論文の内容の要旨

論文題目：Total Synthesis and Structure-Function Relationship Study of Kaikosin E
(カイコシンＥの全合成および構造機能相関研究)

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Introduction

Recently, a novel antimicrobial agent kaikosin E[1] (**1**, Figure 1a) was isolated from a culture supernatant of a lysobacter species by Sekimizu and co-workers, and was showed to be effective against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Peptide **1** had potent antibiotic activity in mouse infection model with ED_{50} value of 0.6 mg/kg. Therefore, this compound is considered as a promising candidate for treatment of multidrug resistant bacterial infection.

The structure of **1** is featured by a 37-membered macrocycle, five D-amino acid residues, *N*-methylation on peptide main chain, and C7 fatty acid moiety. Peptide **1** was suspected to target menaquinone-4 (Figure 1b), which is an essential component of the electron transport system on bacterial membranes, and to dissipate the membrane structure in *S. aureus*. In order to investigate further mechanism of action of **1**, the total synthesis and structure-function relationship study were performed.

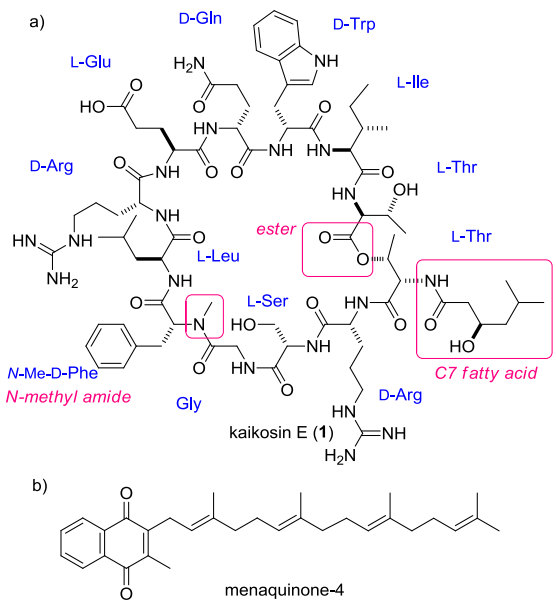


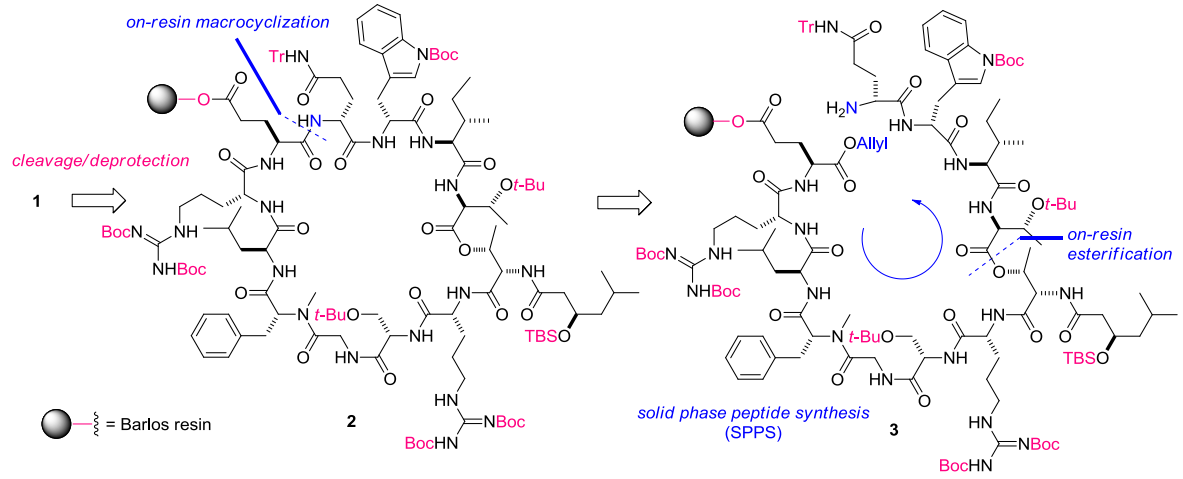
Figure 1. (a) Structure of Kaikosin E (**1**). (b) Structure of menaquinone-4.

Results and Discussion

1. Total synthesis of kaikosin E and its enantiomer

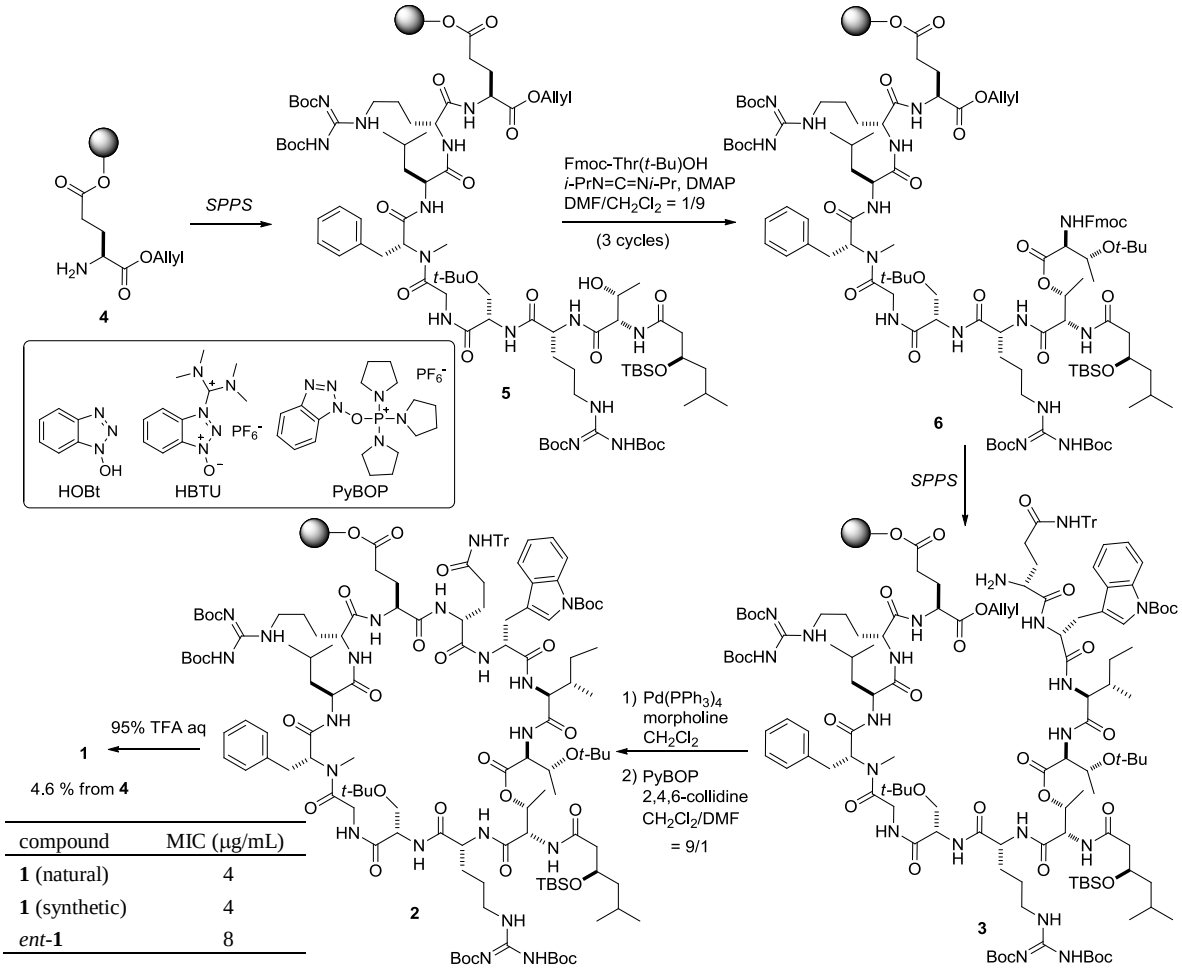
The solid-phase synthesis does not necessitate purification of synthetic intermediates, and thus is advantageous over the solution-phase counterpart, especially for rapid construction of **1**. Moreover, high dilution effect on the solid-phase was expected to facilitate the macrocyclization of the 37-membered ring of **1**. Synthetic strategy to **1** is summarized in Scheme 1. The side chain of allyl glutamate would be anchored to 2-chloro-2-trityl resin (Barlos resin), and the following peptide elongation would give linear depsipeptide **3**, which was to be cyclized to generate the peptide **2**. Then, all the side chain protecting groups (*t*-Bu, Tr, TBS and Boc) would be removed simultaneously with the cleavage of the peptide **2** from the Barlos resin to produce **1**.

Scheme 1. Retrosynthesis of kaikosin E (**1**).



The solid phase peptide synthesis (SPPS) started from allyl glutamate loaded resin **4** (Scheme 2). The attachments of the six Fmoc amino acid residues and threonine fragment, which included the fatty acid moiety on its amine group, were realized by using the HBTU/HOBt activation method under the microwave irradiation, leading to **5**. Then, on-resin esterification of the secondary alcohol of **5** with Fmoc-L-Thr(*t*-Bu)OH was performed. To compete the esterification reaction, the coupling with 10 equiv of Fmoc-L-Thr(*t*-Bu)OH, *N*,*N*'-diisopropylcarbodiimide, and DMAP were used for three cycles to generate **6**. After the subsequent Fmoc cleavage, a microwave-assisted protocol was re-applied for stepwise coupling of the three amino acid residues to furnish acyclic depsipeptide **3**. Deprotection of the allyl group of **3** with catalytic $Pd(PPh_3)_4$ and excess amount of morpholine followed by PyBOP-promoted macrolactamization, resulting in formation of resin bound lactam **2**. Finally, cleavage of **2** from Barlos resin with 95% aqueous TFA completed within 1 hour at room temperature, and then the reaction mixture was stirred for additional 2 hours to remove all the protecting groups attached to the side chains. After purification by the reversed-phase HPLC, kaikosin E (**1**) was obtained in 4.6% yield from the allyl glutamate loaded resin **4**. The NMR spectra and the retention time in the HPLC of synthetic **1** agreed with those of natural **1**. A comparison of MIC values shows synthesized **1** is in good accordance with that of naturally isolated one.

Scheme 2. Total synthesis of kaikosin E (**1**).



compound	MIC (μg/mL)
1 (natural)	4
1 (synthetic)	4
ent-**1**	8

Enantiomer of **1** (*ent*-**1**) was also prepared by the slightly modified conditions from the ones described above. Interestingly, synthesized *ent*-**1** exhibited similar MIC values with that of **1**. Retention of antimicrobial activity of *ent*-**1** supported that **1** targets achiral menaquinone-4.

2. Synthesis and functional analysis of side chain-modified analogues

Scheme 3. Synthesis of side chain modified analogues.

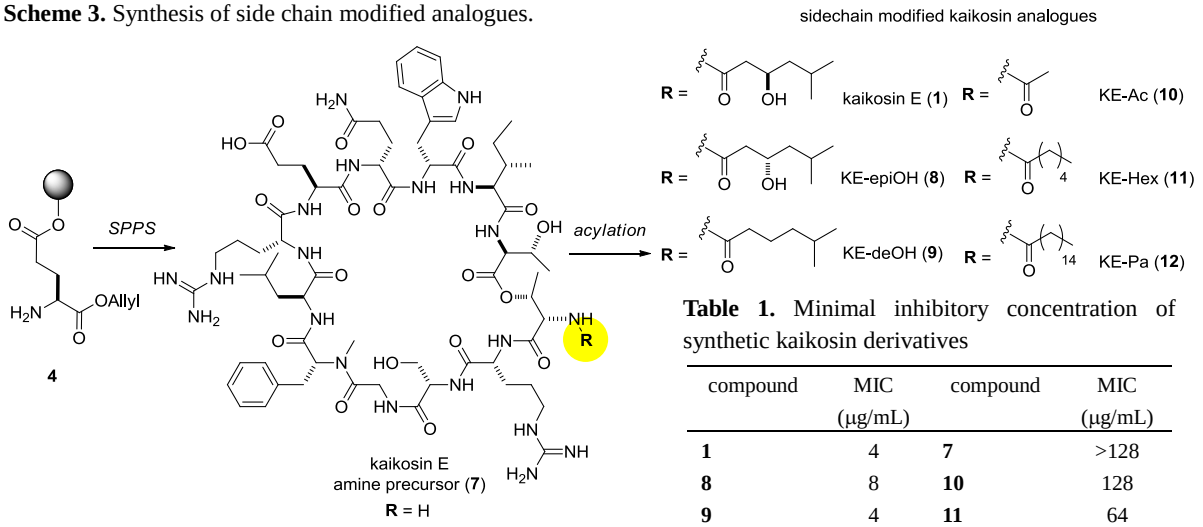


Table 1. Minimal inhibitory concentration of synthetic kaikosin derivatives

compound	MIC (μg/mL)	compound	MIC (μg/mL)
1	4	**7**	>128
8	8	**10**	128
9	4	**11**	64
		12	>128

One of the structural features of **1** is the C7 fatty acid attached to the threonine residue. To evaluate the importance of the fatty acid moiety, alternative synthetic route was designed. A series of side chain-modified kaikosin analogues would be prepared by the last-step acylation of common amine precursor **7** (Scheme 3). Kaikosin E amine precursor **7** was synthesized in 25.6 % yield by similar synthetic procedure for **1,** and the acylation was successfully proceeded only at the amine group of the threonine residue. Antimicrobial activity of the synthesized analogues were examined and summarized in Table 1. These results suggested that particular length (n = 7) of side chain is important to exhibit antimicrobial activity (Table 1, **1**, **8**, **9** vs. **7**, **10-12**). For further investigation of the role of the C7 fatty acid of **1**, the binding affinity of the synthetic analogues to menaquinone-4 are now being explored.

Conclusion

The first total synthesis of kaikosin E (**1**) and its analogues were achieved via macrocyclization on solid support. To investigate the structure-function relationship of **1**, alternative synthetic route via amine precursor **7** was also established. This work demonstrated that the solid phase approach was effective for rapid production of wide range of kaikosin E analogues. MIC values of synthesized kaikosin E derivatives implied the importance of the C7 fatty acid moiety of **1** for its antimicrobial activity.

References

(1) Hamamoto, H.; Urai, M.; Paudel, A.; Horie, R. ; Murakami, K. ; Sekimizu, K. *J. Pharm. Soc. Jpn.*. **2012**, *132*, 79.