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$Pr(OTf)_3$-promoted Chichibabin pyridine synthesis of isodesmosine in H_2O/MeOH

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ABSTRACT

1,2,3,5-Tetrasubstituted pyridinium amino acid isodesmosine is a crosslinking amino acid of elastin and is an attractive biomarker for the diagnosis of chronic obstructive pulmonary disease (COPD). Herein, we report an application of the Chichibabin pyridine synthesis to the total synthesis of isodesmosine. Specifically, the appropriate protected lysine and the corresponding aldehyde were reacted using $Pr(OTf)_3$ in H_2O/MeOH.

In 1905, Chichibabin reported the thermal cyclo-condensation between 1 equiv of ammonia and 3 equiv of aldehyde to form 2,3,5-trisubstituted pyridines.[1] The Chichibabin pyridine synthesis, however, required intense conditions, such as high pressures, high temperatures, and long reaction times.[1,2] In 1997, Wang and co-workers reported that the use of lanthanide trifluoromethanesulfonates (triflates), $Ln(OTf)_3$, as Lewis acids promoted the condensation of amine hydrochlorides and aldehydes for the preparation of 1,2,3,5-tetrasubstituted dihydropyridinium and pyridinium derivatives.[3] In contrast to the original Chichibabin pyridine synthesis, this reaction proceeded at room temperature in aqueous media to give the corresponding products.

Isodesmosine (**1**, Fig. 1) and desmosine (**2**) are 1,2,3,5- and 1,3,4,5-tetrasubstituted pyridinium amino acids, respectively, that are found only in the elastin matrix.[4] It has been proposed that the formation of the crosslinkers **1** and **2** occurs spontaneously via Mannich reactions and aldol condensations after oxidative transformation of the lysine residues of tropoelastin by lysyl oxidase.[5] Elastin, the main component of elastic fibers, is an insoluble extracellular matrix protein that consists of soluble precursor tropoelastin monomers connected in a sophisticated three-dimensional crosslinked network by desmosines.[6,7]

The irreversible degradation of elastin that occurs in chronic obstructive pulmonary disease (COPD) is found to give rise to the elastin crosslinkers **1** and **2**.[8,9] COPD is known as 'tobacco disease' and is currently the fourth leading cause of death worldwide. Both isodesmosine **1** and desmosine **2** can be measured specifically and sensitively in clinical samples, such as plasma, urine, sputum, and bronchoalveolar lavage fluid (BALF), using liquid chromatography–mass spectrometry (LC–MS or LC–MS/MS). Therefore, desmosines are attractive biomarkers for both drug discovery and the rapid diagnosis of COPD.

We recently reported the total synthesis of **2** starting from 4-hydroxypyridine or 3,5-dibromopyridine.[10] We also reported the total synthesis of both **1** and **2** using a room temperature Chichibabin pyridine synthesis with $Ln(OTf)_3$ in H_2O.[11] These syntheses were achieved starting from amine hydrochloride and aldehyde, and the poor solubility of the amine hydrochloride in H_2O resulted in low yields for these reactions. It was envisaged that the addition of organic solvents such as methanol (MeOH) would enhance the solubility of the substrates and potentially afford better yields of the products. It was also thought that the Chichibabin pyridine

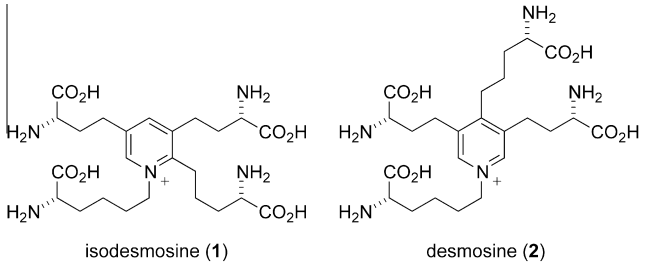

Figure 1. Structures of isodesmosine (**1**) and desmosine (**2**).

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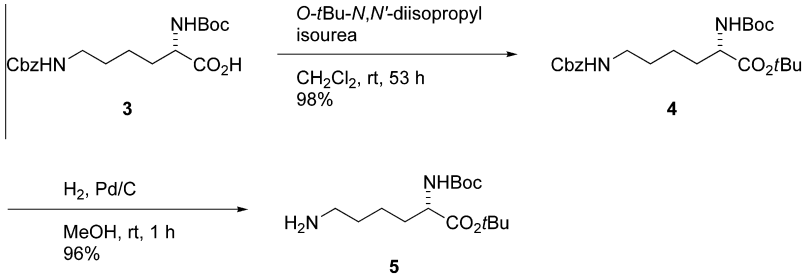
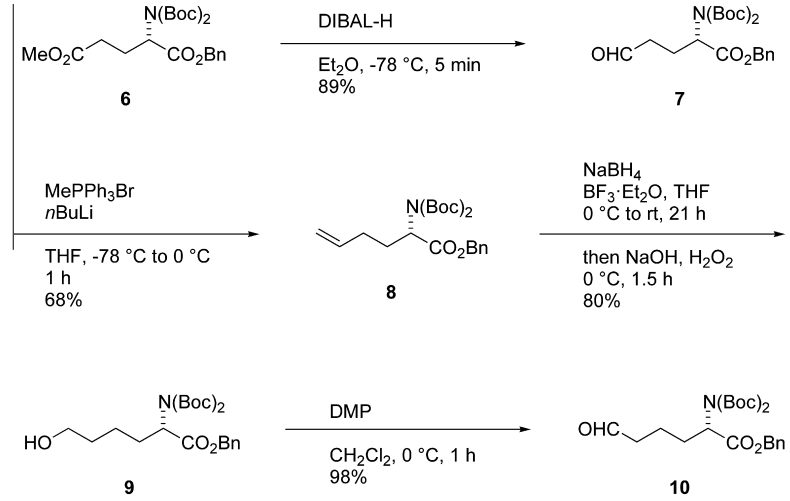


Scheme 1. Synthesis of the protected lysine **5**.



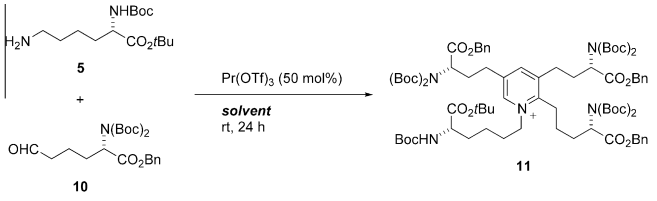
Scheme 2. Synthesis of the aldehyde **10**.

synthesis would allow for the exclusive formation of the 1,2,3,5-tetrasubstituted pyridinium amino acid isodesmosine **1** in the same manner as the original reaction.[1–3] Herein, we report the development of new methods for the preparation of the protected lysine **5** and the corresponding aldehyde **10**, as well as the total synthesis of **11** via the reaction of these two compounds in a Chichibabin pyridine synthesis using $Pr(OTf)_3$ in H_2O and MeOH.

Our synthesis commenced with the preparation of the protected lysine **5** starting from commercially available 6-(benzyloxycarbony)-amino-2-(*S*)-4-[(*tert*-butoxycarbonyl)amino]-hexanoic acid (**3**) (Scheme 1). Protection of the carboxyl group as the corresponding *tert*-butyl ester was achieved using *O*-*tert*-butyl-*N*,*N*′-diisopropyl isourea, which was freshly prepared from *N*,*N*′-diisopropylcarbodiimide.[12] Compound **3** and the isourea were reacted in dichloromethane (CH_2Cl_2) at room temperature to give the *tert*-butyl ester **4** in 98% yield. Subsequent removal of the benzyloxycarbonyl group under Pd/C hydrogenation conditions in MeOH gave the desired amine **5** in 96% yield. The optical rotation of this material ($[\alpha]_D$ +7.75) was in good agreement with the reported value ($[\alpha]_D$ +6.81).[13] Thus, the synthesis of the protected lysine **5**, which is a precursor of the Chichibabin reaction for the synthesis of **1**, was achieved in an overall yield of 94% in two steps from **3**.

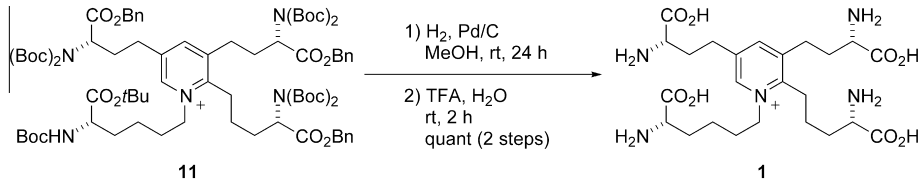
The synthesis of the aldehyde **10** was achieved in four steps beginning with 1-benzyl-5-methyl-2-(*S*)-[bis-(*tert*-butoxycarbonyl)-amino]-pentanedioate (**6**) according to the route shown in Scheme 2. Compound **6** was prepared from commercially available 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanedioic acid 1-benzyl ester according to literature procedures,[14] and the overall yield for the steps from **6** to **9** was slightly improved. Reduction of the methyl ester **6** using diisobutylaluminum hydride (DIBAL-H) in diethyl ether (Et_2O) afforded the aldehyde **7** in 89% yield, which was converted to the *exo*-olefin **8** in 68% yield via a Wittig reaction using *n*-butyllithium (*n*BuLi) and methyltriphenylphosphonium bromide ($MePPh_3Br$) in tetrahydrofuran (THF). Formation of the ylide was carried out at −78 °C rather than 0 °C, which resulted in an improvement of the yield over these two steps from 47% to 61%.[14] Hydroboration–oxidation of **8** using sodium borohydride ($NaBH_4$) and boron trifluoride etherate ($BF_3{\cdot}Et_2O$) in THF, followed

Table 1
Chichibabin pyridine synthesis of **11** from the protected lysine **5** and aldehyde **10**



Entry[a]	H_2O/MeOH ratio	Yield[b] (%)
1	1:0	N.R.
2	1:1	Trace
3	1:2	Trace
4	1:5	Trace
5	2:1	29
6	3:1	26

[a] Reactions between 1 equiv of **5** and 4 equiv of **10** were run at room temperature for 24 h.
[b] Isolated yield.



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

by sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) then afforded the *anti*-Markovnikov product **9** in 80% yield. Treatment of the primary alcohol **9** with Dess–Martin periodinane (DMP) in CH_2Cl_2 led to the desired compound **10** in 98% yield. It is noteworthy that the oxidation of the mono-Boc-protected version of **9** gave the cyclized product.[15] Thus, the aldehyde **10**, which is a precursor of the Chichibabin pyridine synthesis for the preparation of **1**, was prepared from **6** in an overall yield of 47% over four steps.

With the protected lysine **5** and aldehyde **10** in hand, we turned our attention to the $Pr(OTf)_3$-promoted Chichibabin pyridine synthesis. Among the family of $Ln(OTf)_3$ reagents,[16,17] $Pr(OTf)_3$ was found to be the best reagent for the Chichibabin pyridine synthesis, most likely because it possesses the appropriate level of Lewis acidity for the Mannich and aldol condensation reactions that occur.[11] Given that the starting materials for the Chichibabin pyridine synthesis were expected to be poorly soluble in H_2O, the effect of using MeOH as a co-solvent was investigated (Table 1). Reactions between 1 equiv of **5** and 4 equiv of **10** were conducted in the presence of 50 mol % $Pr(OTf)_3$ at room temperature for 24 h. When the reaction was performed in 100% H_2O, it failed to afford any of the desired products (Table 1, entry 1), likely due to the fact that substrates **5** and **10** were insoluble in H_2O. Different amounts of MeOH were then added to the reaction (H_2O/MeOH = 1:1, 1:2, and 1:5 (v/v)) in an attempt to increase the solubility of the substrates (Table 1, entries 2–4, respectively). However, only trace amounts of the desired pyridinium compound **11** were observed in these reactions, along with several byproducts, which had the same *m*/*z* as **11** as determined by mass spectrometry (MS) analysis. Pleasingly, when the reaction was run in a 2:1 (v/v) mixture of H_2O and MeOH, the isodesmosine-type Chichibabin product **11** was obtained in 29% yield (Table 1, entry 5), along with several unidentified byproducts, which were observed by thin layer chromatography (TLC). Interestingly, and in contrast to the previous report,[11] only a trace amount of the desmosine-type pyridinium compound was identified in this particular case. Further increasing the ratio of H_2O to MeOH to 3:1 (v/v) resulted in a slight decrease in the yield of **11** to 26% (Table 1, entry 6). Taken together, these results indicated that the use of a 2:1 (v/v) mixture of H_2O and MeOH (Table 1, entry 5) was optimal for the Chichibabin pyridine synthesis in H_2O/MeOH and suggested that the solubility of starting materials **5** and **10** was a crucial factor for the success of this synthesis. These conditions also promoted the selective synthesis of the isodesmosine-type product.

The protecting groups in compound **11** were then removed as follows. The three benzyl groups were removed by hydrogenation over Pd/C and the seven *tert*-butoxycarbonyl groups and one *tert*-butyl group were removed by trifluoroacetic acid (TFA) in a stepwise manner. These deprotection steps proceeded quantitatively to provide the desired pyridinium amino acid isodesmosine **1** (Scheme 3). Spectroscopic analysis of this material, including ^1H NMR, MS, and optical rotation, afforded data that were in good agreement with those of natural **1**.

In summary, we have developed a new method for the total synthesis of the COPD biomarker isodesmosine **1** via the $Pr(OTf)_3$-promoted Chichibabin pyridine synthesis of the protected lysine **5** and aldehyde **10** in H_2O and MeOH. This new synthesis of **1** was completed in 27% yield (under the conditions of entry 5 in Table 1) over five steps starting from commercially available 6-(benzyloxycarbony)-amino-2-(*S*)-4-[(*tert*-butoxycarbonyl)amino]-hexanoic acid (**3**). The results obtained in the current study suggest that the solubility of compounds **5** and **10** in the H_2O/MeOH solvent system was critical to the success of this Chichibabin pyridine synthesis. Furthermore, this reaction system favored the selective formation of the isodesmosine-type Chichibabin product, with only a trace amount of the desmosine-type Chichibabin product being detected. This selectivity implies that the formation of 1,3,4,5-tetrasubstituted pyridine was prevented in the presence of MeOH. Further studies designed to elucidate the mechanisms involved in the formation of the elastin crosslinkers isodesmosine **1** and desmosine **2** from lysine in nature are required in order to develop a deeper understanding of these processes, and these studies are currently underway in our laboratory. The preparation of isotopically labeled desmosines as internal standards for the analysis of clinical COPD samples by the isotope-dilution LC–MS/MS[9d] is also underway in our laboratory using the synthetic route described above. This route is also being investigated for the preparation of related crosslinking amino acids for the elucidation of the three-dimensional crosslinking structure of elastin.[18]

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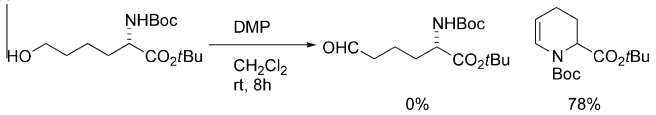
Supplementary data

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.2014.09.097.

References and notes

1. Chichibabin, A. E. *J. Russ. Phys. Chem. Soc.* **1905**, *37*, 1229–1253.
2. (a) Frank, R. L.; Seven, P. P. *J. Am. Chem. Soc.* **1949**, *71*, 2629–2635; (b) Farley, C. P.; Eliel, E. L. *J. Am. Chem. Soc.* **1956**, *78*, 3477–3484.
3. Yu, L.-B.; Chen, D.; Li, J.; Ramirez, J.; Wang, P. G.; Bott, S. G. *J. Org. Chem.* **1997**, *62*, 208–211.
4. (a) Partridge, S. M.; Elsden, D. F.; Thomas, J. *Nature* **1963**, *197*, 1297–1298; (b) Thomas, J.; Elsden, D. F.; Partridge, S. M. *Nature* **1963**, *200*, 651–652.
5. (a) Davis, N. R.; Anwar, R. A. *J. Am. Chem. Soc.* **1970**, *92*, 3778–3782; (b) Akagawa, M.; Suyama, K. *Connect. Tissue Res.* **2000**, *41*, 131–141.
6. (a) Rosenbloom, J.; Abrams, W. R.; Mecham, R. P. *FASEB J.* **1993**, *7*, 1208–1218; (b) Debelle, L.; Tamburro, A. M. *Int. J. Biochem. Cell Biol.* **1999**, *31*, 261–272.
7. Vrhovski, B.; Weiss, A. S. *Eur. J. Biochem.* **1998**, *258*, 1–18.
8. (a) Kaga, N.; Soma, S.; Fujimura, T.; Seyama, K.; Fukuchi, Y.; Murayama, K. *Anal. Biochem.* **2003**, *318*, 25–29; (b) Boutin, M.; Berthelette, C.; Gervais, F. G.; Scholand, M.-B.; Hoidal, J.; Leppert, M. F.; Bateman, K. P.; Thibault, P. *Anal. Chem.* **2009**, *81*, 1881–1887; (c) Albarbarawi, O.; Barton, A.; Lin, Z.; Takahashi, E.; Buddharaju, A.; Brady, J.; Miller, D.; Palmer, C. N. A.; Huang, J. T.-J. *Anal. Chem.* **2010**, *82*, 3745–3750; (d) Miliotis, T.; Lindberg, C.; Semb, K. F.; van Geest, M.; Kjellström, S. *J. Chromatogr., A* **2013**, *1308*, 73–78; (e) Albarbarawi, O.; Barton, A.; Miller, D.; McSharry, C.; Chaudhuri, R.; Thomson, N. C.; Palmer, C. N. A.; Devereux, G.; Huang, J. T.-J. *Bioanalysis* **2013**, *5*, 1991–2001; (f) Lamerz, J.; Friedlein, A.; Soder, N.; Cutler, P.; Döbeli, H. *Anal. Biochem.* **2013**, *436*, 127–136.

9. (a) Ma, S.; Lieberman, S.; Turino, G. M.; Lin, Y. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12941–12943; (b) Ma, S.; Lin, Y. Y.; Turino, G. M. *Chest* **2007**, *131*, 1363–1371; (c) Ma, S.; Turino, G. M.; Lin, Y. Y. *J. Chromatogr., B* **2011**, *879*, 1893–1898; (d) Ma, S.; Turino, G. M.; Hayashi, T.; Yanuma, H.; Usuki, T.; Lin, Y. Y. *Anal. Biochem.* **2013**, *440*, 158–165.
10. (a) Usuki, T.; Yamada, H.; Hayashi, T.; Yanuma, H.; Koseki, Y.; Suzuki, N.; Masuyama, Y.; Lin, Y. Y. *Chem. Commun.* **2012**, *48*, 3233–3235; (b) Yanuma, H.; Usuki, T. *Tetrahedron Lett.* **2012**, *53*, 5920–5922.
11. Usuki, T.; Sugimura, T.; Komatsu, A.; Koseki, Y. *Org. Lett.* **2014**, *16*, 1672–1675.
12. Tietze, L. F.; Brashe, G.; Grube, A.; Böhnke, N.; Stadler, C. *Chem. Eur. J.* **2007**, *13*, 8543–8563.
13. Fekner, T.; Li, X.; Lee, M. M.; Chen, M. K. *Angew. Chem. Int. Ed.* **2009**, *48*, 1633–1635.
14. Koseki, Y.; Yamada, H.; Usuki, T. *Tetrahedron: Asymmetry* **2011**, *22*, 580–586.
15. 

16. For lanthanide triflates in organic synthesis, see the review: Kobayashi, S. *Synlett* **1994**, 689–701.
17. For organolanthanide complexes, see the review: Anwander, R.; Herrmann, W. A. In *Organolanthoid Chemistry: Synthesis, Structure, Catalysis*; Herrmann, W. A., Ed.; Springer: Berlin Heidelberg, 1996; pp 1–32.
18. Brown-Augsburger, P.; Tisdale, C.; Broekelmann, T.; Sloan, C.; Mecham, R. P. *J. Biol. Chem.* **1995**, *270*, 17778–17783.