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Synthesis of desmosine-containing cyclic peptide for the possible elucidation of elastin crosslinking structure

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ABSTRACT

Elastin is a vital extracellular matrix protein, which is known for providing elasticity in numerous tissues. It is formed by the self-assembly and subsequent crosslinking of elastin precursor, tropoelastin. Two tetrafunctional, pyridinium-based amino acids desmosine and isodesmosine are exclusively found in elastin and play an important role as crosslinkers. Structural elucidation of elastin has eluded scientists to date, owing to the highly cross-linked structure and insoluble nature. Therefore, in this study, we aimed to synthesize a desmosine-containing cyclic peptide as a partial elastin mimic, in order to eventually facilitate the elucidation of the crosslinking pattern of elastin by mass spectrometric analysis.

1. Introduction

Elastic fibers in extracellular matrices are significant structural components of skin, blood vessels, alveolus, ligamentum, and other connective tissues, where they are responsible for the retention of mechanical strength and elasticity.[1] Elastin, a major structural component of elastic fibers, is a highly crosslinked insoluble protein formed by oxidative deamination of lysine (Lys) residues in the soluble precursor tropoelastin, catalyzed by lysyl oxidase.[2] Desmosine and isodesmosine (Fig. 1) are two major pyridinium amino acids that serve as important crosslinkers binding the polymeric chains of amino acids in the 3D network of elastin.[3,4]

Amino acid sequence analysis has shown that crosslinking occurs within the hydrophilic (rich in Lys and alanine (Ala)) domains of elastin.[5] Mecham and co-workers reported that one crosslinking structure involved hydrophilic domains between 19 and 25, which are joined by desmosine (Fig. 2, left). However, the detailed structure remains unclear.[6]

Structural identification of crosslinking moieties involving desmosine is difficult using available proteomic mass spectrometric techniques. Recently, a new software, PolyLynx, was developed for the analysis of crosslinking peptides[7]; however, the characterization of crosslinked peptides from mature elastin digests remains a considerable challenge. The degradation of elastin-containing tissues is irreversible and occurs in several widely prevalent diseases, such as atherosclerosis,[8] aortic aneurysms,[8] cystic fibrosis,[9] and chronic obstructive pulmonary disease (COPD).[10] In order to understand the relationship between these diseases and elastin, the detailed crosslinking structure should be elucidated.

Based on the study by Mecham and co-workers, we envisioned a crosslinking structure containing desmosine with Ala-Ala into the 3,4-positions, desmosine with cyclic peptide (**1**, Fig. 2, right). Utilizing our synthetic strategy for the total synthesis of desmosine,[11–13] we aimed to synthesize **1**, which contains a macrocycle with two alanine residues in the 3 and 4 positions, which would be structurally similar to part of elastin. Synthetic studies of cyclic peptides that contain a pyridine ring are unusual,[14] and thus are of considerable interest. Furthermore, we hoped to elucidate the crosslinking structure of elastin by comparative analysis of **1** and degradated elastin peptides. By employing MS/MS as an analytical technique, if we could find a degradated product with the same fragment peak as **1**, a partial structure of elastin could be elucidated. Therefore, in order to contribute to the elucidation of the elastin crosslinking structure by an innovative chemical synthetic approach, we synthesized **1** *via* stepwise Sonogashira and Negishi cross-coupling reactions and intramolecular condensation as key steps.

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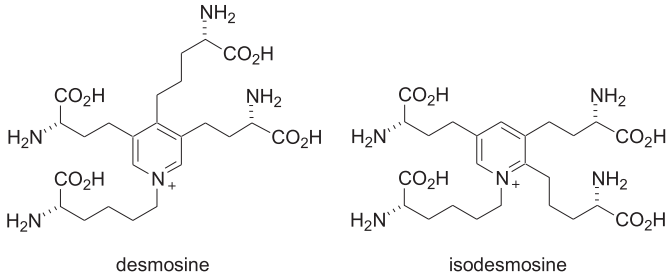


Fig. 1. Structures of desmosine and isodesmosine.

2. Results and discussion

According to the retrosynthetic analysis shown in Scheme 1, **1** could be derived from cyclic peptide **2** or **3**, which contain a pyridine ring, and iodo amino acid (ω-iodobutyl L-glycine) derivative **4** through late stage formation of the pyridinium salt. The macrocycle of **2** or **3** could be constructed by hydrogenation and intramolecular condensation of **5** or **6**. Synthesis of **5** or **6** would involve chemo- and regioselective palladium-catalyzed Sonogashira and Negishi cross-coupling reactions between trihalogenated pyridine **7**,[11c,11d] which can be prepared from commercially available 4-aminopyridine,[15] terminal alkyne **8**, and iodo amino acid **9** or **10**. The protected alkyne **8** could be formed by intermolecular condensation between **11** and **12**.

We commenced the synthesis of **1** by preparing terminal alkyne **8** (Scheme 2). Carboxylic acid **11** was prepared by hydrolysis of **13** quantitatively while amine **12** was synthesized by removal of *tert*-butyl (*t*Bu) group of **14** quantitatively.[16] The intermolecular condensation between **11** and **12** using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as a condensation agent and 1-hydroxybenzotriazole monohydrate

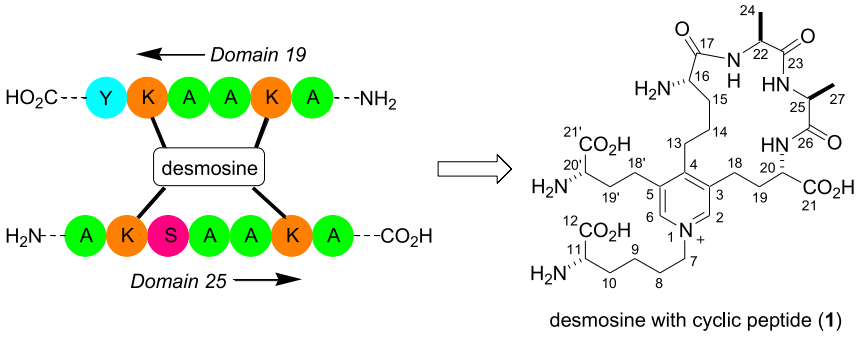
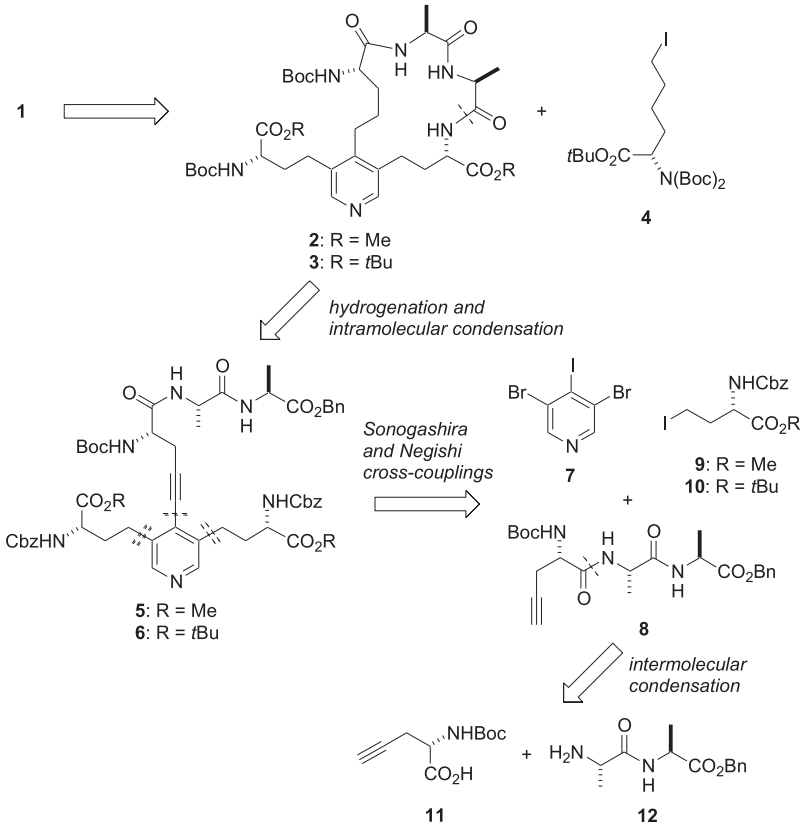


Fig. 2. Proposed schematic model of elastin crosslinking peptide (left, A: alanine, K: lysine, S: serine, Y: tyrosine) and structure of desmosine-containing cyclic peptide (**1**, right).



Scheme 1. Retrosynthesis of **1**.