Organic LETTERS

Letter

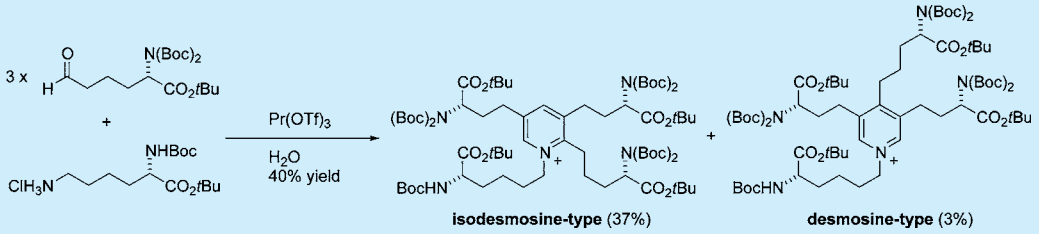
pubs.acs.org/OrgLett

Biomimetic Chichibabin Pyridine Synthesis of the COPD Biomarkers and Elastin Cross-Linkers Isodesmosine and Desmosine

Toyonobu Usuki,* Takanori Sugimura, Akira Komatsu, and Yohei Koseki

Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo 102-8554, Japan

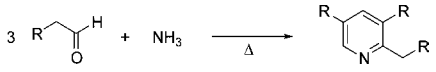
Supporting Information



ABSTRACT: The tetrasubstituted pyridinium amino acids isodesmosine and desmosine are cross-linkers of elastin and are attractive biomarkers for the diagnosis of chronic obstructive pulmonary disease (COPD). In this study, the biomimetic total synthesis of isodesmosine and desmosine via a lanthanide-promoted Chichibabin pyridine synthesis using the corresponding aldehyde and amine hydrochloride is reported.

More than 100 years ago, Chichibabin reported the thermal cyclocondensation of aldehydes (3 equiv) and ammonia to form 2,3,5-trisubstituted pyridines (Scheme 1).[1]

Scheme 1. Typical Chichibabin Pyridine Synthesis



The reaction 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 a catalytic amount of lanthanide trifluoromethanesulfonate (triflate) promoted the condensation of aldehydes and amine hydrochlorides to give 1,2,3,5-tetrasubstituted dihydropyridinium and pyridinium derivatives.[3] The reaction proceeded under mild conditions (room temperature in aqueous media) to afford the Chichibabin products.

Isodesmosine (**1**, Figure 1) and desmosine (**2**) are 1,2,3,5- and 1,3,4,5-tetrasubstituted pyridinium amino acids, respectively, and are found only in the elastin matrix.[4] Elastin is the main component of the elastic fibers that exist in vertebrate tissues and organs, such as the lung, skin, blood vessels, heart, etc., and plays an important role in providing their elasticity and stretchy properties.[5] Elastin is an extremely insoluble extracellular matrix protein that consists of soluble precursor tropoelastin monomers (60−72 kDa) connected in a sophisticated three-dimensional cross-linked network in an inter- and intramolecular fashion by amino acids.[5,6] Isodesmosine **1**, desmosine **2**, desmopyridine,[7] isodesmopyridine,[7] neodesmosine,[8] oxodesmosine,[9] isooxodesmosine,[9] and pentasine,[10] etc. have been identified to date as cross-linker amino acids for elastin.

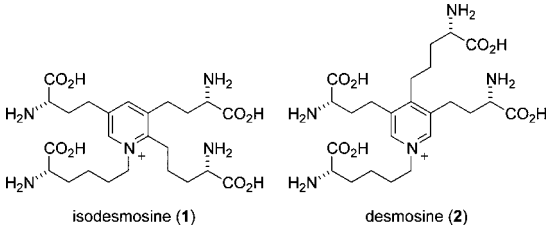


Figure 1. Structures of isodesmosine (**1**) and desmosine (**2**).

The irreversible degradation of lung elastin that occurs in chronic obstructive pulmonary disease (COPD), which is currently the fourth leading cause of death worldwide, is found to give rise to these two amino acids.[11,12] Elastin cross-linkers **1** and **2** can be measured specifically and sensitively in clinical samples, such as plasma, urine, and sputum, using liquid chromatography−mass spectrometry (LC−MS or LC−MS/MS).[11,12] Therefore, isodesmosine **1** and desmosine **2** are attractive biomarkers for both drug discovery and the rapid diagnosis of COPD. According to the World Health Organization, COPD affects over 60 million people worldwide and is estimated to become the third leading cause of death by 2030.[13] 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).[14] Presently, there is a lack of effective therapies and medicines that can prevent progression of the disease, and thus

Received: January 31, 2014
Published: March 5, 2014

dx.doi.org/10.1021/ol500333t | Org. Lett. 2014, 16, 1672−1675

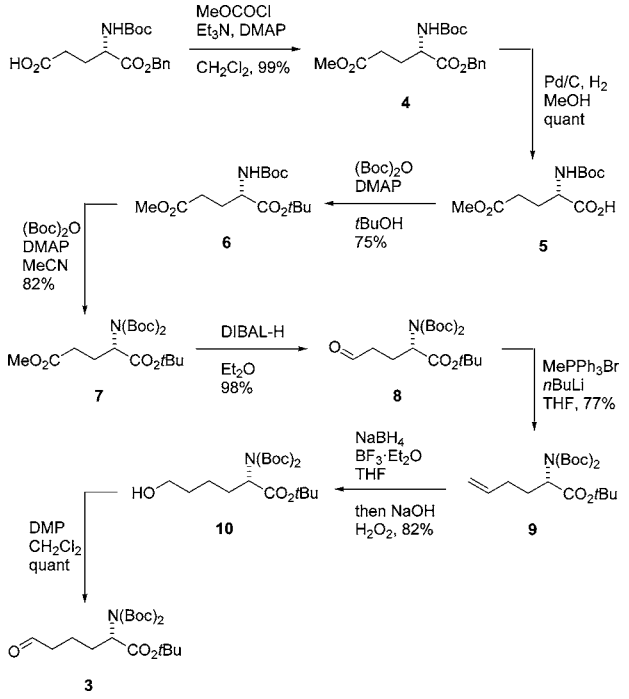
survival rates cannot be improved. As a result, intensive drug discovery activities are underway to find an effective treatment. The development of biomarkers that can act as indicators of the severity of COPD and the level of patient response to therapy would thus aid these efforts.[15]

Many pharmaceutical companies and academic researchers have attempted to establish a methodology for the biomarker analysis of desmosines using LC−MS or LC−MS/MS techniques for the diagnosis of COPD.[11] However, due to a lack of specific internal standards, such as isotopic labeled compounds, the precise analysis of desmosines has not yet been achieved. Recently, we reported the isotope-dilution LC−MS/MS analysis of desmosines using a stable deuterium internal standard that was chemically synthesized.[12d] As a result, the demand for the efficient chemical synthesis of desmosines has increased.

The total synthesis of 1,3,4,5-tetrasubstituted pyridine desmosine **2** starting from 4-hydroxypyridine or 3,5-dibromopyridine was recently achieved in our laboratory.[16] The synthesis relied on palladium-catalyzed cross-coupling reactions between the pyridine cores and the corresponding segments as key steps. However, the chemical synthesis of 1,2,3,5-tetrasubstituted pyridine isodesmosine **1** has not been reported yet. It has been proposed that the formation of the cross-linkers **1** and **2** occurs spontaneously via Mannich reactions and aldol condensations after oxidative transformation of the lysine residues of tropoelastin by lysyl oxidase.[17] Considering the above-mentioned Chichibabin pyridine synthesis,[1−3] we hypothesized that **1** and **2** could be chemically prepared from the corresponding aldehyde and amine hydrochloride. The Chichibabin pyridine synthesis has, in fact, previously been utilized in the synthesis of other natural products, such as juliprosine[18] and haouamine A,[19] etc.[20] Herein we report the biomimetic total synthesis of isodesmosine **1** and desmosine **2** in the aqueous phase via a lanthanide-promoted Chichibabin pyridine synthesis.

Our synthesis commenced with preparation of aldehyde **3** starting from commercially available 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanedionic acid 1-benzyl ester (Scheme 2).[21] Protection of the carboxyl group via reaction with methyl chloroformate in the presence of 4-(*N*,*N*′-dimethylamino)pyridine (DMAP) in triethylamine gave methyl ester **4**, glutamic acid derivative, in 99% yield.[22] Removal of the benzyl group under Pd/C hydrogenation conditions provided carboxylic acid **5** quantitatively. Reaction of **5** with *tert*-butyl alcohol (*t*-BuOH), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and DMAP afforded compound **6** in 67% yield. However, the specific rotation of the product was found to be $[\alpha]^{20}_{D}$ −10.2, indicating that partial racemization occurred (literature data: $[\alpha]^{20}_{D}$ −28.2[23]). Fortunately, the use of di-*tert*-butyl dicarbonate ($(Boc)_2O$), DMAP, and *t*-BuOH gave the desired product **6** in 75% yield with a good optical rotation value ($[\alpha]^{20}_{D}$ −32.5). Additional Boc protection of the amine group was then achieved using $(Boc)_2O$ and DMAP in acetonitrile (MeCN), providing compound **7** in 82% yield. Reduction of the methyl ester using diisobutylaluminum hydride (DIBAL-H) afforded aldehyde **8** in 98% yield, followed by formation of the terminal olefin **9** in 77% yield via a Wittig reaction. Hydroboration−oxidation of **9** then afforded the anti-Markovnikov alcohol **10** in 82% yield. Oxidation of **10** with Dess−Martin periodinane (DMP) led to the desired compound **3** quantitatively. Thus, aldehyde **3**, a precursor for the Chichibabin pyridine synthesis, was prepared from 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanedionic acid 1-benzyl ester in 38% yield over eight steps.

Scheme 2. Synthesis of Aldehyde 3

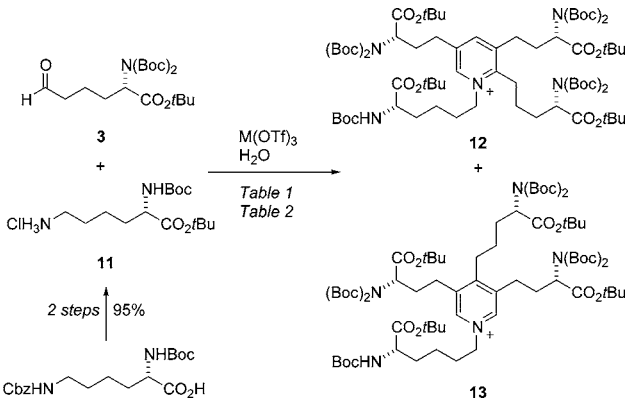


Next, the synthesis of amine hydrochloride **11**, lysine derivative, was achieved in 95% yield over two steps starting from commercially available 6-(benzyloxycarbonyl)amino-2-(*S*)-[(*tert*-butoxycarbonyl)amino]hexanoic acid.[24] With aldehyde **3** and amine hydrochloride **11** in hand, we then turned our attention to the lanthanide-promoted Chichibabin pyridine synthesis (Table 1). Reactions with 4 equiv of aldehyde **3** and 1 equiv of amine hydrochloride **11** were run using 0.5 equiv of several types of lanthanide triflates ($Ln(OTf)_3$)[25] in H_2O for 24 h at room temperature (entries 1−7). Although more than 10 spots were identified via thin-layer chromatography (TLC) (CH_2Cl_2/MeOH = 10/1) analysis, both isodesmosine-type pyridinium **12** and desmosine-type pyridinium **13** were generated and could be separated and isolated using column chromatography. Of the $Ln(OTf)_3$ catalysts investigated, $Pr(OTf)_3$ gave the best yields (34% for **12**, 3% for **13**, total 37%). When the reaction was run with $Sc(OTf)_3$ and $Y(OTf)_3$, which are typical Lewis acids in the aqueous phase, the total yields of **12**/**13** were 10% and 20%, respectively (entries 8 and 9).

The milder Lewis acids (Sc > Y > Yb > Er > Dy > Gb > Nd > Pr > La)[26] gave better yields in this reaction, probably suggesting that the Mannich reaction and aldol condensation were promoted under milder conditions. While Wang and co-workers reported generation only of the 1,2,4,5-tetrasubstituted pyridine skeleton in the lanthanide-promoted reaction of hexanal and benzylamine hydrochloride,[3] desmosine-type 1,3,4,5-tetrasubstituted pyridines were also obtained from the reaction of **3** and **11**. The cross-linkers isodesmosine **1** and desmosine **2** are formed naturally in elastin in approximately a 1:1 ratio. Although the ratio of **12** and **13** obtained in the present reaction did not follow the same trend as that of the Lewis acidity of the catalysts, a ratio close to 1:1 was observed for $Dy(OTf)_3$, $Sc(OTf)_3$, and $Y(OTf)_3$. Therefore, a route

dx.doi.org/10.1021/ol500333t | Org. Lett. 2014, 16, 1672−1675

Table 1. Chichibabin Pyridine Synthesis of 12 and 13 from Aldehyde 3 and Amine Hydrochloride 11



			yield (%)			
entry[a]	$M(OTf)_3$[b]	temp (°C)	**12**	**13**	total[c]	ratio (**12**/**13**)
1	La	rt	21	7	28	3
2	Pr	rt	34	3	37	11.3
3	Nd	rt	18	6	24	3
4	Gb	rt	11	5	16	2.2
5	Dy	rt	12	8	20	1.5
6	Er	rt	15[d]	2[d]	17	7.5
7	Yb	rt	16[d]	1[d]	17	16
8	Sc	rt	6[d]	4[d]	10	1.5
9	Y	rt	12	8	20	1.5

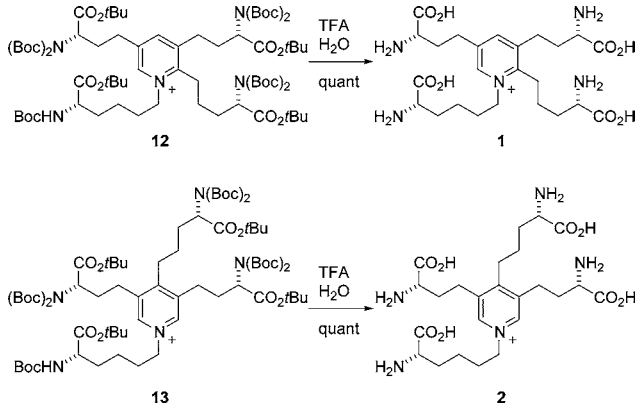
[a]Reactions between 4 equiv of **3** and 1 equiv of **11** were run in H_2O for 24 h. [b]0.5 equiv for each reaction. [c]Isolated yield. [d]Determined by ^1H NMR analysis.

employing one of these catalysts can be regarded as a biomimetic synthesis.

Optimization of the reaction conditions using $Pr(OTf)_3$ in H_2O was then investigated (Table 2). When the temperature was increased to 40 °C, the total yield was slightly decreased to 30% (entry 2). However, when the reaction was run at 80 and 100 °C, isodesmosine-type product **12** was obtained in 32% and 15% yield, respectively, along with a trace amount of **13** (entries 3 and 4). Increasing the equivalents of $Pr(OTf)_3$ from 0.5 to 2.0 led to a decrease in the product yield (entry 5), suggesting that $Pr(OTf)_3$ may act as a catalytic reagent. In fact, when the reaction was run without $Pr(OTf)_3$, TLC analysis indicated the presence of the two starting materials and numerous other compounds, but not the desired products (entry 6). Notably, when the reaction time was extended from 24 to 48 h, the total yield reached a maximum of 40% (entry 7). Changing the pH of the solution to acidic or basic conditions, however, had a negative effect. Under acidic conditions (AcOH), the yield of products dropped to 21% (entry 8), whereas under basic conditions (added K_2CO_3 or $NaHCO_3$) the desired products were obtained in 20% and 33% yields, respectively (entries 9 and 10). Not surprisingly, when the ratio of aldehyde **3** and amine hydrochloride **11** was changed from 4:1 to 3:1, 2:1, or 1:1, the total yield steadily decreased to 13%, 9%, and 8%, respectively (entries 11−13). As a result, reaction conditions used for entry 7 were determined to be the optimum conditions for the synthesis of isodesmosine-type product **12**.

The seven *tert*-butoxycarbonyl groups and four *tert*-butyl groups of **12** and **13** were quantitatively and simultaneously removed using trifluoroacetic acid (TFA) to provide the natural products isodesmosine **1** and desmosine **2**, respectively (Scheme 3, counter anions: TFA). Spectroscopic data obtained for **1** and **2** were in good agreement with those of naturally derived **1** and **2**.

Scheme 3. Total Synthesis of 1 and 2



In summary, the biomimetic total synthesis of isodesmosine **1** and desmosine **2** was achieved for the first time via a

Table 2. Optimization of the Chichibabin Pyridine Synthesis of 12 and 13 from Aldehyde 3 and Amine Hydrochloride 11

						yield (%)		
entry[a]	$Pr(OTf)_3$ (equiv)	ratio (**3**/**11**)	time (h)	additive	temp (°C)	**12**	**13**	total[b]
1[c]	0.5	4:1	24		rt	34	3	37
2	0.5	4:1	24		40	24	6	30
3	0.5	4:1	24		80	32	0	32
4	0.5	4:1	24		100	15	0	15
5	2.0	4:1	24		rt	10	0	10
6	0	4:1	24		rt	0	0	0
7	0.5	4:1	48		rt	37	3	40
8	0.5	4:1	24	AcOH	rt	15	6	21
9	0.5	4:1	24	K_2CO_3	rt	16	4	20
10	0.5	4:1	24	$NaHCO_3$	rt	30	3	33
11	0.5	3:1	24		rt	11	2	13
12	0.5	2:1	24		rt	7[d]	2[d]	9
13	0.5	1:1	24		rt	6[d]	2[d]	8

[a]Reactions were run in H_2O. [b]Isolated yield. [c]Same as Table 1, entry 2. [d]Determined by ^1H NMR analysis.

dx.doi.org/10.1021/ol500333t | *Org. Lett.* 2014, 16, 1672−1675

lanthanide-promoted Chichibabin pyridine synthesis using the corresponding aldehyde and amine hydrochloride. The total synthesis of **1** and **2** was achieved in 35% (under the condition of entry 7 in Table 2) and in 8% (under the condition of entries 5 and 9 in Table 1) yields over four steps starting from commercially available 6-(benzyloxycarbonyl)amino-2-(*S*)-[(*tert*-butoxycarbonyl)amino]hexanoic acid, respectively. These results may provide insight into possible mechanisms for elastin reformation (repair) from degraded elastin peptides that are detected in patients with elastin-related diseases. In addition, this lanthanide-promoted pyridine synthesis in water can be applied as an environmentally benign approach to the synthesis of other natural products. Most notably, the synthetic route described above can provide access to isotopically labeled desmosines as internal standards for the precise isotope-dilution LC−MS/MS analysis of clinical COPD samples and should also enable the preparation of related cross-linking amino acids for the elucidation of the three-dimensional cross-linking structure of elastin.[27]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: t-usuki@sophia.ac.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Yong Y. Lin (Columbia University) for invaluable discussions and Prof. Sergei V. Dzyba (Texas Christian University) for suggestions regarding the manuscript. This work was supported by a Grant-in-Aid for Young Scientists (B) from JSPS (KAKENHI Grant Nos. 22710224 and 25750388), the Shimadzu Science Foundation, and the Naito Science Foundation.

■ REFERENCES

(1) Chichibabin, A. E. *J. Russ. Phys. Chem. Soc.* **1905**, *37*, 1229−1253.
(2) (a) Frank, R. L.; Seven, R. 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) 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.
(6) Vrhovski, B.; Weiss, A. S. *Eur. J. Biochem.* **1998**, *258*, 1−18.
(7) Umeda, H.; Takeuchi, M.; Suyama, K. *J. Biol. Chem.* **2001**, *276*, 12579−12587.
(8) (a) Nagai, Y. *Connect. Tissue* **1983**, *14*, 112−113. (b) Nakamura, F.; Suyama, K. *Agric. Biol. Chem.* **1991**, *55*, 547−554.
(9) Suyama, K.; Nakamura, F. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1767−1770.
(10) Starcher, B.; Cook, G.; Gallop, P.; Hensen, E.; Shoulders, B. *Connect. Tissue Res.* **1987**, *16*, 15−25.
(11) (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) Miliotis, T.; Lindberg, C.; Semb, K. F.; van Geest, M.; Kjellström, S. *J. Chromatogr. A* **2013**, *1308*, 73−78. (d) 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. (e) Lamerz, J.; Friedlein, A.; Soder, N.; Cutler, P.; Döbeli, H. *Anal. Biochem.* **2013**, *436*, 127−136. (f) Ongay, S.; Hendriks, G.; Hermans, J.; van den Berge, M.; ten Hacken, N. H.T.; van de Merbel, N.; Bischoff, R. *J. Chromatgr. A* **2014**, *1326*, 13−19.
(12) (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.
(13) WHO. Chronic obstructive pulmonary disease (COPD), http://www.who.int/respiratory/copd/en/ (accessed Jan 31, 2014).
(14) (a) Yoshida, T.; Tuder, R. M. *Physiol. Rev.* **2007**, *87*, 1047−1082. (b) Mannino, D. M.; Buist, A. S. *Lancet* **2007**, *370*, 765−773.
(15) (a) Turino, G. M.; Ma, S.; Lin, Y. Y.; Cantor, J. O.; Luisetti, M. *AJRCCM* **2011**, *184*, 637−641. (b) Martinez, F. J.; Donohue, J. F.; Rennard, S. I. *Lancet* **2011**, *378*, 1027−1037.
(16) (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.
(17) (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.
(18) Baran, P. S.; Burns, N. Z. *J. Am. Chem. Soc.* **2006**, *128*, 3908−3909.
(19) Burns, N. Z.; Baran, P. S. *Angew. Chem., Int. Ed.* **2008**, *47*, 205−208.
(20) Dagorn, F.; Yan, L.-H.; Gravel, E.; Leblanc, K.; Maciuk, A.; Poupon, E. *Tetrahedron Lett.* **2011**, *52*, 3523−3526.
(21) Koseki, Y.; Yamada, H.; Usuki, T. *Tetrahedron: Asymmetry* **2011**, *22*, 580−586.
(22) Kim, S.; Kim, Y. C.; Lee, J. I. *Tetrahedron Lett.* **1983**, *24*, 3365−3368.
(23) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **1999**, *10*, 775−781.
(24) (a) Fekner, T.; Li, X.; Lee, M. M.; Chan, M. K. *Angew. Chem., Int. Ed.* **2009**, *48*, 1633−1635. (b) Rosenberg, A. J.; Clark, D. A. *Org. Lett.* **2012**, *14*, 4678−4681.
(25) For lanthanide triflates in organic synthesis, see the following review: Kobayashi, S. *Synlett* **1994**, 689−701.
(26) Anwander, R.; Herrmann, W. A. iIn *Organolanthoid Chemistry: Synthesis, Structure, Catalysis*; Herrmann, W. A., Ed.; Springer Verlag: Berlin, Heidelberg, 1996; pp 1−32.
(27) Brown-Augsburger, P.; Tisdale, C.; Broekelmann, T.; Sloan, C.; Mecham, R. P. *J. Biol. Chem.* **1995**, *270*, 17778−17783.