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Total Synthesis of Aureobasidin A, an Antifungal Cyclic Depsipeptide

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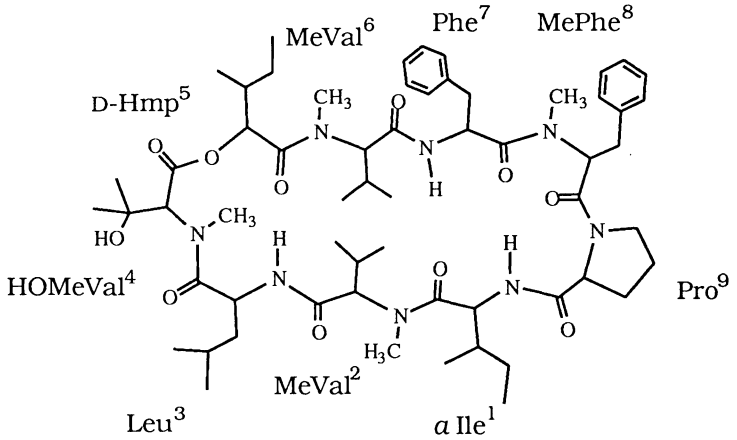
A total synthesis of an antifungal cyclic depsipeptide aureobasidin A was first achieved mainly using PyBroP[1] as a coupling reagent. The synthetic cyclized product was completely identical with the natural antibiotic in all respects. Some unexpected reactions due to *N*-methylamino acid were also described.

A new cyclic depsipeptide aureobasidin A (**1**), isolated as a major component from the culture medium of the black yeast *Aureobasidium pullulans* R106, exhibits a strong antifungal activity against pathogenic fungi such as *Candida albicans*, *Cryptococcus neoformans*, and some species of *Aspergillus* with a low toxicity.[2] A whole structure of the peptide was determined mainly by the HMBC technique and chemical degradation.[3] The structures of more than twenty congeners of aureobasidin were determined in comparison with aureobasidin A.[4]



Aureobasidin A (**1**)

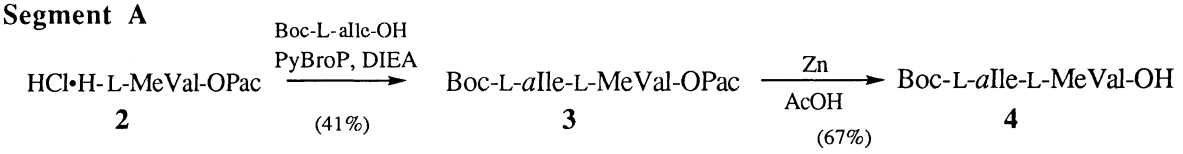
For purpose of investigation of a structure-activity relationship of the aureobasidin family antibiotic, we attempted a total synthesis of aureobasidin A aiming an establishment of a synthetic technique of the cyclic depsipeptide containing *N*-methyl amino acids, which is known to be difficult to condense by usual peptide-coupling methods.

The linkage between *a*Ile[1] and Pro[9] was chosen as a site of the final cyclization to avoid the coupling at an *N*-methyl amino acid as an amine component, which otherwise may diminish a yield of the cyclization reaction

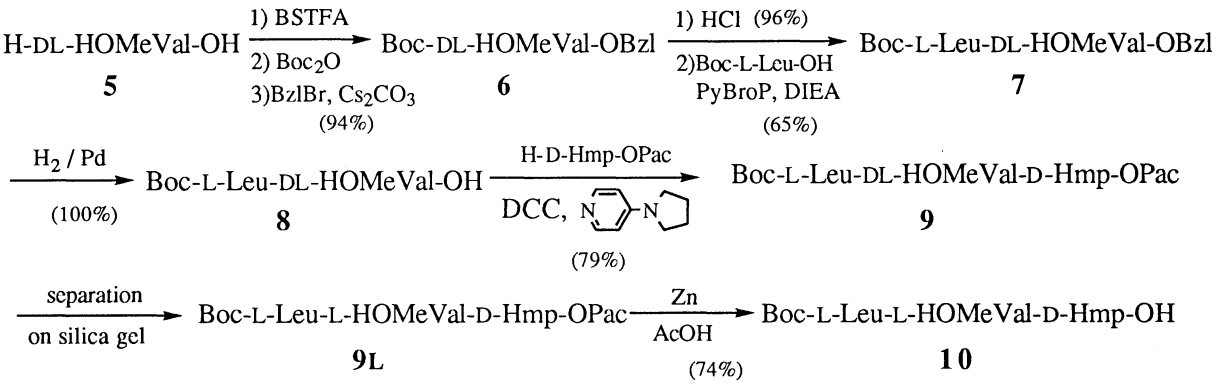
due to its steric characters. A linear nonapeptide(1-9) was synthesized according to the Boc strategy. Thus, a coupling between Leu^3-HOMeVal^4-Hmp^5 (Segment B) and MeVal^6-Phe^7-MePhe^8-Pro^9 (Segment C) was first attempted, followed by condensation of the coupling product (3-9) with *a*Ile^1-MeVal^2 (Segment A) by the fragment condensation as shown below.

Segment C was prepared by a stepwise elongation method starting from Boc-L-Pro-OPac (**11**) as a carboxyl terminus by the successive condensation using PyBroP[5] and DIEA in dichloromethane.[6]

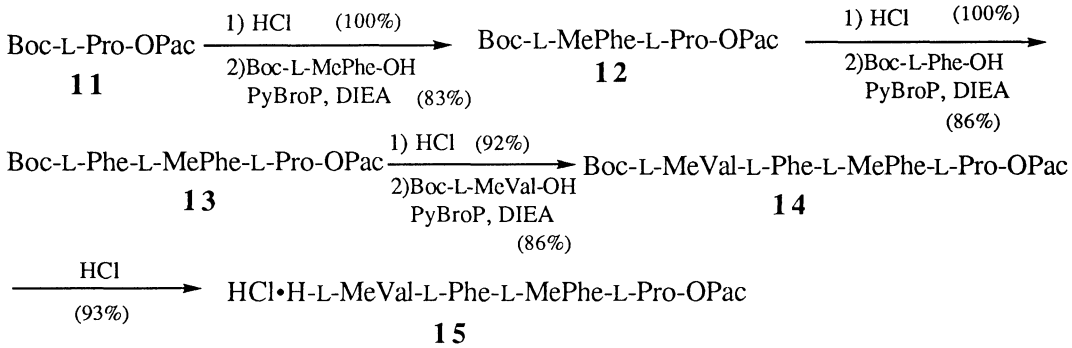
Segment A



Segment B

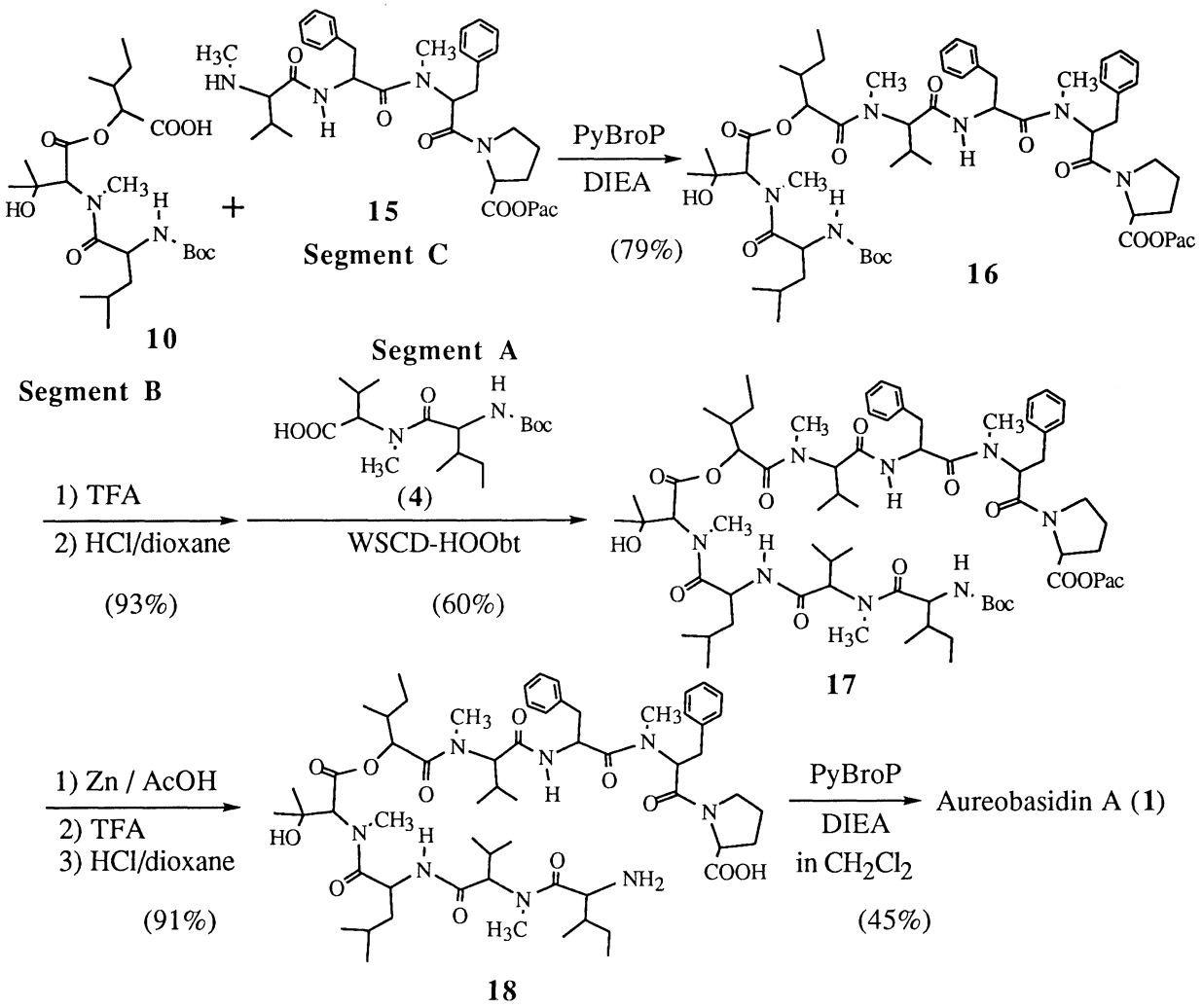


Segment C



DL-β-Hydroxymethylvaline (HOMeVal) in Segment B was prepared according to the method by Izumiya *et al.*[7] Although HOMeVal could not be protected with the Boc group by the usual manner, the protection could be achieved after activation and solubilization by trimethylsilylation with BSTFA. The introduction of the benzyl group to its carboxyl function and the removal of the Boc group were carried out, followed by coupling with Boc-L-Leu-OH using PyBroP without protection of β-OH group in HOMeVal to afford **7** in a moderate yield. After deprotection of Bzl group, a mixture of the diastereomers of the product **8** was coupled with H-D-Hmp-OPac with DCC and 4-pyrrolidinopyridine as an acylation catalyst. In this condensation reaction, DCC was

superior to PyBroP in the yield. Both coupling reagents without the base catalyst did not afford the desired product owing to the formation of the oxazolinium compound of Boc-Leu-HOMeVal as a main intermediate.[8] The diastereomers of the product **9** were separated each other by silica gel column chromatography,[9] and the L-L-D compound (**9L**) was deprotected with zinc/acetic acid to give **10** (Segment B). The coupling of **10**, whose *C*-terminus is a hydroxy acid, Hmp, with **15** was carried out using PyBroP to avoid a racemization. After removal of the Boc group in the peptide **16** with TFA , a fragment condensation with **4** (Segment A) by the WSCD-HOOBt method was carried out to give the protected linear nonapeptide **17** with a slight racemization as shown in the scheme, although the same condensation by the PyBroP method gave a 1:1 mixture of the completely racemized diastereomers.



The protecting groups of both carboxyl and amino groups in the linear nonapeptide **17**, which was purified diastereomerically, were removed successively, and then the free peptide **18** was cyclized with PyBroP in CH_2Cl_2 under a high-dilution condition (10^{-3} M) to afford predominantly the cyclic monomeric peptide (PD-MS: Found, M^+ 1100; Calcd for cyclic monomer, 1100). The cyclization of *N*-hydroxysuccinimide ester of the nonapeptide at the same concentration in CH_2Cl_2 as in that of PyBroP reaction gave a cyclic dimer as the major product. In case of PyBroP, the reaction seemed to proceed promptly compared to that in the active ester. There-

Table 1. Minimum Inhibitory Concentrations (μg/ml) of Natural and Synthetic Aureobasidin A

	Natural	Synthetic
Candida albicans TIMM 0136	≤0.0125	≤0.0125
Candida albicans TIMM 0171	≤0.0125	≤0.0125
Candida kefyr TIMM 0301	0.4	0.4
Candida glabrata TIMM 1062	0.1	0.2
Cryptococcus neoformans TIMM 0354	0.8	0.8
Saccharomyces cerevisiae ATCC 9763	0.2	0.2
Aspergillus fumigatus F48	>25	>25

fore, a condensation in a high-dilution condition was actually achieved preventing the dimerization in that case. On the other hand, the cyclization of the active ester in DMF proceeded very slowly even though the cyclic monomer was obtained as a major product besides the dimer. Such difference depending on the solvent seemed to arise from the solvation feature of the peptide molecule in DMF which may be advantageous to the monomeric cyclization.

The synthetic cyclic monomer thus obtained is completely identical with the natural antibiotic in all respects (TLC, HPLC, ^{1}H-NMR, and antifungal activities). Thus we now achieved the first total synthesis of aureobasidin A with the unique pattern of the antifungal activity.

References

1) Abbreviations: PyBroP: bromotris(pyrrolidino)phosphonium hexafluorophosphate; HMBC: heteronuclear multiple-bond connectivity; Boc: *t*-butoxycarbonyl; Pac: phenacyl; DIEA: diisopropylethylamine; BSTFA: *N, O*-bistrimethylsilyltrifluoroacetamide; DCC: dicyclohexylcarbodiimide; Hmp: *(2R)*-hydroxy-*(3R)*-methylpentanoic acid; WSCD: water-soluble carbodiimide [*N*-ethyl-*N'*-(dimethylaminopropyl)carbodiimide (EDC)]; HOObt: 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine; PD-MS: plasma-desorption mass spectroscopy; HOBt: 1-hydroxybenzotriazole; 1M=1mol dm^{-3}.
2) K. Takesako, K. Ikai, F. Haruna, M. Endo, K. Shimanaka, E. Sono, T. Nakamura, I. Kato, and H. Yamaguchi, *J. Antibiot.*, **44**, 919 (1991).
3) K. Ikai, K. Takesako, K. Shiomi, M. Moriguchi, Y. Umeda, J. Yamamoto, I. Kato, and H. Naganawa, *J. Antibiot.*, **44**, 925 (1991).
4) K. Ikai, K. Shiomi, K. Takesako, S. Mizutani, J. Yamamoto, Y. Ogawa, and I. Kato, *J. Antibiot.*, **44**, 1187 (1991); K. Ikai, K. Shiomi, K. Takesako, and I. Kato, *ibid.*, **44**, 1199 (1991).
5) J. Coste, E. Frérot, P. Jouin, and B. Castro, *Tetrahedron Lett.*, **32**, 1967 (1991).
6) The coupling reaction of Boc-L-MePhe-OH with HCl•H-L-Pro-OPac by the WSCD-HOBt method gave the product in a yield of 87%, accompanying L-D isomer (A ratio of L-L and L-D isomers was 10 : 3). This racemization was due to the intramolecular formation of the charged Schiff base in Pro residue between the imino group and the carbonyl function of the Pac ester. (H. Kuroda, S. Kubo, N. Chino, T. Kimura, and S. Sakakibara, *Int. J. Peptide Protein Res.*, **40**, 114 (1992). The reaction with PyBroP afforded the desired product without racemization.
7) N. Izumiya and A. Nagamatsu, *J. Chem. Soc. Jpn.*, **72**, 336 (1951).
8) N. L. Benoiton and F. M. F. Chen, *Can. J. Chem.*, **59**, 384 (1981).
This oxazolinium-forming phenomenon was recently reported in case of *N*-MeVal and some *N*-methylamino acids by B. Castro *et al.* independently. (E. Frérot, P. Jouin, J. Coste, and B. Castro, *Tetrahedron Lett.*, **33**, 2815 (1992).
9) These diastereomeric peptides (L-L-D or L-D-D) after separation were hydrolyzed with 6N HCl for 19 h to give respectively L-HOMeVal and D-HOMeVal, which were assigned in comparison with the authentic amino acids on Daicel Chiralpak WH.

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