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Determination of absolute stereochemistry, total synthesis, and evaluation of peptides from the myxomycete *Physarum melleum*

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Abstract—The absolute stereochemistry of melleumin A (**1**) and B (**2**), novel peptide compounds isolated from the myxomycete *Physarum melleum*, was determined by synthesis of their segments and by a modified Mosher's method. Total synthesis of melleumin B (**2**) was achieved by a stereoselective method, which provided further evidence for all the absolute stereochemistries of melleumin B (**2**). The Wnt signal inhibitory activities of **2** and its 10*R*-epimer **19** were evaluated. Compound **19** showed moderate inhibition of Wnt signal transcription, which suggests that melleumin analogues might be useful as Wnt signal inhibitors.

Myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes. Our analysis of myxomycetes has led us to isolate new compounds.[1–3] A novel peptide lactone, melleumin A (**1**), and its *seco* acid methyl ester, melleumin B (**2**), were isolated from cultured plasmodium of the myxomycete *Physarum melleum* (Fig. 1).[1] The stereochemistry of **2** at the chiral centers was established as 3*S*, 10*S*, and 11*R* configurations. However, the absolute stereochemistry of the C-4 position of **2** remained undetermined. Here, we describe the determination of the absolute stereochemistry at the C-4 position of **2** on the basis of the synthesis of partial structures of **2** in optically active forms. The total synthesis of melleumin B (**2**) provided unequivocal evidence for all the stereochemistry of melleumin B (**2**). The 10*R*-epimer of melleumin B (**19**) exhibited Wnt signal inhibition.

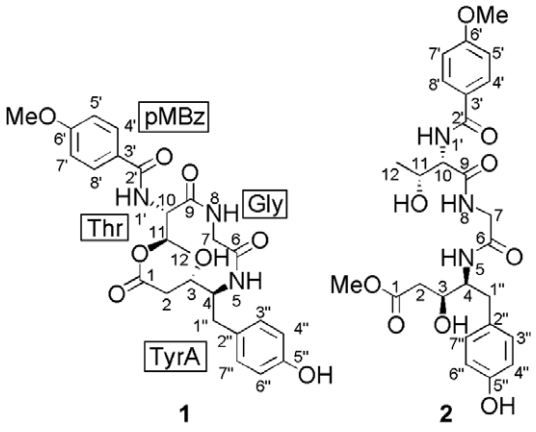

Figure 1. Structure of melleumin A (**1**) and B (**2**). Stereochemistry at the C-4 position was determined in this study.

Melleumin A (**1**) and B (**2**) consist of four residues, *p*-methoxybenzoic acid (pMBz), L-threonine (L-Thr), glycine (Gly), and an unusual amino acid, a tyrosine-attached acetic acid (TyrA).

The undefined chiral center at the C-4 position was included in TyrA unit. To determine the absolute configuration, we synthesized two diastereomers of TyrA and compared them by HPLC analysis with the unit obtained from hydrolysis of melleumin B (**2**).

Synthesis of the two diastereomers of the TyrA unit is shown in Scheme 1. L-Tyrosine methyl ester hydrochloride was protected with *t*-butoxycarbonyl (Boc) ester to give **3**.[4] Reduction of the protected tyrosine **3** with DIBAL gave aldehyde **4**, which was treated with a lithium enolate of ethyl acetate to give two diastereomers **5a** and **5b**.[5–7] Their stereochemistries were determined by the modified Mosher's method (see Supplementary data, Fig. 1).[8] Hydrolysis and deprotection of the Boc group of **5a** and **5b** gave **7a** and **7b**, respectively.

Keywords: Myxomycete; *Physarum melleum*; Melleumin; Peptide; Wnt signal inhibitor.
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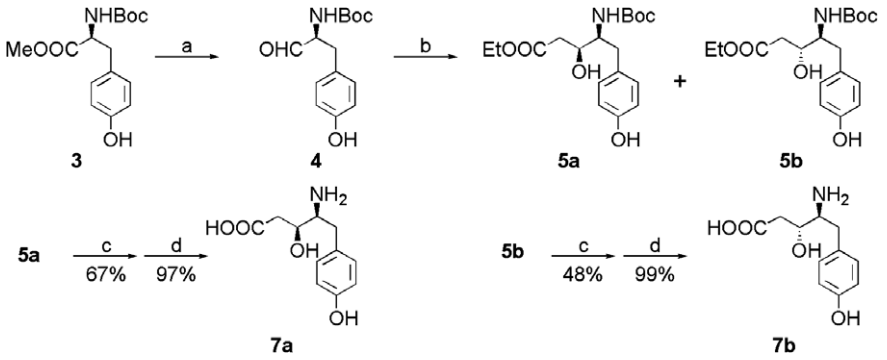


Scheme 1. Reagents and conditions: (a) DIBAL, toluene, −78 °C, 6 min; (b) EtOAc, LDA, THF, −78 °C, 2 h, 11% (two steps), **5a/5b** = 54/46; (c) LiOH, THF/MeOH/H_2O = 3/1/1, rt, 1 h; (d) TFA, CH_2Cl_2, rt, 1 h.

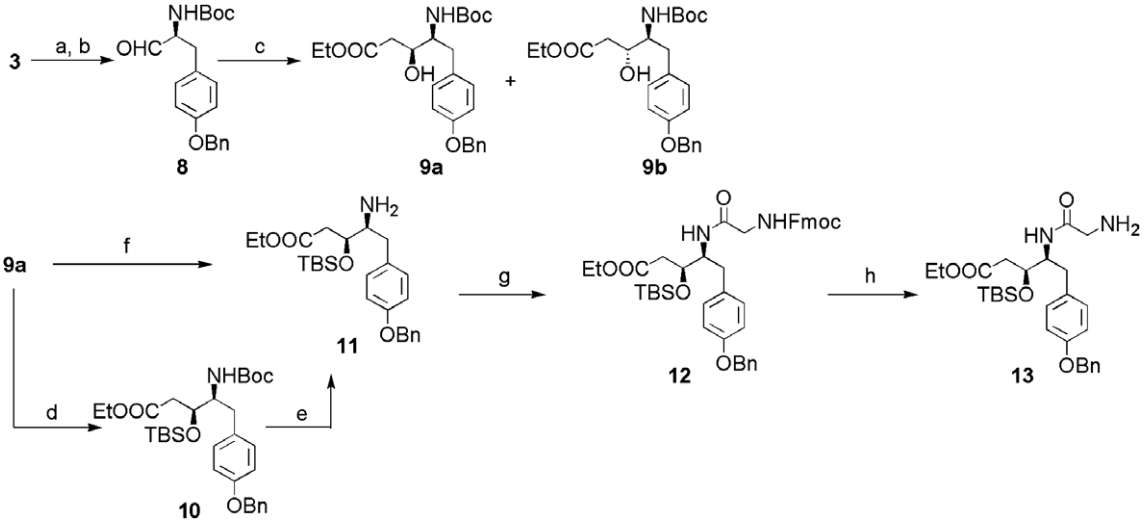
Comparison of the reverse-phase HPLC retention time between melleumin B (**2**) hydrolysate and the synthetic TyrA unit (**7a** and **7b**) was performed. The retention time of **7a** matched that of the melleumin B (**2**) hydrolysate (see Supplementary data, Fig. 2).[9] Melleumin A (**1**) and B (**2**) have the same stereochemistry because treatment of **1** with 28% MeONa afforded **2**.[1] The result suggested that the stereochemistry of melleumin A (**1**) and B (**2**) has the same structure as **7a** (3*S*,4*S*) and all the absolute stereochemistries of melleumin A (**1**) and B (**2**) were determined as 3*S*, 4*S*, 10*S*, and 11*R*.

Next, we studied the total synthesis of melleumin B (**2**). As shown in Scheme 2, the protected TyrA units (**9a** and **9b**) were prepared from **3** in the same manner as **3** to **5a** and **5b**, and the phenolic hydroxyl group was protected with benzyl (Bn) ether.[4] The absolute stereochemistries of **9a** and **9b** were confirmed by comparison of ^{1}H NMR spectral data with **5a** and **5b** after cleavage of the Bn group.

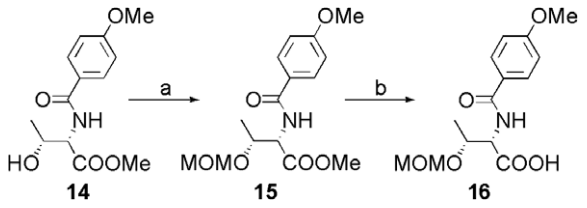
Compound **9a**, having the desired stereochemistry of the TyrA unit, was protected with TBS ether using TBSCl,[10] followed by deprotection of the Boc group with TBSOTf[11] to give a free amine **11**. This method generally requires two steps and purification after each step. This step was improved as a one-pot reaction according to procedure reported by Corey et al. First, **9a** was treated with TBSOTf for cleavage of the Boc group, and then 2,6-lutidine was added to give the TBS ether.[12] The reaction mixture, containing free amine **11**, was evaporated and the residue was subjected to coupling with Fmoc-Gly to give compound **12** with a good yield (78%, two steps from **9a**). Subsequently, deprotection of the Fmoc group gave the desired TyrA-Gly unit **13**.

The L-Thr-pMBz unit **16** was prepared from the known compound **14**.[13] MOM protection and hydrolysis of the methyl ester gave the desired L-Thr-pMBz unit **16** (Scheme 3).

Coupling of the TyrA-Gly unit **13** with the L-Thr-pMBz unit **16** gave tripeptide **17** with a good yield (67%, two steps from **12**) including the 10*R*-epimer **18** (**17/18** = 92/8, determined by ^{1}H NMR),



Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, acetone, reflux, 24 h, 99%; (b) DIBAL, toluene, −78 °C, 0.5 h, 86%; (c) EtOAc, LDA, THF, −78 °C, 2 h, 80%, **9a/9b** = 1/1; (d) TBSCl, imidazole, DMF, rt, 54 h, 81%; (e) TBSOTf, CH_2Cl_2, 2 h; (f) TBSOTf, CH_2Cl_2, 0 °C, 2 h, then 2,6-lutidine, 2 h; (g) Fmoc-Gly, EDC, HOBt, 4-DMAP, CH_2Cl_2, rt, overnight, 78% (two steps from **9a**); (h) piperidine, CH_2Cl_2, 0 °C, 1 h.

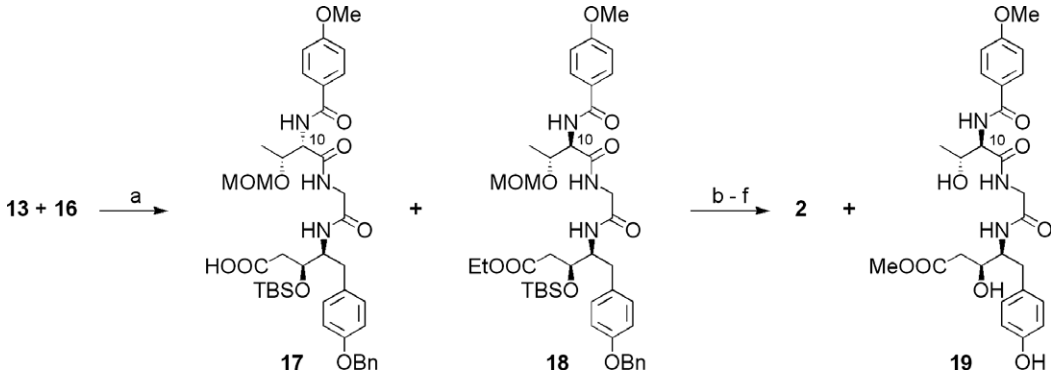


Scheme 3. Reagents and conditions: (a) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, rt, 48 h, 97%; (b) LiOH, THF/H_2O = 1/1, 0 °C, 45 min, 95%.

and we used a mixture of **17** and **18** for next steps (Scheme 4).

Deprotection of the TBS and MOM groups on the tripeptide, followed by hydrolysis of the ethyl ester with LiOH, afforded the *seco* acid, which was converted to a methyl ester with TMS-diazomethane. The benzyl group was finally removed by hydrogenolysis to give melleumin B (**2**) as a mixture with its 10*R*-epimer **19** (**2**/**19** = 91/9, determined by reversed-phase HPLC).[14] Melleumin B (**2**) and **19** were separated as a pure form by reversed-phase HPLC.[15] The ^{1}H and ^{13}C NMR and FABMS spectra of synthetic **2** were compared with those of a natural product specimen, showing that they were completely identical (see Supplementary data). This result provided unequivocal evidence of the whole structure of melleumin B (**2**) as well as melleumin A (**1**), including absolute stereochemistry.[1]

We examined the Wnt signal inhibitory activity of the two synthesized compounds (**2** and **19**) using a luciferase reporter gene assay.[16] Wnt signaling activates gene transcription by forming a complex between DNA-binding proteins of the Tcf/LEF family and β-catenin. SuperTOP-Flash, a β-catenin-responsive reporter plasmid with multiple TCF-binding sites (CCTTTGATC), was activated in cells.[17,18] SuperFOP-Flash has eight mutated TCF-binding sites (CCTTTGGCC), therefore a selective inhibitor would not show enhanced



Scheme 4. Reagent and conditions: (a) EDC, HOOBt, 4-DMAP, CH_2Cl_2, 0 °C, overnight, 67% (two steps from **12**), **17**/**18** = 92/8; (b) TBAF, THF, rt, 3 h; (c) HCl, EtOH, 60 °C, 45 min; (d) LiOH, THF/H_2O = 1/1, 0 °C, 45 min; (e) TMS-diazomethane, MeOH, rt, overnight; (f) H_2, 10% Pd/C, MeOH, rt, overnight, 56% (five steps), **2**/**19** = 91/9.

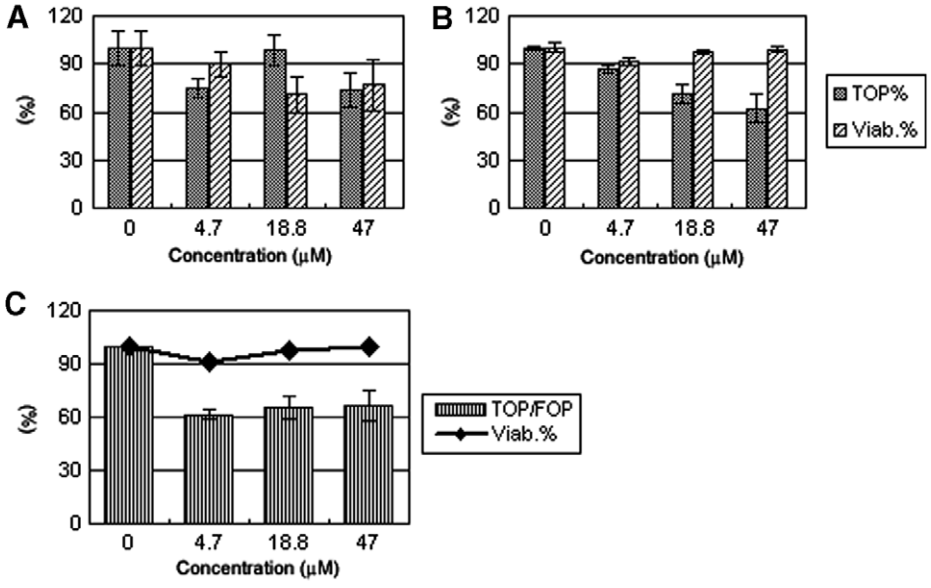


Figure 2. Wnt signal inhibitory activity: (A) SuperTOP-Flash transfected cells inhibition by **2**; (B) SuperTOP-Flash transfected cells inhibition by **19**; (C) TOP/FOP-Flash reporter activation ratio of **19**.

transcription in SuperFOP-Flash transfected cells; thus, these cells provide a negative control for the assay and the TOP/FOP-Flash reporter activation ratio provides a measure of the selectivity of Wnt signal inhibition.

The results are shown in Figure 2, along with the cell viability in order that a decrease in cell number should not resemble inhibition. 10-*epi*-Melleumin B (**19**) exhibited moderate dose-dependent SuperTOP-Flash-transfected cell inhibition (Fig. 2B), whereas melleumin B (**2**) had no effect (Fig. 2A). Subsequently, the ratio of the TOP/FOP-Flash reporter activation by **19** was examined, as shown in Figure 2C. Compound **19** exhibited moderate Wnt signal inhibition. We reported bisindole alkaloids were potent inhibitors from myxomycetes.[16] Since few small molecules are known as Wnt signal inhibitors, we believe that these results are interesting for the identification of Wnt signal inhibitors from natural products.

We determined the absolute configurations of melleumin A (**1**) and B (**2**) as 3*S*, 4*S*, 10*S*, and 11*R*. Total synthesis of melleumin B (**2**) was accomplished and the whole structure of **2** was identified. 10-*epi*-Melleumin B (**19**) showed inhibition of Wnt signal transcription. The novel structure of the melleumin group may be included as a candidate for a small-molecule Wnt signal inhibitor.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.005.

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15. HPLC condition: Develosil ODS-HG-5; 20 × 250 mm; eluent, 50% MeOH; flow rate, 5.0 mL/min; detection, UV at 275 nm. Retention time: **2** (24.0 min), **19** (27.0 min).
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