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MIDDLE-SCALE ISOCHICHIBABIN DESMOSINE SYNTHESIS

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Abstract – Desmosine is a 1,3,4,5-tetrasubstituted pyridinium amino acid that crosslinks elastin, and is considered to be a useful biomarker for elastin-degraded diseases such as chronic obstructive pulmonary disease (COPD). In this work, starting from the corresponding aldehyde and amine, the isoChichibabin pyridinium synthesis of protected desmosine was achieved on a middle-scale in H_2O/CH_2Cl_2 (1/6) with $Pr(OTf)_3$. A shorter route for the synthesis of the aldehyde was also developed.

Desmosine (**1**) and isodesmosine (**2**), shown in Figure 1, are 1,3,4,5- and 1,2,3,5-tetrasubstituted pyridiniums, which exist in elastin in an approximately 1:1 ratio as crosslinking amino acids.[1,2] Elastin is an insoluble protein located in the extracellular matrix, with the essential role of expressing elasticity and resilience of internal organs and tissues such as ligaments, skin, lungs, etc.[3] These crosslinking pyridinium amino acids are formed via oxidative deamination of lysine in soluble tropoelastin, the monomer of elastin, by lysyl oxidase, followed by a sequence of dehydration, condensation, aromatization with imine formation, Michael addition, and Mannich reaction.[4,5]

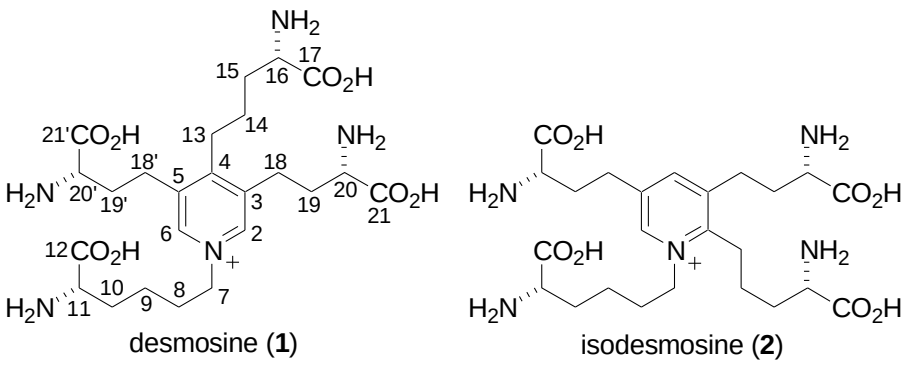


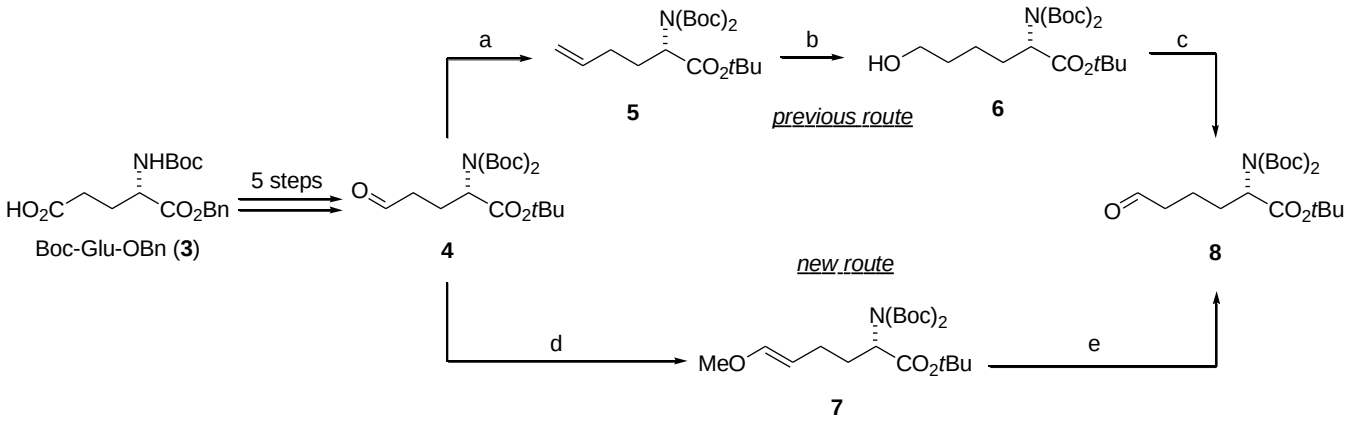
Figure 1. Structures of desmosine (**1**) and isodesmosine (**2**)

Recent studies suggest that **1** and **2** are useful biomarkers for several diseases related to elastin degradation, such as chronic obstructive pulmonary disease (COPD)[6,7] and atherosclerosis.[8] COPD is the 3rd leading cause of death worldwide, but a fundamental remedy or therapeutic medication of the disease has not been established yet. Desmosine **1** has been expected to be a biomarker of COPD, and can be detected by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Clinical, pharmacological, and medicinal research, along with drug discovery for treating these diseases, require a continuous chemical supply of the desmosines, including isotopically labeled compounds. In addition to this, we recently achieved the quantitative LC-MS/MS analysis of desmosine **1** and isodesmosine **2** in human skin[9] and stroke patient's plasma samples.[10]

For the past ten years, we have explored the total synthesis of **1** and **2** as well as isotopically labeled compounds utilizing either stepwise and regioselective palladium-catalyzed cross-coupling reactions[11-13] or biomimetic Chichibabin pyridinium synthesis.[14] $La(OTf)_3$-promoted Chichibabin pyridinium synthesis,[15] which was modified from the original reaction conditions that form 2,3,5-trisubstituted pyridines from aldehydes and ammonia (Chichibabin pyridine synthesis),[16] was achieved starting from the corresponding aldehyde and amine hydrochloride.[14]

The selective synthesis of **1**-type and **2**-type products was achieved in different solvent systems. The synthesis using protected allysines and lysine with 50 mol% $Pr(OTf)_3$ in H_2O/DMF (2/1) afforded only protected isodesmosine (**2**-type). In contrast, the selective synthesis in H_2O/CH_2Cl_2 (1/6) gave protected desmosine (**1**-type) in 29% yield along with 2% of **2**-type.[14] We named this reaction the "isoChichibabin pyridinium synthesis". In this study, isoChichibabin "desmosine" synthesis on a middle-scale along with modified synthesis of the allysine aldehyde are reported.

In our previous synthesis, as shown in Scheme 1, protected allysine aldehyde **8** was obtained in eight steps from commercially available 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanedionic acid 1-benzyl ester **3** (*N*-Boc-Glu-OBn).[14] The synthesis involved a Wittig reaction of aldehyde **4** to form terminal olefin **5** in 77% yield, which underwent hydroboration and double oxidation in 82% yield. In this study, optimization of the substrate in the Wittig reaction of **4** was performed to reduce the synthetic steps. As a result, $MeOCH_2PPh_3Cl$ with lithium hexamethyldisilazane (LiHMDS) was used for the reaction instead of $MePPh_3Br$ to give enol ether **7** in 78% yield.[17] **7** was then acidified with 2 M HCl to give desired aldehyde **8** in 95% yield,[17] which is the substrate for the isoChichibabin pyridinium synthesis. It is worth noting that this route is shorter than the previous route and requires only one simple step after the Wittig reaction. Over 4 g of **8** was thus obtained using the modified route. The overall yield and steps from substrate **3** to **8** were improved from 38% in eight steps[14a] to 57% in seven steps. The optical rotation values of **8** via these two routes were in good agreement (previous route: $[\alpha]_D^{20}$ -192 (*c* 0.1, MeOH); current route: $[\alpha]_D^{20}$ -229 (*c* 0.1, MeOH)).



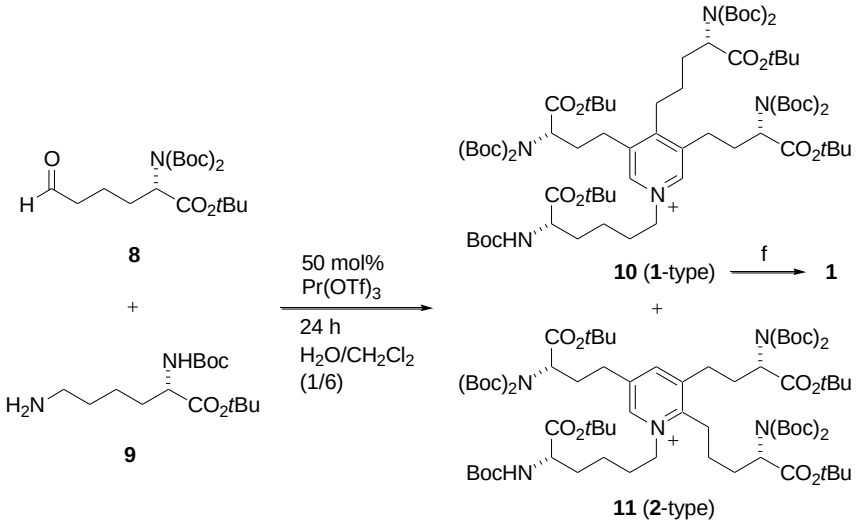
Scheme 1. Previous and modified syntheses of aldehyde **8**. Reagents and conditions: previous route, a) $MePPh_3Br$, *n*BuLi, THF, 77%; b) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, then NaOH, H_2O_2, 82%; c) DMP, CH_2Cl_2, quant; current route, d) $MeOCH_2PPh_3Cl$, LiHMDS, THF, 78%; e) 2 M HCl, THF, 95%.

Next, we conducted the isoChichibabin pyridinium synthesis on different scales starting from prepared **8** and **9**[14] using 50 mol% $Pr(OTf)_3$ in H_2O/CH_2Cl_2 (1/6) for 24 hours (Table 1). Although 0.3 g of **8**, which is five times more than the previous attempt (entry 0), gave a similar total yield and selectivity (entry 1), middle-scale isoChichibabin pyridinium synthesis proceeded in a selective manner to afford **1**-type product **10** (entry 2) in 36% yield without a detectable amount of **2**-type product **11**. Finally, global deprotection of the obtained **10** was performed to give **1**.

A detailed reaction mechanism of the synthesis of **10** (**1**-type product) and **11** (**2**-type product) is shown in Scheme 2 based on the proposed pathway of formation of elastin crosslinkers[18] as well as a model substrate study.[19] In addition, we have previously reported that the preference for certain pyridinium types changes depending on the solvent system.[19] Since the selectivity of the reaction is sensitive to solvent, it is possible that volatile CH_2Cl_2 and the biphasic organic-aqueous system affected the selectivity in the small scale synthesis. Therefore, further optimization of the solvent system is needed to better understand the reaction mechanism for the multigram-scale synthesis of **1**.

In summary, starting from the corresponding allysine aldehyde **8** and amine **9**, we achieved the middle-scale isoChichibabin protected demosine **10** synthesis using 50 mol% $Pr(OTf)_3$ in H_2O/CH_2Cl_2 (1/6) from 1 g of **8**, which is seventeen times more than the previous synthesis. Moreover, we confirmed that only 1,3,4,5-tetrasubstituted pyridinium **10** was obtained selectively on this scale, whereas the small scale isoChichibabin pyridinium synthesis gave both **10** and 1,2,3,5-tetrasubstituted pyridinium **11**. The optimization of the substrate in the Wittig reaction reduced the synthetic steps toward **8**, which enabled an efficient synthesis of desmosine **1**. Finally, the overall yield from **3** was increased from 38% in eight steps to 57% in seven steps.

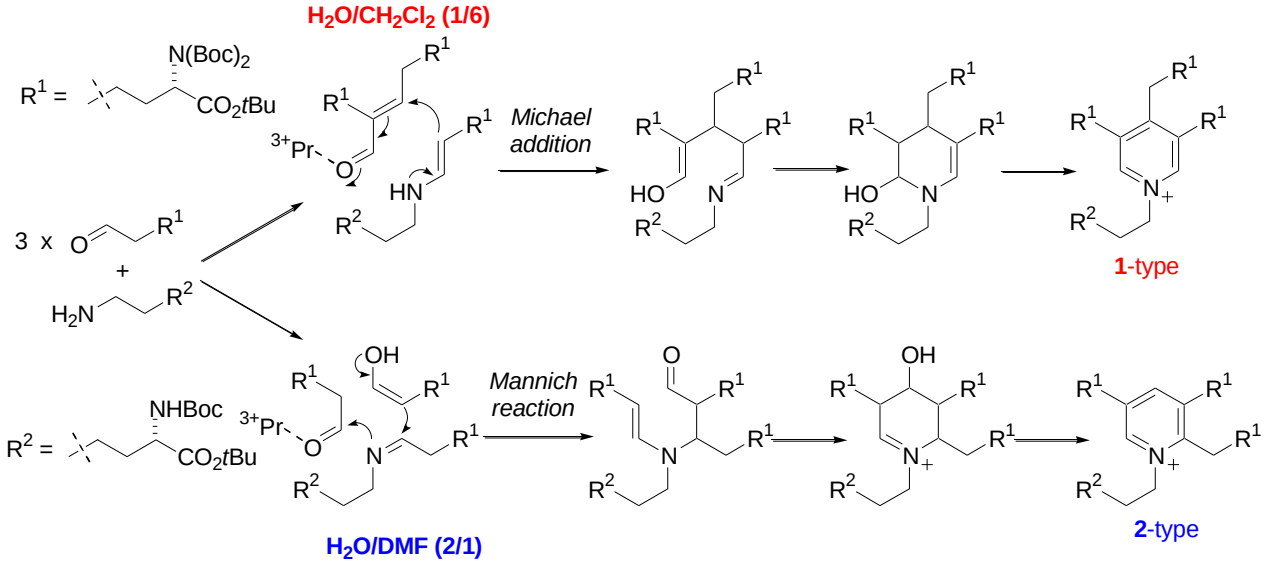
Table 1. IsoChichibabin pyridinium synthesis of **8** and **9** to produce **10** and **11**. Reagents and conditions: f) TFA, H_2O, 99%.



Entry	Scale (g)		Yield (%)			Selectivity (**10**/**11**)[a]
	8	**9**	**10**	**11**	total	
0[b]	0.06	0.01	29	2	31	14.5
1	0.3	0.056	29	4	33	7.5
2	1.0	0. 188	36	0	36	**10** only

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

[b] Data of ref 14d.



Scheme 2. Proposed reaction mechanisms of formation of **1**-type (**10**) and **2**-type (**11**) products

EXPERIMENTAL

General procedures:

All non-aqueous reactions were conducted under an atmosphere of nitrogen with magnetic stirring unless otherwise indicated. With regards to reactions conducted at room temperature, the established range includes temperatures between 24 and 28 °C. Solvents, such as dichloromethane (CH_2Cl_2), and tetrahydrofuran (THF), were purchased from commercial suppliers and stored over activated molecular sieves. All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates produced by Merck. Column chromatography was performed with acidic Silica gel 60 (spherical, 40-50 μm) or neutral Silica gel 60N (spherical, 40-50 μm) produced by Kanto Chemicals (Tokyo, Japan). Removal of small amounts of solvent was performed by a Smart Evaporator CEV1B-SQ/SU/GR/SK-V1A-GR-P2 produced by BioChromato (Kanagawa, Japan).

Optical rotations were measured on a JASCO P-2200 digital polarimeter at the sodium lamp (λ = 589 nm) D line and are reported as follows: $[\alpha]_D^T$ (*c* g/100 mL, solvent). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA 500 spectrometer (500 MHz). ^{1}H NMR data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (*J*) in Hz, assignments. ^{13}C NMR data are reported in terms of chemical shift (δ, ppm). Electrospray ionization-mass spectrometer (ESI-MS) spectra with time-of-flight (TOF) detection for high resolution measurements were recorded on a JEOL JMS-T100LC instrument and are reported as mass-to-charge ratios (*m*/*z*).

16-(*S*)-[Bis-(*tert*-butoxycarbonyl)amino]-13-en-12-methoxyhexanoic acid *tert*-butyl ester (7):

To a stirred suspension of (methoxymethyl)triphenylphosphonium chloride (3.10 g, 9.039 mmol) in anhydrous THF (72 mL) at -78 °C was added dropwise LiHMDS (1.3 M in THF, 6.26 mL). The reaction mixture was stirred at 0 °C for 10 min and then cooled again to -78 °C, and aldehyde **4** (1.401 g, 3.616 mmol) in anhydrous THF (33.8 mL) was added. The reaction mixture was warmed to room temperature and stirred overnight. Saturated aqueous NH_4Cl (30 mL) was added, and the aqueous layer was extracted with Et_2O (70 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by silica gel column chromatography (hexane/EtOAc = 20:1) afforded **7** (1.1757 g, 2.83 mmol, 78%) as a colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.32-6.29 (1H, d, *J* = 12.6 Hz, CH), 4.78-4.72 (1H, m, CH), 4.71-4.62 (1H, m, CH), 3.52-3.45 (3H, s, CH_3), 1.72-1.60 (4H, m, CH_2CH_2), 1.50 (18H, s, *t*Bu), 1.43 (9H, s, *t*Bu); ^{13}C NMR (125 MHz, $CDCl_3$)

δ 170.1, 152.6, 147.9, 101.6, 82.8, 81.2, 58.3, 55.9, 30.6, 28.2, 28.1, 24.8; ESI-HRMS (*m*/*z*) calcd for $C_{75}H_{128}N_5O_{22}Na$ $[M+Na]^+$ 438.2468, found 438.2474.

16-(*S*)-[Bis-(*tert*-butoxycarbonyl)amino]-2-oxohexanoic acid *tert*-butyl ester (8):

The reaction was conducted on scales from 0.136-3.87 g (91-95%) and a representative procedure follows. 2 M HCl (1.56 mL) and anhydrous THF (10.93 mL) were added to **7** (136 mg, 0.328 mmol). After stirring at room temperature for 42 h, the reaction mixture was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by silica gel column chromatography (hexane/EtOAc = 4:1) afforded **8** (124.6 mg, 0.310 mmol, 95%) as a colorless oil. R_f 0.60 (hexane/EtOAc = 2:1); $[\alpha]_D^{20}$ -229 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (1H, t, *J* = 1.7 Hz, CHO), 4.70 (1H, dd, *J* = 9.7, 5.2 Hz, CH), 2.56-2.41 (2H, m, CH_2), 2.12-2.03 (1H, m, CH), 1.96-1.87 (1H, m, CH), 1.74-1.63 (2H, m, CH_2), 1.51 (18H, s, *t*Bu), 1.45 (9H, s, *t*Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.4, 170.0, 152.9, 83.3, 81.7, 58.9, 43.8, 29.0, 28.4, 28.3, 19.4 ; ESI-HRMS (*m*/*z*) calcd for $C_{75}H_{128}N_5O_{22}Na$ $[M+Na]^+$ 424.2199, found 424.2225.

2-{16-(*tert*-Butoxycarbonyl)-16-(*S*)-[bis-(*tert*-butoxycarbonyl)amino]butyl}-3,5-bis-{20,24-(*tert*-butoxycarbonyl)-20,24-(*S*)-[bis-(*tert*-butoxycarbonyl)amino]propyl}-1-{11-(*tert*-butoxycarbonyl)-11-(*S*)-[(*tert*-butoxycarbonyl)amino]pentyl}pyridinium (10):

H_2O (1.11 mL), $Pr(OTf)_3$ (0.183 g, 0.311 mmol, 50 mol%), and a solution of **8** (1.0 g, 249 mmol, 4.0 equiv) in CH_2Cl_2 (6.23 mL) were added to a solution of **9** (0.188 g, 0.623 mmol, 1.0 equiv) in CH_2Cl_2 (2.0 mL). After stirring for 24 h at room temperature, the reaction mixture was diluted with EtOAc (10 mL) and H_2O (5.0 mL). The aqueous layer was then extracted with EtOAc (30.0 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by neutral silica gel column chromatography (hexane/EtOAc/MeOH = 1:1:0, 0:1:0, 0:10:1) afforded **10** (332.7 mg, 0.223 mmol, 36%) as a yellow oil; **10**: R_f 0.18 (CH_2Cl_2/MeOH = 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (2H, s, H2/6), 5.20-5.18 (1H, m, NH), 4.71-4.50 (5H, m, H7/16/20/20'), 4.13-4.12 (1H, m, H11), 3.08-2.88 (6H, m, H13/18/18'), 2.42-2.35 (2H, m, H19/19'), 2.27-2.20 (1H, m, H15), 2.10-2.03 (5H, m, H10/15/19/19'), 1.85-1.67 (6H, m, H8/9/14), 1.51-1.48 (54H, s, $(Boc)_2$), 1.46-1.43 (45H, s, *t*Bu, NHBoc); ESI-HRMS (*m*/*z*) calcd for $C_{75}H_{128}N_5O_{22}$ $[M]^+$ 1450.9051, found 1450.9029.

4-[16-(*S*)-Amino-16-carboxybutyl]-1-[11-(*S*)-amino-11-carboxypentyl]-3,5-bis-[20,20'-(*S*)-amino-20,20'-carboxypropyl]pyridinium, **desmosine (1):**

TFA and distilled water (1.850 mL, TFA/water = 95:5) were added to **10** (212.8 mg, 0.1427 mmol, 1.0 equiv) at room temperature and the mixture was stirred for 3 h. The solvent was removed by rotary evaporator. Purification by C18 silica gel column chromatography (0.1% TFA in distilled water) yielded desmosine **1** as a colorless solid (74 mg, 0.141 mmol, 99%); R_f 0.22 [MeOH (0.1% TFA)/H_2O (0.1% TFA) = 1:9]; ^{1}H NMR (D_2O, 500 MHz) δ8.50 (2H, s, H2/6), 4.45 (2H, t, J = 7.5 Hz, H7), 3.96 (2H, m, H20/20'), 3.90 (1H, m, H16), 3.84 (1H, m, H11), 3.00 (2H, m, H13), 2.85 (4H, m, H18/18'), 2.15 (4H, m, H19/19'), 2.05 (2H, m, H8/15), 1.97 (2H, m, H10), 1.87 (2H, m, H23), 1.55-1.34 (4H, m, H9/14).

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