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Convergent synthesis and in vivo inhibitory effect on fat accumulation of (−)-ternatin, a highly N-methylated cyclic peptide

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Abstract—(−)-Ternatin (**1**), a highly N-methylated cyclic heptapeptide, is a potent inhibitor of fat accumulation against 3T3-L1 murine adipocytes (EC_{50} = 0.14 μg/mL) [Shimokawa, K.; Mashima, I.; Asai, A.; Yamada, K.; Kita, M.; Uemura, D. *Tetrahedron Lett*. **2006**, *47*, 4445]. Compound **1** was synthesized from Boc-protected amino acids in solution. Upon treatment with **1** at 5 mg/kg/day, increases in body weight and fat accumulation in high-fat-fed mice were both significantly suppressed.

Obesity is a serious problem that affects nearly 30% of the adult population in developed countries, and may cause serious lifestyle-related diseases such as cancer, cardiovascular disease, hypertension, hyperlipidemia, and diabetes.[2] In general, obesity is associated with each individual's appetite, relative food preference, storage of energy, thermogenesis, and metabolism. This can make it difficult to develop effective anti-obesity drugs. However, there may be other approaches[3] to solve this problem. Recently, several natural materials that may prevent diet-induced increases in body weight have been a focus of attention worldwide.[4]

In a previous work, we described the isolation of a highly N-methylated cyclic heptapeptide (−)-ternatin (**1**) from the bracket fungus *Coriolus versicolor*, which significantly suppressed the fat accumulation against 3T3-L1 murine adipocytes (EC_{50} = 0.14 μg/mL). The structure of **1** was confirmed to be a cyclo [D-*allo*-Ile[1]-L-(*N*Me)Ala[2]-L-(*N*Me)Leu[3]-L-Leu[4]-L-(*N*Me)Ala[5]-D-(*N*Me) Ala[6]-(2*R*,3*R*)-3-hydroxy-Leu[7]] by spectroscopic analyses and solid-phase peptide synthesis (SPPS) of **1**.[1]

However, due to the limitation of the natural source and the high cost of the SPPS method, a more effective large-scale synthesis of **1** is required to further evaluate its biological and physiological activities. We describe here the solution-phase synthesis of **1** and its in vivo inhibitory effect on fat accumulation in diet-induced obese mice.

Figure 1 illustrates the two key fragments **2** and **3** that are involved in our synthetic route. We thought amide bond formation could be achieved between the amino group in the D-*allo*-Ile[1] moiety of **3** and the carboxylic acid group in the β-OH-D-Leu[7] moiety of **2**. Cyclization of the linear peptide would then occur between the amino group in the L-(*N*Me)Ala[5] moiety and the carboxylic acid group in the L-Leu[4] moiety, which worked well as a reactive coupling site in our previous synthesis.[5]

For effective coupling reactions, we selected phosphonium and uronium salt-based coupling reagents such as PyBOP, PyBroP, and HATU, which have advantages for fast and high-yield coupling reactions with low racemization.[6]

The left fragment **2** was synthesized by stepwise coupling of Boc-*N*Me-D-Ala-OH and Boc-*N*Me-L-Ala-OH to β-OH-D-leucine ethyl ester (**5**), which was easily prepared from the known azide **4**[7] by hydrogenolysis (Scheme 1). First, **5** was coupled with Boc-*N*Me-D-Ala-OH in the presence of PyBroP to yield dipeptide

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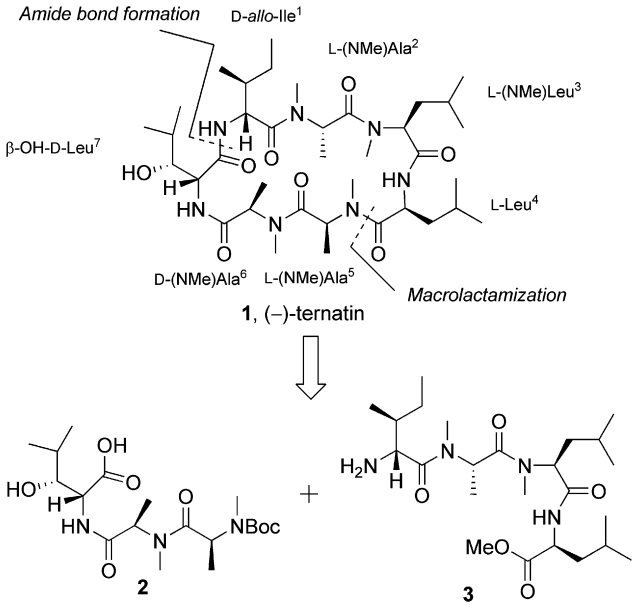
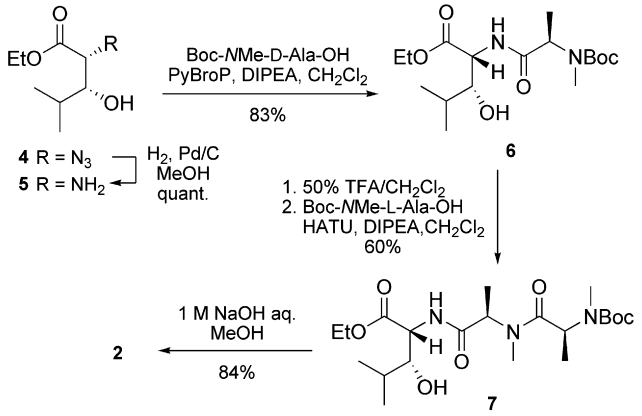


Figure 1. Retrosynthetic analysis of (−)-ternatin (**1**).

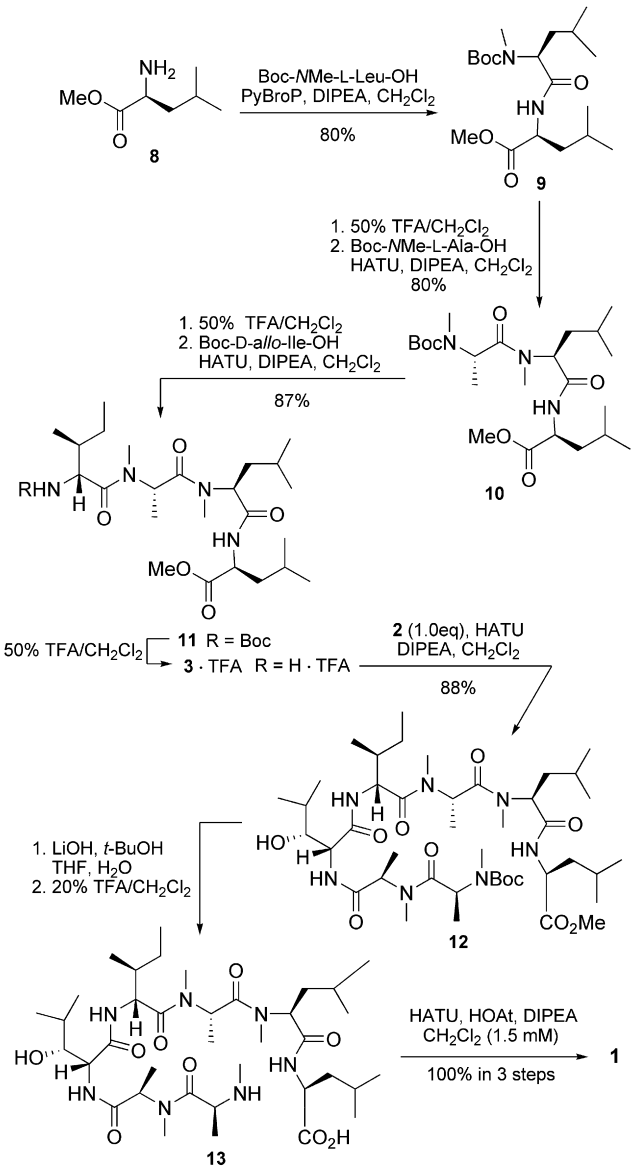
6. Removal of the Boc group of **6** followed by coupling with Boc-*N*Me-L-Ala-OH and alkaline hydrolysis of ethyl ester gave **2**.

The right fragment **3** was prepared in the same fashion (Scheme 2). L-Leucine methyl ester (**8**) was coupled with Boc-*N*Me-L-Leu-OH to give dipeptide **9**. Removal of the Boc group of **9** followed by coupling with Boc-*N*Me-L-Ala-OH in the presence of HATU afforded tripeptide **10**. Sequential removal of the Boc group of **10** followed by coupling with Boc-D-*allo*-Ile-OH gave tetrapeptide **11**. Finally, treatment of **11** with 50% TFA/CH_2Cl_2 gave **3** as a TFA salt. HATU-mediated fragment coupling between a TFA salt of **3** and the left fragment **2** gave heptapeptide **12** in good yield (88%). Methyl ester hydrolysis of **12** and deprotection of the Boc group provided linear peptide **13**.

The final conversion of **13** to **1** was achieved by HATU (2.0 equiv)/HOAt (2.0 equiv)-mediated macrolactamization at low concentration (1.5 mM), which gave the product in quantitative yield from **12**. This successful



Scheme 1. Synthesis of the left fragment **2**.



Scheme 2. Synthesis of the right fragment **3** and completion of the synthesis of (−)-ternatin (**1**).

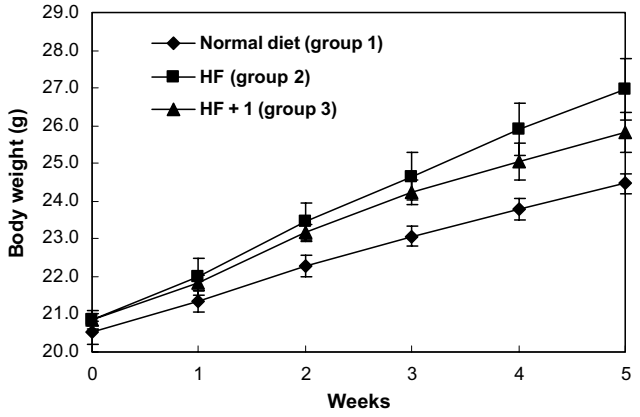


Figure 2. Changes in body weight of C57BL/6J mice fed normal diet (group 1; $n = 8$), high-fat diet (group 2; $n = 8$) or high-fat diet with **1** (group 3; $n = 4$). All values are means ± SE.

Table 1. Body and organ weights of mice after the experiment

Tissues	Group 1	Group 2 (HF)	Group 3 (HF+**1**)
Body weight (g)	24.5 ± 0.30	27.0 ± 0.80	25.8 ± 0.50*
Liver (g)	1.13 ± 0.02	0.91 ± 0.04	0.93 ± 0.03
Fat tissues of internal organs			
Perirenal (g)	0.10 ± 0.01	0.21 ± 0.01	0.16 ± 0.03
Around genitals (g)	0.30 ± 0.02	0.81 ± 0.06	0.50 ± 0.10
Subcutaneous fat tissues			
Inguinal (g)	0.11 ± 0.02	0.25 ± 0.01	0.16 ± 0.03
Dorsal (g)	0.13 ± 0.03	0.29 ± 0.03	0.23 ± 0.04*

All values represent means ± SE, $n = 8$ for groups 1 and 2, $n = 4$ for group 3. Values without asterisk are significantly different at $P < 0.05$.

macrolactamization is notable compared to previous examples of cyclization in the synthesis of cyclic peptides.[8] In conclusion, **1** was synthesized in 10 steps (longest sequence) in high yield (49%) from compound **8**. Synthetic **1** inhibited fat accumulation against 3T3-L1 murine adipocytes at a concentration similar to that of the natural compound.

The fat accumulation-inhibitory effect of (−)-ternatin (**1**) in C57BL/6J mice (in vivo) has been examined extensively.[9]

Mice were fed normal diet (group 1; $n = 8$), high-fat diet (HF) (group 2; $n = 8$) or HF with **1** (5 mg/kg/day) (group 3; $n = 4$) for 5 weeks. After this period, the body weights of group 1 and group 2 mice were significantly different, with means of 24.5 and 27.0 g, respectively (Fig. 2). Meanwhile, the increase in body weight in group 3 was drastically suppressed (25.8 g) compared to that in group 2. Interestingly, food consumption in group 3 mice (2.2 g/day/head) was similar to that in group 2 mice (2.2 g/day/head). This suggested that **1** suppressed the increase in body weight but did not affect food consumption or appetite.

Furthermore, after these experiments, average weights of the liver and the fat tissues of internal organs (perirenal and around the genitals) and subcutaneous parts (inguinal and dorsal tissue) in each group of mice were measured (Table 1). In all cases, the weight of fat tissue in group 3 was greatly reduced compared to that in group 2, and was close to that in normal (group 1) mice. Thus, **1** significantly suppressed fat accumulation in both subcutaneous fat tissues and in those around internal organs.

In summary, (−)-ternatin (**1**) was synthesized from Boc-protected amino acids via a convergent route in solution. Compound **1** significantly inhibited both the increase in body weight and fat accumulation in high-fat-fed mice. This work will enable further studies on the derivatization of **1** and the evaluation of its structure–activity relationships.

Acknowledgments

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5. In our previous synthesis, cyclization was performed using 0.77 mM linear peptide in CH_2Cl_2, HATU (2 equiv), HOAt (2 equiv), DIPEA (4.5 equiv) (73% yield). However, the hydroxyl group in the β-OH-D-Leu[7] moiety was protected with the Bn group.
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9. Animals and diets; 4-week-old male C57BL/6J mice were purchased from Charles River Japan Co. Ltd (Kanagawa, Japan). The diets, MF (standard), and high-fat diet (composed of 40% beef suet, 10% cornstarch, 9% glucose, 4% AIN-76™ with minerals, AIN-76™ with vitamins, and 36% casein) were purchased from Oriental Yeast Co., Ltd (Tokyo, Japan). The mice were individually housed under a controlled temperature (24 ± 3 °C) and a 12-h light–dark cycle. The mice had free access to water and standard rodent diet for 8 days before the experiment. The mice were then randomly divided into three groups for the study. (−)-Ternatin (**1**) (5 mg/kg/day) was administered by oral injection in group 3. Food was changed twice a week. Daily food intake and weekly body weight for each mouse were measured throughout the study. After the 5-week feeding period, mice were anesthetized with ether and killed by cardiac puncture. Blood samples were obtained using heparinized syringes. Livers and fat tissues were collected and immediately weighed.