{1}H-NMR ($CDCl_3$) δ 5.24 (d, 1H, J=9.8 Hz), 4.60 (dd, 1H, J=9.5, 9.3 Hz, α-CH *a*Ile), 4.50 (d, 1H), 3.12 (s, 3H, *N*-CH_3 MeVal), 2.38 (m, 1H), 1.72 (m, 1H), 1.43 (s, 9H, Boc), 1.23 (m, 1H), 1.06 (d, 3H, J=6.6 Hz), 0.96 (t, 3H, J=7.3 Hz, δ-CH_3 *a*Ile), 0.90 (d, 3H, J=6.8Hz), 0.89 (d, 3H, J=6.6 Hz), Anal. Calcd. for $C_{17}H_{32}N_2O_5$: C, 59.28; H, 9.36; N, 8.13. Found: C, 59.29; H, 9.40; N, 7.80.

Boc-DL-HOMeVal-OBzl

To a suspension of H-DL-HOMeVal-OH (**7**) (4.0 g, 27.4 mmol) in DMF (60 ml) was added BSTFA (21.8 ml, 82.1 mmol) at 0°C. The mixture was stirred at room temperature for 1 h. Di-*t*-butyl dicarbonate (7.54 ml, 32.8 mmol) was then added at 0°C, and the mixture was stirred at room temperature for 1.5 h. After concentration under reduced pressure, the residue was dissolved in EtOAc. The EtOAc solution was washed successively with 10 % aq. AcOH and saturated aq. NaCl, and extracted with saturated aq. $NaHCO_3$. The extract was adjusted to pH 4 with citric acid. The obtained solution was extracted with EtOAc and the extract was washed with saturated aq. NaCl, dried over $MgSO_4$, and concentrated under reduced pressure to dryness. The residue was dissolved in ethanol/H_2O (40/10 ml), and cesium carbonate (Cs_2CO_3) (5.34 g, 16.4 mmol) was added to the solution. The suspension was stirred until it became a homogenous solution. The solution was concentrated under reduced pressure and lyophilized from H_2O.

The residue was dissolved in DMF (60 ml), and benzyl bromide (4.23 ml, 35.6 mmol) was added at room temperature. The mixture was stirred at room temperature for 6.5 h. After concentration under reduced pressure, the residue was dissolved in EtOAc, washed successively with 10% aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$, and saturated aq. NaCl, and then dried over $MgSO_4$. Concentration under reduced pressure gave Boc-DL-HOMeVal-OBzl in a crude state. Part of this crude residue was purified by preparative silica gel TLC (developed with a mixture of $CHCl_3$/MeOH (19:1)) to give Boc-DL-HOMeVal-OBzl as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 7.35 (s, 5H), 5.21 (m, 2H, Bzl), 4.65 (s) and 4.54 (s)(1H, α-CH), 3.74 (br. s), 3.49 (br. s, 1H, OH), 2.85 (s, 3H, *N*-CH_3), 1.38 (s, 3H, γ-CH_3), 1.22(s, 3H, γ-CH_3), Anal. Calcd. for $C_{18}H_{27}NO_5$: C, 64.08; H, 8.07; N, 4.15. Found: C, 63.94; H, 8.13; N, 4.54.

*HCl·H-DL-HOMeVal-OBzl (**8**)*

The crude product of Boc-DL-HOMeVal-OBzl (10.9 g) was mixed with 5.5 N HCl/dioxane solution (99.5 ml, 0.55 mol), and the mixture was allowed to stand at room temperature for 40 min. After concentration under reduced pressure, the residue was crystallized from ethyl ether and washed with ethyl ether to give **8** as colorless crystals (7.05 g, 94% yield from **7**). mp. 164-166°C, ^{1}H-NMR (DMSO-d_6) δ 9.17 (m, 1H, NH), 8.90 (m, 1H, NH), 7.46-7.36 (m, 5H), 5.85 (s, 1H, OH), 5.32 (d, 1H, J=12.2 Hz, CH_2 Bzl), 5.25 (d, 1H, J=12.2 Hz, CH_2 Bzl), 4.02 (s, 1H, α-CH), 2.57 (s, 3H, *N*-CH_3), 1.31 (s, 3H, γ-CH_3), 1.12 (s, 3H, γ-CH_3), HR-FABMS m/z: 238.1441 (M+H, calcd. for $C_{13}H_{20}NO_3$, 238.1443).

*Boc-L-Leu-DL-HOMeVal-OBzl (**9**)*

Compound **9** was synthesized as described for the synthesis of **5**, using Boc-L-Leu-OH·H_2O (1.34 g, 5.39 mmol), HCl·H-DL-HOMeVal-OBzl (981 mg, 3.59 mmol), PyBroP (2.52 g, 5.39 mmol), DIEA (2.50 ml, 14.4 mmol) and CH_2Cl_2 (10 ml). The product (**9**) was obtained as a colorless oil (1.05 g, 65% yield). $[\alpha]_D^{20}$ -25.0° (*c* 0.20, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.32 (m, 5H), 5.20 (m, 2H, Bzl), 4.96 (s, 1H, α-CH HOMeVal), 4.68 (m, 1H, α-CH Leu), 3.75 (br. s, 1H, OH), 3.08 (s) and 3.06 (s) (3H, *N*-CH_3 HOMeVal), 1.43 (s, 9H, Boc), 1.40 (s, 3H, γ-CH_3 HOMeVal), 1.25(s) and 1.15 (s) (3H, γ-CH_3 HOMeVal), 0.98 (d, 3H, J=6.6 Hz, δ-CH_3 Leu), 0.91 (d, 3H, J=6.6 Hz, δ-CH_3 Leu), Anal. Calcd. for $C_{24}H_{38}N_2O_6 \cdot 0.2H_2O$: C, 63.47; H, 8.52; N, 6.17. Found: C, 63.49; H, 8.56; N, 6.19.

*Boc-L-Leu-DL-HOMeVal-OH (**10**)*

To a solution of Boc-L-Leu-DL-HOMeVal-OBzl (43.5 mg, 96.5 μmol) in MeOH (40 ml) was added palladium black (40 mg). Hydrogen gas was bubbled into the mixture at room temperature for 50 min. After filtration of the catalyst, the filtrate was concentrated under reduced pressure to give **10** as a colorless oil (30.6

mg, 88% yield). $[\alpha]_D^{20}$ -21.6° (*c* 0.37, MeOH), ^{1}H-NMR ($CDCl_3$) δ 6.96 and 6.87 (d, 1H, J=8.3 Hz, NH Leu), 4.96 and 4.84 (s, 1H, α-CH HOMeVal), 4.45 (m, 1H, α-CH Leu), 3.21 and 3.19 (s, 3H, *N*-CH_3 HOMeVal), 2.94 and 2.89 (s, 1H, OH HOMeVal), 1.35 (s, 9H, Boc), 1.07 (s, 3H, γ-CH_3 HOMeVal), 0.87 (d, 6H, J=6.6 Hz, δ-CH_3 Leu), FAB-MS m/z 359 (M-H).

H-D-Hmp-OH

D-Isoleucine (3.0 g, 22.9 mmol) was dissolved in a mixture of 1 N HCl (22.9 ml)/H_2O (91.6 ml)/AcOH (45.8 ml) mixture. To the solution was added dropwise a solution of sodium nitrite (16.1 g, 0.23 mmol) in H_2O (27.6 ml) at 0°C within 30 min. The mixture was stirred at 0°C for 20 min and then at room temperature for 15 h. After concentration under reduced pressure, the residue was coevaporated successively with 1 N HCl and H_2O. The residue obtained was dissolved in H_2O, and the solution was extracted with ethyl ether. The extract was washed with saturated aq. NaCl, dried over $MgSO_4$ and concentrated under reduced pressure to give H-D-Hmp-OH as a colorless oil (1.95 g, 83% yield).

H-D-Hmp-OPac

H-D-Hmp-OPac was synthesized as described for the synthesis of **4** using a solution of H-D-Hmp-OH (1.07 g, 8.09 mmol) in EtOAc (16 ml), triethylamine (1.24 ml, 8.90 mmol) and phenacyl bromide (1.77 g, 8.90 mmol). H-D-Hmp-OPac was obtained as a colorless oil (1.52 g, 75% yield). $[\alpha]_D^{20}$ -5.3° (*c* 0.94, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.92 (d, 2H, J=7.1 Hz), 7.62 (d, 1H, J=7.3 Hz), 7.50 (t, 2H, J=7.1, 7.3 Hz), 5.51 (d, 1H, J=16.4 Hz), 5.40 (d, 1H, J=16.4 Hz), 4.30 (dd, 1H, J=5.7 Hz, α-CH), 2.67 (d, 1H, J=6.1 Hz, OH), 1.97 (m, 1H, β-CH), 1.56 (m, 1H, γ-CH_2), 1.34 (m, 1H, γ-CH_2), 1.08 (d, 3H, J=6.8 Hz, γ-CH_3), 0.96 (t, 3H, J=7.3 Hz, δ-CH_3), Anal. Calcd. for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.14; H, 8.11; N, 7.31.

*Boc-L-Leu -L-HOMeVal-D-Hmp-OPac (**11L**)*

Boc-L-Leu-DL-HOMeVal-OH (744 mg, 2.15 mmol), H-D-Hmp-OPac (589 mg, 2.36 mmol) and 4-pyrrolidinopyridine (95.6 mg, 0.65 mmol) were dissolved together in THF (4.3 ml). To the mixture, DCC (487 mg, 2.36 mmol) was added at 0°C. The mixture was stirred at 0°C for 1 h, and then allowed to return slowly to room temperature and stirred for an additional 18 h. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc and the insoluble material was filtered. The filtrate was washed successively with 10% aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by silica gel column chromatography (silica gel: 80 g, eluted with a mixture of toluene/EtOAc (5:1)) to give Boc-L-Leu-DL-HOMeVal-D-Hmp-OPac (**11DL**) as a colorless oil (1.00 g, 79% yield).

The diastereomers **11DL** were separated from each other by silica gel column chromatography (silica gel: 200 g, eluted with a mixture of toluene/EtOAc (15:1 and 10:1)) to give the L-L-D isomer as a colorless oil (357 mg, 56% calculated from the L-L isomer in Boc-L-Leu-DL-HOMeVal-OH **10**). **L-L-D isomer (11L)** $[\alpha]_D^{20}$ -141.7° (*c* 0.12, MeOH), Anal. Calcd. for $C_{31}H_{48}N_2O_9$: C, 62.82; H, 8.16; N, 4.73. Found: C, 62.91; H, 8.11; N, 4.80. ^{1}H-NMR ($CDCl_3$) δ 7.91 (d, 2H, J=7.1 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.50 (t, 2H, J=7.6 Hz), 5.58 (d, 1H, J=16.4 Hz), 5.36 (s, 1H, α-CH HOMeVal), 5.28 (d, 1H, J=16.4 Hz), 5.14 (d, 1H, J=7.5 Hz, NH Leu), 5.12 (d, 1H, J=4.2 Hz, α-CH Hmp), 4.72 (m, 1H, α-CH Leu), 3.48 (s, 1H, OH), 3.26 (s, 3H, *N*-CH_3 HOMeVal), 2.13 (m, 1H, β-CH Hmp), 1.58 (s, 3H, γ-CH_3 HOMeVal), 1.42 (s, 9H, Boc), 1.20(s, 3H, γ-CH_3 HOMeVal), 1.07 (d, 3H, J=7.5 Hz, γ-CH_3 Hmp), 0.97 (t, 3H, J=7.5 Hz, δ-CH_3 Hmp), 0.97 (d, 3H, J=6.4 Hz, δ-CH_3 Leu), 0.91 (d, 3H, J=6.4 Hz, δ-CH_3 Leu). **L-D-D isomer (11D)** δ 7.90 (d, 2H, J=7.1 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.50 (t, 2H, J=7.3, 7.1 Hz), 5.59 (d, 1H, J=16.4 Hz), 5.28 (d, 1H, J=16.4 Hz), 5.24 (s, 1H, α HOMeVal), 5.18 (br. d, 1H, NH Leu), 5.18 (d, 1H, J=4.2 Hz, α-CH Hmp), 4.73 (m, 1H, α Leu), 3.64 (br. s, 1H, OH), 3.27 (s, 3H, *N*-CH_3 HOMeVal), 2.17 (m, 1H, β-CH Hmp), 1.52 (s, 3H, γ-CH_3 HOMeVal), 1.43 (s, 9H, Boc), 1.18 (s, 3H, γ-CH_3, HOMeVal), 1.09 (d, 3H, J=7.5 Hz, γ-CH_3 Hmp), 1.00 (t, 3H, J=7.5 Hz, δ-CH_3 Hmp), 0.97 (d, 3H, J=6.4 Hz, δ-CH_3 Leu), 0.94 (d, 3H, J=6.4 Hz, δ-CH_3 Leu).

Boc-L-Leu-L-HOMeVal-D-Hmp-OH (12)

Compound **12** was synthesized as described for the synthesis of **6** using Boc-L-Leu-L-HOMeVal-D-Hmp-OPac (220 mg, 0.37 mmol), zinc dust (3.60 g, 55.5 mmol) and 90% aq. AcOH (18.5 ml). Compound (**12**) was obtained as a colorless oil (133 mg, 74% yield). mp. 45-48°C, $[\alpha]_D^{20}$ -86.1° (*c* 0.36, MeOH), ^{1}H-NMR ($CDCl_3$) δ 5.41 (m), 5.12 (m) and 4.74 (m) (total 3H), 3.32 (br. s, 3H, *N*-CH_3 HOMeVal), 1.47 (s, 3H, γ-CH_3 HOMeVal), 1.42 (s, 9H, Boc), 1.22 (s, 3H, γ-CH_3 HOMeVal). Anal. Calcd. for $C_{23}H_{42}N_2O_8 \cdot 0.5H_2O \cdot CH_3CO_2H$: C, 55.23; H, 8.71; N, 5.15. Found: C, 55.33; H, 8.36; N, 5.45.

Boc-L-Pro-OPac (13)

To a solution of Boc-L-Pro-OH (1.08 g, 5.00 mmol) in acetone (10 ml) were added triethylamine (0.76 ml, 5.50 mmol) and phenacyl bromide (1.09 g, 5.50 mmol) at 0°C. The mixture was stirred for 5 min and then at room temperature for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc. The EtOAc was washed successively with 10% aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl and then dried over $MgSO_4$. After concentration under reduced pressure, the residue was crystallized from hexane and washed with cold hexane to give **13** as colorless crystals (1.54 g, 92% yield). mp. 80-82°C, $[\alpha]_D^{20}$ -81.7° (*c* 0.55, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.90 (d, 2H, J=7.6 Hz), 7.60 (d, 1H, J=8.1 Hz), 7.49 (t, 2H, J=7.6 Hz), 5.37 (2H), 4.43 (t) and 4.48 (m) (1H, α-CH), 3.45 (m) and 3.58 (m) (2H, β-CH_2), 2.32 (m, δ-CH_2), 1.93 (m) and 2.06 (m) (2H, γ-CH_2).

Boc-L-MePhe-L-Pro-OPac (14)

Boc-L-Pro-OPac (**13**) (12.71 g, 38.1 mmol) was treated with 5.5 N HCl/dioxane solution (139 ml) as described for the synthesis of **4** to give HCl·H-L-Pro-OPac as colorless crystals (10.3 g, 99% yield). mp. 156-158°C, $[\alpha]_D^{20}$ -24.6° (*c* 0.57, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.98 (d, 2H, J=7.0 Hz), 7.67 (d, 1H, J=7.3 Hz), 7.55 (t, 2H, J=7.5 Hz), 5.66 (2H), 4.64 (t, 1H, J=7.5 Hz, α-CH), 3.45 (m) and 3.41 (m) (δ-CH_2), 2.49 (m, β-CH_2), 2.17 (m, γ-CH_2). The compound (**14**) was synthesized by the method described for the synthesis of **5** using Boc-L-MePhe-OH (1.00 g, 3.58 mmol), HCl·H-L-Pro-OPac (644 mg, 2.39 mmol), CH_2Cl_2 (3.58 ml), PyBroP (1.67 g, 3.58 mmol) and DIEA (1.67 ml, 9.56 mmol). Compound **14** was obtained as colorless crystals (951 mg, 83% yield). mp. 121-122°C, $[\alpha]_D^{20}$ -118.6° (*c* 0.56, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.92 (d, 2H, J=6.8 Hz), 7.62 (d, 1H, J=7.3 Hz), 7.51 (t, 2H, J=6.7 Hz), 7.19-7.28 (m, 5H, MePhe), 4.95 (m) and 5.33 (m) (1H, α-CH Pro), 4.66 (m, 1H, α-CH MePhe), 3.03 (m) and 3.18 (m) (2H, β-CH_2 MePhe), 2.83 (s) and 2.85 (s) (3H, *N*-CH_3 MePhe), 1.20 (s) and 1.35 (s) (9H, Boc). Anal. Calcd. for $C_{28}H_{34}N_2O_6$: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.05; H, 7.04; N, 5.56.

Boc-L-Phe-L-MePhe-L-Pro-OPac (15)

Boc-L-MePhe-L-Pro-OPac (**14**) (12.3 g, 25.7 mmol) was treated with 5.5 N HCl/dioxane solution (140 ml) as described for the synthesis of **4** to give HCl·H-L-MePhe-L-Pro-OPac as colorless crystals (11.2 g, 100% yield). mp. 190-192°C, $[\alpha]_D^{20}$ -41.2° (*c* 0.51, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.90 (d, 2H), 7.62 (d, 1H), 7.50 (t, 2H), 7.44 (d, 1H), 7.33 (s, 3H), 7.28 (d, 1H), 5.59-5.29 (2H), 2,73 and 2.82 (s, 3H, *N*-CH_3 MePhe). Compound **15** was synthesized as described for the synthesis of **5** using Boc-L-Phe-OH (424 mg, 1.60 mmol), CH_2Cl_2 (2.9 ml), HCl·H-L-MePhe-L-Pro-OPac (600 mg, 1.39 mmol), PyBroP (745 mg, 1.60 mmol) and DIEA (921 μl, 5.29 mmol). Compound **15** was obtained as an amorphous powder (767 mg, 86% yield). $[\alpha]_D^{20}$ -83.7° (*c* 0.49, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.89 (d, 2H, J=7.1 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.50 (t, 2H, J=7.4 Hz), 7.14-7.26 (m, 10H, MePhe, Phe), 4.80 (m, 1H, α-CH Pro), 4.80 (br. d, 1H, NH Phe), 4.53 (q, 1H, α-CH MePhe), 3.15-3.30 (m, 2H, δ-CH_2 Pro), 2.80-3.10 (m, 4H, β-CH_2 MePhe, Phe), 2.94 (s, 3H, *N*-CH_3 MePhe), 1.80-2.30 (m, 4H, β and γ-CH_2 Pro), 1.42 (s, 9H, Boc), Anal. Calcd. for $C_{37}H_{43}N_3O_7$: C, 69.25; H, 6.75; N, 6.55. Found: C, 69.29; H, 6.80; N, 6.82.

Boc-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (16)

Boc-L-Phe-L-MePhe-L-Pro-OPac (**15**) (10.8 g, 16.8 mmol) was treated with 5.5 N HCl/dioxane solution

(122 ml) as described for the synthesis of **4** to give HCl·H-L-Phe-L-MePhe-L-Pro-OPac as colorless crystals (8.91 g, 92% yield). mp. 108-111°C, $[\alpha]_D^{20}$ -38.6° (*c* 0.57, MeOH), ^{1}H-NMR ($CDCl_3$) δ 8.57 (br. s, 2H, NH Phe), 7.87 (d, 2H, J=7.1 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.50 (t, 2H, J=7.6 Hz, 7.4 Hz), 7.10-7.28 (m, 10H, ar. MePhe, Phe), 5.40 (m, 1H, α-CH MePhe), 5.27 (2H), 4.74 (m, 1H, α-CH Phe), 4.54 (m, 1H, Pro), 3.49 (m, 2H, β-CH_2 Phe), 3.28 (m, 2H, β-CH_2 Pro), 2.97 and 2.86 (s, 3H, *N*-CH_3 MePhe), 2.24 (m, 2H, β-CH_2 Pro), 1.85 (m, 2H, γ-CH_2 Pro). Compound **16** was synthesized as described for the synthesis of **5** using Boc-L-MeVal-OH (192 mg, 0.83 mmol), CH_2Cl_2 (1.6 ml), HCl·H-L-Phe-L-MePhe-L-Pro-OPac (400 mg, 0.69 mmol), PyBroP (387 mg, 0.83 mmol) and DIEA (500 μl, 2.87 mmol). Compound **16** was obtained as a colorless oil (450 mg, 86% yield). $[\alpha]_D^{20}$ -132.2° (*c* 0.52, MeOH), ^{1}H-NMR ($CDCl_3$) δ 7.10-7.30 (m, 10H, MePhe, Phe), 6.41 (br. d, 1H, NH Phe), 5.62 (dd, 1H, J=7.1, 8.3 Hz, α-CH MePhe), 5.10 (q, 1H, α-CH Phe), 4.56 (m, 1H, α-CH Pro), 4.02 (br. d, 1H, α-CH MeVal), 2.97 (s, 3H, *N*-CH_3 MePhe), 2.57 (s, 3H, *N*-CH_3 MeVal), 1.49 (s, 9H, Boc), 0.87 (d, 3H, J=5.6 Hz, γ-CH_3 MeVal), 0.83 (d, 3H, J=6.4 Hz, γ-CH_3 MeVal), Anal. Calcd. for $C_{43}H_{54}N_4O_8$: C, 68.41; H, 7.21; N, 7.42. Found: C, 68.60; H, 7.40; N, 7.50.

HCl·H-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (17)

Compound **17** was synthesized as described for the synthesis of **4** using Boc-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (1.08 g, 1.43 mmol) and 5.5 N HCl/dioxane solution (13.0 ml). Compound **17** was obtained as colorless crystals (922 mg, 93% yield). mp. 125-127°C, $[\alpha]_D^{20}$ -67.8° (*c* 0.59, MeOH), ^{1}H-NMR (DMSO-d_6) δ 8.95 (d, 2H, J=7.3 Hz, NH MeVal), 8.58 (m, 1H, NH Phe), 7.97 (d, 2H, J=7.3 Hz), 7.71 (t, 1H, J=7.3 Hz, 7.4 Hz), 7.57 (t, 2H, J=7.3 Hz), 7.28-7.10 (total 10H, ar. Phe, MePhe), 5.54 (m, 1H, α-CH MePhe), 5.49 (d, 2H, J=3.3 Hz), 5.42 (br. t, 1H, α-CH Phe), 5.06 (m, 1H, α-CH MeVal), 4.45 (m, 1H, α-CH Pro), 3.08 (m, 2H), 3.00 (s, 3H, *N*-CH_3), 2.80 (m, 2H), 2.18 (m, 2H), 0.86 (d, 3H, J=6.9 Hz, γ-CH_3 MeVal), 0.80 (d, 3H, J=6.9 Hz, γ-CH_3 MeVal).

Boc-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (18)

Compound **18** was synthesized as described for the synthesis of **5** using Boc-L-Leu-L-HOMeVal-D-Hmp-OH (128 mg, 0.26 mmol), CH_2Cl_2 (720 μl), HCl·H-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (270 mg, 0.39 mmol), PyBroP (158 mg, 0.34 mmol) and DIEA (186 μl, 1.07 mmol). Compound **18** was obtained as a colorless oil (228 mg, 79% yield). ^{1}H-NMR ($CDCl_3$) δ 5.63 (t, 1H, J=7.7 Hz, α-CH MePhe), 5.35 (s, 1H, α-CH HOMeVal), 5.19 (m, 1H, α-CH Phe), 4.91 (d, 1H, J=5.6 Hz, α-CH Hmp), 4.79 (m, 1H, α-CH Leu,), 4.56 (m, 1H, α-CH Pro), 4.66 (d, 1H, J=10.5 Hz, α-CH MeVal), 4.51 (dd, 1H, J=8.4, 3.8 Hz, α-CH Pro), 3.43 (s, 3H, *N*-CH_3 HOMeVal), 2.82 (s, 3H, *N*-CH_3 MePhe), 2.72 (s, 3H, *N*-CH_3 MeVal), 1.53 (s, 3H, γ-CH_3 HOMeVal), 1.20 (s, 3H, γ-CH_3 HOMeVal), 1.03 (d, 3H, J=7.6 Hz, γ-CH_3 Hmp), 1.01 (d, 3H, J=7.1 Hz, δ-CH_3 Leu), 0.98 (d, 3H, J=7.1 Hz, δ-CH_3 Leu), 0.95 (d, 3H, J=6.6 Hz, γ-CH_3 MeVal), 0.89 (t, 3H, J=7.3 Hz, δ-CH_3 Hmp), 0.80 (d, 3H, J=6.6 Hz, γ-CH_3 MeVal), Anal. Calcd. for $C_{61}H_{86}N_6O_{13}$: C, 65.92; H, 7.80; N, 7.56. Found: C, 65.89; H, 7.84; N, 7.54.

Boc-L-alle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (19)

To Boc-L-Leu-L-HOMeVal-L-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (**18**) (196 mg, 0.18 mmol) was added TFA (1.11 ml, 14.1 mmol), and the mixture was allowed to stand at 0°C for 30 min. After concentration under reduced pressure, the residue was dissolved in ethyl ether. To the solution, 5.5 N HCl/dioxane solution (49 μl, 0.27 mmol) was added at 0°C and the resulting mixture was allowed to stand at 0°C for 30 min to give HCl·H-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac as colorless crystals (197 mg, 93% yield). mp. 118-121°C, $[\alpha]_D^{20}$ -125.0° (*c* 0.36, MeOH), ^{1}H-NMR (DMSO-d_6) δ 8.16 (br. s, 3H, NH Leu), 7.96 (d, 2H, J=7.3 Hz), 7.70 (d, 1H, J=6.9 Hz), 7.56 (t, 2H, J=7.8 Hz), 7.27-7.11 (m, total 10H, ar. Phe, MePhe), 5.46 (s, 2H, Pac), 5.27 (m, 1H), 4.48-4.37 (m, total 3H), 3.25 (s), 3.20 (s), 2.90 (s), 2.89 (s), 2.87 (s) and 2.81 (s) (total 12H, *N*-CH_3), 2.12 (m, 3H), 1.81 (m, 3H), 1.41 (s, 3H, γ-CH_3 HOMeVal), 1.18 (s, 3H, γ-CH_3 HOMeVal).

HCl·H-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (197 mg, 0.19 mmol),

obtained as described above, and Boc-L-aIle-L-MeVal-OH (97.2 mg, 0.28 mmol) were dissolved in DMF (400 μl). To the solution, HOObt (36.9 mg, 0.23 mmol) and a solution of WSCD (36.7 μl, 0.21 mmol) in DMF (100 μl) were added at 0°C. The mixture was stirred at 0°C for 2 h and then at room temperature for 2 h. Next EtOAc was added to the mixture, which was then washed successively with 10% aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl, and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by silica gel column chromatography (silica gel: 10 g, eluted with a mixture of toluene/EtOAc (6:1)) to give **19** as colorless rods (150 mg, 60% yield). mp. 108-110°C, $[\alpha]_D^{20}$ -153.1° (*c* 0.32, MeOH), ^{1}H-NMR ($CDCl_3$) δ 5.61 (t, 1H, J=8.1 Hz, α-CH MePhe), 5.25 (s, 1H, α-CH HOMeVal), 5.15 (m, 1H, α-CH Phe), 5.09 (m, 1H, α-CH Leu), 4.96 (d, 1H, J=5.6 Hz, α-CH Hmp), 4.71 (d, 1H, J=11.0 Hz, α-CH MeVal), 4.69 (d, 1H, J=10.5 Hz, α-CH MeVal), 4.55 (m, 1H, α-CH *a*Ile), 4.52 (m, 1H, α-CH Pro), 3.42 (s, 3H, *N*-CH_3 HOMeVal), 2.83 (s, 3H, *N*-CH_3 MePhe), 2.74 (s, 3H, *N*-CH_3 MeVal), 1.52 (s, 3H, γ-CH_3 HOMeVal), 1.42 (s, 9H, Boc), 1.17 (s, 3H, γ-CH_3 HOMeVal), Anal. Calcd. for $C_{73}H_{108}N_8O_{15}\cdot H_2O$: C, 64.68; H, 8.18; N, 8.27. Found: C, 64.80; H, 8.25; N, 8.20.

HCl·H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (20)

Boc-nonapeptide phenacyl ester (**19**) (59 mg, 44.1 μmol) was treated with zinc dust (577 mg, 8.8 mmol) in 90% aq. AcOH (2.2 ml) to give Boc-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH as a crude oil (53.9 mg). This product (53.0 mg, 43.5 μmol) was treated with TFA (2.89 ml, 37.5 mmol) and 5.5 N HCl/dioxane solution (11.9 μl, 63.5 mmol) to give compound **20** as colorless crystals (45.8 mg, 91% yield). mp. 146-148°C, $[\alpha]_D^{20}$ -149° (*c* 0.53, MeOH), ^{1}H-NMR (DMSO-d_6) δ 8.44 (br d, 1H), 7.97 (m, 2H), 7.62 (br d, 1H), 7.15-7.23 (m, 10H), 5.26 (br t, 1H), 5.24 (m, 1H), 5.24 (s, 1H), 4.72 (m, 1H), 4.70 (br. d, 1H), 4.49 (br. d, 1H), 4.32 (m, 1H), 4.13 (m, 1H), 3.27, 2.99, 2.89, 2.87, 2.78 (total 12H, *N*-CH_3), 1.35 (s, 3H), 1.24 (s, 3H), 0.71-0.96 (total 27H, CH_3), Anal. Calcd. for $C_{60}H_{95}N_8O_{12}Cl\cdot H_2O\cdot CF_3CO_2H$: C, 57.82; H, 7.67; N, 8.70. Found: C, 57.85; H, 7.60; N, 8.38.

Aureobasidin A (1)

HCl·H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH (**20**) (5.0 mg, 4.3 μmol) and DIEA (1.5 μl, 8.6 μmol) were dissolved together in CH_2Cl_2 (2.15 ml). The solution was added dropwise to a solution of PyBroP (10.0 mg, 21.5 μmol) and DIEA (1.5 μl, 8.6 μmol) in CH_2Cl_2 (2.15 ml) within 2 h at room temperature. The mixture was stirred at room temperature for 15 h. After concentration under reduced pressure, the residue was dissolved in EtOAc and the mixture was washed successively with 10% aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl and dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by HPLC (column: YMC A-323 ODS, 10 x 250 mm, eluted with 80% MeCN/H_2O) followed by lyophilization to give the major product **1** (aureobasidin A) as an amorphous powder (2.1 mg, 45% yield) and cyclo(L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-D-Pro) ([D-Pro^9]-aureobasidin) (0.4 mg, 7.6% yield). **1**: mp. 128-130°C, $[\alpha]_D^{20}$ -247° (*c* 0.21, MeOH), PD-MS m/z 1100.6 (M+H), ^{1}H-NMR ($CDCl_3$) δ 8.90-7.59 (total 3H, NH), 7.32-7.10 (m, 9H, ar. MePhe, Phe), 6.52 (d, 1H, J=7.5 Hz, ar. MePhe), 5.80 (s) and 5.77 (s) total 1H, α-CH Hmp), 4.20 (s, 1H, HO HOMeVal), 3.41 (s, 1H, α-CH HOMeVal), 3.31 (s), 3,18 (s), 3.16 (s), 3.07 (s), 2.67 (s) and 2.50 (s) (total 12H, *N*-CH_3), 1.39 (s, 3H, γ-CH_3 HOMeVal), 1.19 (s, 3H, γ-CH_3 HOMeVal), Anal. Calcd. for $C_{60}H_{92}N_8O_{11}\cdot 2H_2O$: C, 63.36; H, 8.51; N, 9.85. Found: C, 63.64; H, 8.34; N, 9.82. *cyclo(L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-D-Pro) [D-Pro^9]-aureobasidin A:* ^{1}H-NMR ($CDCl_3$) δ 8.60, 8.57, 7.90, 7.74 (total 3H, NH), 7.39-7.14, 6.76 (total 10H, ar. Phe, MePhe), 3.40, 3.39, 3.30, 3.25, 2.82, 2.57 (total 12H, *N*-CH_3), FAB-MS m/z 1101 (M+H).

TFA·H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-ONSu (21)

To a solution of Boc-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OH obtained from Boc-nonapeptide (**19**) as described for the synthesis of **20** (22.9 mg, 18.8 μmol) in DMF (40 μl) were added *N*-hydroxysuccinimide (3.3 mg, 28.2 μmol) and WSCD·HCl (5.4 mg, 28.2 μmol) at 0°C. The

mixture was stirred at 0°C for 15 h. The resulting mixture was extracted with EtOAc and the extract was washed successively with 10 % aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl and then dried over $MgSO_4$. Concentration under reduced pressure gave Boc-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-ONSu in a crude form (23 .5 mg, 95% yield). To a solution of this product (23.0 mg, 17.5 μmol) was added TFA (1.17 ml, 15.2 mmol) at 0°C. The mixture was allowed to stand at 0°C for 30 min. After removal of the solvent under reduced pressure, the residue was crystallized from ethyl ether and washed with cold ether to give **21** as a colorless powder (17.4 mg, 75% yield). ^{1}H-NMR ($CDCl_3$) δ 8.01 (m, 1H, NH), 7.29-7.12 (m, 10H, ar. Phe, MePhe), 5.40-4.73 (m, α-CH), 4.38 (m, 4H, ONSu), 3.32, 3.19, 3.06, 2.85 (total 12H, *N*-CH_3), 1.05-0.74 (CH_3), PD-MS: 1218.8.

Cyclization of N-hydroxysuccinimide ester (21)

Method A: A solution of TFA·H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-ONSu (**21**) (5.0 mg, 3.76 μmol) in CH_2Cl_2 (1.0 ml) was added dropwise to a solution of DIEA (0.72 μl, 4.13 μmol) in CH_2Cl_2 (2.8 ml) within 3 h at room temperature. The mixture was stirred at room temperature for 24 h. After concentration under reduced pressure, the residue was dissolved in EtOAc and the mixture was washed successively with 10 % aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl and dried over $MgSO_4$. After concentration under reduced pressure, the residue obtained was purified by HPLC (column: YMC A-502 S-5 CN, 4.6 X 150 mm, a 20-min gradient elution of 55→75% MeCN/H_2O, 0.1% TFA) followed by lyophilization to give **1** (t_R 6.4 min) (0.6 mg, 14% yield) and cyclic dimer (t_R 12.0 min) (1.6 mg, 36% yield). Cyclic dimer: PD-MS 2201.1 (M+H).

Method B: A solution of TFA·H-L-aIle-L-MeVal-L-Leu-L-HOMeVal-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-ONSu (**21**) (5.0 mg, 3.76 μmol) in DMF (1.0 ml) was added dropwise to a solution of DIEA (0.72 μl, 4.13 μmol) in DMF (2.8 ml) within 3 h at room temperature. The mixture was stirred at room temperature for 24 h. After concentration under reduced pressure, the residue was dissolved in EtOAc and the mixture was washed successively with 10 % aq. citric acid, saturated aq. NaCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl and then dried over $MgSO_4$. Concentration under reduced pressure gave a crude oil (2.9 mg). The cyclic monomer (**1**) and cyclic dimer were detected in yields of 16% and <4%, respectively (HPLC, PD-MS).

Coupling between Boc-DL-HOMeVal-OH and H-D-Hmp-OPac by PyBroP method (Run 1 in Table 5)

Boc-DL-HOMeVal-OH (10.0 mg, 40.4 μmol) and H-D-Hmp-OPac (6.7 mg, 26.9 μmol) were dissolved in CH_2Cl_2 (100 μl). To the mixture, PyBroP (18.8 mg, 40.4 μmol) and DIEA (16.4 μl, 94.2 μmol) were added. The mixture was stirred at 0°C for 3.5 h and then at room temperature for 20 h. The reaction mixture was purified by silica gel TLC (developed with a mixture of benzene/EtOAc (30:1)) to give Boc-D-Hmp-OPac (**22**) (Rf 0.5) as a colorless oil (2.7 mg, 29% yield). ^{1}H-NMR ($CDCl_3$) δ 7.91 (d, 2H, J=7.1 Hz), 7.60 (d, 1H, J=7.6 Hz), 7.49 (t, 2H, J=7.7 Hz), 5.54 (d, 1H, J=16.4 Hz), 5.30 (d, 1H, J=16.4 Hz), 4.71 (d, 1H, J=4.6 Hz, α-CH), 2.10 (m, 1H), 1.70 (m, 1H), 1.50 (s, 9H, Boc), 1.11 (d, 3H, J=6.8 Hz, γ-CH_3), 0.83 (t, 3H, J=7.5 Hz, δ-CH_3).

Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac

Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac was synthesized as described for the synthesis of **5** using Boc-D-Hmp-OH (20.2 mg, 87.0 μmol), CH_2Cl_2 (200 μl), HCl·H-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (40.0 mg, 58.0 μmol), PyBroP (40.6 mg, 87.0 μmol) and DIEA (40.4 μl, 0.23 mmol). Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac was produced as a colorless oil (33.6 mg, 67% yield). ^{1}H-NMR ($CDCl_3$) δ 7.88 (d, 2H, J=7.1 Hz), 7.60 (d, 1H, J=7.3 Hz), 7.49 (t, 2H, J=7.6 Hz), 7.25-7.16 (m, 10H, MePhe, Phe), 5.60 (t, 1H, J=7.7 Hz), 5.39 (d, 1H, J=16.4 Hz), 5.21 (d, 1H, J=16.4 Hz), 5.09 (m, 1H), 4.92 (d, 1H, J=6.1 Hz), 4.49 (m, 1H), 4.45 (d, 1H, J=11.0 Hz), 2.87 (s, 3H, *N*-CH_3), 2.86 (s, 3H, *N*-CH_3), 1.46 (s, 9H, Boc), 1.00 (d, 3H, J=6.8 Hz), 0.81 (d, 3H, J=6.8 Hz).

Deprotection of Boc group of Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac

Method A (HCl/dioxane solution): Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (30.0 mg, 34.4 μmol) was mixed with a 5.5 N HCl/dioxane solution (1.13 ml, 6.21 mmol) and the mixture was allowed to stand at room temperature for 5.5 h. After concentration under reduced pressure, the residue was purified by silica gel TLC (developed with a mixture of $CHCl_3$/MeOH/AcOH (18:2:1)) to give **25** (Rf 0.4) (8.0 mg, 41% yield). ^{1}H-NMR ($CDCl_3$) of **25** was identical with that of HCl·H-L-Phe-L-MePhe-L-Pro-OPac described in the synthesis of **16**.

Method B (TFA): Boc-D-Hmp-L-MeVal-L-Phe-L-MePhe-L-Pro-OPac (9.0 mg, 10.3 μmol) was mixed with TFA (47.8 μl, 0.62 mmol) at -10°C. The mixture was allowed to stand at -10°C for 10 min and at room temperature for 35 min. After concentration under reduced pressure, the residue was purified by silica gel TLC (developed with a mixture of benzene/EtOAc (1:1)) to give **23** (Rf 0.3-0.4) (5.5 mg, 69% yield). ^{1}H-NMR ($CDCl_3$) δ 7.89 (d, 2H, J=7.1 Hz), 7.61 (d, 1H, J=7.6 Hz), 7.50 (t, 2H, J=7.5 Hz), 6.48 (d, 1H, J=9.28 Hz), 5.68 (m, 1H), 5.47 (d, 1H, J=16.6 Hz), 5.23 (d, 1H, J=16.6 Hz), 5.14 (m, 1H), 4.59 (m, 1H), 4.32 (d, 1H, J=11.2 Hz), 4.14 (br. d, 1H), 3.08 (s, 3H, *N*-CH_3), 2.48 (s, 3H, *N*-CH_3), 1.08 (d, 3H, J=6.8 Hz), 0.74 (d, 3H, J=6.8 Hz).

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