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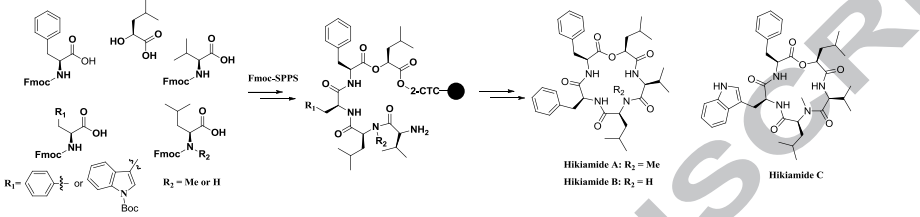

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First total synthesis of cyclic pentadepsipeptides Hikiamides A−C

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ABSTRACT

The first total syntheses of naturally occurring cyclodepsipeptides Hikiamides **A−C** are described. The key linear pentapeptide precursors, prepared efficiently *via* Fmoc-solid-phase synthesis, were cyclized in dilute solution to provide the target Hikiamides A−C. The structures of the synthetic Hikiamides **A−C** were characterized by NMR and HRMS spectroscopy which were in agreement with those of natural products.

Introduction

Diabetes is one of the fastest growing global public health concerns.[1] Type II diabetes mellitus (T2DM), once known as non-insulin-dependent diabetes mellitus, accounts for more than 90% diabetes cases. It was reported that T2DM is becoming a serious threat to human health.[2] Antidiabetics in clinical application include: insulin and its analogues, insulin secretagogues (sulfonylureas and glinides), insulin sensitizers (Metformin and Thiazolidinediones (PPARγ agonists), α-glucosidase inhibitors (acarbose) et al.[3] Compared to other anti-diabetic agents, PPARγ agonists have the following advantages: (a) improvement in insulin sensitivity, (b) modulation of cytokine secretion from adipocytes, (c) preserve cell mass and insulin secretion capability, (d) reduction in cardiovascular morbidity and mortality in high-risk T2DM patients.[4] Thiazolidinediones (TZDs) are most extensively employed PPARγ agonists to treat type II diabetes. However, several serious side-effects such as macular edema and hepatotoxicity restricted their clinical application.[5] Succedaneous drugs without such deleterious effects are peremptorily needed. Hikiamides **A-C** (Figure 1), cyclo depsipeptides, were isolated and identified from *Fusarium* species. TAMA 456 in 2015.[6] Structurally, Hikiamide **A** and **B** contain four identical residues: L-Phe, L-Phe, L-Val and S-OLeu. The different residue is N-methylated L-Leu of Hikiamide **A**. Hikiamide **A** and **C** also contain four residues in common: L-Phe, N-Me-L-Leu, L-Val and S-OLeu. Hikiamide **A** differs from **C** by substitution of a single amino acid residue L-Phe instead of L-Trp. These cyclodepsipeptides induce the differentiation of murine ST-13 preadipocytes into mature adipocytes at 2 μM and adiponectin mRNA expression (5 to 13 fold stronger than TZDs).[6] To date, this rare family of cyclic

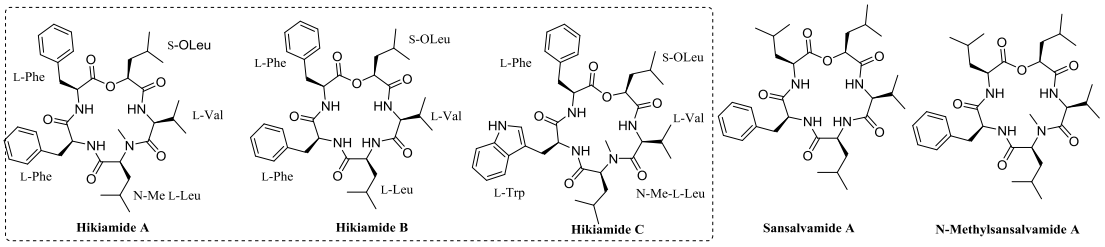

Figure. 1. Structures of Hikiamides (A-C) and Sansalvamide A.

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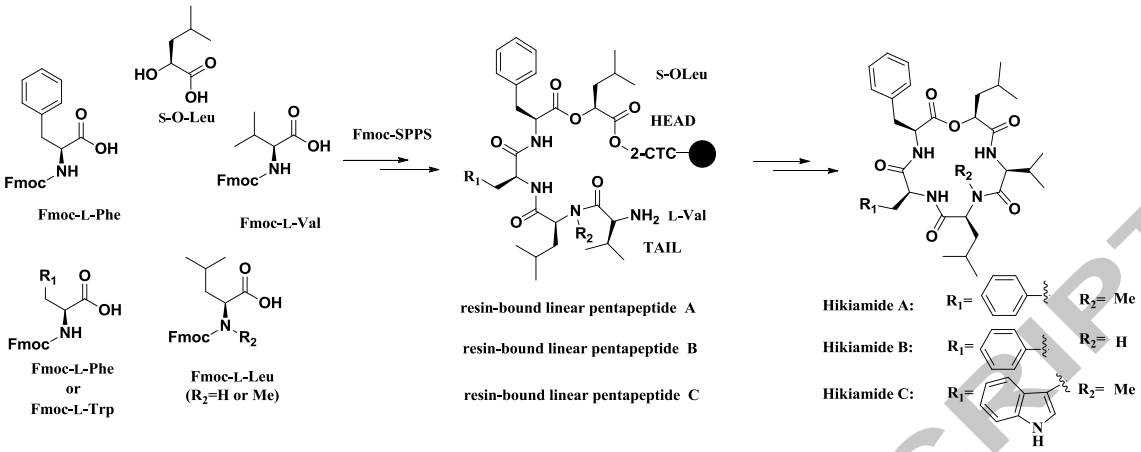


Figure. 2. synthetic strategy of Hikiamides **A-C**.

peptides also include sansalvamide **A** and N-methylsansalv amide **A** as metabolites from *Fusarium* species.[7] (Figure 1) Hikiamides (**A-C**) were the only cyclic peptide PPARγ agonists isolated from *Fusarium* species as potential lead compounds. Our interest toward these natural products also relied on their intriguing structural features. It was reported that the construction of large cyclopeptides containing more than seven amino acids is not problematic whereas cyclization of smaller peptides with tetra- or pentapeptides are usually challenging.[8] Moreover, N-alkyl or β-branched amino acids which are extensively existed in Hikiamides (**A-C**) could indeed hamper the intramolecular cyclization process.[9] Considering the fact that synthesis of Hikiamides (**A-C**) has not been reported ever since their first identification in 2015, in the present study, we want to challenge the first total synthesis of Hikiamides (**A-C**).

Solid-phase peptide synthesis (SPPS), pioneered by Merrifield[10], is a practical approach to resolve some disadvantages inherent in solution-phase-synthesis. One of the benefits is that it could be applied for the preparation of complex peptide without additional purification. The strategy of SPPS has been extensively utilized to prepare several important cyclodepsipeptides.[11-14]

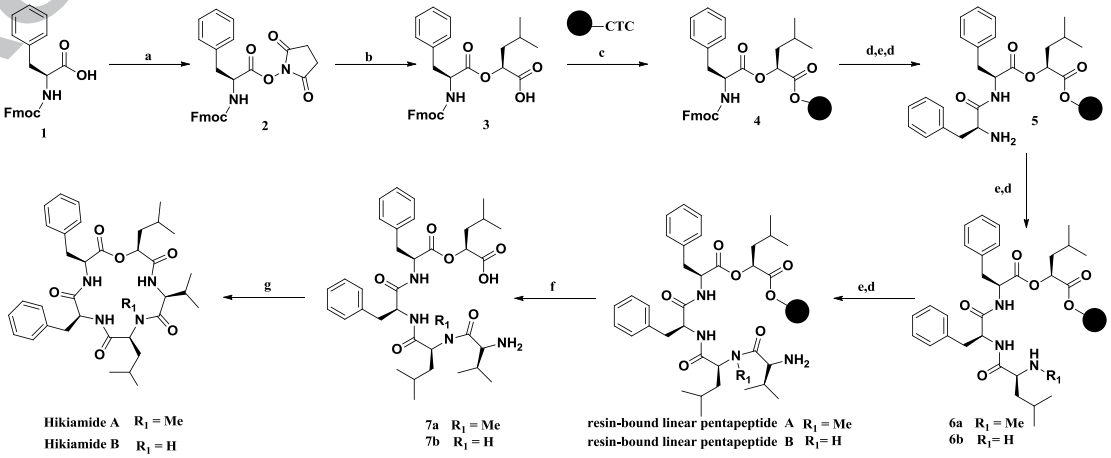
Herein, we report the first total synthesis of Hikiamides **A-C**. The key linear pentapeptide analogs were first constructed by solid-phase peptide synthesis which were then subjected to a solution phase assisted cyclization to provide Hikiamides **A−C**.

Results and discussion

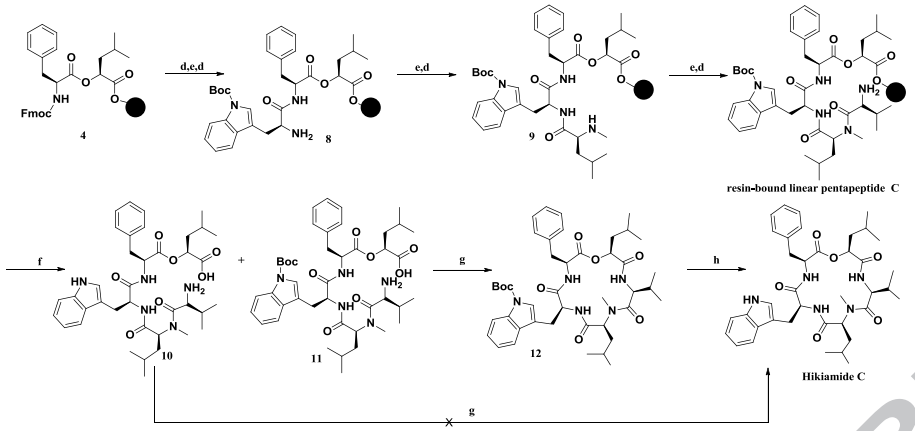
The synthetic strategy of Hikiamides A-C was depicted in Figure 2. Four amino acid Fmoc-L-Phe, Fmoc-L-phe(Trp), Fmoc-L-Leu(R_2), Fmoc-L-Val as well as S-OLeu were used to assemble resin-bound linear pentapeptides **A-C** through SPPS. After cleavage of 2-chlorotrityl chloride (2-CTC) resin, the resulted linear pentapeptides were subjected to head-to-tail macrolactamization[8,15] to provide target Hikiamides **A-C** in solution phase.

*Total synthesis of Hikiamide **A** and **B***

Firstly, the Fmoc-L-Phe-O-Leu ester (**3**) was produced by EDCI and N-Hydroxysuccinimide (NHS) mediated esterification of Fmoc-L-Phe (**2**) and S-OLeu in 81% yield.[16] **3** was then treated with 2-CTC resin in the presence of N,N'-diisopropylethylamine (DIPEA) to give resin-attached intermediate (**4**). Sequence elongation of Fmoc-L-Phe-OLeu-resin (**4**) with Fmoc-L-Phe, N-Me-Fmoc-L-Leu, Fmoc-L-Val in a C to N direction was then carried out to provide resin-bound linear pentapeptide (**A**). Each addition reaction involved deprotection and coupling procedures. Fmoc protection was removed with 20% 4-methylpiperidine in DMF. The target resin-bound linear pentapeptide (**A**) was assembled by using HBTU/DIPEA, which was reported as an efficient coupling reagent in SPPS. [15,17]



Scheme 1. Reagents and conditions: (a) EDCI, NHS, DCM, 24h, rt.; (b) DMAP, DCM, 18h, rt., 81% (over 2 steps); (c) DIPEA, DMF, 2h, rt., 56%; (d) Piperidine(20%), DMF, 30min, rt; (e) HBTU, DIPEA, DMF, 6h, rt.; (f) TFA, DCM, 1h, rt., (**7a**, 35% from **4**) or (**7b**, 32% from **4**); (g) HATU, EDPBT, TBTU, DIPEA, DCM, 72h, rt., 25.7% (Hikiamide **A**) or 22.6% (Hikiamide **B**).



Scheme 2. Reagents and conditions: (d) Piperidine (20%), DMF, 30min, rt.; (e) HBTU, DIPEA, DMF, 6h, rt.; (f) TFA (1%), DCM, 1h, rt., (**11**, 51% from **4**); (g) HATU, EDPBT, TBTU, DIPEA, DCM, 72h, rt, 45%., (h) TFA (50%), DCM, 30min, rt, 90%.

All of the amino acid coupling and deprotection reactions were monitored by Kaiser or chloranil test.[18] The cleavage between linear peptide and 2-CTC resin was achieved by using a 5% trifluoroacetic acid (TFA) in dichloromethane (DCM) solution and the linear pentapeptide (**7a**) was obtained with the total yield of 35% from **4**. Finally, the key cyclization to afford Hikiamide **A** was successfully performed by the treatment of linear pentapeptide (**7a**) with a mixture of HATU, DEPBT and TBTU.[19] The cyclization process was finished within 72 h and the desired Hikiamide **A** was obtained with the isolated yield of 25.7%. The spectroscopic data of synthetic Hikiamide **A** were in agreement with those of the natural product.[6, 24]

When Fmoc-L-Leu other than N-Me-Fmoc-L-Leu was used as peptide building block, the above synthetic strategy was also effective for the synthesis of Hikiamide **B**. Thus, the linear pentapeptide (**7b**) was obtained in the total yield of 32% from **4**. The subsequent intramolecular cyclization of **7b** performed under similar condition successfully provided the desired Hikiamide **B** in 22.6% yield (Scheme 1). The ^{1}H NMR and ^{13}C NMR spectrum of synthetic Hikiamide **B** were also in accord with those of the natural product.[6, 24]

Total synthesis of Hikiamide C

The above synthetic strategy which successfully provided us Hikiamide **A** and **B** was then used for the preparation of Hikiamide **C**. In a similar manner, 2-CTC resin attached intermediate (**4**) was first assembled with Boc-protected L-Trp, N-Me-L-Leu and L-Val to give the resin-bound linear pentapeptide (**C**). The cleavage of 2-CTC resin was carried out under the same reacting condition discussed above. Interestingly, deprotection of Boc group on L-Trp fragment also occurred simultaneously to give the linear pentapeptide (**10**) in 30% yield together with Boc-protected linear peptide precursor (**11**) in 10% yield as minor product. Since previous reports indicated the participation of unprotected tryptophan moiety into intramolecular cyclization.[20-23] The major product (**10**) was first subjected to macrolactamization. Unfortunately, the reacting system turned out to be a complex mixture and the desired Hikiamide **C** could not be detected even in trace amount. When the same cyclization reaction was applied to minor product (**11**), the corresponding Boc-protected cyclic peptide (**12**) could be obtained with the isolation yield of 45%, indicating that Boc-protection on L-Trp residue was critical for the intramolecular cyclization of linear pentapeptide. Thus, different reacting conditions were scanned to optimize the reaction yield of **11**. When TFA solution was diluted to 1% concentration, Boc-protected linear peptide (**11**) could be obtained with the total yield of 51% from **4**. Finally, the Boc-protected cyclicpeptide (**12**) was treated with 50% TFA in DCM solution to remove Boc protection, giving Hikiamide **C** in 90% yield. The spectroscopic data ^{1}H NMR and ^{13}C NMR of synthetic Hikiamide **C** were consistent with those of the natural product.[6, 24]

The determination of an appropriate disconnection site is critical for the construction of cyclic peptide.[8] It has to be pointed out that different ring-closure strategies have been attempted during our synthetic process and most of them were proven to be ineffective.[25] As for the construction of Hikiamide families, ring closure at ester residue should be first excluded from the potential cyclization protocol, owing to a reported risk of racemization.[26] In addition, the methylation of Leu moiety, as in the case of Hikiamides **A** and **C**, made the certain area more sterically encumbered which could also potentially hamper the cyclization reaction. The intramolecular cyclization between the N-terminus L-Phe and C-terminus L-Phe was also unsuccessful which might be rationalized by sterical hindrance arising from the attachment of the adjacent benzyl group.

Conclusions

In summary, we have established a suitable strategy for the preparation of Hikiamides **A**-**C** by a combination of solid-phase and solution-phase synthesis. SPPS was found to be efficient to assemble different single amino acids to afford linear pentapeptide intermediates which could then macrocyclized smoothly in solution-phase system to furnish the target natural product Hikiamide **A**-**C**. Different experiments were attempted in order or determine an appropriated ring-closure site which might be constructive for the synthesis of similar cyclic peptide derivatives. Further structural modification and biological evaluation of Hikiamide **A**-**C** will be presented in due course.

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Supplementary data

Supplementary data associated with this article can be found

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Highlights

- First total syntheses of natural cyclodepsipeptides Hikiamides A−C are described.
- Solid-phase peptide synthesis was found effective to assemble linear pentapeptides.
- Cyclization of linear pentapeptides in solution phase provided Hikiamides **A−C.**

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