A Facile Synthesis of Orthogonally Protected Stereoisomeric Lanthionines by Regioselective Ring Opening of Serine β-Lactone Derivatives

Hui Shao, Susan H. H. Wang, Chang-Woo Lee, George Ösapay, and Murray Goodman*

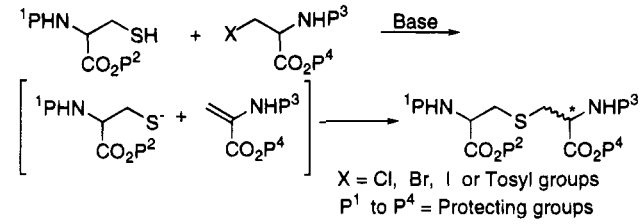
Department of Chemistry and Biochemistry, University of California—San Diego, La Jolla, California 92093

Received January 31, 1995

Lanthionine is an unusual amino acid composed of two alanine-like residues linked by a thioether bridge. It is the monosulfide analog of cystine. Lanthionine was isolated from wool, human hair, lactalbumin, and feathers.[1] The biosynthesis of lanthionine derivatives involves Michael addition of cysteine to dehydroalanine.[2] Both lanthionine and its 3-methyl homolog have received wide attention because they are building blocks in a family of unusual bioactive polypeptides called "lantibiotics",[3] including nisin (an efficient food preservative), subtilin, epidermin (active against staphylococcus and streptococcus), ancovenin (an enzyme inhibitor), and other compounds having immunostimulant and antitumor properties.[4] Compared to the labile disulfide bridge of cystine in natural peptide sequences, the monosulfide bridge of lanthionine provides more constrained peptide structures and greater stability toward enzymatic degradation. We therefore introduced lanthionines as novel peptidomimetic building blocks into two drug families.[5]

Several methods have been developed to prepare lanthionine derivatives and natural lanthionine-containing peptides. The first method involves the oxidation of two cysteine residues to generate cystine, followed by reversible sulfur extrusion with tris(dialkylamino)phosphine from the disulfide bridge to produce the monosulfide structure without regioselectivity.[3,6,7] Another strategy to prepare lanthionines is based on the nucleophilic attack of cysteine derivatives on β-haloalanines, β-tosylated serine (or threonine) (Scheme 1), amino acrylates or aziridines.[7,8] All of these multistep methods proved to be difficult because of tedious purification, poor yields, or lack of stereochemical control. However, Shiba and co-workers successfully accomplished the total synthesis of nisin using sulfur extrusion method to prepare cyclic lanthionine fragments followed by fragment condensations.[8e]

Scheme 1



Our desire to prepare the orthogonally protected lanthionine derivatives in a more efficient, stereoselective fashion led us to explore a new route with an appropriate chiral intermediate as an electrophile which can react with a nucleophilic thiol group of protected cysteine. Vederas and co-workers[9] reported the reaction of a number of nucleophiles with various derivatives of enantiomerically pure *N*-protected and unprotected serine β-lactones, 3-amino-2-oxetanone derivatives, to give the corresponding stereochemically pure products. The synthesis of unprotected symmetrical lanthionine by the ring opening of the serine β-lactone with (*R*)-cysteine in water (pH = 5.0–5.5) has been investigated by Vederas. No desired compound was formed when the protected *N*-[benzyloxycarbonyl(Cbz)]-serine β-lactone was treated with (*R*)-cysteine in 1:1 acetonitrile/water. They presumed that the organic solvent, necessary to dissolve the *N*-protected lactone, suppressed the thiolate anion formation.

The investigation of Vederas's results encouraged us to discover a new way to synthesize lanthionine derivatives with the protected serine β-lactone, *N*-Cbz-(*S*)-3-amino-2-oxetanone.[10] The ring opening of this lactone by the thiolate anion of methyl Boc-(*S*)-cysteinate involves two possible pathways (Scheme 2): pathway A leads to thioester formation by the *O*-acyl fission; pathway B yields the desired lanthionine through *O*-alkyl fission. Our early efforts to optimize the ring opening reaction of the β-lactone catalyzed by different bases (including normal organic and inorganic bases) in THF, CH_3CN, alcoholic,[11] or aqueous solutions at various temperatures showed exclusive formation of thioester.

* To whom correspondence should be addressed.

(1) (a) Horn, M. J.; Jones, D. B.; Ringel, S. J. *J. Biol. Chem.* **1941**, *138*, 141–149. (b) Horn, M. J.; Jones, D. B. *Ibid.* **1941**, *139*, 473. (c) Horn, M. J.; Jones, D. B.; Ringel, S. J. *J. Biol. Chem.* **1942**, *144*, 87–91.

(2) Zervas, L.; Ferderigos, N. *Isr. J. Chem.* **1974**, *12*, 139–152.

(3) (a) *Nisin and Novel Lantibiotics*; Jung, G., Sahl, H. G., Eds.; ESCON Science Publishers B. V.: Leiden, 1988. (b) Gross, E.; Morell, J. L. *J. Am. Chem. Soc.* **1971**, *93*, 4634–4635. (c) Fukase, K.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2505–2508. (d) Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Fijita, H.; Horimoto, S.; Wakamiya, T.; Shiba, T. *Tetrahedron Lett.* **1988**, *29*, 795–798. (e) Gross, E.; Kiltz, H. H.; Nebelin, E. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 810–812. (f) Allgaier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zahner, H. *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 1051–1053. (g) Allgaier, H.; Jung, G.; Werner, R. G.; Schneider, U.; Zahner, H. *Eur. J. Biochem.* **1986**, *160*, 9–22. (h) Wakamiya, T.; Ueki, Y.; Shiba, T.; Motoki, Y. *Tetrahedron Lett.* **1985**, *26*, 665–668. (i) Shiba, T.; Wakamiya, T.; Fukase, K.; Sano, A.; Shimbo, K.; Ueki, Y. *Biopolymers* **1986**, *25*, 11–19.

(4) (a) Migliore-Samour, D.; Bouchaudon, J.; Floc'H, F.; Zerial, A.; Ninet, L.; Werner, G.; Jolles, P. *Life Sci.* **1980**, *26*, 883–888. (b) Izumi, S. *J. Antibiot.* **1983**, *36*, 566–574. (c) Wiesmüeller, K. H.; Bessler, W.; Jung, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1983**, *364*, 593–606.

(5) (a) Polinsky, A.; Cooney, M. G.; Toy-Palmer, A.; Ösapay, G.; Goodman, M. *J. Med. Chem.* **1992**, *35*, 4185–4194. (b) Ösapay, G.; Goodman, M. *J. Chem. Soc. Chem. Commun.* **1993**, 1599–1600. (c) Lee, C. W.; Zhu, Q.; Shao, H.; Wang, S. H.-H.; Ösapay, G.; Goodman, M. In *Peptides: Proceedings of the 23rd European Peptide Symposium*; Maia, F., Ed.; ESCOM: Leiden, 1995; in press. (d) Goodman, M.; Ösapay, G.; Melacini, G.; Zhu, Q. *Ibid.*

(6) (a) Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1971**, *36*, 73–80. (b) Harpp, D. N.; Gleason, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 2437–2445. (c) Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1970**, *35*, 3259–3263. (d) Kitazawa, M.; Fukase, K.; Wakamiya, T.; Shiba, T. *Pept. Chem.* **1986**, 317–322.

(7) Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. *Tetrahedron Asymm.* **1992**, *3*, 85–94.

(8) (a) Photaki, I. *J. Am. Chem. Soc.* **1963**, *85*, 1123–1126. (b) Nakajima, K.; Oda, H.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 520–522. (c) Photaki, I.; Samouilidis, I.; Caranikas, S.; Zervas, L. *J. Chem. Soc. Perkin. Trans. 1* **1979**, 2599–2605. Cavelier-Frontin *et al.* were unsuccessful in repeating this synthesis.[7] We were able to obtain the desired product with comparable yields. (d) Wakamiya, T.; Oda, Y.; Fukase, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 536–539. (e) Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227–2240.

(9) (a) Pansare, S. V.; Huyer, G.; Arnold, L. D.; Vederas, J. C. *Org. Synth.* **1991**, *70*, 1–26 and references therein. (b) Zhou, Q. X.; Kohn, J. *Macromolecules* **1990**, *23*, 3399–3406.

(10) The enantiomers of *N*-Cbz-serine β-lactone were prepared based on the published procedure (yield, 40–50%).[10a] The modified method with diethyl azodicarboxylate (DEAD)[10] did not provide better yields because of the difficulty in separating the β-lactone from the corresponding hydrazine derivative from DEAD.

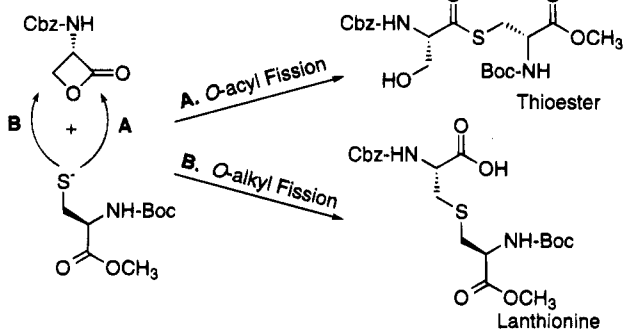
0022-3263/95/1960-2956$09.00/0

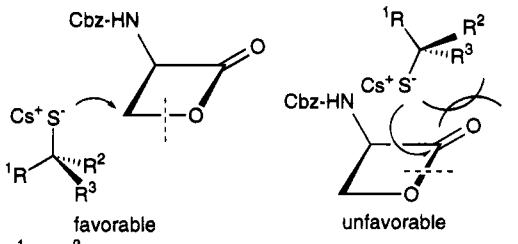
Table 1. Regioselective Ring-Opening of *N*-Cbz-Serine β-Lactones with Cysteine Derivatives

entry	*N*-Cbz-serine-β-lactones	cysteine derivatives[a]	lanthionines (yield, %)[b]
1	(*S*)	(*S*), R_1=R_2=H	**1** (2*R*, 2'*S*) (50)
2	(*S*)	(*R*), R_1=R_2=H	**2** (2*R*, 2'*R*) (50)
3	(*R*)	(*S*), R_1=R_2=H	**3** (2*S*, 2'*S*) (50)
4	(*R*)	(*R*), R_1=R_2=H	**4** (2*S*, 2'*R*) (50)
5	(*R*)	(*R*), R_1=R_2=CH_3	**5** (2*S*, 2'*R*) (78)
6	(*R*)	(*S*), R_1=R_2=CH_3	**6** (2*S*, 2'*S*) (84)
7	(*R*)	(±)[c], R_1, R_2= -$(CH_2)_5$-	**7**[c] (2*S*) (92)

[a] Methyl esters of cysteine derivatives were directly prepared by the standard diazomethane method. To avoid air oxidation of the thiol group, they were used immediately to prepare the lanthionines. [b] The yields for compounds **1** to **4** were based on reactions carried out on various scales. [c] Note: R_1 and R_2 form a cyclic structure.

Scheme 2





R^1 to R^3 = alkyl groups on β-carbon of cysteine derivatives

Figure 1. The steric effect of β-substituents of cysteine derivatives on the regioselective ring opening of *N*-Cbz-serine β-lactone.

We observed that cesium is a very good counterion for the nucleophile in dimethylformamide (DMF). Thiols were found to be deprotonated readily by Cs_2CO_3 or $CsHCO_3$ in DMF to form reasonably soluble cesium thiolates. With 1 equiv of the cesium reagent in DMF at room temperature, the orthogonally protected lanthionine derivative was formed in 50% overall yield (entries 1–4, Table 1).[12]

Suppression of the thioester formation was achieved in the synthesis of β-substituted lanthionine derivatives. When the methyl esters of (*R*)- and (*S*)-Boc-penicillamines (β,β-dimethylcysteine analogs) were allowed to react with *N*-Cbz-(*R*)-serine β-lactone under the condition described above, β,β-dimethyl lanthionines (**5**, **6**) were formed in about 80% yield. With the more constrained cysteine analog, β,β-pentamethylenecysteine,[13] the lanthionine **7** was formed in as high as 92% yield (Table 1). In both cases, only traces of the thioesters were detected. A schematic explanation for the high regioselectivity of these reactions is shown in Figure 1. The primary thiolate anions of methyl *N*-Boc-cysteinates (entry 1–4 in Table 1) can attack both the carbonyl and the β-methylene positions with almost the equal accessibility to form thioesters and lanthionines. The access of the tertiary thiolate anions of β,β-disubstituted cysteine derivatives (entries 5–7 in Table 1) to the carbonyl carbon of the β-lactone is blocked by steric hindrances. This significantly suppresses the formation of thioester *via* *O*-acyl fission (pathway A in Scheme 2). However, the approach of these hindered thiolate anions to the β-methylene group of the β-lactones remains unhindered, leading to *O*-alkyl fission (pathway B in Scheme 2) to produce lanthionine compounds in high yields.

In summary, we have developed an efficient route to prepare orthogonally protected stereoisomeric lanthionine derivatives. The simplicity, the high yields, and the optical purity in this approach are attractive features compared with previous methods. This method is also an appealing strategy to prepare other substituted lanthionine analogs for peptide and peptidomimetic synthesis. In our continuing research effort on lanthionine chemistry, we have synthesized cyclolanthionines based on compounds **1**–**6**, and X-ray diffractions of these molecules proved the correct chiralities of all of the asymmetric centers.[14]

Acknowledgment. We wish to thank Drs. William Craig and Soan Chen of Telios Pharmaceuticals for a sample of the pentamethylenecysteine derivative. We also thank Dr. Richard Kondrat (UC, Riverside) for mass spectroscopic analyses. This work was supported by NIHDK-15410.

Supplementary Material Available: General experimental procedure, characterization data, and copies of ^{1}H NMR spectra of **1**–**7** (16 pages).

JO950183L

(11) We were not able to obtain lanthionines based on the nucleophilic reaction condition developed by D. Seebach and co-workers. See: (a) Griesbeck, A.; Seebach, D. *Helv. Chim. Acta.* **1987**, *70*, 1326–1332. (b) Breitschuh, R.; Seebach, D. *Synthesis-Stuttgart* **1992**, 83–89.

(12) Under this condition, the thioesters were formed in about 30–40% yields. The lanthionine products can be isolated from the reaction mixture because lanthionines are more polar compounds than the thioesters. In the ^{1}H NMR spectra, the chemical shifts of β-H of lanthionines are in the range of 2.60–3.00 ppm while the chemical shifts of β-H of the thioesters are in the region of 3.00–4.00 ppm.

(13) The pentamethylenecysteine analog can be prepared by published methods, see: (a) Stanfield, C. F.; Cody, W. L.; Hruby, V. J. *J. Org. Chem.* **1986**, *51*, 5153–5156. (b) Stanfield, C. F.; Hruby, V. J. *Synth. Commun.* **1988**, *18*, 531–543.

(14) Ösapay, G.; Zhu, Q.; Shao, H.; Chadha, R. K.; Goodman, M., submitted to *Int. J. Pept. Protein Res.*