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Total synthesis of the COPD biomarker desmosine via Sonogashira and Negishi cross-coupling reactions

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ABSTRACT

Desmosine, a crosslinking pyridinium amino acid of elastin, is an attractive biomarker for the diagnosis of chronic obstructive pulmonary disease (COPD). In this study, the total synthesis of (+)-desmosine is reported utilizing chemo- and regioselective Sonogashira and Negishi cross-coupling reactions in 15% yield over six steps.

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder and is a leading cause of morbidity and mortality. According to the World Health Organization (WHO), COPD, affecting over 60 million people, is currently the fourth leading cause of death worldwide, and is estimated to become the third leading cause by 2030. The risk for COPD is related to the interaction between many environmental factors, such as tobacco smoking, and a genetic factor that is known as α_1-antitrypsin deficiency (AATD).[1] Presently, there is a lack of effective therapies and medicines that can prevent progression of the disease, and thus survival rates cannot be improved. As a result, intensive drug discovery activities are underway to find an effective treatment. The development of biomarkers that can act as indicators of the severity of COPD and the level of patient response to therapy would thus aid these efforts.[2]

Elastin fibers are a part of the extracellular matrix and are an essential structural component of the lungs, ligaments, skin, blood vessels, and so on. Desmosine (**1**) and isodesmosine (**2**), which exist only in the elastin matrix, are pyridinium amino acids that serve as the crosslinking molecules that bind the polymeric chains in elastin into a sophisticated 3-D network (Fig. 1).[3,4] The irreversible degradation of lung elastin that occurs in COPD is known to give rise to these two amino acids, which can be measured specifically and sensitively in clinical samples, such as plasma, urine, and sputum, by liquid chromatography–mass spectrometry (LC–MS) analysis.[5–7] Therefore, the elastin crosslinking amino acids **1** and **2** are attractive biomarkers for both drug discovery and rapid diagnosis of COPD.

Despite the potential application of these compounds as biomarkers for COPD, rigid quantitation of **1** and **2** has not been achieved due to a lack of promising internal standards for strict analysis by LC–MS.[5,6] The total synthesis of desmosine **1** has been recently achieved in 13 steps in 11% overall yield in our laboratory.[8] The synthesis relied on stepwise and regioselective Sonogashira cross-coupling reactions between the pyridine core and the corresponding alkynes. After the preparation of the tri-alkylated pyridine, several steps were required for conversion from the oxazolidine rings into the corresponding amino acid moieties. Herein, utilizing Sonogashira and Negishi cross-coupling reactions as key steps in order to avoid further conversions, we report a

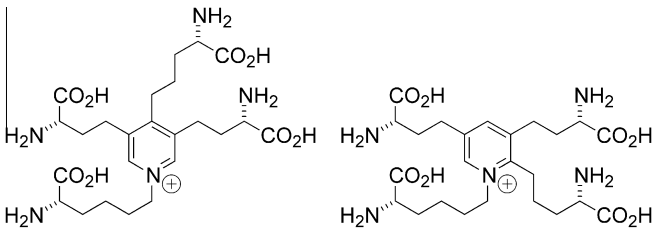

Figure 1. Structures of desmosine (**1**) and isodesmosine (**2**).

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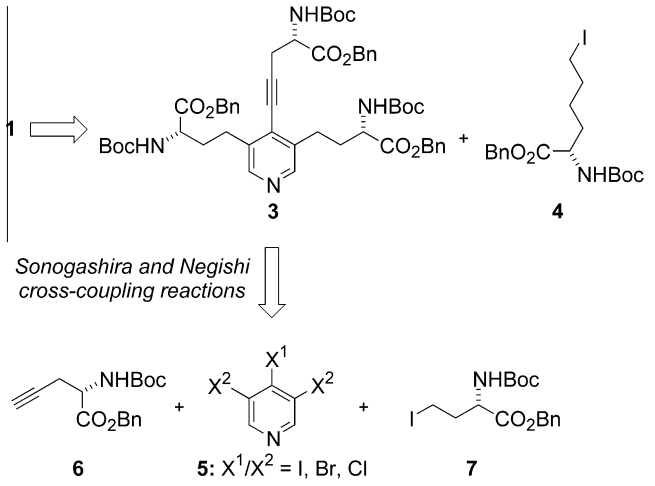


Scheme 1. Retrosynthetic analysis of desmosine (**1**).

shortened and improved total synthesis of the COPD biomarker desmosine **1** that crosslinks elastin.

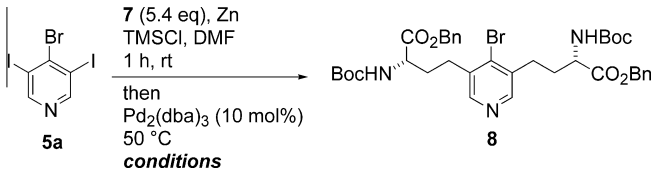
As illustrated in Scheme 1, it was envisioned that **1** would be derived from 3,4,5-tri-alkylated pyridine **3** and ω-iodoalkylated L-glycine **4**[9] through late-stage formation of the pyridinium salt. Synthesis of **3** would then involve chemo- and regioselective palladium-catalyzed Sonogashira and Negishi cross-coupling reactions of trihalogenated pyridines **5**, the corresponding terminal alkyne **6**,[8] and γ-iodoalkylated L-glycine **7**.[9] The key strategy was to utilize the different reactivities of each compound as determined by the substituent position and the halogen species on the pyridine ring.

Firstly, the Negishi cross-coupling reaction between 4-bromo-3,5-diiodopyridne **5a**[8] and γ-iodoalkylated L-glycine **7** was investigated (Table 1). 3,5-Chemo- and regioselective introduction of **7** into **5a** using 10 mol % $Pd_2(dba)_3$ with 40 mol % P(2-furyl)$_3$ at 50 °C for 20 h afforded the desired di-coupled product **8** in 44% yield (entry 1). In the Negishi reaction, we have applied a modified protocol in which an extra Zn from the organozinc reagent can be removed via centrifugation.[10] When the reaction was shortened to 5 h under the same conditions, the yield was slightly improved to 49% (entry 2). Replacing the P(2-furyl)$_3$ ligand with $AsPh_3$, which is known to be more effective in the palladium-catalyzed reaction,[11] led to a decrease in the yield to 33% when the reaction was run for 20 h, but a reaction time of just 5 h resulted in a significant increase in the yield to 71% (entries 3 and 4, respectively). With $AsPh_3$, the longer reaction time led to a lower yield, suggesting that the ligand may cause the decomposition of the substrates and/or product. When the Negishi cross-coupling reaction between 3,4,5-triiodopyridne and iodo amino acid **7** was carried out under the same conditions as used in entry 1 (Table 1), however, no coupling products were observed.

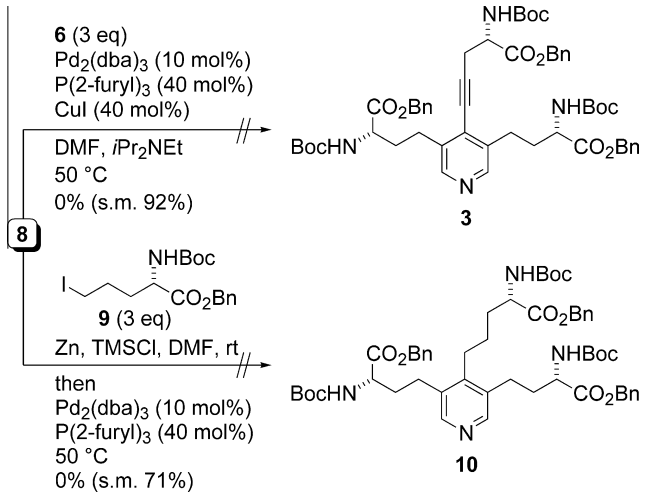
Toward the total synthesis, a Sonogashira cross-coupling reaction was then attempted with compound **8** and alkyne **6** using 10 mol % $Pd_2(dba)_3$, 40 mol % P(2-furyl)$_3$, and 40 mol % CuI in DMF and iPr$_2$NEt at 50 °C with the hope of obtaining 3,4,5-trialkylated pyridine **3** (Scheme 2). However, recovery of the starting material was confirmed in 92% yield, while the desired coupling product was not observed. Meanwhile, in order to obtain the trialkylated pyridine **10**, the Negishi cross-coupling reaction between **8** and δ-iodoalkylated L-glycine **9**[9] under the same conditions as entry 2 (Table 1) did not proceed. Instead, starting material **8** was recovered in 71% yield. These results are probably due to steric repulsion of the 3,5-di-sp^3-carbon atom in the oxidative addition of palladium to the 4-bromo-position on the pyridine ring, while the Sonogashira cross-coupling reaction to 4-bromo-3,5-dialkynylpyridine (i.e., 3,5-di-sp-carbon) was successful.[8] This synthetic route was thus ruled out.

As an alternative strategy, 3,5-dibromo-4-iodopyridine **5b** and commercially available 3,5-dichloro-4-iodopyridine **5c** were selected as the substrates for the cross-coupling reactions (Scheme 3). Preparation of **5b** was accomplished in 70% yield from 3,5-dibromopyridine **11** by regioselective iodination using LDA and I_2.[12] Chemo- and regioselective Sonogashira cross-coupling reactions between alkyne **6** and the 4-position of **5b** and **5c** were carried out using 10 mol % $Pd_2(dba)_3$ with 40 mol % P(2-furyl)$_3$ to afford the corresponding monoalkynes **12b** and **12c** in 58% and 80% yield, respectively.[13] The different reactivities might be due to the extents of steric hindrance of the neighboring iodine groups, which prevents the oxidative addition of the palladium. Incorporation of γ-iodoalkylated L-glycine **7** into the 3,5-positons of the obtained **12b** and **12c** was attempted via a Negishi cross-coupling reaction using Pd-PEPPSI-IPr, which was developed by Organ and co-workers.[14] When the reaction was conducted with 3,5-dibromopyridine **12b**, the desired tri-alkylated pyridine **3** was obtained in 60% yield, along with the mono coupling product **13** in 10% yield.[15] However, the Negishi cross-coupling with 3,5-dichloropyridne **12c** using Pd-PEPPSI-IPr did not provide the product, while the starting material was recovered in 44% yield. These results suggested that the reactivity of the chloropyridine is reduced compared to those of the bromopyridine and iodopyridine.[10]

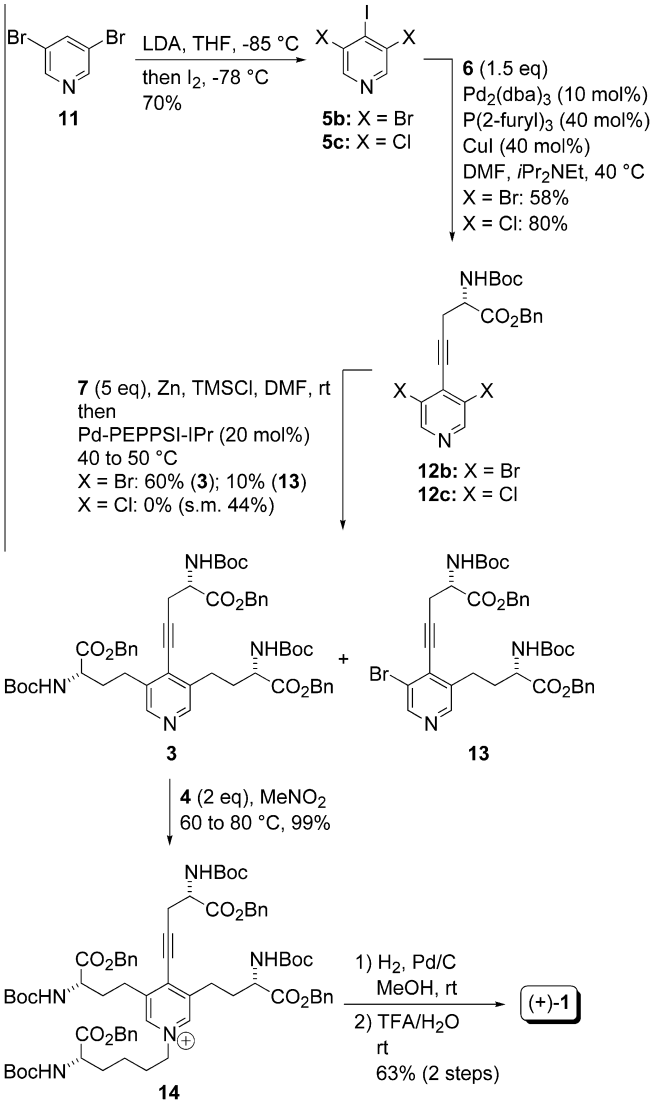
Table 1
Negishi cross-coupling of **5a** and **7**.



Entry	Ligand (40 mol %)	Time (h)	Yield (%)
1	P(2-furyl)$_3$	20	44
2	P(2-furyl)$_3$	5	49
3	$AsPh_3$	20	33
4	$AsPh_3$	5	71



Scheme 2. Attempts at the cross-coupling reactions of **8**.



Scheme 3. Total synthesis of desmosine **1**.

Formation of the pyridinium salt of **3** with ω-iodoalkylated L-glycine **4** in MeNO2 at 60 to 80 °C was then conducted to produce **14** in 99% yield.[16] After reduction of the benzyl and alkyne groups with H_2 and Pd/C, the *t*-butoxycarbonyl protecting groups were successfully removed using TFA to give crude **1**. Purification on C_{18} column chromatography afforded pure desmosine **1** in 63% yield over two steps. Spectroscopic data for obtained **1** including the optical rotation were in good agreement with those measured for natural **1**.[8]

Conclusion

In summary, the total synthesis of (+)-desmosine **1**, a crosslinking pyridinium amino acid of elastin, has been achieved via chemo- and regioselective Sonogashira and Negishi cross-coupling reactions as a key transformation in 15% yield over six steps starting from 3,5-dibromopyridine **11**. The straightforward synthetic route is greatly shortened compared to the previous synthesis of **1** (11% yield over 13 steps). The improved synthetic strategy described above is currently being applied to the preparation of other crosslinking amino acids[4] in order to elucidate the 3-D structure of elastin fiber, including the structure of the degraded elastin peptides that are generated due to COPD that can be indicative of the severity of the disease.

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Supplementary data

Supplementary data (experimental procedures and characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.08.084.

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