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Total Synthesis of Leucinostatin D

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Abstract: Peptide antibiotic, Leucinostatin D, was synthesized by stepwise elongation method, starting from β-alanine *t*-butyl ester. Z-octapeptide ester was converted to amide derivative and finally coupled with (4S,E)-4-methylhex-2-enoyl-L-4-methylproline.

Leucinostatins are peptide antibiotics produced by *Paecilomyses* fungi. Until now, 7 congeners are isolated in nature and determined their structures; A,B,C,D,F,H and K.[1] They are active against gram-positive bacteria, yeast and fungi. They are also known as strong mycotoxins and, therefore, they called Paecilotoxins, too.[2] The structural feature of these compounds are the presence of many unusual amino acids, as well as chiral unsaturated aliphatic acid as N-terminal acyl component and various chiral diamines as an amide part. High contents of leucine and α-aminoisobutyric acid (Aib) are also their characteristic feature. Recently, Aib-rich peptides have provoked a great deal of interest in their biological activities and conformations. Therefore, to examine the structure-activity relationship of these antibiotics, we have planned to synthesize leucinostatin D, a minor component of this antibiotic's family, which has relatively simple structure among them.

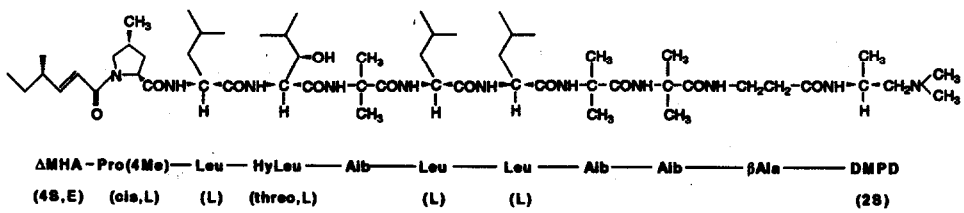
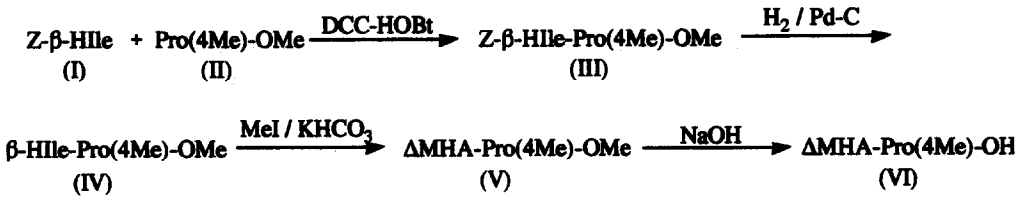


Fig. 1. Structure of Leucinostatin D[3]

cis-L-4-Methylproline (Pro(4Me)) was synthesized by catalytic hydrogenation of L-4-methylene-proline obtained by Wittig reaction of L-4-oxoproline derived from L-4-hydroxyproline.[4] *threo*-L-β-Hydroxyleucine (HyLeu) was obtained by optical resolution of racemic form which was synthesized by condensation of 2-methylpropanal with glycine and purified by recrystallization from water several times to remove the *erythro* compound.[4,5] (2S)-N^1,N^1-Dimethylpropane-1,2-diamine (DMPD) was synthesized by alkylating

dimethylamine with O-tosyl-N-Z-L-alaninol.[4)]

As to the synthesis of the N-acyl part of this peptide, (4S,E)-4-methylhex-2-enoyl-L-proline (ΔMHA-Pro-OH) was tried to be synthesized as a model compound. Mild Hofmann degradation[6)] and successive hydrolysis of L-β-homoisoleucyl-L-proline methyl ester afforded this compound in optically pure state.[7)] Then, (4S,E)-4-methylhex-2-enoyl-L-4-methylproline (VI) was prepared by the same synthetic route. Z-L-β-homoisoleucine (I) was condensed by DCC-HOBt method with *cis*-L-4-methylproline methylester (II) to Z-L-β-homoisoleucyl-L-4-methylproline methyl ester (III), (yield 88%, mp 63-65°C, $[\alpha]^{25}_{D}$ -58.5° (c1.03, MeOH)). After removal of N-protecting group by catalytic hydrogenation and successive mild Hofmann degradation (MeI, $KHCO_3$, MeOH, room temperature 2 days), (4S,E)-4-methylhex-2-enoyl-L-4-methylproline methyl ester (V) (yield 72%, oil, $[\alpha]^{25}_{D}$ -72.6° (c 1.2, MeOH)) was obtained. By hydrolysis of this ester with 1M aqueous sodium hydroxide, (4S,E)-4-methylhex-2-enoyl-L-4-methylproline (ΔMHA-Pro(4Me)-OH) (VI) (yield 81%, oil, $[\alpha]^{25}_{D}$ -62.2° (c 0.92, MeOH)) could be synthesized (Scheme 1.).



(I) (II) (III) (IV) (V) (VI)

Scheme 1. Synthetic Route for (4S,E)-4-Methylhex-2-enoyl-L-4-methylproline (VI)

For the synthesis of the C-terminal octapeptide, stepwise elongation starting from β-alanine using methyl ester as a C-terminal protecting group followed by conversion of methyl ester to amide derivative was first tried. Condensation was mainly carried out by DCC-HOBt method. However, in the case of the introduction of *threo*-β-hydroxyleucine, condensation reaction could not proceed well by DCC-HOBt method when no protection was applied to the side chain of hydroxyleucine. Therefore, Z-HyLeu-OH was derived to mixed anhydride with isobutylchloroformate and condensed with hexapeptide methyl ester. By this method, Z-heptapeptide methyl ester could be obtained in a moderate yield and elongated to Z-octapeptide methyl ester without problem. The ester, thus obtained, was tried to hydrolyze with sodium hydroxide to convert to amide derivative. However, in this case, splitting of the N-terminal protecting group and formation of urea derivative[8)] have occurred during alkaline hydrolysis. Therefore, to avoid this side reaction, C-terminal protecting group was changed as an acid-labile *t*-butyl group and the same synthesis was tried again (Fig.2).[9)]

In this case, Z-octapeptide acid could be obtained by usual TFA treatment of the *t*-butyl ester. Condensation of this peptide with (2S)-N^1,N^1-dimethylpropane-1,2-diamine was carried out using DCC-HOBt as a coupling reagent. After removal of the Z group of protected C-terminal octapeptide, (4S,E)-4-methylhex-2-enoyl-L-4-methylproline was condensed by DCC-HOBt method. The raw product was purified by preparative TLC (Wakogel B-5F; developing solvent, MeOH : AcOEt = 4 : 1) and leucinostatin D was obtained as a hydrochloride.

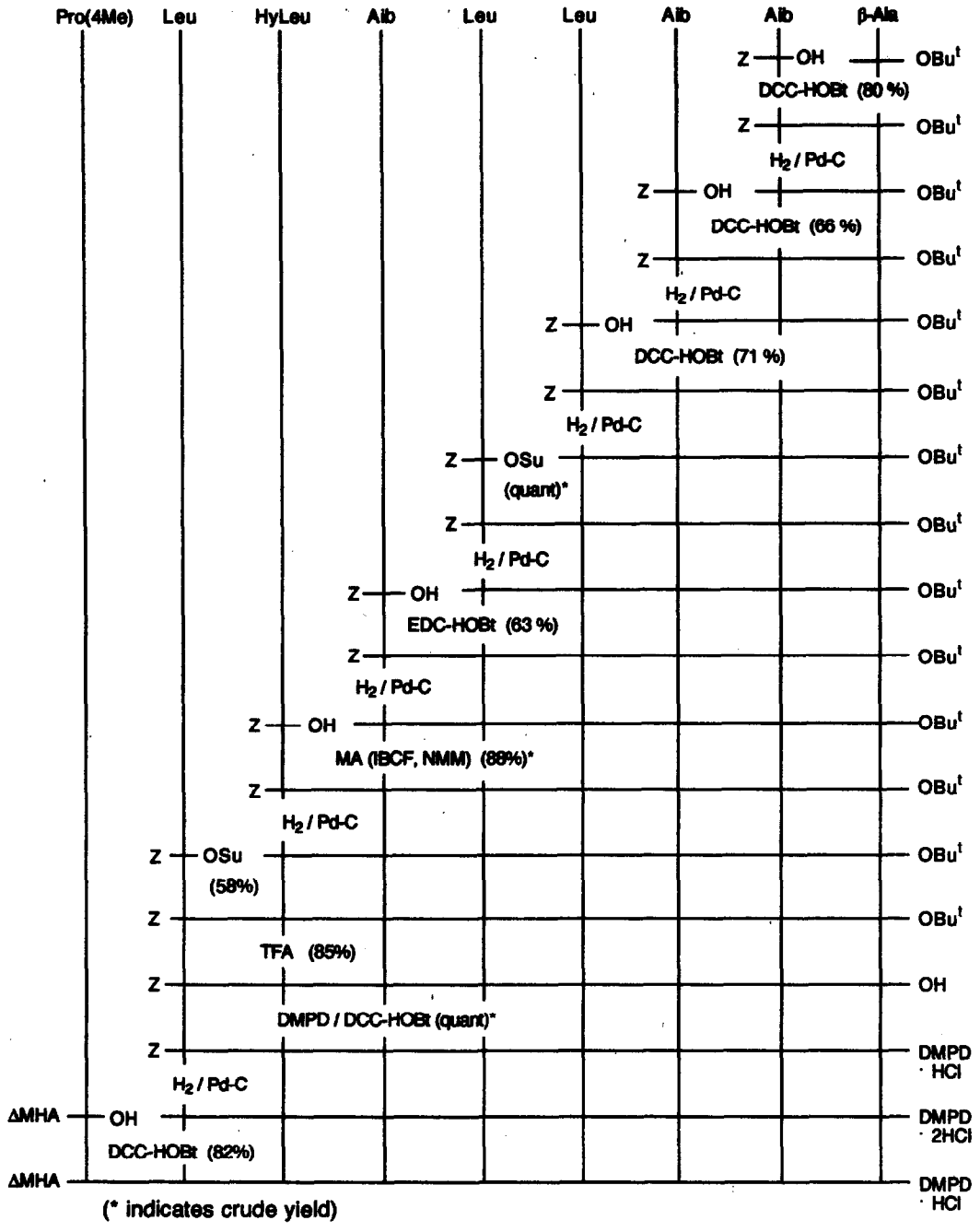


Fig. 2. Synthetic Scheme for Leucinostatin D

The validity of the structure was confirmed by SIMS (m/z 1119 MH^{+}) and NMR (^{1}H, ^{13}C) analyses.[10] Furthermore, synthetic leucinostatin D, thus obtained, showed similar biological activities as natural one reported in the literature.[3a]

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9. Abbreviations: DCC, dicyclohexylcarbodiimide; EDC, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; IBCF, isobutylchloroformate; MA, mixed anhydride; NMM, N-methylmorpholine; OSu, N-hydroxysuccinimide ester; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl.
10. ^{1}H NMR ($CDCl_3$) -CH=C<u>H</u>CO- δ 6.36 (dd, J=1Hz, 15.4Hz, 1H); -C<u>H</u>=CHCO- δ 6.67 (dd, J=7.3Hz, 15.4Hz, 1H); -CO-N<u>H</u>- δ 8.95 (d, J=6.6Hz, 1H): δ 8.72 (d, J=4.5Hz, 1H): δ 8.35 (dd, J=3.9Hz, J=9.2Hz, 1H): δ 8.28 (s, 1H): δ 8.12 (s, 1H): δ 8.07 (d, J=5.3Hz, 1H): δ 7.70 (s, 1H): δ 7.47 (d, J=14.5Hz, 1H): δ 7.26 (d, J=6.3Hz, 1H); -N^{+}<u>H</u>$(CH_3)_2$ δ 8.02 (br, 1H); -N^{+}H$(C$<u>H</u>$_3)_2$ δ 3.16 (d, J=3.1Hz, 3H): δ 3.07 (d, J=2.8Hz, 3H).

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