Whole Structure−Activity Relationships of the Fat-Accumulation Inhibitor (−)-Ternatin: Recognition of the Importance of Each Amino Acid Residue

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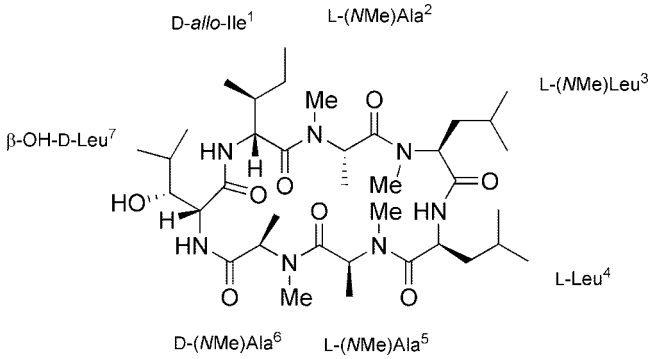
Abstract: A series of Ala and Aoc analogues of (−)-ternatin were prepared, and their bioactivities were assessed by a fat-accumulation inhibition assay using 3T3-L1 adipocytes, which led to the discovery of key structure−activity relationships (SAR).

Since obesity is becoming a pandemic public-health problem[1] and leads to lifestyle-related diseases, e.g., diabetes, hyperlipidemia, and hypertension, both the development of therapeutic treatments for obesity and the discovery of potential antiobesity drugs are needed.[2] In our continuing search for new fat-accumulation inhibitors from natural sources (a set of screening and isolation experiments guided by the fat-accumulation-inhibition assay), we found the novel cyclic heptapeptide (−)-ternatin (**1**) in the mushroom *Coriolus versicolor* as a candidate for a potential drug.[3] The IC_{50} value of **1** for fat accumulation against 3T3-L1 murine adipocytes was 0.027 μM. Further biological assessments revealed that **1** potently inhibited fat accumulation in vivo as well as in vitro.[4] However, the biological mechanism of action of **1** and its cellular target remained unknown. Toward the identification of the molecular target of **1**, we planned for the affinity purification using biotinylated derivatives.



(−)-Ternatin (**1**)

To clarify the best location for chemical modifications, we began to study the structure−activity relationships (SAR) of **1** with regard to its inhibitory effect on fat accumulation in 3T3-L1 murine adipocytes. Recently, we reported first-generation analogues that were assembled via systematic replacement of the unusual amino acid residue β-OH-D-Leu[7] [(2*R*,3*R*)-3-hydroxyleucine] with normal amino acids.[5] Biological evaluation of these synthetic analogues revealed that the side chain of the Leu[7] moiety (isobutyl group) is necessary for potent activity. The hydroxy group is also an important factor for a single-peptide conformation in solution. We describe here the synthesis and biological activities of a new series of ternatin analogues that led to the discovery of key SAR of **1**.

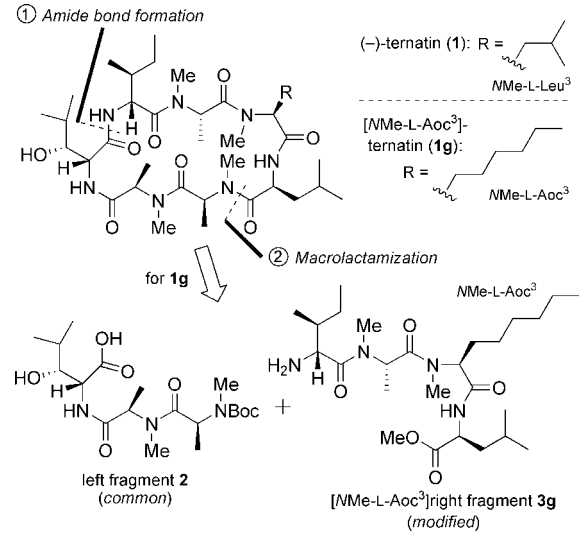


Figure 1. General strategy for the synthesis of ternatin analogues (e.g., retrosynthetic analysis of [*N*Me-L-Aoc[3]]ternatin (**1g**).

Our current SAR study was aimed at identifying essential amino acid moieties, i.e., the recognition of amino acids required in the "natural form" for potent bioactivity of **1**. To determine whether a slight change in side chains would have critical or little influence on the potency of bioactivity, we planned to install two amino acids that were distinct with regard to side chain length. Since (i) **1** contains high levels of Leu and Ile (side chain length, R = C_3H_6 + additional CH_3) and Ala moieties (R = CH_3) and (ii) a slight modification in side chain length may be enough for this purpose, we selected two amino acids, alanine (Ala, R = CH_3) and 2-aminooctane carboxylic acid (Aoc, R = C_6H_{13}), as new substituents for each position (1 → 7) in the structure of **1**. The SAR profile of the Aoc analogues was also thought to provide useful information for the installation of new functionalities, e.g., biotin and a fluorescent unit, for further biological investigations.

On the basis of our convergent strategy developed for the total synthesis of **1**, we thought that ternatin analogues could be synthesized from two key fragments with high efficiency (Figure 1). For example, [*N*Me-L-Aoc[3]]ternatin (**1g**), an Aoc analogue modified at the 3-position, could be prepared from the common left fragment **2** and the modified right fragment **3g** via four steps, including amide bond formation and macrolactamization.

First, Aoc derivatives [Boc-Aoc-OH (**4**), Boc-*N*-Me-Aoc-OH (**5**), Aoc-OMe (**6**)], which are key building blocks for the synthesis of Aoc analogues, were prepared in chiral form starting from L- or D-serine hydrochloride salt (**7**) (Scheme 1). Boc-β-iodoalanine methyl ester (**8**), which is easily prepared from **7** in three steps,[6] was treated with zinc metal activated by a catalytic amount of iodine.[7] The resulting organozinc reagent **9** was subjected to a Negishi coupling reaction[8] with vinyl iodide

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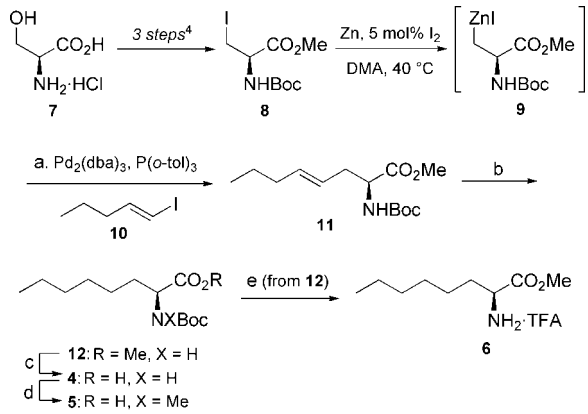
Scheme 1. Synthesis of Aoc Derivatives **4**−**6**[a]



[a] Reagents and conditions: (a) $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, 1-iodo-1-pentene (**10**), DMA, room temp, 77%; (b) H_2, PtO_2, MeOH, room temp, 87%; (c) LiOH, *t*-BuOH, THF, H_2O, room temp, quant; (d) MeI, NaH, THF, 0 °C, 83%; (e) 50% TFA/CH_2Cl_2, 0 °C, quant.

10,[9] in accordance with Jackson's protocol using $Pd_2(dba)_3$ and P(*o*-tol)$_3$ as a standard catalyst. The desired product **11** was obtained in high yield (77%) by optimizing the amount of **9** (2.0 equiv). The olefin moiety of **11** was reduced by Pt-catalyzed hydrogenation. The methyl ester of **12** was then cleaved with LiOH to provide Boc-Aoc-OH (**4**). The N-methylation of **4** gave Boc-*N*-Me-Aoc-OH (**5**), and Aoc-OMe (**6**) was prepared as a TFA salt from **12** by Boc deprotection.

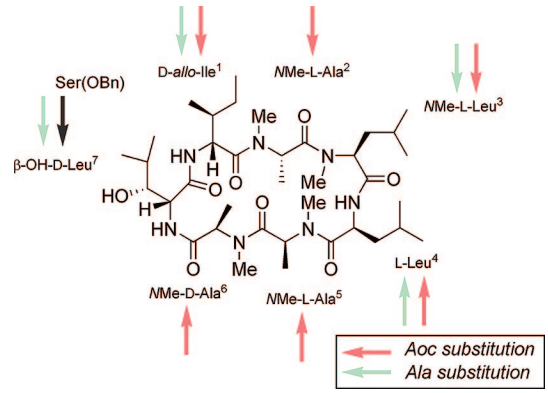
Finally, Ala and Aoc ternatin analogues (**1a**−**d** and **1e**−**j**, respectively) were assembled in solution by exploiting our synthetic strategy toward **1** (for detailed schemes and synthetic procedures, see the Supporting Information).[4]

The results of the in vitro fat-accumulation-inhibition assay for the synthetic analogues **1a**−**k** against 3T3-L1 murine adipocytes along with two controls, (−)-ternatin (**1**) and (−)-noradrenaline (+)-bitartrate salt, are shown in Table 1. Cell viability was calculated independently to exclude undesired fat-accumulation inhibition arising from the toxicity of the tested compounds (for detailed data, see the Supporting Information). In the experiments, no cell toxicity was observed for any of the synthetic analogues at a concentration that gave 50% fat-accumulation inhibition (IC_{50}). On the basis of the current results, all but four of the analogues (**1a**, **1c**, **1e**, and **1h**) showed potent activity compared to (−)-noradrenaline (IC_{50} = 260 μM). Both analogues that were modified at the 1-position with Ala and Aoc (**1a** and **1e**) showed poor activity (no activity and 1700-fold less activity compared to natural **1**, respectively). Similarly, analogues that were modified at the 4-position (**1c** and **1h**) did not show any activity. These findings suggested that the side chain lengths of the natural Ile[1] and Leu[4] moieties are essential for bioactivity (Figure 2). Hence, the Leu[3] and β-OH-Leu[7] moieties were tolerant of chemical modification to some extent based on the relative activities of corresponding compounds (**1b** and **1g** for the 3- position, **1d** and **1k** for the 7-position, respectively).

Surprisingly, none of the Aoc analogues that were modified at the Ala moieties (2-, 5-, and 6- position; **1f**, **1i**, and **1j**) showed a significant loss of activity. In addition, **1j** showed a slightly more potent inhibitory effect than natural **1** (IC_{50} = 0.016 μM, 1.7-fold more potent activity).

These results defined the key Ile[1] and Leu[4] moieties that may interact with critical binding sites in the still unknown cellular target (Figure 3). The SAR profile of Aoc analogues suggested that the Ala[2,5,6] and Leu[3] moieties are promising locations for chemical modification. Particularly, Ala[6] moiety is thought to be the best position for further functionalization to explore more bioactive analogues and to attach the probe tags such as biotin and fluorescent units.

Table 1. Fat-Accumulation Inhibitory Effects of Ala and Aoc Ternatin Analogues and Their Relative Potencies[a]



synthetic compound	fat-accumulation inhibitory effect, IC_{50} (μM)	relative potency
[D-Ala[1]]ternatin (**1a**)	>130	
[*N*Me-L-Ala[3]]ternatin (**1b**)	1.6 ± 0.1	1/60
[L-Ala[4]]ternatin (**1c**)	>130	
[D-Ala[7]]ternatin (**1d**)[b]	14 ± 4.0	1/520
[D-Aoc[1]]ternatin (**1e**)	46 ± 2.6	1/1700
[*N*Me-L-Aoc[2]]ternatin (**1f**)	1.1 ± 0.07	1/40
[*N*Me-L-Aoc[3]]ternatin (**1g**)	0.38 ± 0.08	1/14
[L-Aoc[4]]ternatin (**1h**)	>130	
[*N*Me-L-Aoc[5]]ternatin (**1i**)	0.12 ± 0.01	1/4
[*N*Me-D-Aoc[6]]ternatin (**1j**)	0.016±0.004	1.7
[D-Ser(OBn)[7]]ternatin (**1k**)[b,c]	5.9 ± 1.0	1/220
(−)-ternatin	0.027±0.003	1
(−)-noradrenaline (+)-bitartrate	260	1/9600

[a] Values are the mean of quadruplicate determinations. [b] Reported in ref 5. [c] Shown instead of Aoc analogues modified at the 7-position.

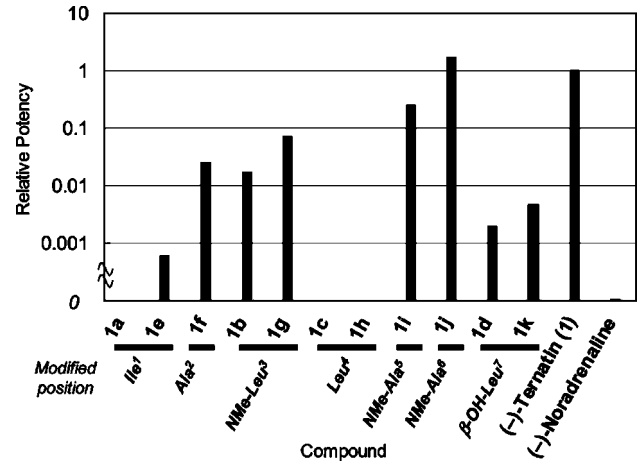


Figure 2. Classification of fat-accumulation inhibitory effect of ternatin analogues with modified position. Compound **1** was set as standard (=1). Relative potencies of analogues **1a**, **1c**, and **1h** are under 2 × 10^{-3}.

In addition, we found that all analogues except for three analogues (**1b**, **1d**, and **1k**) afforded single conformers in ^{1}H NMR spectra (see the Supporting Information). **1d** and **1k** showed the mixture of two conformers in ^{1}H NMR spectra, which were thought to be due to the significant loss of the hydroxy group at the β-OH-Leu[7] moiety. As we mentioned in a previous paper,[5] compound **1** was proposed to adopt β-turn

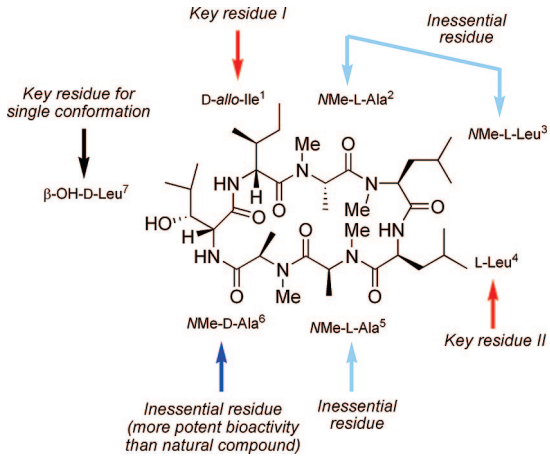


Figure 3. SAR profile of (−)-ternatin.

structure in the region of L-Leu[4] and β-OH-D-Leu[7] moieties with the assistance of three intramolecular H-bonds in solution. The observation implies that the hydroxy group in the β-OH-D-Leu[7] moiety may play an important role on stabilizing and/or restricting peptide conformation by forming an intramolecular H-bond between the OH proton and the C=O in the D-*allo*-Ile[1] moiety. The reason for the existence of two conformers in **1b** (Ala analogue modified at the *N*-Me-L-Leu[3] moiety) remained unknown, though it did not provide critical decrease in the potency of biological activity. Therefore, in our current SAR study, it can be said that the drastic changes in the relative potencies of bioactivity are not induced by conformational changes of analogues.

In summary, we have constructed Ala and Aoc ternatin analogues. Our current findings have demonstrated the critical SAR of **1** and have clearly revealed the essential and inessential amino acid moieties for fat-accumulation inhibition as shown in Figure 3. This result should enable investigations on the molecular-target identification and the mode of action responsible for fat-accumulation inhibition. Further bioorganic studies on this bioactive molecule are ongoing.

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Supporting Information Available: Synthetic schemes, experimental procedures, ^{1}H NMR spectra of analogues, and compound characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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