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Macrolactonization of Peptide Thioesters Catalyzed by Imidazole and Its Application in the Synthesis of Kahalalide B and Analogues

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Abstract

The macrolactonization of peptide thioester to yield cyclic depsipeptides was developed using imidazole as a catalyst. This strategy was applied to the synthesis of kahalalide B and its analogues.

Bioactive cyclic peptides and depsipeptides that are isolated from natural sources provide a range of lead structures for the design of new drugs.[1] Kahalalide compounds, A-F, are cyclic depsipeptides isolated from a sacoglossan mollusk, *Elysia rufescens*, and its algal diet *Bryopsis* sp. They range from a C_{31} tetrapeptide to a C_{75} octapeptide.[2] Kahalalide compounds have similar structures with an endocyclic depsipeptide bond and fatty acids at the N-termini. Kahalalides exhibit a diverse spectrum of biological activities, including antiviral, antimicrobial, and antitumor activity.[3] Though the pharmaceutical potential of these natural compounds is high, isolation and purification of larger quantities of this class of products is difficult. The development of synthetic strategies for natural compounds and their analogues is essential for the discovery of lead compounds of this type for use in drug discovery.[4]

The most commonly used methodology for the synthesis of cyclic depsipeptide involes cyclization of a linear depsipeptide containing an internal ester bond. The intramolecular macrolactamization can be carried out either in solution or on resin.[5] Problematically, the endoester bond may break during the acidic cleavage when a typical solid-phase synthetic strategy is applied to make the linear precursor and/or cyclic product. The choice of resins is thus limited, and some of the widely used resins, which require strong acidic cleavage conditions, cannot be successfully used. To overcome this limitation, macrolactonization is a favorable alternative. Since the lactonization is much less efficient than the lactamization under conventional coupling conditions, synthesis of cyclic depsipeptides by this strategy is rarely reported and remains a challenge.[6]

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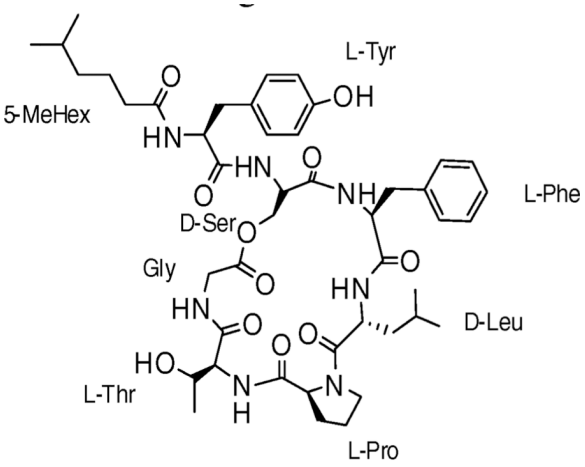
Supporting Information Available: Experimental detail, compound characterization, copies of ^{1}H NMR, ^{13}C NMR spectra, HRMS, and LC–MS for cyclic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Biosynthesis of cyclic depsipeptides through peptide synthetase has been reported by using peptide thioester as substrate.[7] Studies of different peptide synthetase systems suggest the possibility that a histidine residue functions as a catalyst of condensation/elongation/cyclization reaction in the peptide synthesis.[8] Imidazole has been reported as a catalyst mimicking the histidine residues of enzymes hydrolyzing the ester bond and the thioester bond.[9] Our recent studies have shown that a cyclic peptide can be formed through direct aminolysis of a peptide benzylthioester catalyzed by imidazole.[10] The generally accepted mechanism of the imidazole catalysis involves the reaction intermediates of acyl imidazole and the acyl imidazolium cation, which are formed by the direct attack of imidazole on the carbonyl group. This mechanism can also be envisioned for the formation of a cyclic depsipeptide by macrolactonization. The mild imidazole-catalytic condition may also eliminate the risk of racemization during acyl activation. Herein, we report a imidazole-catalytic approach for the synthesis of cyclic depsipeptides by macrolactonization of peptide thioesters and the use of this application in the synthesis of kahalalide B and its analogues.



Kahalalide B

The stepwise synthesis of the linear peptides starts with functionalized mercaptomethylphenyl silica gel **1** as the "volatilizable" support (Scheme 1).[11] PyBOP/DIEA was used as the protected amino acid activation reagent to generate resin bound **2**. Boc-glycine was coupled on the resin as the C-terminal residue based on the structure of kahalalide B. After removal of the Boc group with 55% TFA, threonine, proline, D-leucine, phenylalanine, D-serine, tyrosine, and 5-methylhexanoic acid were coupled stepwise to form the on-resin synthetic precursor of kahalalide B **3a**. The resin-bound **3a** was then treated with anhydrous HF for 2 h at 0 °C. Following evaporation of the anhydrous HF with a gaseous nitrogen stream, the unprotected peptide thioester **4a** was obtained following lyophlization.

The cyclization to the depsipeptide was performed by macrolactonization in acetonitrile using imidazole as a catalyst. The effect of imidazole on catalytic esterification and cyclization was first tested using an *N*-acetyl pentapeptide thioester Ac-Xxx-Ala-Phe-Tyr-Gly-SCH_2Ph, where Xxx was Ser or Thr. It was found the concentration of imidazole significantly accelerated the macrolactonization. Macrolactonization by the hydroxyl on the serine residue was complete after a 24 h reaction at room temperature gave a yield over 95% when the concentration of imidazole was 1.5 M, but failed even after reacting for 10 days when the concentration was 0.15 M. The effect of the concentration of imidazole on macrolactonization by peptide threonine had a similar result but a lower yield of 70% after reacting for 24 h. The need for a high concentration of imidazole likely suggests that the

formation of the imidazolyl intermediate was rate-limiting (Scheme 2). Increasing the concentration of imidazole thus drives the formation of the imidazolyl intermediate. The formation of kahalalide B (**5a**) was tested by dissolving **4a** to a concentration of 1 mM in 1.5 M imidazole in acetonitrile at room temperature for 24 h. A portion of the reaction mixture was examined by LC–MS. It was found that the cyclic depsipeptide of kahalalide B was quantitatively formed. When compared to the reported strategy of PyBOP-DIEA activation for macrolactonization, the yield of cyclization of the same compound is much higher using the present experimental approach (reported, 28%; current, quantitative).[12] To accecelarate the cyclization, microwave reaction was tested under the same concentrations but at 65 °C in a separate experiment. It was found that the cyclization was complete within 2 h with a quantitative conversion.

Because there are also hydroxyl groups on the residue of serine or threonine at position aa_1 and tyrosine at position aa_5, the cyclic product identified in LC–MS analysis could be either the expected kahalalide B or the esterification product of C-terminal thioester by the hydroxyl groups of these residues. To distinguish these possible products, semipreparative reverse-phase HPLC was used to purify the cyclic product. The purified product was analyzed by NMR spectroscopy and found to be identical to the natural kahalalide B reported earlier.[12] In addition, linear peptides CH_3CO-Tyr-Ala-Phe-Tyr-Gly-SCH_2Ph, CH_3CO-Ser-Gly-SCH_2Ph, and CH_3CO-Thr-Gly-SCH_2Ph were also synthesized, and cyclizations were attempted under the same conditions as the cyclization of kahalalide B. No cyclization products were detected in the three cases even after reacting for 3 days.

Analogues of kahalalide B (**5b–e**) were synthesized by selectively changing residues at positions aa_1, aa_2, aa_3, aa_4, and aa_5. Residues of glycine and proline were reserved in the cyclic product. The yield and purity of kahalalide B and its analogues are shown in Table 1.

In summary, we present here a novel method for the synthesis of cyclic depsipeptides by macrolactonization of peptide thioesters. The esterifaction/cycliczation was greatly faciliated with catalysis by imidazole. Kahalalide B and its analogues were obtained in high yields and purities by this method. This strategy is promising for the synthesis of other natural cyclic depsipeptides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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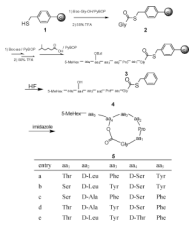
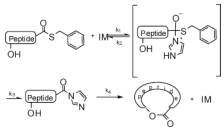


entry	aa$_1$	aa$_2$	aa$_3$	aa$_4$	aa$_5$
a	Thr	D-Leu	Phe	D-Ser	Tyr
b	Ser	D-Leu	Tyr	D-Ser	Tyr
c	Ser	D-Ala	Phe	D-Ser	Phe
d	Thr	D-Ala	Tyr	D-Ser	Phe
e	Thr	D-Leu	Tyr	D-Thr	Phe

Scheme 1.

Synthesis of Kahalalide B and Its Analogues through Macrolactonization of Peptide Thioester Catalyzed by Imidazole



Scheme 2.
Mechanism of Imidazole Catalysis of Macrolactonization

Table 1

Kahalalide B and Analogues Synthesized by Macrolactonization

	linear peptide thioesters				cyclic products		
entry	yield (%)[a]	yield (%)[b]	MW (calcd)[c]	MW (obsd)[d]	yield (%)[e]	MW (calcd)[c]	MW (obsd)[d]
a	90	42	1002.5	1002.5	98	878.5	878.5
b	92	40	1004.5	1004.5	90	880.4	880.4
c	90	57	930.4	930.4	85	806.4	806.4
d	94	51	960.5	960.5	80	836.4	836.4
e	90	46	1016.5	1016.5	95	892.5	892.5

[a] Yield is based on the weight of crude product and the amount of resin used.

[b] Yield is based on the weight of purified product (purity >95%) and the amount of resin used.

[c] Caclulated from [M + H].

[d] $[M + H]^+$ by ESI-MS.

[e] Yield is based on the weights of purified cyclic products and their purified linear peptide thioesters.