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Catalytic Asymmetric Total Synthesis and Stereochemical Revision of Leucinostatin A, a Modulator of Tumor-Stroma Interaction

Hikaru Abe, Hitoshi Ouchi, Chiharu Sakashita, Manabu Kawada, Takumi Watanabe,* Masakatsu Shibasaki*

Abstract: Total synthesis of leucinostatin A, a modulator of tumor-stroma interactions, using an asymmetric catalysis, a nitroaldol reaction, thioamide-aldol reaction, Strecker-type reaction, and alcoholysis of 3-methylglutaric anhydride, is described. We demonstrated the applicability of the established catalytic asymmetric process to the synthesis of molecules with a complex structure. Careful analysis of the NMR data, HPLC profiles, and biological activity revealed that the correct structure of leucinostatin A is the epimeric form of the reported structure; the secondary alcohol within the AHMOD residue has an *R*-configuration.

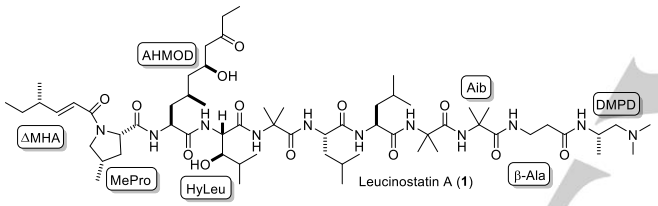

Figure 1. Reported structure of leucinostatin A

Tumor cells never exist in isolation, and are surrounded by normal cells such as fibroblasts (stromal cells).[1] Tumor cell proliferation is regulated by the stromal cells through secreted factors and adhesion:[2] an intercellular communication of this type is termed tumor-stroma interactions. The signals involved in tumor cell growth emitted from stromal cells are a promising target in the current anticancer lead generation process as normal cells are expected to be less prone to mutation of proteinous factors and acquisition of resistance due to the stable nature of the genes. We have investigated modulators of tumor-stroma interactions among natural products, especially those produced by microorganisms and fungi. In our assay system, the hit compounds should selectively suppress the proliferation of tumor cells cultured in the presence of the corresponding stromal cells as opposed to their absence.[3] During screening, leucinostatin A (Figure 1) produced by fungi,[4] which had been originally reported as an effective antibacterial and cytotoxic peptidyl natural product,[5] was found to inhibit the growth of DU-145 prostate cancer cells more potently under coculture with prostate stromal cells (PrSC) than under monoculture conditions. Intriguingly, leucinostatin A exerts antitumor activity in vivo in mouse only when coinoculated with DU-145 cells and PrSC.

Leucinostatin A is comprised of 4-methyl-2-hexenoic acid (ΔMHA) 4-methylproline (MePro), 2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD), *threo*-β-hydroxyleucine (HyLeu), three 2-aminoisobutyric acid (Aib), two leucines (Leu), β-alanine (β-Ala), and N^1,N^1-dimethylpropane-1,2-diamine (DMPD).[6],[7] Cerrini and co-workers reported the crystal structure of leucinostatin A to assign the *S*-configuration of the secondary alcohol within the AHMOD residue,[8] which could not be unveiled in the previous degradation study. Recently, Brimble and co-workers reported the total synthesis of peptibol antibiotics containing the AHMOD residue such as culicinin D,[9] and trichoderin A,[10] however, no precedent for leucinostatin A has been reported to date. Herein we disclose the first catalytic asymmetric total synthesis of leucinostatin A, which exemplifies the general applicability of four catalytic asymmetric processes consisting of three C-C bond-forming reactions as well as one C-O bond formation developed in this laboratory in order to access complicated chemical entities.

Scheme 1 illustrates our synthetic strategy focusing on the unnatural amino acid components, HyLeu and AHMOD. A nitroaldol process could set up the carbon framework of HyLeu from inexpensive substrates **4** and **5**. It was proposed that the AHMOD unit could be constructed with a three-catalyst protocol: the the 4-methyl substituent could be furnished by asymmetric methanolysis of 3-methylglutaric anhydride **13** to provide the corresponding half ester **12**, and *S*-configuration of the α-carbon atom should be delivered by Strecker-type reaction from **11** to **10**, followed by a thioamide-aldol reaction of **8** and **9** to install the (*S*)-hydroxy group at 6-position in the adduct **7**. The two aldol-type methodologies exemplify direct catalytic asymmetric reactions; in the C-C bond-forming process, only proton-transfer from the prenucleophile to the hydroxy group of the product occurs and no pre-activation of the substrate by derivatization is required, which fulfils perfect atom economy.

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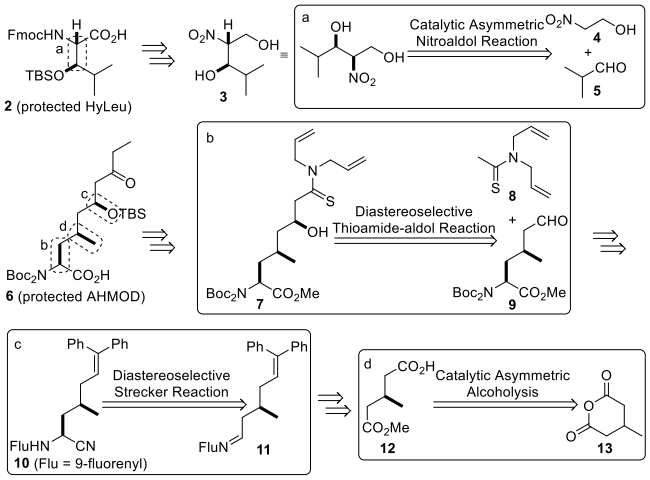
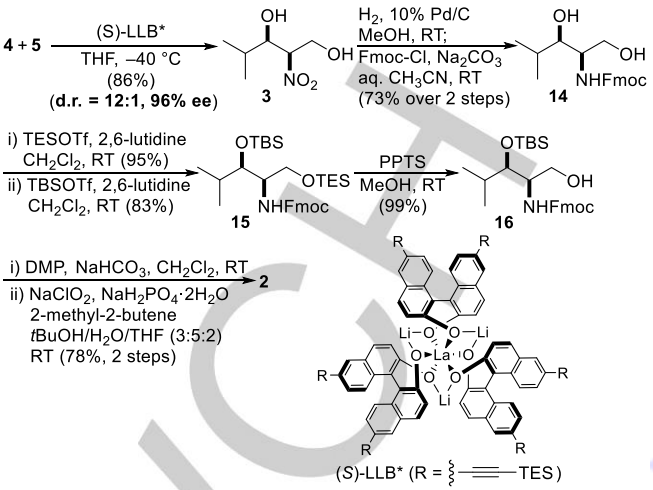


Scheme 1. Strategy toward catalytic asymmetric total synthesis of leucinostatin A.

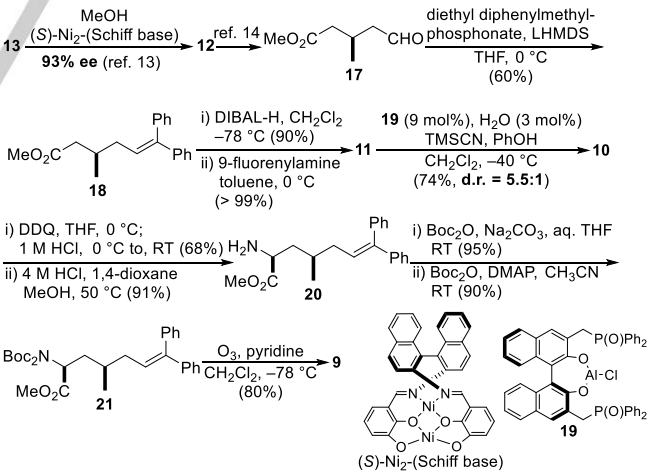


Scheme 2. Synthesis of HyLeu segment.

As detailed in Scheme 2, the present total synthesis commenced with preparation of HyLeu. Although this segment is commercially available, we envisioned that a catalytic asymmetric *syn*-selective nitroaldol reaction could be used to construct the requisite carbon skeleton with high enantioselectivity. Indeed, LLB* (a modified LLB complex as shown in Scheme 2) is a particularly effective catalyst to facilitate highly enantioselective nitroaldol reactions using 2-nitroethanol **4** as a substrate.[11] In the original report, no α-branched aliphatic aldehyde was examined. Therefore, we optimized the reaction conditions for isobutyraldehyde **5** (Table S1). It was found that 9 mol % of LLB* catalyst with 3:1 ratio of **4** and **5** in THF at −40 °C for 140 h afforded a good yield (86%) and satisfactory diastereo- (12:1) and enantioselectivity (96% ee) of the corresponding nitroaldol adduct **3**. At this stage, the enantiopurity of **3** could be enriched to > 99% ee by recrystallization.

Progression to HyLeu derivative **2**, which may be used in Fmoc-based peptide synthesis, was performed without trouble. The nitro group of **3** was reduced to a primary amine, followed by Fmoc-protection to give **14**. The secondary alcohol was then selectively masked as a TBS ether to give **16** via bissilyl ether **15**. Finally, a series of oxidation protocols (Dess-Martin, and Pinnick oxidations) completed the catalytic asymmetric synthesis of **2**.

The next synthetic task was preparation of the AHMOD segment: several synthetic studies related to AHMOD distinct from leucinostatin A have been reported.[9,12] Our synthesis was initiated (Scheme 3) from the central part of AHMOD; the 4-methyl group was furnished with good enantiocontrol in the first step by catalytic asymmetric methanolysis of 3-methylglutaric anhydride **13**, which led to the half ester **12** as reported previously (93% ee).[13] The carboxyl functionality of **12** was readily converted to a formyl group (**17**) according to the reported protocol,[14] which was followed by Wittig-type chemistry to provide trisubstituted olefin **18**. The remaining ester moiety was subsequently transformed into imine to give the substrate (**11**) of a Strecker-type reaction.

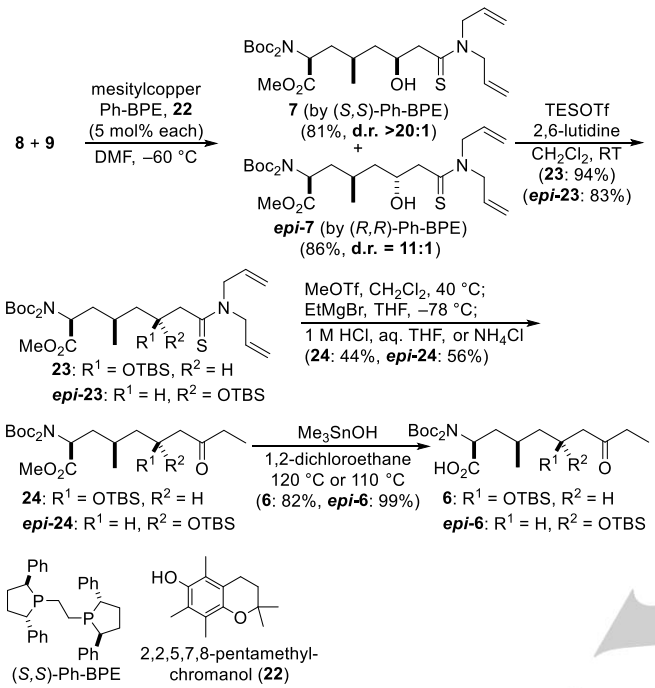


Scheme 3. Synthesis of the substrate of the thioamide-aldol reaction.

After optimization (Table S2) of the Strecker-type reaction, the highest diastereoselectivity up to 5.5:1 (74% yield) was obtained when 9 mol % of catalyst **19**, 3 mol% of H_2O, 0.2 equiv of phenol, and 2 equiv of TMSCN were employed. Subsequently (Scheme 4), the cyano group of **10** was directly converted into methyl ester

10.1002/chem.201703239

20 by treatment with 4 M HCl in a 1,4-dioxane-MeOH system under gentle warming. The amino functionality was then bis-protected by Boc groups (**21**) to avoid participation of the nitrogen in an undesired cyclization with the aldehyde after the subsequent transformation. Finally, ozonolysis afforded **9** in 80% yield.



Scheme 4. Synthesis of AHMOD segments.

For installation of the β-hydroxyketone moiety of AHMOD, a catalytic asymmetric direct thioamide-aldol reaction condition was effective (Scheme 4).[15] In this reaction, Cu^{I} complexed with a chiral bidentate phosphine ligand, Ph-BPE, works as the soft Lewis acid to activate thioamide **8** to promote deprotonation at the α-carbon, even in the presence of a more acidic proton adjacent to the formyl group of **9**. Interestingly, facial selectivity in this case was opposite to the prediction based on the original report, which led us to use (*R,R*)-Ph-BPE to set the *S*-configuration for the 6-hydroxy functionality. After optimization (Table S3), the use of 5 mol% catalyst with chromanol as additive (**22**) in DMF at −60 °C was revealed to give the best result. In the previous study, the thioamide functionality could only be converted to methylketone or β-ketoester via the addition of lithium reagents to the methylthioiminium intermediate generated by the treatment of MeOTf.[15] In the present synthesis, EtMgBr was successfully used for the first time, after protection of the hydroxy group as a TBS ether (**23**). Although addition of Grignard reagent took place without issue, subsequent hydrolysis by the original procedure was slow: acid-treatment (1 M HCl/THF or NH_4Cl) was required instead of wet silica gel (original) to afford **24** in reasonable yield (43%). The conversion of methyl ester to carboxylic acid under conventional methods caused predominatly β-elimination of the siloxy functionality and epimerization at the α-carbon. The desired transformation proceeded by Me_3SnOH[16] giving **6** with no trace of undesired byproducts.

With the requisite monomer units in hand, hexapeptide **25** was synthesized on solid support (Supporting Information), and progression to leucinostatin A was performed with HATU-mediated[17] peptide coupling in liquid phase (HATU: 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate). Firstly, condensation with DMPD (**26**) afforded **27** (Scheme 5). After cleaving the Fmoc group, HyLeu-segment **2** was attached giving **28**, then sequential deprotection and coupling with AHMOD-segment **6** provided **29** (56%). Lewis acidic Boc-deprotection mediated by BF_3·OEt was found the most preferable[18] in order to avert undesired β-elimination of the siloxy moiety on AHMOD which was observed when using standard TFA conditions. The final coupling reaction with an acylated MePro, **30**, accompanied deprotection to complete the synthesis of leucinostatin A (**1**) in 43% yield. The NMR data of the synthetic sample was barely distinguishable from that of the natural compound (Figure S2). The antiproliferative activity against DU-145 cells cocultured with PrSC (Table 1) of the synthesized leucinostatin A, however, was 2-fold less potent than the natural sample (IC_{50}; natural: 0.028 ± 0.004 μg mL^{-1}, synthetic: 0.063 ± 0.015 μg mL^{-1}), and the HPLC retention time differed slightly (Figure S3). In the synthesis of culicinin D and trichodermin A, Brimble concluded that the 6-position of AHMOD bearing a hydroxy group has an *R*-configuration,[9,10] which is opposite to the reported configuration. In each case, the configuration was determined by comparing similarity of NMR data of the *R*- and *S*-isomers to the reported values: in fact, exact match was not observed for both the isomers. Although complicated by the close similarity of the NMR data of the synthetic and natural samples in our case and general reliability of the configuration determined by crystallography,[8] we synthesized the epimer of the reported leucinostatin A (***epi*-1** in Scheme 5) for the analysis.

Table 1. Antiproliferative activity (IC_{50} (μg mL^{-1})) of nominal leucinostatin A and epimer toward DU-145 cells cocultured with PrSC cells.

Compound	Cocultured	Monocultured
leucinostarin A (natural)	0.028 ± 0.004	~1
1	0.063 ± 0.015*	~1
***epi*-1**	0.029 ± 0.001	~1

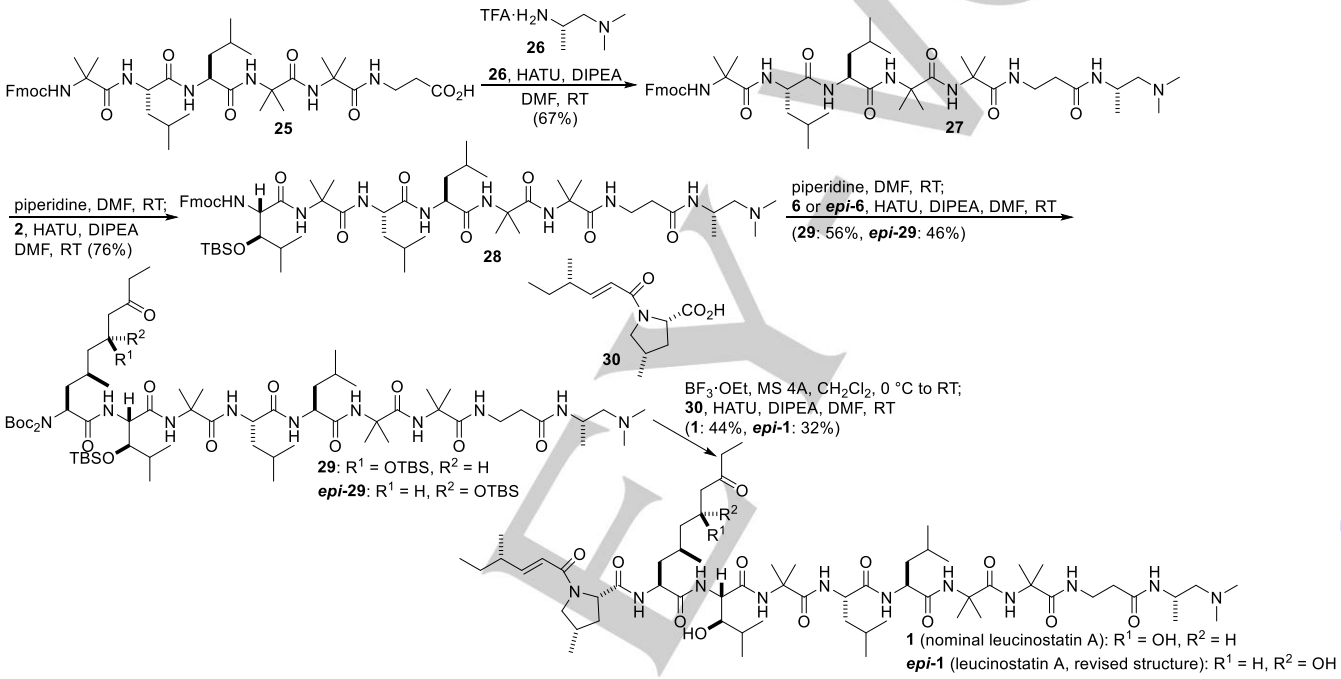
The values are means ± s.d. of three independent experiments with similar results. *P < 0.01 versus the values of leucinostatin A (natural) (Student's *t*-test).

Performing the diastereoselective thioamide-aldol reaction of **8** and **9** using (*R,R*)-Ph-BPE (Scheme 4) gave aldol adduct ***epi*-7** (86%) with a slightly compromised but still excellent diastereoselectivity of 11:1. Subsequent transformations were executed in the same manner as for **6** to afford ***epi*-6**, which was coupled with the Fmoc-removed form of heptapeptide **28** to give ***epi*-29** (46%). The final coupling with **30** proceeded with concomitant deprotection to provide an epimeric form of the

10.1002/chem.201703239

reported structure of leucinostatin A, ***epi*-1** (32%). The physicochemical properties of ***epi*-1** exactly matched those of natural leucinostatin A including NMR data (Figure S4, also almost very similar to that of **1** in Figure S2!). The retention times of ***epi*-1** and naturally occurring leucinostatin A based on HPLC analysis were completely identical (Figure S5). Moreover, these two samples exhibited the same growth inhibitory activity toward DU-145 cells in the presence of PrSC (IC_{50}; ***epi*-1**: 0.029 ± 0.001 μg mL^{-1}, Table 1). Together, this evidence led us to conclude that the correct absolute configuration of the secondary alcohol of AHMOD should be *R*, which is epimeric to the originally proposed configuration.

In summary, the first total synthesis of leucinostatin A, a modulator of tumor-stroma interactions, was achieved by applying four catalytic asymmetric reactions developed in this laboratory, which exemplify the adaptability of our processes with fine-tuning to the synthesis of molecules with a complex structure. Careful analysis of the NMR data, HPLC profiles, and biological activity unequivocally indicated that the correct structure of leucinostatin A is the epimer of the reported structure, with *R*-configuration at the secondary alcohol portion of the AHMOD residue. Further SAR studies to generate anticancer leads and probe compounds to explore molecular targets upon intervening with tumor-stroma interactions are in high demand and are currently underway.



Scheme 5. Completion of the total synthesis of leucinostatin A and stereochemical revision.

Acknowledgements

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Keywords: Asymmetric catalysis • Total synthesis • Configuration determination • Natural products• Tumor–stroma interaction

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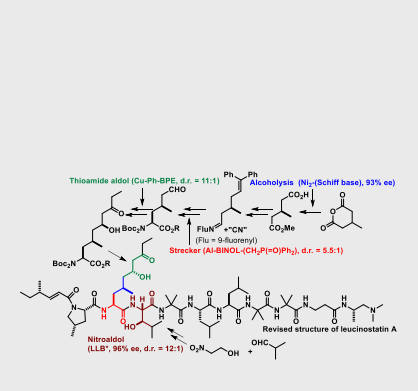

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Page No. – Page No.

Catalytic Asymmetric Total Synthesis and Stereochemical Revision of Leucinostatin A, a Modulator of Tumor-Stroma Interaction