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SYNTHESIS OF ELASTIN CROSSLINKER DESMOSINES

Hiroaki Tanaka and Toyonobu Usuki*

Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan. Email: t-usuki@sophia.ac.jp

Abstract – Desmosine (**1**) and isodesmosine (**2**) are 1,3,4,5- and 1,2,3,5-tetrasubstituted pyridiniums and exist only in the extracellular matrix protein elastin as major crosslinking amino acids. They are formed by the condensation and cyclization of one lysine and three allysines, generated from lysine by lysyl oxidase. Since the discovery of desmosines in 1964, there have been no reports on their chemical synthesis for decades. The first total synthesis of **1** was reported in 2012 and since then, isodesmosine (**2**), desmopyridine (**3**), isodesmopyridine (**4**), neodesmosine (**5**), and merodesmosine (**6**) have been synthesized. Isotopically labeled desmosines have also been synthesized for the precise analysis of desmosines using isotope-dilution LC–MS/MS analysis. Synthesis of conjugates with desmosines and carrier proteins was achieved for antibody production. This review summarizes a series of desmosine syntheses based on cross-coupling reactions and Chichibabin pyridinium synthesis as synthesis methodologies. The obtained results would help in the development of novel diagnostic and therapeutic methodologies for elastin-degradation-related diseases.

CONTENTS

1. Introduction

1.1 Elastin

Elastin is an insoluble protein present in the extracellular matrix of vertebrate tissues, including in the ligamentum flavum, lungs, skin, blood vessels, and heart, and is responsible for their elasticity and stretchiness (Figure 1).[1] Elastin is the core extracellular matrix protein (>90%) of elastic fibers comprising soluble precursor tropoelastin monomers crosslinked by specific amino acids. Hydrophobic domains in the rubber-like protein make elastin easily stretchable under an externally applied tensile force and the stretching can be released without any external action. Therefore, elastin crosslinkers are important amino acids owing to their elasticity.

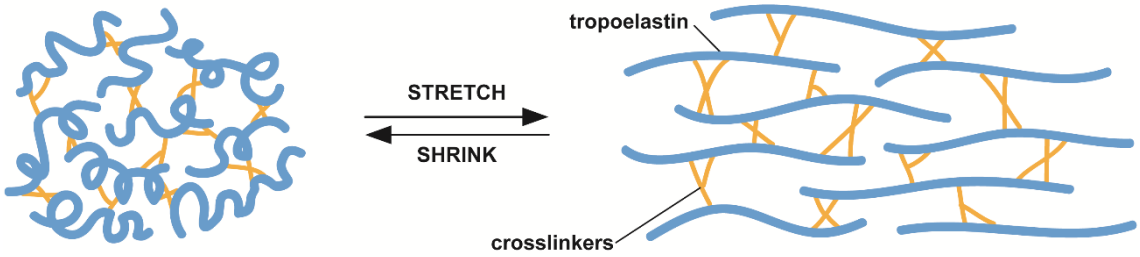


Figure 1. Elastin fibers

Elastin, with a half-life of 74 years in healthy tissues and organs, is known to be the longest-lasting protein in the body. Although the mechanism of the formation of elastin fibers is not yet fully understood, they are believed to be formed through the following process: 1) the water-soluble precursor tropoelastin, the elastin monomer with a molecular weight of approximately 72 kDa, is secreted by dermal fibroblast cells; 2) molecular interactions between tropoelastin and microfibril proteins such as fibrillin-1 and fibulin-5 align the crosslinking domains of tropoelastin molecules; 3) desmosine (Figure 2, **1**) and isodesmosine (Figure 2, **2**), the crosslinking pyridinium amino acids, are formed via oxidative deamination of lysine in soluble tropoelastin by lysyl oxidase, followed by dehydration, condensation, and aromatization.[2] Mecham and co-workers reported that crosslinkers **1** and **2** are located in hydrophilic segments between the 19th and 25th domains of alanine-rich areas of tropoelastin.[3] The detailed three-dimensional structures of elastin have not yet been elucidated because of the extremely insoluble nature of elastin, which does not allow instrumental analyses such as nuclear magnetic resonance (NMR),[4] X-ray diffraction,[5] circular dichroism (CD),[6] and scanning electron microscopy (SEM),[7] although the model structures of the crosslinking moiety of elastin have been proposed.[8]

Irreversible degradation of elastin-containing tissues causes excretion of desmosines—pyridinium amino acids—in bodily fluids. Such degradation occurs in several widely prevalent diseases such as atherosclerosis,[9] aortic aneurysm,[9] cystic fibrosis,[10] and chronic obstructive pulmonary disease (COPD).[11] Therefore, desmosines, the elastin crosslinker, can be utilized as a useful biomarker for elastin degradation.

1.2 Desmosine and Related Amino Acids

Desmosine (**1**), 1,3,4,5-tetrasubstituted pyridiniums, was first isolated and structurally analyzed by acid hydrolysis of the elastin obtained from the bovine nuchal ligament in 1963 by Thomas and co-workers (Figure 2).[12] Desmosine (**1**) and isodesmosine (**2**), 1,2,3,5-tetrasubstituted pyridinium amino acids, occur in elastin at a ratio of approximately 1:1. They are the major crosslinking amino acids that bind the polymeric chains in elastin into a sophisticated three-dimensional network.[12,13] The etymology of

desmosine **1** is derived from the ancient Greek word "*desmos*," which means "to join."[12,14] Suyama and co-workers in 2001 isolated elastin crosslinkers desmopyridine (**3**) (3,4,5-trisubstituted pyridine) and isodesmopyridine (**4**) (2,3,5-trisubstituted pyridine) through acid hydrolysis of the bovine ligamentum nuchae,[15] while Nagai in 1971 isolated 1,3,4-trisubstituted pyridine neodesmosine (**5**) from bovine ligamentum nuchae and eggshell membrane.[16] Merodesmosine (**6**), which was isolated from the aortae of young ducklings by Starcher and co-workers in 1967,[17] is one of the possible crosslinking amino acids of elastin.

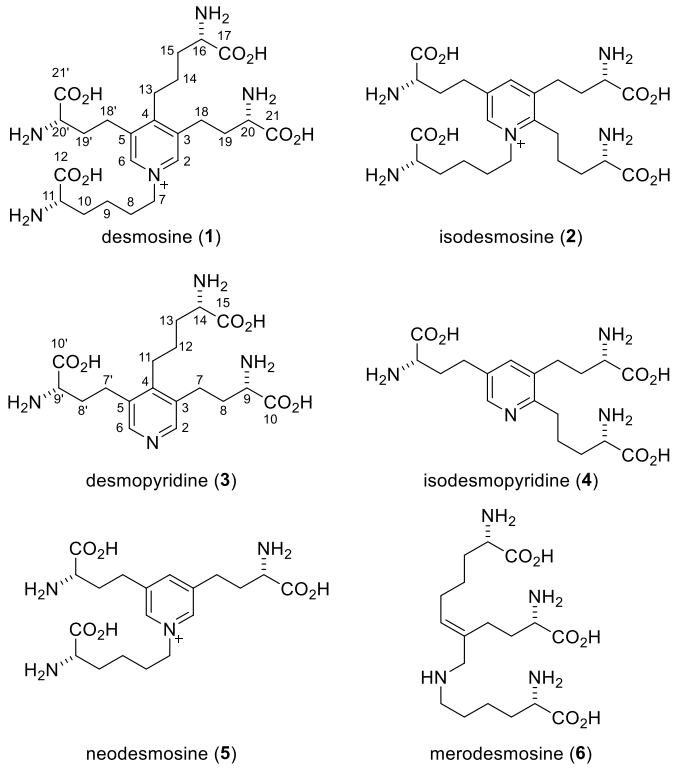


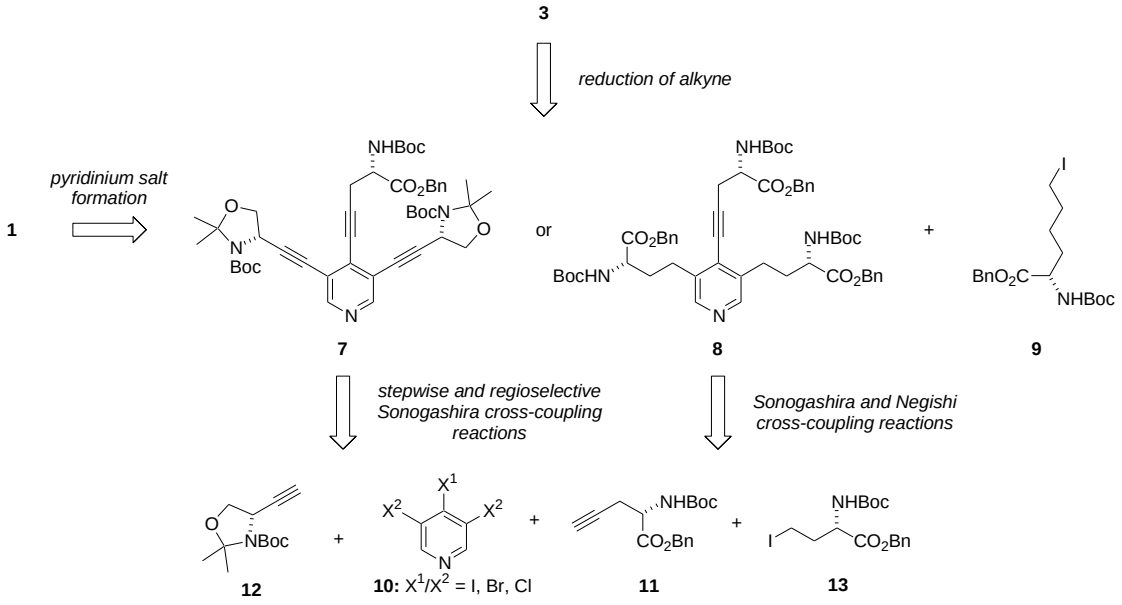
Figure 2. Structures of elastin crosslinkers desmosine (**1**), isodesmosine (**2**), desmopyridine (**3**), isodesmopyridine (**4**), neodesmosine (**5**), and merodesmosine (**6**)

The total syntheses of desmosine (**1**)[18,19] and its analogs (**2**–**6**)[19-24] have been achieved by our research group over the past decade. Based on established synthesis methodologies such as the palladium-catalyzed cross-coupling reaction and Chichibabin pyridine/pyridinium synthesis, an array of desmosine-related compounds has been synthesized, including isotopically labeled desmosines[25,26] and —conjugates with carrier proteins.[27,28] This review summarizes various efforts at the synthesis of **1**, its analogs (**2**–**6**), and related compounds.

2. Synthesis of Desmosines via Cross-Coupling Reactions

2.1 Synthesis Strategies

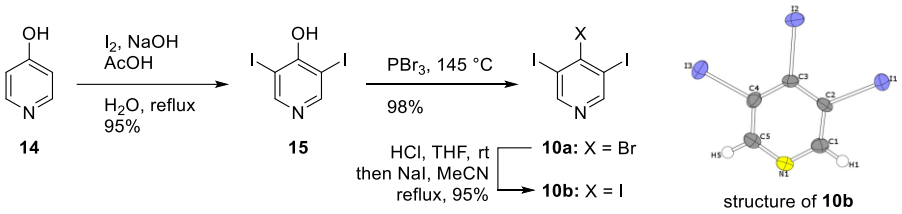
Desmosine (**1**) comprises a 1,3,4,5-tetrasubstituted pyridine core and four amino acid residues with different carbon chains. As illustrated in the retrosynthetic analysis (Scheme 1), **1** was derived from 3,4,5-trisubstituted pyridine **7** or **8** and iodo amino acid (or ω-iodoalkylated L-glycines) **9**[29] through late-stage formation of the pyridinium salt. Tri-serial-substituted pyridine **7** or **8** could be obtained through two possible strategies: (1) by conducting regioselective and stepwise palladium-catalyzed Sonogashira cross-coupling reactions[30] between trihalogenated pyridine **10** and terminal alkynyl amino acids **11** and **12**; and (2) by conducting chemo- and regioselective palladium-catalyzed Sonogashira and Negishi cross-coupling reactions between trihalogenated pyridine **10**, terminal alkyne **11**, and γ-iodoalkylated L-glycine **13**. Protected alkynyl amino acids **11**, **12**, and **13** were derived from L- and D-serine and L-glycine,[29] respectively. For the Sonogashira and Negishi cross-coupling path, the key strategy was to take advantage of the different reactivities of each compound, as determined by the substituent position and halogen species on the pyridine ring. In contrast, desmopyridine (**3**) is a 3,4,5-trisubstituted pyridine and has the same pattern as that of **1** except for the substituent at the 1-position of the pyridine ring. Therefore, **3** could be accessed from **7** or **8**, which have the same intermediates as that for **1**, through the reduction of the alkyne and deprotection.



Scheme 1. Retrosynthetic analysis of desmosine (**1**) and desmopyridine (**3**)

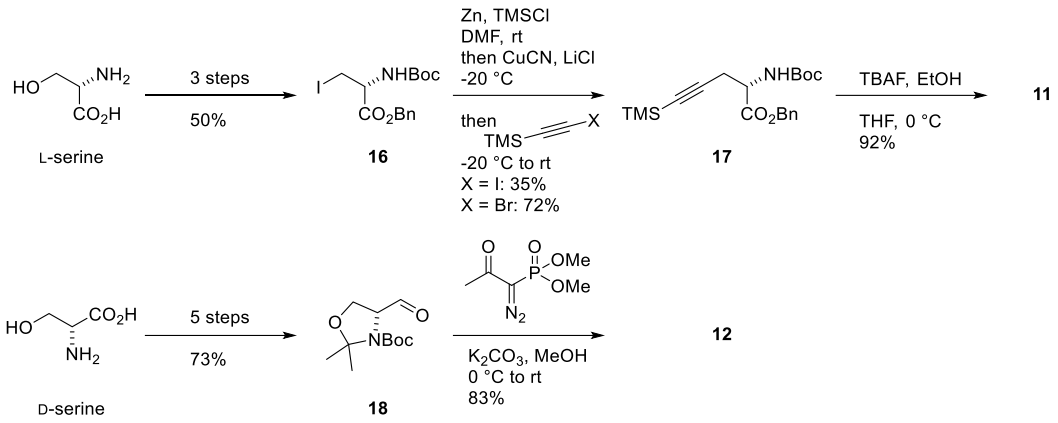
2.2 Stepwise Sonogashira Cross-Coupling Reaction

As outlined in Scheme 2, the synthesis of **1** via stepwise Sonogashira cross-coupling reactions commences with the formation of 3,4,5-trihalopyridines **10a** and **10b** starting from commercially available 4-hydroxypyridine (**14)**. The iodides were furnished at the 3- and 5-positions of the pyridine ring in a regioselective manner using I_2 to produce 4-hydroxy-3,5-diiodopyridine (**15**) in 95% yield.[31] Then, **15** was treated with PBr_3 to obtain 4-bromo-3,5-diiodopyridine (**10a**) in 98% yield, where the hydroxy group of **15** was converted to a bromine group. The bromine of **10a** was replaced with iodide using NaI to produce 3,4,5-triiodopyridine (**10b**) in 95% yield.[32] In this reaction, the pyridinium salt was predominantly formed using HCl so that it could increase the reactivity of the bromine substituent. The structure of **10b** was unambiguously determined using single-crystal X-ray analysis.



Scheme 2. Synthesis of trihalopyridines **10a** and **10b**

Amino acids **11** and **12**, which contain terminal alkynyl groups, were synthesized from L- and D-serines, respectively (Scheme 3). Following a previously reported procedure, iodo amino acid **16** was prepared from L-serine in 50% yield in three steps.[29] Then, **16** was converted to organocopper compound RCu(CN)ZnI,[33] which was used in a coupling reaction with TMS-protected bromo-ethyne to obtain **17** in 72% yield. When TMS-protected iodo-ethyne was used instead of the bromo-ethyne, the product was obtained in 35% yield because of the formation of the exo-olefin side product owing to β-elimination. The TMS group was removed by TBAF to obtain the desired terminal alkynyl amino acid **11** in 92% yield.[34] For the synthesis of **12**, Garner's aldehyde **18** was selected as a precursor of the cross-coupling substrate because it is difficult to prepare protected ethynyl glycine owing to its instability.[35] Compound **18**, reported previously, was prepared from D-serine in 73% yield over five steps.[36] The terminal alkynyl amino acid **12** was obtained from aldehyde **18** using the Ohira–Bestmann reagent,[37] dimethyl (1-diazo-2-oxopropyl)phosphonate, to form enantiomerically pure terminal alkynyl amino acid **12** in 83% yield.[38]



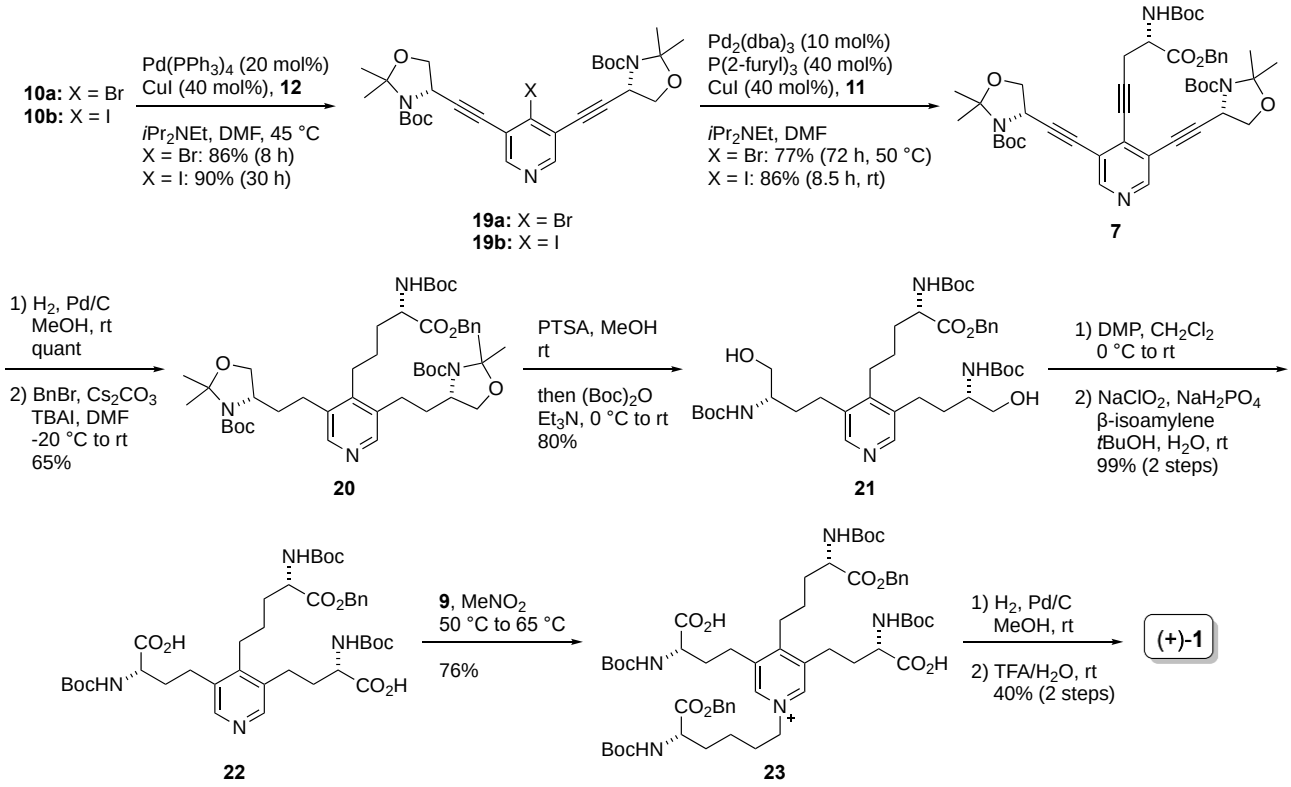
Scheme 3. Synthesis of terminal alkynyl amino acids **11** and **12**

With the obtained pyridine derivatives **10a**/**10b** and terminal alkynyl amino acids **11** and **12**, C–C bond formation between the pyridine core and amino acids was performed through palladium-catalyzed stepwise and regioselective Sonogashira cross-coupling reactions (Scheme 4). The first Sonogashira cross-coupling reaction between **10a**/**10b** and alkynyl amino acid **12** in the presence of 20 mol% of $Pd(PPh_3)_4$ and 40 mol% of CuI stereoselectively afforded the corresponding 3,5-disubstituted pyridines **19a**/**19b** in 86/90% yield. In contrast, the cross-coupling reaction between **10b** and **11** using 5 mol% of $PdCl_2(PPh_3)_2$ in the presence of 10 mol% of CuI gave a mixture of 3-monosubstituted and 3,5-disubstituted pyridines in moderate yields without the formation of 4-monosubstituted pyridine (separate experiment).[18(b)] Therefore, it was suggested that the reactivity of the 4-iodo-position in **10b** was less than that of the 3- and/or 5-iodo-positions because of the steric hindrance owing to the neighboring iodine groups, which prevents the oxidative addition of palladium. A significantly shortened reaction time was observed when 4-bromo-3,5-diiodopyridine **10a** was used instead of **10b**, probably because of the reduced steric hindrance at the 3- and 5-positions (bromine vs. iodine).

The second Sonogashira cross-coupling reaction between disubstituted pyridine **19b** and alkynyl amino acid **11** was accomplished using 10 mol% $Pd_2(dba)_3$ and 40 mol% CuI with 40 mol% of $P(2\text{-furyl})_3$[39] to provide the desired trisubstituted pyridine **7** in 86% yield. In this reaction, 4-iodo-3,5-disubstituted pyridine **19b** proceeded faster (8.5 h) at a lower temperature (room temperature) and gave the product in higher yields (85%) than that obtained with **19a**, probably because palladium better enabled insertion into the C–I bond compared to that in the C–Br bond at the oxidative addition stage.

The alkynes of trisubstituted pyridine **7** were reduced by hydrogenation to furnish alkanes along with deprotection of the Bn ester, followed by re-protection with BnBr to afford **20** in 65% yield. The oxazolidine rings of **20** were cracked into amino alcohols by treatment with PTSA. The resulting amines were protected with $(Boc)_2O$, producing diol **21** in 80% yield. Diol **21** was then subjected to Dess–Martin periodinane (DMP) and Pinnick oxidation to obtain carboxylic acid **22** in 99% overall yield. The pyridine core was

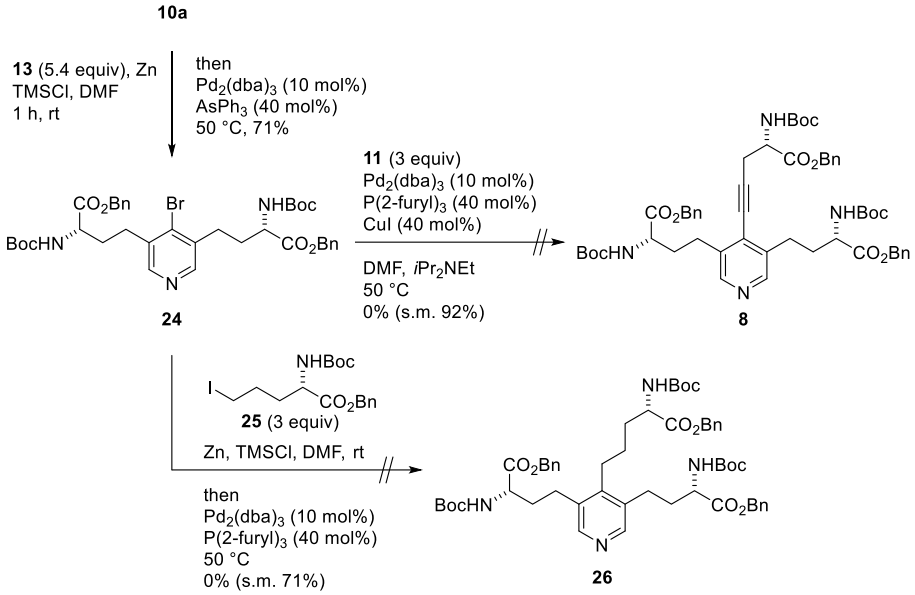
alkylated with iodo amino acid **9** to produce **23** in 76% yield.[40] Finally, the Bn- and Boc-protecting groups were removed in a stepwise manner through hydrogenation with Pd/C and TFA to produce **1** in 40% yield.



Scheme 4. Total synthesis of desmosine (**1**) via stepwise Sonogashira cross-coupling reaction

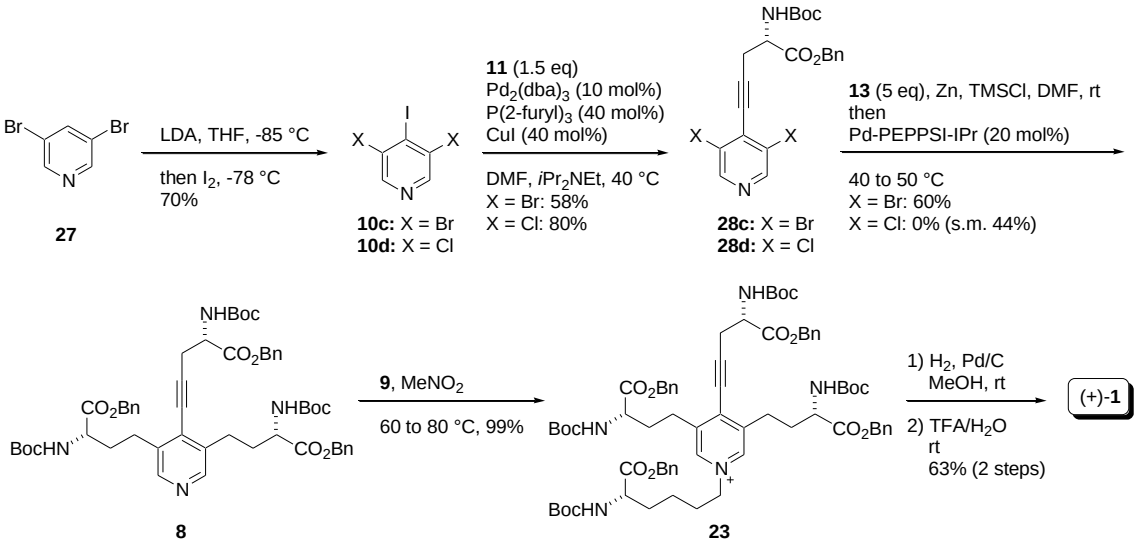
2.3 Sonogashira and Negishi Cross-Coupling Reactions

Synthesis of **1** via Sonogashira and Negishi cross-coupling reactions proceed through the same intermediate described above as the starting point—3,4,5-trihalopyridine **10a** (Scheme 5). The Negishi cross-coupling reaction between 4-bromo-3,5-diiodopyridne (**10a**) and γ-iodoalkylated L-glycine **13** was performed in the presence of the $AsPh_3$ ligand, which is known to be more effective in the palladium-catalyzed reaction.[39] The desired dicoupled product **24** was obtained in 71% yield. In the initial attempt, the Sonogashira cross-coupling reaction between **24** and alkyne **11** using 10 mol% of $Pd_2(dba)_3$, 40 mol% of P(2-furyl)$_3$, and 40 mol% of CuI resulted in the recovery of the starting material in 92% yield; however, the desired 3,4,5-trialkylated pyridine **8** was not observed. The Negishi cross-coupling reaction between **24** and δ-iodoalkylated L-glycine **25**[29] did not afford trialkylated pyridine **26**, although it led to the recovery of the starting material **24** in 71% yield. These results suggest that steric repulsion of the 3,5-di-sp^3-carbon atom in the oxidative addition of palladium to the 4-bromo-position hampered the reaction, while the Sonogashira cross-coupling reaction of 4-bromo-3,5-dialkynylpyridine (i.e., 3,5-di-sp-carbon) proceeded successfully.[18(a)]



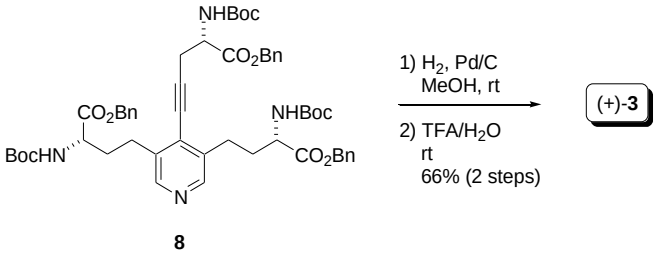
Scheme 5. Attempts at the cross-coupling reactions of **10a**

As 4-bromo-3,5-dialkynylpyridine **24** was found to be an inappropriate intermediate for the synthesis of **1**, 3,5-dibromo-4-iodopyridine (**10c**) and commercially available 3,5-dichloro-4-iodopyridine (**10d**) were selected as alternative substrates for the cross-coupling reactions (Scheme 6). Iodine was regioselectively introduced to commercially available 3,5-dibromopyridine (**27**) using LDA and I_2 to produce **10c** in 70% yield.[41] Although the chemo- and regioselective Sonogashira cross-coupling reaction between alkyne **11** and the 4-position of **10d** successfully afforded the corresponding monoalkynes **28d** in 80% yield by using 10 mol% of $Pd_2(dba)_3$ with 40 mol% of P(2-furyl)$_3$, the incorporation of γ-iodoalkylated L-glycine **13** into the 3,5-positions of **28d** via the Negishi cross-coupling reaction using Pd-PEPPSI-IPr[42] resulted in the recovery of the starting material in 44% yield. When 3,5-dibromo-4-iodopyridine **10c** was subjected to the Sonogashira cross-coupling reaction, monoalkyne **28c** was obtained in 58% yield, which is lower than that obtained with **28d**, presumably because of the large steric hindrance from the neighboring iodine groups that prevents the oxidative addition of the palladium. The Negishi cross-coupling reaction of **28c** and **13** was then carried out using a Pd-PEPPSI-IPr catalytic system to afford the desired trialkylated pyridine **8** in 60% yield, along with the mono-coupling product in 10% yield. It was observed that bromopyridine **28c** has a higher reactivity than that of chloropyridine **28d**. This reaction was performed by adopting the improved experimental protocol,[43] where the extra Zn atom was removed from the organozinc reagent through centrifugation. The obtained trialkylated pyridine **8** was converted to the pyridinium salt with ω-iodoalkylated L-glycine **9** to produce **23** in 99% yield.[40] The reduction of the Bn and alkyne groups with H_2 and Pd/C and the subsequent deprotection of the Boc-protecting groups using TFA gave **1** in 63% yield.



Scheme 6. Total synthesis of desmosine (**1**) via Sonogashira and Negishi cross-coupling reactions

Deprotection of the Bn and reduction of alkyne groups in **8** were catalyzed by Pd/C under a H_2 atmosphere, followed by TFA treatment to obtain desmopyridine (**3**) in 66% yield (Scheme 7).



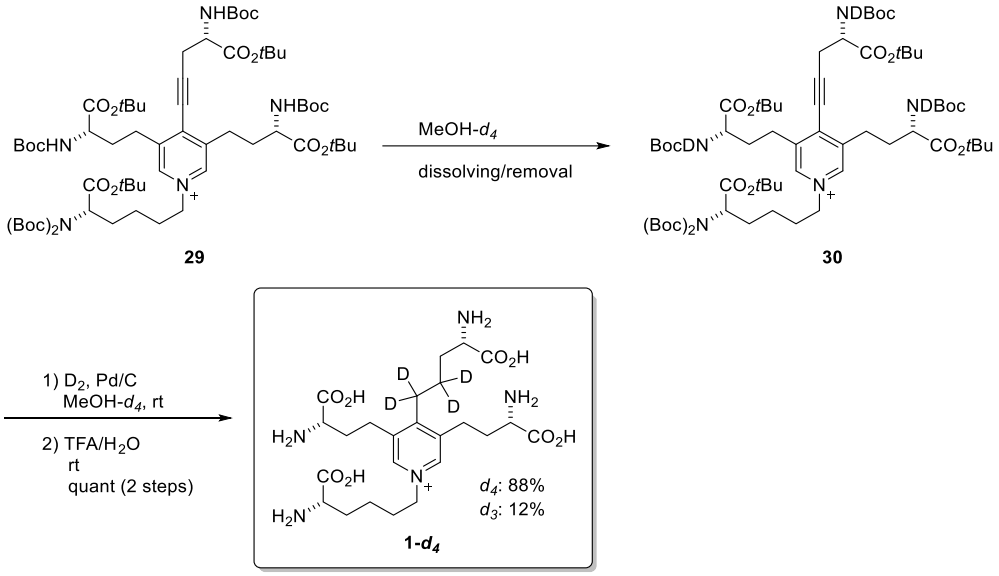
Scheme 7. Total synthesis of desmopyridine (**3**)

2.4 Synthesis of Isotopically Labeled Desmosine-d_n

Irreversible degradation of lung elastin that occurs in COPD is known to form elastin-crosslinking amino acids, particularly **1** and **2**.[13,44,45] Because they can be measured specifically and sensitively in clinical samples such as plasma, urine, and sputum via liquid chromatography-mass spectrometry (LC–MS or LC–MS/MS) analysis, they are considered useful biomarkers for both drug discovery and rapid diagnosis of diseases related to elastin degradation, such as COPD. However, the accurate quantification of desmosines through isotope-dilution LC–MS or LC–MS/MS requires the use of isotopically labeled internal standards, which are analogs of the target molecules. Isotopically labeled desmosine-d_n was synthesized using the established synthesis methodologies utilizing stepwise chemo- and regioselective Sonogashira and Negishi cross-coupling reactions.[25]

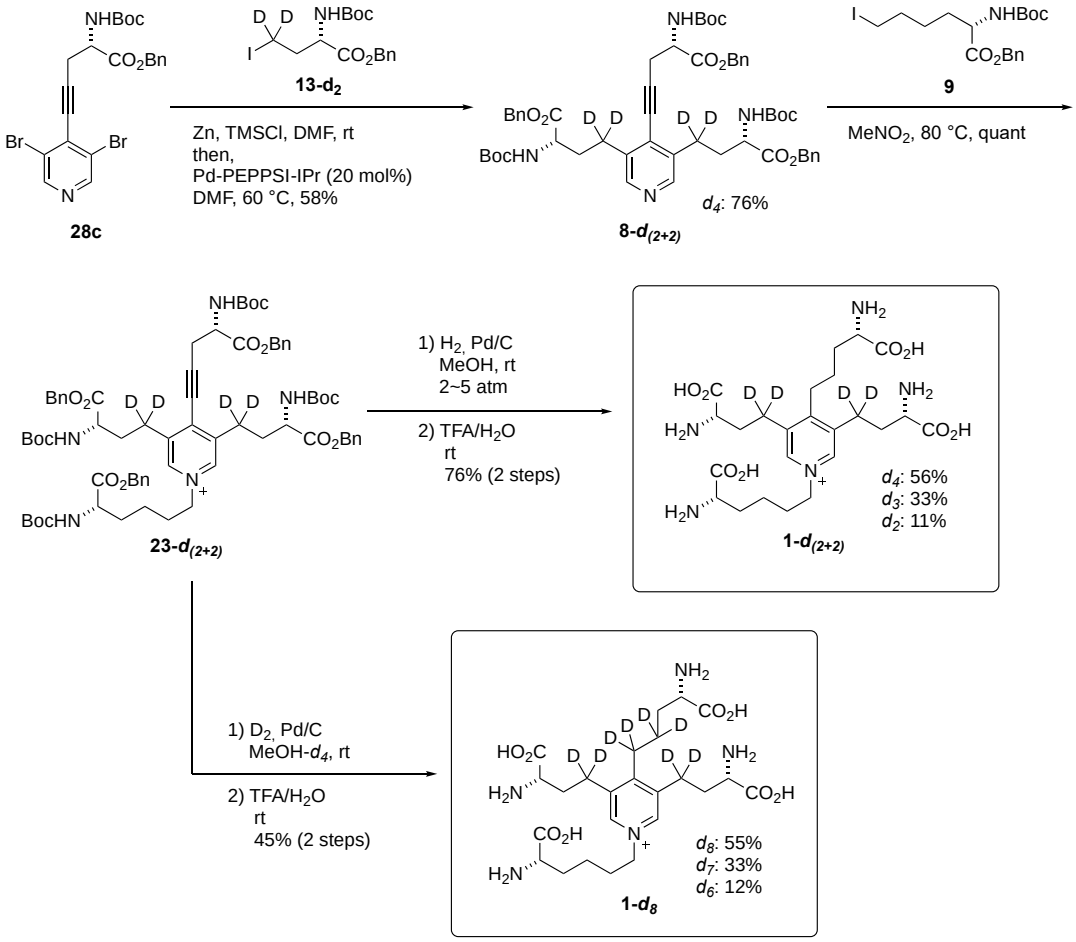
Deuterogenation with methanol-d_4 and the subsequent global deprotection of the Boc and *tert*-Bu groups using TFA were performed on pyridinium salt **29**, which was synthesized via Sonogashira and Negishi

cross-coupling reactions, to quantitatively form **1-*d*4** in two steps with the deuteration ratios of d_4 = 88% and d_3 = 12% (Scheme 8). The protons of the amino groups in pyridinium salt **29** were replaced with deuterium to form **30** by dissolving **29** in MeOH-d_4 and removing the solvent MeOH-d_4 five times prior to deuterogenation because the protons of **29** were assumed to reduce the isotopic purity.



Scheme 8. Synthesis of desmosine-d_4

As illustrated in Scheme 9, deuterated desmosines (**1-*d*(2+2)** and **1-*d*8**) were also prepared. The deuterated iodo amino acid **13-*d*2**[25(a)] was incorporated into the 3- and 5-positions of the pyridine core of **28c** via the Negishi cross-coupling reaction to produce **8-*d*(2+2)** in 58% yield with d_4 = 76%. After the formation of pyridinium salt **23-*d*(2+2)** using **9**, the deuterated desmosine (**1-*d*(2+2)**) was synthesized following the hydrogenation and deprotection of the Boc groups using TFA in 76% yield over two steps (d_4 = 56%, d_3 = 33%, and d_2 = 11%). In addition, deuterogenation with methanol-d_4 and deprotection using TFA/D_2O gave the deuterated desmosines (**1-*d*8**) in 45% yield over two steps (d_8 = 55%, d_7 = 33%, and d_6 = 12%). Because the reaction mixture had no proton source, intramolecular D–H exchange could be performed for reduced isotopic purity. Deuterogenation of alkynes with unprotected carboxylic acid substituents results in moderately deuterated ratios.[46] In such cases, the cleavage of the Bn group and the reduction of the alkyne occur simultaneously, and the resulting free carboxyl groups may cause D–H exchange.

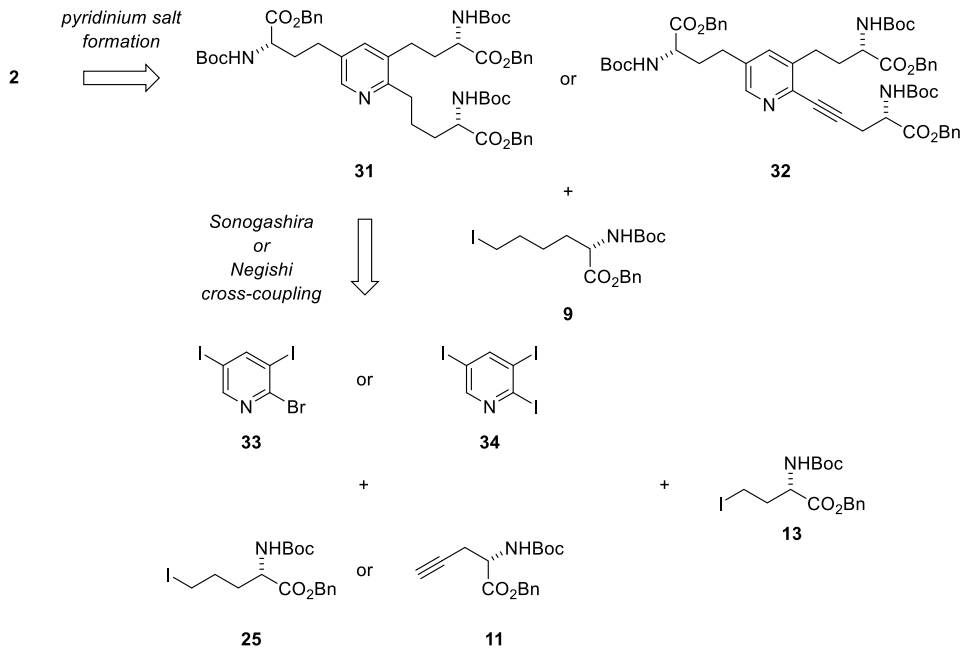


Scheme 9. Synthesis of desmosine-$d_{(2+2)}$ and desmosine-d_8

3. Synthesis of Isodesmosine

3.1 Synthesis Strategy

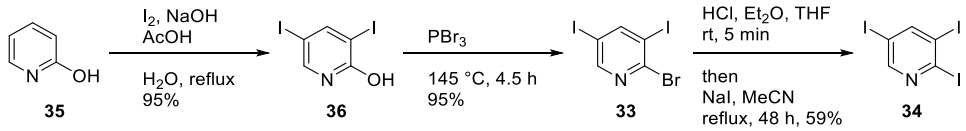
In contrast to the synthesis of **1**, the asymmetric structure of **2**, which is derived from the substituent at the 2-position of the molecule, requires stepwise and regioselective reactions to build up substituents. Therefore, the key strategy is to understand and utilize different reactivities of each compound substituted with different halogen species/positions on the pyridine ring. As illustrated in Scheme 10, the quaternary pyridinium salt is formed at the last stage via *N*-alkylation between 2,3,5-trialkylated precursor **31** or **32** and iodo amino acid **9**.[29] Following the palladium-catalyzed Sonogashira and/or Negishi cross-coupling strategy, **31** or **32** could be obtained from trihalogenated pyridines **33** or **34** by coupling with γ-iodoalkylated L-glycine **13**[29] and δ-iodoalkylated L-glycine **25**[29] or terminal alkyne **11**, respectively.



Scheme 10. Retrosynthetic analysis of isodesmosine (**2**)

3.2 Sonogashira and Negishi Cross-Coupling Reactions

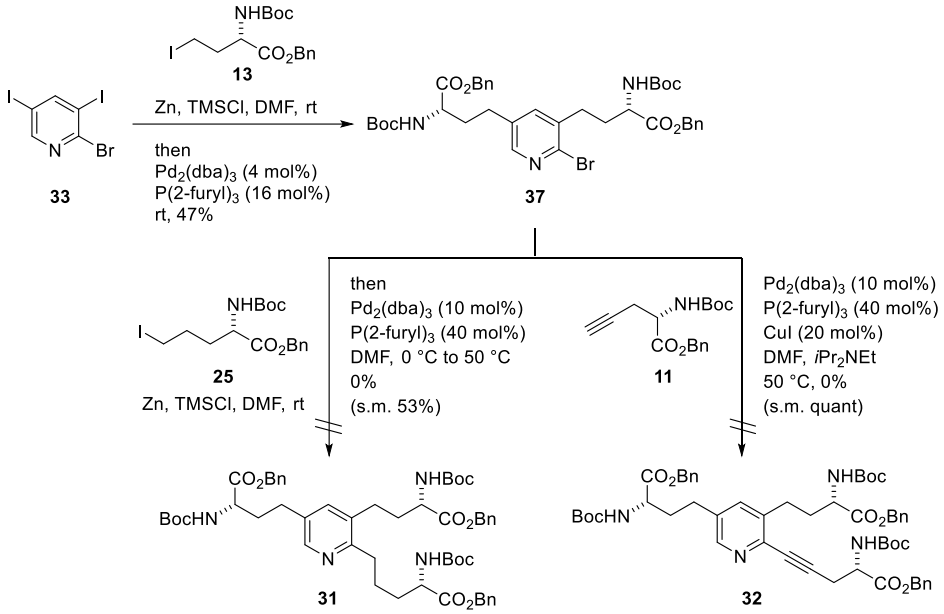
The synthesis began with the preparation of trihalogenated pyridines **33** and **34** using 2-hydroxypyridine (**35**) as the starting material (Scheme 11). 3,5-Regioselective iodination of **35** with I_2 gave 2-hydroxy-3,5-diiodopyridine (**36**) in 95% yield.[31] Conversion of the hydroxy group of **36** to bromine by PBr_3 afforded 2-bromo-3,5-diiodopyridine (**33**) in 95% yield. After the formation of the pyridinium salt using HCl, bromine in **33** was replaced with iodine using NaI, following the procedure described for the synthesis of **10b**, to produce 2,3,5-triiodopyridine (**34**) in 59% yield.[32] The structures of **33** and **34** were also determined unambiguously by single-crystal X-ray analysis.



Scheme 11. Synthesis of trihalogenated pyridines **33** and **34**

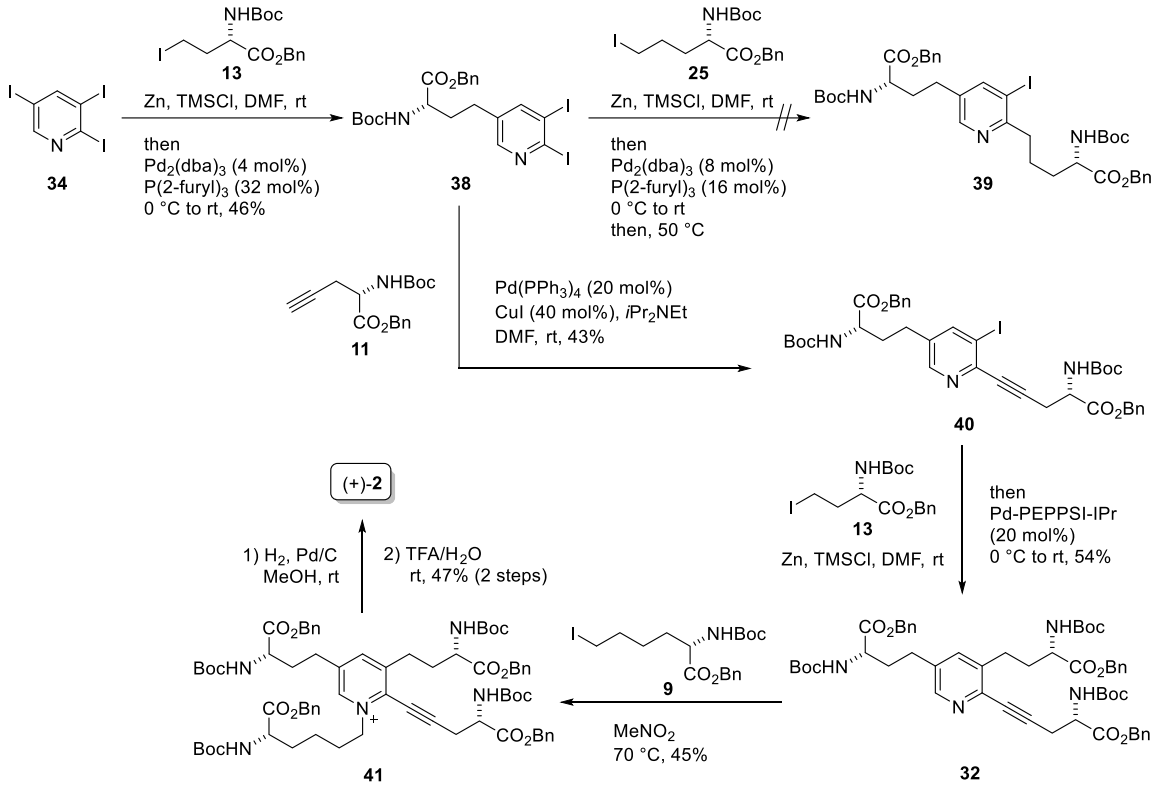
Initially, attempts were made to realize the regioselective incorporation of alkyl chains into the pyridine ring. The Negishi cross-coupling reaction between **33** and iodo amino acid **13** was carried out (Scheme 12). Using 4 mol% of $Pd_2(dba)_3$ with 16 mol% of P(2-furyl)$_3$ gave the desired 3,5-dicoupled product **37** in 47% yield. However, the second Negishi cross-coupling reaction between **37** and **25** did not proceed, resulting in the recovery of the starting material in 53% yield. Moreover, the Sonogashira cross-coupling reaction between **37** and the less hindered alkynyl amino acid segment **11** using 10 mol% of $Pd_2(dba)_3$, 40 mol% of

P(2-furyl)$_3$, and 40 mol% of CuI did not proceed, and **37** was recovered quantitatively, accompanied by the quantitative homocoupling of **11**. As the reduction of bromine of **37** using Zn in DMF afforded the recovery of the starting material (separate experiment), it was suggested that oxidative addition of palladium to the carbon–bromide bond at the 2-position of **37** could be prevented by the lower reactivity of bromide and steric hindrance by the alkyl chain, including sp^3 carbon at the 3-position.



Scheme 12. Attempt at stepwise cross-coupling reaction for **31** or **32**

Considering that the reactivity of bromine at the 2-position of the pyridine ring was insufficient for the palladium-catalyzed cross-coupling reaction, the strategy was changed and 2,3,5-triiodopyridine (**34**) was used with iodine at the 2-position of the pyridine ring, which generally had a higher reactivity than that of bromine (Scheme 13). The Negishi cross-coupling reaction between **34** and **13** was conducted using 4 mol% of Pd$_2$(dba)$_3$ and 32 mol% of P(2-furyl)$_3$ to afford the desired 5-alkylated product **38** in 46% yield along with a 2,5-dicoupled product and a 3,5-dicoupled product in 11% and 5% yields, respectively. It was thus suggested that the reactivity at the 5-position of 2,3,5-triiodoyridine, which was regarded as more reactive (vs. 2-position) and less hindered (vs. 3-position), was reflected in the preference of the cross-coupling reaction. Because the 2-position in the pyridine ring was considered to be more reactive than the 3-position,[30b] the cross-coupling reaction at the 2-position of **38** was investigated.



Scheme 13. Total synthesis of isodesmosine (**2**) via Negishi–Sonogashira–Negishi cross-coupling strategy

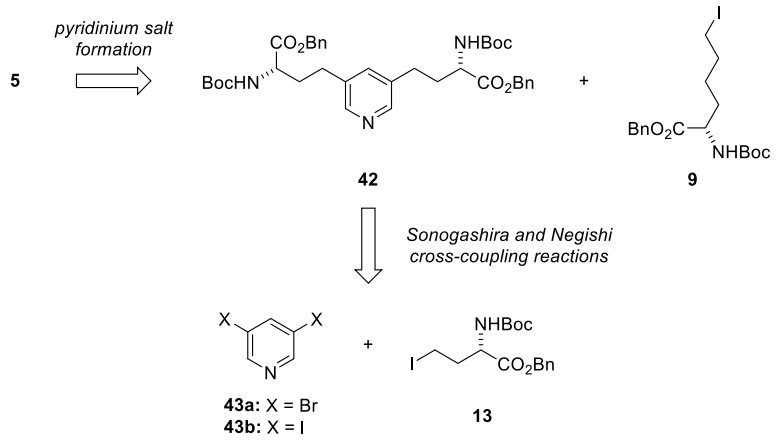
Although the regioselective Negishi cross-coupling reaction between the 2-position of **38** and iodo amino acid **25** produced complex mixtures without the desired product **39** using 8 mol% of $Pd_2(dba)_3$ and 16 mol% of P(2-furyl)$_3$, the Sonogashira cross-coupling reaction with less hindered alkyne **11** using 20 mol% of $Pd(PPh_3)_4$ and 40 mol% of CuI afforded **40** in 43% yield. In this reaction, the undesired 3-alkylated product and 2,3-dialkylated product were also obtained in 16% and 34% yields, respectively.

γ-Iodoalkylated L-glycine **13** was then incorporated into the 3-position of the obtained **40** via the Negishi cross-coupling reaction using 20 mol% of Pd-PEPPSI-IPr to provide the desired tricoupled pyridine **32** in 54% yield. Consequently, 2,3,5-trisubstituted pyridine **32** was successfully obtained by controlling the reactivity of the trihalogenated pyridines with the corresponding coupling substrates. Finally, pyridinium salt **41** was obtained in 45% yield using ω-iodoalkylated L-glycine **9**, which was followed by the reduction of the Bn and alkyne groups and deprotection of the Boc-protecting groups to form **2** in 47% yield over two steps.

4. Synthesis of Neodesmosine and Merodesmosine

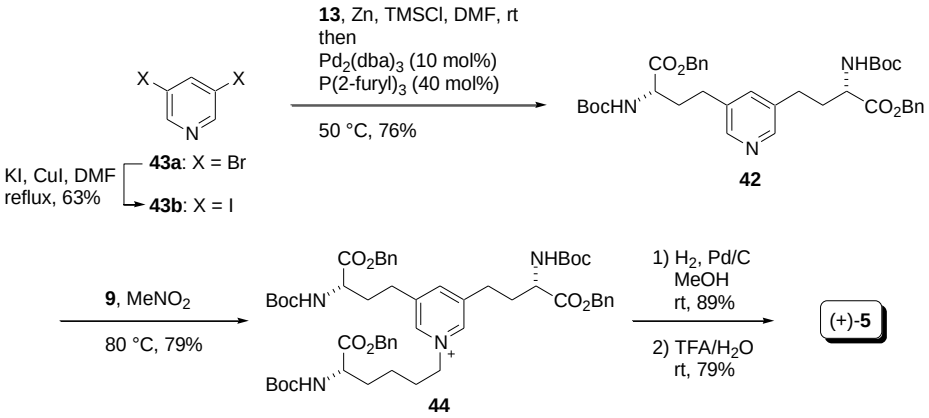
4.1 Synthesis of Neodesmosine

Neodesmosine (**5**) is a 1,3,5-trisubstituted pyridinium salt consisting of a pyridine core and two types of amino acids. Having established a synthesis strategy to introduce alkyl amino acid moieties at the 3- and 5-positions of the pyridine ring via the Negishi cross-coupling reaction of 3,5-dihalogenated pyridines, retrosynthetic analysis of **5** was conducted after that of **1** and **3**. As outlined in Scheme 14, **5** was synthesized by late-stage formation of the pyridinium salt using 3,5-disubstituted pyridine **42** and iodo amino acid **9**. Disubstituted pyridine **42** was obtained by the Negishi cross-coupling reaction between dihalogenated pyridine **43a** or **43b** and γ-iodoalkylated L-glycine **13**.



Scheme 14. Retrosynthetic analysis of neodesmosine (**5**)

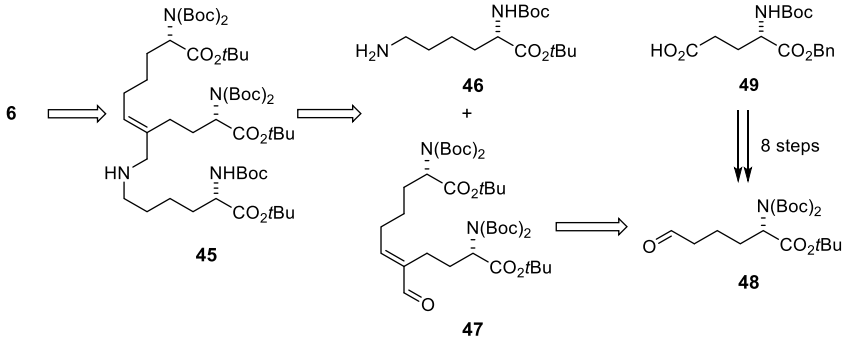
The synthesis began with the preparation of 3,5-diiodopyridine (**43b**) from commercially available 3,5-dibromopyridine (**43a**) using excess KI in the presence of CuI,[47] providing **43b** in 63% yield (Scheme 15). Next, the Negishi cross-coupling reaction between **43b** and **13** was conducted using 10 mol% of $Pd_2(dba)_3$ with 40 mol% of P(2-furyl)$_3$ to successfully produce **42** in 76% yield. Finally, formation of the pyridinium salt of the obtained **42** with protected ω-iodoalkylated L-glycine **9** gave **44** in 79% yield. After reduction of the three Bn groups with H_2 and Pd/C (89% yield), the Boc-protecting groups were successfully removed by the TFA treatment to produce neodesmosine (**5**) in 79% yield.



Scheme 15. Total synthesis of neodesmosine (**5**)

4.2 Synthesis of Merodesmosine

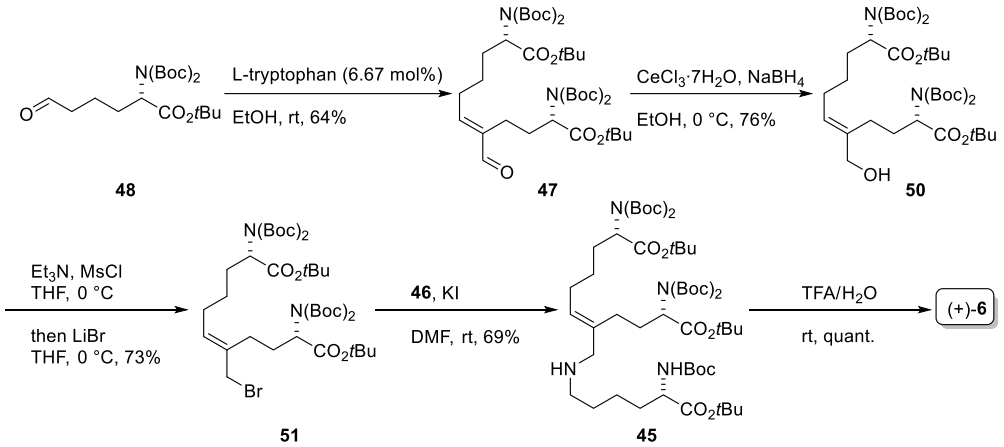
Unlike **1** and other elastin crosslinking amino acids, merodesmosine (**6**) does not contain pyridinium/pyridine rings; instead, it contains a secondary allylic amine that can be derived from one molecule of lysine and two molecules of allysine. As depicted in Scheme 16, **6** would be produced by the global deprotection of the Boc and *tert*-Bu groups of **45**, which would be synthesized from protected lysine **46** and enal **47**. Enal **47** would be obtained by the aldol condensation of allysine **48**, which can be prepared from glutamic acid **49**.



Scheme 16. Retrosynthetic analysis of merodesmosine (**6**)

To begin the synthesis, protected allysine **48** was prepared from commercially available 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanedionic acid mono-benzyl ester (**49**) (*N*-Boc-Glu-OBn).[19(a),48] As outlined in Scheme 17, enal **47** was synthesized via amino acid-catalyzed aldol condensation because the two chiral centers of **47** were regarded as unamenable to acidic or basic conditions. When L-tryptophan[49] was used as the catalyst, the condensation successfully proceeded to give **47** in 64% yield. The obtained enal **47** was subjected to Luche reduction,[50] whereupon selective 1,2-reduction with $CeCl_3 \cdot 7H_2O$ was observed, affording allylic alcohol **50** in 76% yield. Then, a one-pot sequence of mesylation and Finkelstein reaction[51]

gave allylic bromide **51**, followed by coupling with lysine **46**, prepared from commercially available 6-(benzyloxycarbonyl)amino-2-(*S*)-4-[(*tert*-butoxycarbonyl)amino]hexanoic acid,[52] to form protected merodesmosine **45** in 69% yield. Finally, global deprotection of **45** using TFA completed the quantitative synthesis of merodesmosine (**6**).[53]

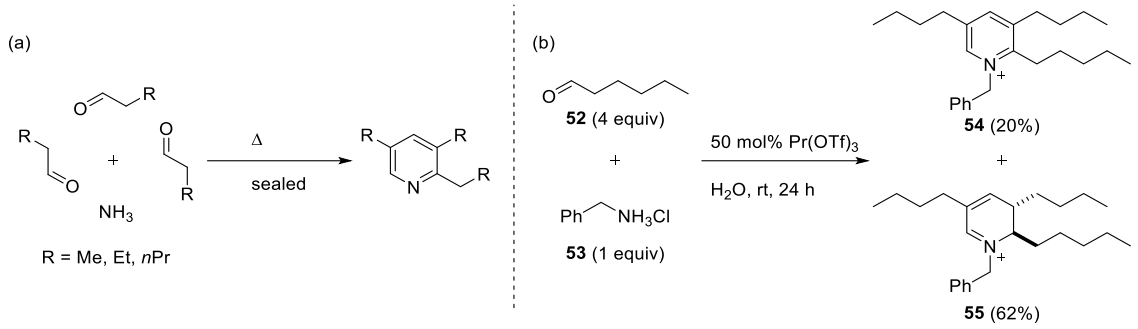


Scheme 17. Total synthesis of merodesmosine (**6**)

5. Synthesis of Desmosines via Chichibabin/isoChichibabin Pyridinium Synthesis

5.1 Background

In 1905, the Russian chemist Aleksei Yevgen'evich Chichibabin reported thermal cyclo-condensation between one equivalent of ammonia and three equivalents of aldehydes to form 2,3,5-trisubstituted pyridines (Scheme 18 (a)).[54] The Chichibabin pyridine synthesis, however, requires intense reaction conditions such as high pressures, high temperatures, and long reaction times.[54,55] Almost a century later, Wang and co-workers extended the Chichibabin pyridine synthesis and reported in 1997 that lanthanide trifluoromethanesulfonates (triflates), $Ln(OTf)_3$, promoted the condensation of amine hydrochlorides and aldehydes for the preparation of 1,2,3,5-tetrasubstituted dihydropyridinium and pyridinium derivatives.[56] In contrast to the original Chichibabin pyridine synthesis, the reaction proceeded at room temperature in aqueous media. A representative of the synthesis included the condensation of four equivalents of hexanal (**52**) and one equivalent of benzylamine hydrochloride (**53**) in the presence of 50 mol% of $Pr(OTf)_3$ in H_2O at room temperature for 24 h to afford 1,2,3,5-tetrasubstituted pyridinium (**54**) in 20% yield and its 2,3-dihydropyridinium (**55**) in 62% yield, respectively (Scheme 18 (b)).[56]

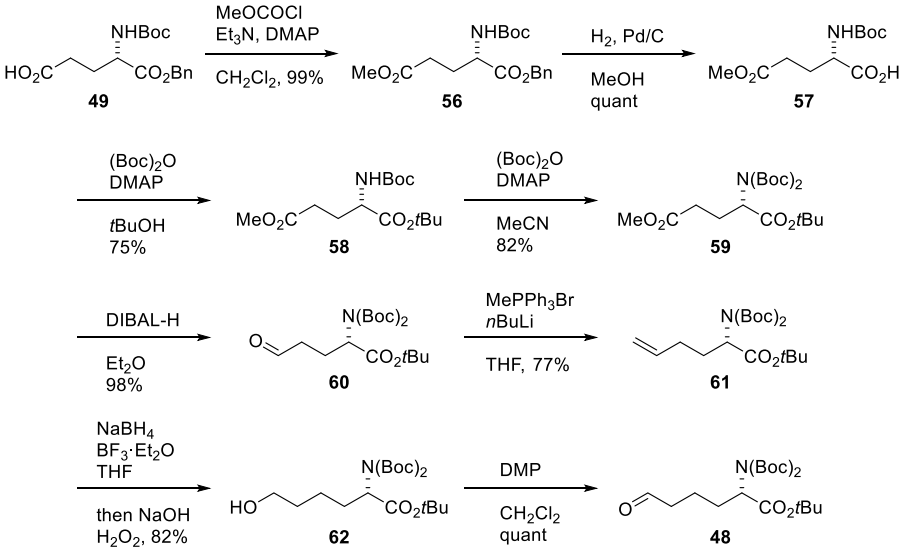


Scheme 18. (a) Original Chichibabin pyridine synthesis; (b) $Pr(OTf)_3$-promoted Chichibabin pyridinium synthesis

Motivated by these reports, we extensively studied the reaction by applying it to the total synthesis of **2** and 1,2,3,5-tetrasubstituted pyridinium derivatives.[19] The efforts led to the discovery of the unique selectivity of the reaction to provide **1** and 1,3,4,5-tetrasubstituted pyridinium derivatives, which were not conventionally formed by Chichibabin pyridinium synthesis (herein named "iso"-Chichibabin pyridinium synthesis). Herein, we discuss the selectivity of ring formation and mechanistic studies of Chichibabin pyridinium synthesis.

5.2 Chichibabin/isoChichibabin Pyridinium Synthesis

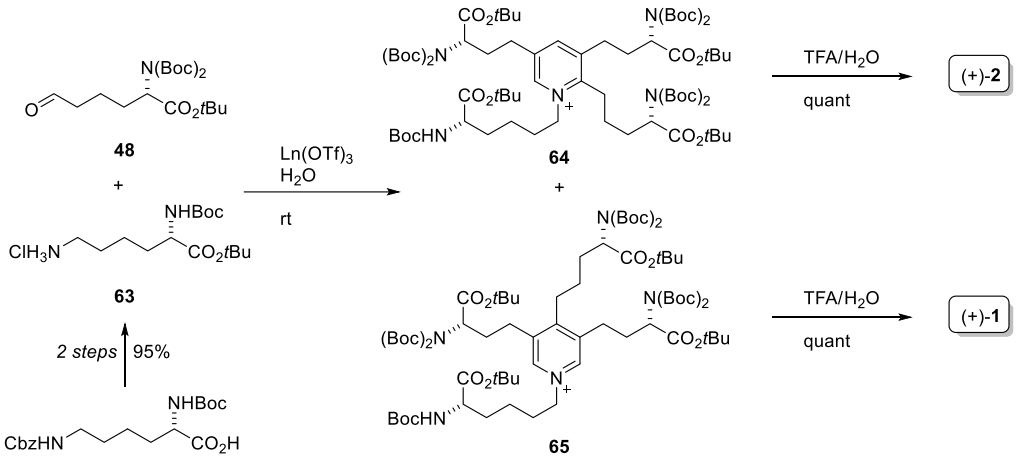
Aldehyde **48** bearing protecting groups for the synthesis of Chichibabin pyridinium was prepared according to Scheme 19. Starting from commercially available 5-benzoxyl-(*S*)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**49**), the carboxyl group was protected with the methyl group using chloroformate to form methyl ester **56** in 99% yield.[57] The Bn ester was then converted to the *tert*-Bu ester by removing the Bn group under hydrogenation conditions with Pd/C and esterification with $(Boc)_2O$ to afford compound **58** in 75% yield. Additional Boc protection of the amine group was carried out with $(Boc)_2O$ to form compound **59** in 82% yield. Reduction of methyl ester by DIBAL-H afforded aldehyde **60** in 98% yield, which was subjected to Wittig reaction to form *exo*-olefin **61** in 77% yield. Hydroboration–oxidation of **61** gave the *anti*-Markovnikov alcohol **62** in 82% yield, and the subsequent DMP oxidation led to the desired aldehyde **48** quantitatively.



Scheme 19. Synthesis of aldehyde **48**

Preparation of amine hydrochloride **63** was carried out from commercially available 6-(benzyloxycarbonyl)amino-2-(*S*)-[(*tert*-butoxycarbonyl)amino]hexanoic acid in 95% yield over two steps.[58] With aldehyde **48** and amine hydrochloride **63** in hand, the lanthanide-promoted Chichibabin pyridine synthesis was examined (Table 1). Reactions between four equivalents of aldehyde **48** and one equivalent of amine hydrochloride **63** were performed with 0.5 equivalent of various lanthanide triflate ($Ln(OTf)_3$)[59] in H_2O for 24 h at room temperature (entries 1–9). Although more than 10 spots were identified in the TLC (CH_2Cl_2/MeOH = 10/1) analysis, both **2**-type pyridinium **64** and **1**-type pyridinium **65** were observed, which could be separated and isolated by column chromatography. Among the examined $Ln(OTf)_3$, $Pr(OTf)_3$ gave the best yields (34% for **64**, 3% for **65**, total 37%). When the reaction was performed with $Sc(OTf)_3$ and $Y(OTf)_3$, typically used Lewis acids in aqueous media, the total yields of **64** and **65** were 10% and 20%, respectively (entries 8 and 9). Considering the trend of Lewis acidity (Sc > Y > Yb > Er > Dy > Gb > Nd > Pr > La),[60] milder Lewis acids were expected to give better yields, probably because the Mannich reaction and aldol condensation are promoted under milder conditions (discussed in Section 5.4). Even though Wang and co-workers reported that only the 1,2,4,5-tetrasubstituted pyridine skeleton was generated in the lanthanide-promoted reaction of hexanal and benzylamine hydrochloride,[56] **1**-type 1,3,4,5-tetrasubstituted pyridines were observed in this case. The seven Boc groups and four *tert*-Bu groups of **64** and **65** were quantitatively removed using TFA to produce **2** and **1**, respectively. As crosslinkers **2** and **1** are formed naturally in elastin in an approximately 1:1 ratio, a route employing $Dy(OTf)_3$, $Sc(OTf)_3$, and $Y(OTf)_3$ as catalysts, which gave a ratio close to 1:1, can be regarded as a biomimetic synthesis.

Table 1. Chichibabin pyridinium synthesis of **64** and **65** with different $Ln(OTf)_3$



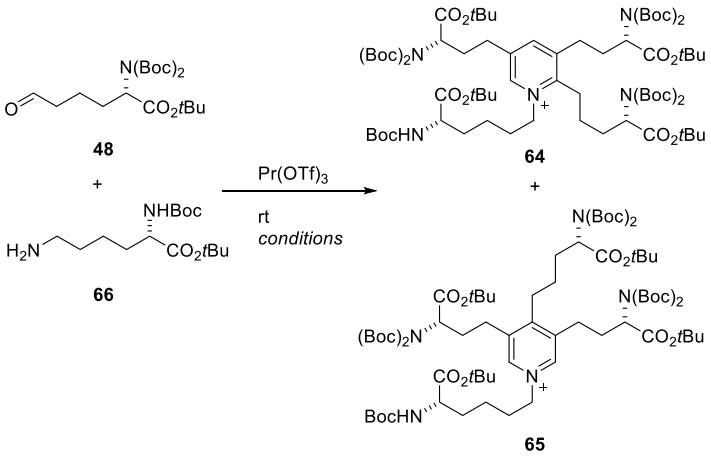
entry[a]	$Ln(OTf)_3$[b]	yield (%)			ratio (**64**/**65**)
		64	**65**	total[c]	
1	La	21	7	28	3
2	Pr	34	3	37	11.3
3	Nd	18	6	24	3
4	Gb	11	5	16	2.2
5	Dy	12	8	20	1.5
6	Er	15[d]	2[d]	17	7.5
7	Yb	16[d]	1[d]	17	16
8	Sc	6[d]	4[d]	10	1.5
9	Y	12	8	20	1.5

[a]Reactions between four equivalents of **48** and one equivalent of **63** were performed in H_2O for 24 h. [b]0.5 equivalent for each reaction. [c]Isolated yield. [d]Determined by ^1H NMR.

Next, optimization of solvent systems was conducted through a protocol utilizing $Pr(OTf)_3$ as a catalyst.[19(b)] It was previously suggested in separate experiments of Chichibabin pyridinium synthesis using $Pr(OTf)_3$ that the solubilities of aldehyde and amine were critical to the success of the reaction, resulting in the discovery of H_2O/MeOH (2/1) that provided 29% of **2**-type product with a trace amount of **1**-type product.[52] Thus, the utility of other water-miscible solvents was first examined as 2:1 mixtures with H_2O (Table 2, entry 1–4). Combinations of H_2O and organic solvents, including THF, dioxane, DMSO, and DMF, gave **64** in 22–35% yield; **65** was obtained in small amounts. Among the tested solvent mixtures, the reaction in H_2O/DMF (2/1) provided **64** in the highest yield (35%, entry 4).

As the solvent ratio of H_2O and DMF at 1/6 gave products with reduced selectivities (entry 5), the mixtures of H_2O and an organic solvent at the same ratio (1/6) were investigated using toluene, dioxane, THF, and $MeNO_2$ (entries 6–9). Although the nonpolar solvent toluene resulted in a low product yield, **1**-type product **65** was obtained as the major product (entry 6). Dioxane gave **65** in 10% yield almost exclusively (entry 7),

Table 2. Chichibabin pyridinium synthesis of **64**/**65** with different solvent systems



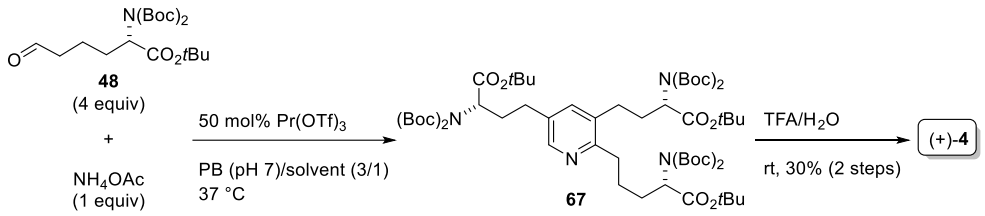
entry	solvent	yield (%)			selectivity	ratio
		64	**65**	total	(**64**/**65**)	(major/minor)
1	H_2O/THF (2/1)	29	2	31	**64**	14.5
2	H_2O/dioxane (2/1)	29	trace	29	**64**	>99
3	H_2O/DMSO (2/1)	22	1	23	**64**	22
4	H_2O/DMF (2/1)	35	trace	35	**64**	>99
5	H_2O/DMF (1/6)	9	4	13	**64**	2.3
6	H_2O/toluene (1/6)	5[a]	13[a]	18	**65**	2.6
7	H_2O/dioxane (1/6)	trace	10	10	**65**	>99
8	H_2O/THF (1/6)	2[a]	2[a]	4	-	1
9	H_2O/$MeNO_2$ (1/6)	2[a]	4[a]	6	**65**	2
10	H_2O/$CHCl_3$ (1/6)	3[a]	11[a]	14	**65**	3.7
11	H_2O/DCE (1/6)	9[a]	21[a]	30	**65**	2.3
12	H_2O/CH_2Cl_2 (1/6)	3[a]	27[a]	30	**65**	9
13[b]	H_2O/CH_2Cl_2 (1/6)	0	27	27	**65**	>99

[a]Determined by ^{1}H NMR analysis.

[b]1.0 g of **48** was used.

while the use of aprotic polar solvents THF and $MeNO_2$ afforded the lowest yields (entries 8 and 9), probably because of the deactivation of $Pr(OTf)_3$ induced by the large donor effects of these solvents.[59] Chlorinated solvents such as $CHCl_3$, DCE, and CH_2Cl_2 were also examined (entries 10–12). Although the use of $CHCl_3$ resulted in a low yield, reactions in DCE and CH_2Cl_2 afforded better results. In particular, **65** was obtained in 27% yield in H_2O/CH_2Cl_2 (1/6) with excellent selectivity, along with 3% of **64** (entry 12). Moreover, gram-scale isoChichibabin pyridinium synthesis proceeded in a selective manner to afford **65** without a detectable amount of **64** (entry 13).[48] Because the selectivity of the reaction is sensitive to the solvent, it is possible that volatile CH_2Cl_2 and the biphasic organic–aqueous system affected the selectivity in small-scale synthesis. Notably, the present selective reaction gave an isoChichibabin pyridinium product with a 1,3,4,5-tetrasubstituted pyridinium core, in contrast to the conventionally obtained Chichibabin pyridinium product with a 1,2,3,5-tetrasubstituted pyridinium core.

Following the established $Pr(OTf)_3$-assisted isoChichibabin pyridinium synthesis, isodesmopyridine (**4**) was synthesized starting from protected allysine **48** and NH_4OAc, as shown in Scheme 20.[22] The reaction between four equivalents of **48** and one equivalent of NH_4OAc in a mixture of pH 7 phosphate buffer (PB) and CH_2Cl_2 successfully proceeded to form isoChichibabin pyridinium product **67**, followed by the deprotection of Boc groups and *tert*-Bu groups using TFA to provide isodesmopyridine (**4**) in 30% yield in two steps.

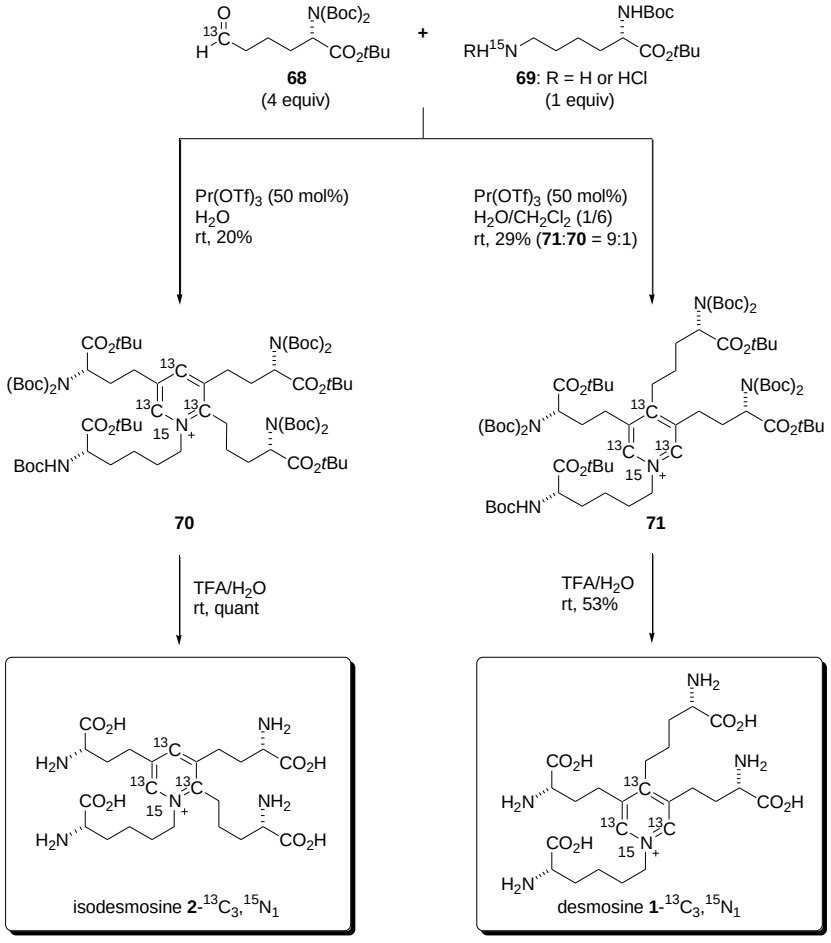


Scheme 20. Total synthesis of isodesmopyridine (**4**)

5.3 Synthesis of [$^{13}C_3$,$^{15}N_1$]-Labeled Desmosine/Isodesmosine

Although the deuterated desmosines (**1-*d*$_4$**, **1-*d*$_{(2+2)}$**, and **1-*d*$_8$**) were stable and retained their structures under acid hydrolysis conditions, the distributions of deuteration ratios were observed in the mass spectrum analyses.[25] The synthesized **1-*d*$_4$** involved complex multistep reactions and resulted in reduced deuterium isotope purity (88%) because of deuterium atom scrambling during the reactions, while **1-*d*$_{(2+2)}$** and **1-*d*$_8$** also had isotopic distributions. However, precise LC–MS/MS analysis of **1** and **2** during elastin degradation requires isotopically pure internal standards. To overcome this problem, [$^{13}C_3$,$^{15}N_1$]-labeled desmosine/isodesmosine, which does not have an isotopic distribution, was synthesized via the Chichibabin/isoChichibabin pyridinium synthesis.[26]

After obtaining ^{13}C-labeled aldehyde **68** and ^{15}N-labeled amine hydrochloride **69**, Chichibabin pyridine synthesis using $Pr(OTf)_3$ in H_2O was performed to form the protected isodesmosine **70** in 20% yield, where no **1**-type 1,3,4,5-tetrasubstituted pyridine with isotope labeling was obtained (Scheme 21).[26(a)] Then, Boc groups and *tert*-Bu groups were removed quantitatively using TFA to provide [$^{13}C_3$,$^{15}N_1$]-labeled isodesmosine **2-$^{13}C_3$,$^{15}N_1$**, which showed an isotopically pure mass spectrum obtained by ESI-HRMS analysis. In the same way, isoChichibabin pyridinium synthesis using $Pr(OTf)_3$ in H_2O/CH_2Cl_2 (1/6) was conducted to form 1,3,4,5-tetrasubstituted pyridinium **71** in 29% yield. The subsequent global deprotection of the Boc groups and *tert*-Bu groups in TFA produced [$^{13}C_3$,$^{15}N_1$]-labeled desmosines **1-$^{13}C_3$,$^{15}N_1$** in 53% yield.[26(b)]



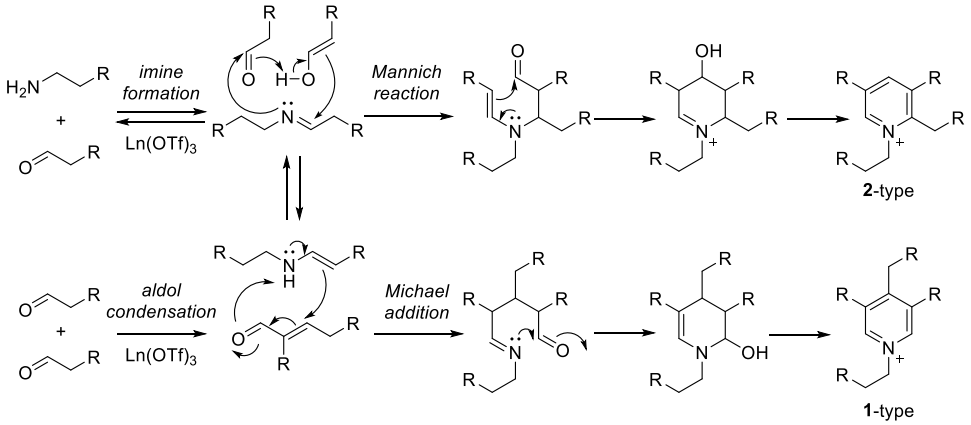
Scheme 21. Synthesis of isodesmosine **2-$^{13}C_3$** and desmosine **1-$^{13}C_3$,$^{15}N_1$**

The obtained [$^{13}C_3$,$^{15}N_1$]-labeled desmosine (**1-$^{13}C_3$,$^{15}N_1$** or isodesmosine **2-$^{13}C_3$,$^{15}N_1$**) was used as an internal standard for isotope-dilution LC–MS/MS analysis of **1** and **2** in the human skin.[26(b)] The analysis of the human skin indicated that the epidermis and fat layer contained almost no **1** and **2**, whereas they were clearly detected in the dermis. Furthermore, the first quantitative analysis of **1** and **2** in the human skin dermis using LC–MS/MS was reported, where approximately 1.43 μg/mg of **1** and **2** (based on 1 mg of dry

human skin) was observed. This is the first report to demonstrate the quantitative analysis of elastin-crosslinker desmosines in the dermis of the human skin. In addition, quantitative analysis of desmosines in the plasma of stroke patients, food materials of skipjack tsuna "Katsuo" elastin, and clinical samples of spinal stenosis patients was conducted.[61]

5.4 Mechanistic Insights

The mechanism of the Chichibabin reaction is shown in Scheme 22 based on the proposed pathway for the formation of elastin crosslinkers reported by Anwar and Suyama.[62,63] For the **2**-type product, imine is formed from amine and aldehyde prior to the Mannich reaction, which is followed by intramolecular cyclization to form an iminium product. Dehydration and aromatization afforded **2**-type product. Polar solvent systems, such as H_2O/DMF (2/1), may contribute to the stabilization of iminium intermediates. When the reaction was performed in only H_2O, **1**-type compound was obtained as a minor product (Table 1, entry 1), suggesting that the enamine would be dissolved in the substrate aldehyde phase, which resulted in a reaction with aldol condensates to form **1**-type product. Application of the solvent system of H_2O/DMF (2/1) would easily reverse the enamine, which can be dissolved in the solvents, back to imine or aldehyde and amine, preventing the generation of **1**-type product. In contrast, for the formation of the **1**-type product, aldol condensation with two aldehydes proceeds first to form α,β-unsaturated aldehyde, followed by Michael addition with enamine and subsequent cyclization. Dehydration and aromatization gave **1**-type products. The best selectivity ratio observed in the solvent system of H_2O/CH_2Cl_2 (1/6) can be explained as follows: a smaller amount of H_2O would prevent the hydrolysis of imine and promote the formation of enamine, which is easily soluble in CH_2Cl_2.

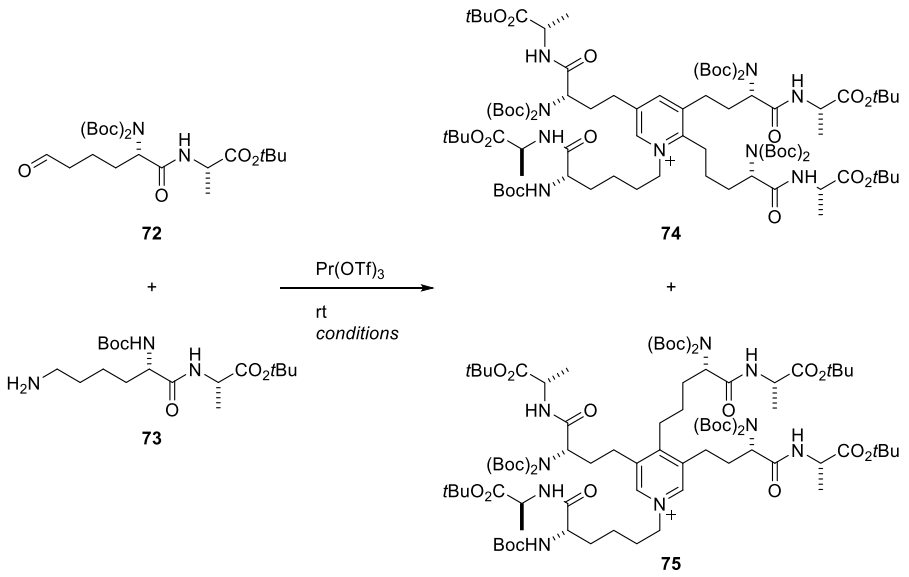


Scheme 22. Proposed mechanisms for the formation of **2**-type and **1**-type products

To further understand the reaction mechanism, Chichibabin pyridinium synthesis of allysine-Ala **72** and lysine-Ala **73** was examined (Table 3).[19(b)] When Chichibabin pyridinium synthesis of **72** and **73** was

carried out using $Pr(OTf)_3$ in H_2O/DMF (2/1), **2**-type product **74** and **1**-type product **75** were obtained in 13% and 8% yields, respectively, whereas the reaction in H_2O/CH_2Cl_2 (1/6) afforded **74** and **75** in 24% and 22% yields, respectively, in a similar manner. The obtained ratios of **74** and **75** were approximately 1:1 in both cases, similar to the ratio of **2** and **1** in natural elastin.[13] This result suggests that there may be a correlation between the number of hydrogen bonds in a substrate or the solubility (hydrophobicity or hydrophilicity) of the substrate and the ratio of the products obtained by Chichibabin/isoChichibabin pyridinium syntheses. It has also been suggested that the characteristics of allysine-Ala and lysine-Ala peptide substrates are similar to those of natural tropoelastin, which enable the imitation of *in vivo* crosslinking formation with a mixture of **2**-type and **1**-type products.

Table 3. Chichibabin pyridine synthesis of **74**/**75** with different solvent systems



entry	solvent	yield (%)			selectivity	ratio
		74	**75**	total	(**74**/**75**)	(major/minor)
1	H_2O/DMF (2/1)	13	8	21	**74**	1.6
2	H_2O/CH_2Cl_2 (1/6)	24	22	46	**75**	1.1

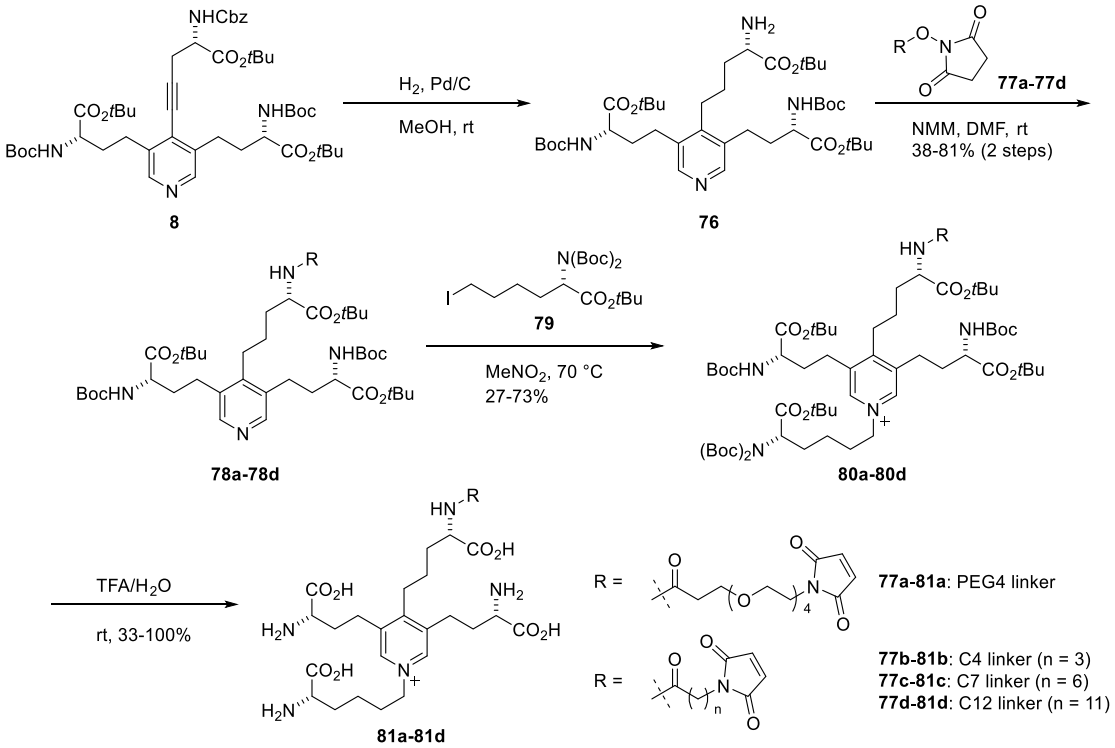
6. Synthesis of Carrier Protein Conjugate

6.1 Synthesis of Desmosine–BSA/KLH Conjugate

LC–MS or LC–MS/MS analysis requires expertise and highly specialized and expensive equipment. Hence, an enzyme-linked immunosorbent assay (ELISA) system has been proposed as an alternative method for the analysis of **1**. ELISA systems are plate assay methods that can detect certain compounds, including

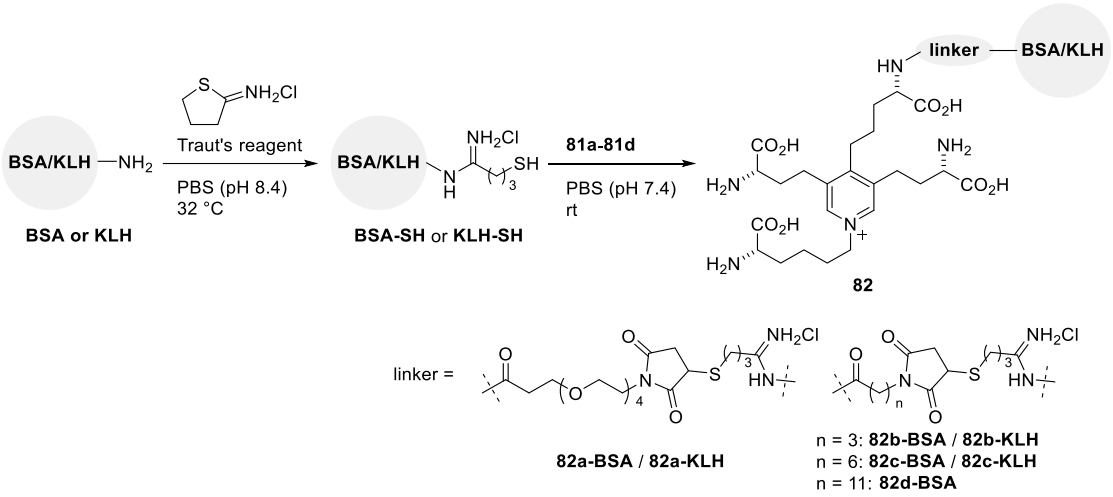
antigens and proteins, with high sensitivity and selectivity in an easy and affordable manner. These systems require mono- or polyclonal antibodies capable of detecting the antigen of interest with high specificity; however, the small molecular weight (526 Da) of **1** is not readily amenable to acting as an epitope for antibodies.[64] Hence, it is necessary to conjugate a carrier protein to **1** so that it can have immunogenicity to acquire antibodies with higher sensitivity and selectivity. The antigen was designed with a linker at the 4-position of the pyridine ring to expose the pyridinium moiety. In addition to keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) was selected as a carrier protein for ELISA.

Synthesis of desmosine conjugates with BSA or KLH is outlined in Scheme 23, which relies on chemo- and regioselective cross-coupling reactions.[27] Trisubstituted pyridine **8** was prepared following the synthesis route described in Scheme 6, where a Cbz-protected terminal alkyne was used instead of **11**. After selective deprotection of the Cbz group and reduction of the alkyne in **8** under hydrogen atmosphere, the resulting amine **76** and maleimide linkers **77a**–**77d** were coupled to form **78a**–**78d** in 38–81% yields over two steps. Subsequently, **79** was attached to **78a**–**78d** via *N*-alkylation to afford pyridiniums **80a**–**80d** in 27–73% yields. Removal of the Boc and *tert*-Bu groups of **80a**–**80d** under acidic conditions gave linker-attached desmosines **81a**–**81d** in 33–100% yield as a single diastereomer.



Scheme 23. Synthesis of desmosine with maleimide linkers **81a**–**81d**

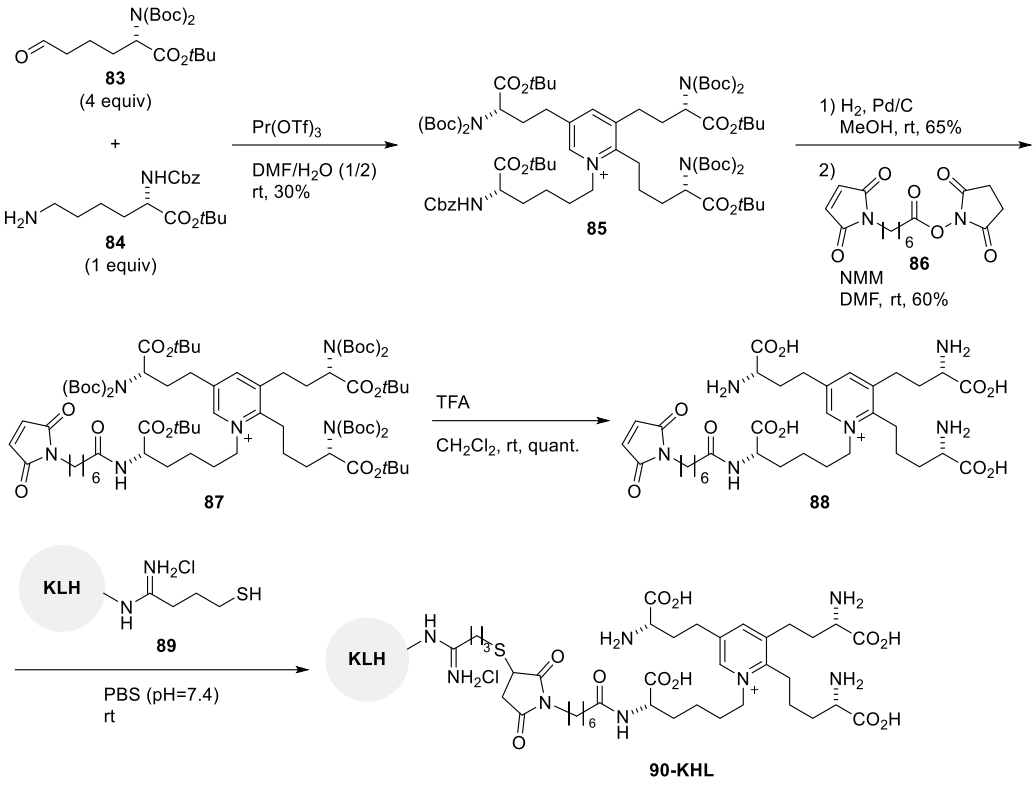
Next, thiolation of carrier proteins BSA and KLH was conducted using Traut's reagent in phosphate-buffered saline (PBS) to obtain BSA-SH and KLH-SH, respectively, followed by Michael addition with the maleimide linkers of **81a**–**81d** to form the corresponding conjugates **82a**–**82d** (Scheme 24).[65] Mass spectrometric analysis of **82b-BSA** by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) measurement showed a peak of 69,546 Da as the average value, while the average MS of BSA was 66,952 Da, which suggests that approximately three desmosines with a C4 linker were attached to BSA based on the mass of desmosine with a linker (795 Da). In contrast, in the case of **82a-BSA**, approximately six desmosines with PEG4 linkers were attached to BSA, as determined by MALDI-TOFMS analysis. As a result, seven types of desmosine–BSA/KLH conjugates with different maleimide linkers (**82a-BSA/82a-KLH**, **82b-BSA/82b-KLH**, **82c-BSA/82c-KLH**, and **82d-BSA**) were successfully prepared.



Scheme 24. Synthesis of a series of desmosine–BSA/KLH conjugates **82**

6.2 Synthesis of Isodesmosine–KLH Conjugate

Based on Chichibabin pyridinium synthesis, a selective sulfhydryl-based linkage at the 1-position of isodesmosine (**2**) was accomplished that enabled full exposure of hapten.[28] As described in Scheme 25, the Chichibabin pyridinium synthesis of **83** and **84**, where the Cbz group was used as a protecting group for the amine, was conducted in the presence of $Pr(OTf)_3$ in H_2O/DMF (2/1) to form the desired tetrasubstituted pyridinium **85** in 30% yield. Then, selective deprotection of the Cbz group in **85** under hydrogen atmosphere gave the free amine in 65% yield, which was treated with maleimide segment **86** to obtain **87** in 60% yield. After the deprotection of the Boc and *tert*-Bu groups in isodesmosinemaleimide intermediate **87**, **88** was subjected to Michael addition with thiolated KLH protein **89** to provide isodesmosine–KLH conjugate **90-KLH**.



Scheme 25. Synthesis of a series of isodesmosine–KLH conjugates **90-KLH**

7. Conclusion

This review summarizes the synthesis of desmosine (**1**) and its analogs (**2**–**6**) by focusing on cross-coupling strategies and Chichibabin/isoChichibabin pyridinium synthesis. A series of desmosine-related compounds, obtained using these strategies, are reported, including the synthesis of isotopically labeled desmosines, desmosine/isodesmosine-Ala, isodesmosine–KLH conjugate, and desmosine–BSA/KLH conjugate. This review also provides mechanistic insights into the biological functions of elastin, which may help in the development of novel diagnostic and therapeutic methodologies for elastin-degradation-related diseases.

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Hiroaki Tanaka received his B.Eng. and M.Eng. degrees from Waseda University, Tokyo, Japan, in 2008 and 2010 under the supervision of Professor Kuniaki Tatsuta. Afterwards, he joined Astellas Pharma Inc. and had worked as a medicinal chemist for 9 years. In 2019, he joined Fujifilm Corporation and has been working as a chemist for 4 years. He is currently a Ph.D. candidate at Sophia University, Tokyo, Japan under the supervision of Professor Toyonobu Usuki while working at Fujifilm Corporation. His research interests include medicinal chemistry, peptide synthesis, conjugation chemistry and fluorescent dyes for diagnostic use.

Toyonobu Usuki obtained his B.S., M.S., and Ph.D. degrees in chemistry under the supervision of Professor Masahiro Hirama and Professor Masayuki Inoue from Tohoku University, Sendai, Japan, in 2000, 2002, and 2005, respectively. After postdoctoral studies in the laboratory of Professor Koji Nakanishi at the Department of Chemistry, Columbia University, New York, USA, from 2005 to 2007 as a JSPS Postdoctoral Fellow for Research Abroad, he joined the Department of Materials and Life Sciences, Sophia University, Tokyo, Japan, as a Principal Investigator in 2008. Currently, he is a Professor at the same university. In 2022, he was a Visiting Professor at Fraunhofer IMWS Halle (Saale), Germany, with Professor Christian Schmelzer. His research interests include natural products chemistry, organic chemistry, medicinal chemistry, bioorganic chemistry and elastin chemistry.