Lantibiotic Synthesis

DOI: 10.1002/anie.200802919

Solid-Supported Synthesis and Biological Evaluation of the Lantibiotic Peptide Bis(desmethyl) Lacticin 3147 A2**

*Vijaya R. Pattabiraman, Shaun M. K. McKinnie, and John C. Vederas**

Lantibiotics are a class of bacteriocins (antimicrobial peptides from bacteria) that undergo extensive post-translational processing.[1] Their biosynthesis involves enzymatic dehydration of serine and/or threonine residues with subsequent intramolecular Michael addition of cysteine thiols to form lanthionine or β-methyllanthionine rings.[2] Lantibiotics are produced by Gram-positive bacteria either as single peptide antibiotics (e.g., nisin A) or as two peptide systems.[3] Many lantibiotics bind lipid II, the precursor of peptidoglycan, thereby hindering bacterial cell wall formation, and in some cases, creating pores in the membrane at nanomolar concentrations.[4] They are generally nontoxic to mammals, and some are very active against Gram-positive bacteria that are resistant to methicillin (e.g., methicillin-resistant *Staphylococcus aureus* (MRSA)) and vancomycin (e.g., vancomycin-resistant enterococcus (VRE)).[1] They are already used in food preservation and have considerable potential in human medicine. The two-component lantibiotic, lacticin 3147, consists of A1 (**1**) and A2 (**2**) peptides[5] that exhibit synergistic antimicrobial activity in nanomolar concentration (Figure 1). The mechanism involves initial binding of A1 (**1**) to lipid II.[4b] This complex is then recognized by lacticin A2 (**2**) to give a three-component assembly that promotes the formation of pores in the cell membrane. Studies on structure–activity relationships of these lantibiotics are being pursued to uncover the principles for designing new antibiotics.[6] In this respect, the development of chemical methods for the synthesis of lantibiotics and their analogues has interested a number of research groups.[7,8] A solution-phase synthesis of nisin A represents the only total chemical construction of a lantibiotic.[9] Recently, we reported a solid-supported synthesis of an inactive analogue of lacticin 3147 A2 wherein all of the lanthionine bridges were replaced by larger carbocyclic rings.[10] We now describe a solid-phase synthesis of bis(desmethyl) lacticin 3147A2 (Lan-A2, **3**), an analogue of A2 (**2**) that has the two β-methyllanthionine bridges replaced by lanthionines. Biological evaluation of **3** shows that it unexpectedly retains potent synergistic activity with A1, but loses its inherent independent antimicrobial activity, which indicates two independent mechanisms for the natural A2 peptide.

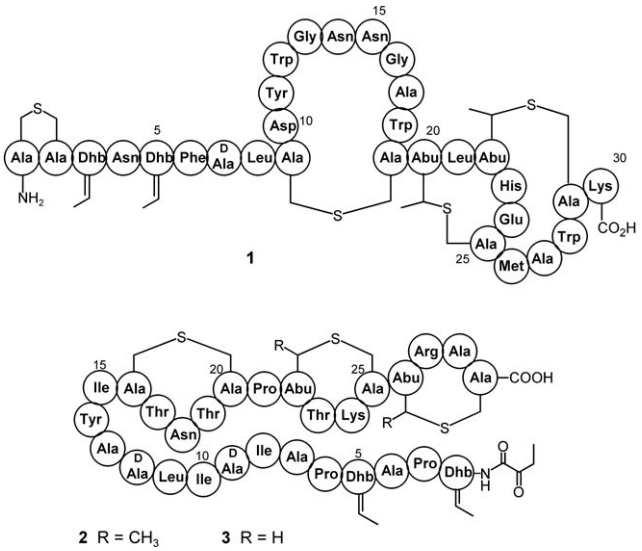


Figure 1. Lacticin 3147 components A1 (**1**) and A2 (**2**). The bis(desmethyl) lanthionine analogue (R = H) of lacticin A2 is Lan-A2 (**3**). It is proposed that the α-carbon atoms of residues 26, 22, and 16 in **2**, and alanine residues 9 and 12 have the D configuration.[5]

The synthetic approach to **3** utilizes solid-supported (9*H*-fluoren-9-ylmethoxy)carbonyl (Fmoc) peptide synthesis with an orthogonally protected lanthionine precursor, which is coupled to the growing chain and eventually deprotected at the distal sites for intramolecular ring formation (Figure 2). The N-terminal residues (1–5) are synthesized in solution and coupled as a unit onto the peptide. The lanthionine protection was inspired by elegant studies by Tabor and co-workers for the preparation of the monocyclic ring of nisin.[8]

To obtain multigram quantities of orthogonally protected lanthionine[7–9,11] in a minimum number of steps, a combination of the phase-transfer conditions to make lanthionines reported by Zhu and Schmidt[12] was used with the orthogonal protection scheme of Bregant and Tabor.[8a] Reaction of Aloc/allyl-protected β-bromo-D-alanine (**4**) (Aloc = allyloxycarbonyl) as the electrophile with Fmoc/*t*Bu-protected L-cysteine (**5**) as the nucleophile[13] in the presence of $(Bu)_4NBr$ in EtOAc and $NaHCO_3$ (0.5 M, pH 8.5), gave orthogonally protected lanthionine, with the desired isomer predominating in a 9:1 ratio based on the ^{13}C NMR spectrum (Scheme 1).

[*] V. R. Pattabiraman, S. M. K. McKinnie, Prof. J. C. Vederas
Department of Chemistry, University of Alberta
Edmonton, Alberta T6G 2G2 (Canada)
Fax: (+1) 780-492-2134
E-mail: john.vederas@ualberta.ca

[**] This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Alberta Heritage Foundation for Medical Research (AHFMR), the Advanced Food & Materials Network (AFMNet), and the Canada Research Chair in Bioorganic and Medicinal Chemistry. We thank Randy Whittal and Jing Zheng (University of Alberta) for help with mass spectrometry studies.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200802919.

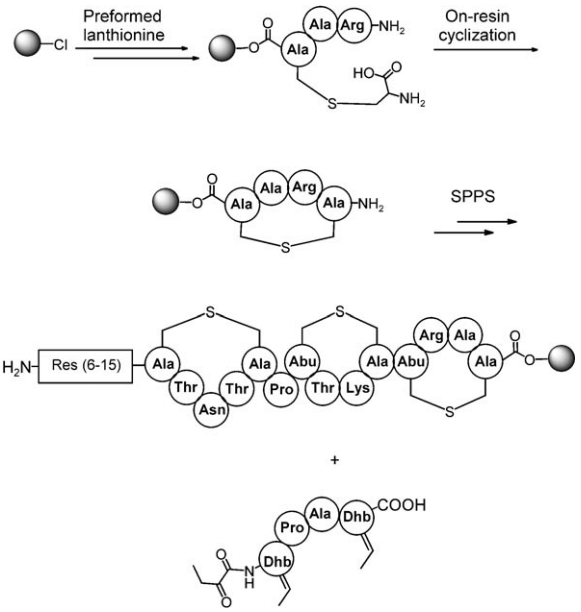
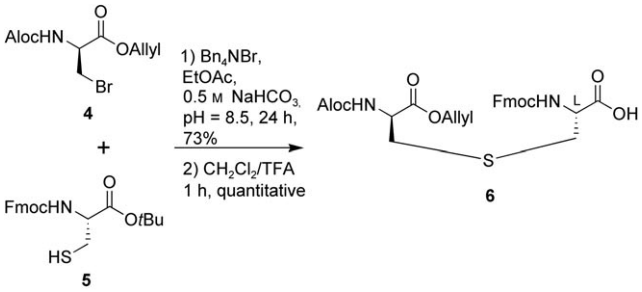


Figure 2. Synthetic approach to **3**, a lanthionine analogue of lacticin A2.

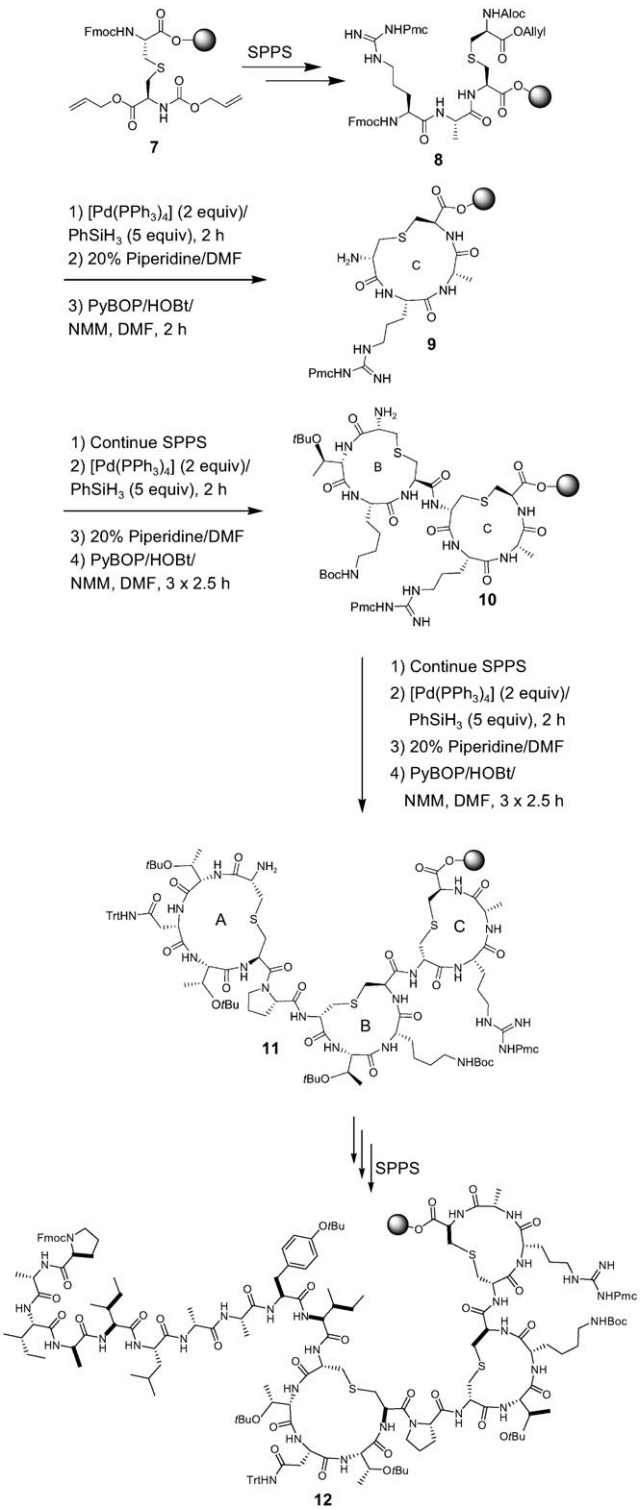
Removal of the *tert*-butyl group under acidic conditions gave **6** in quantitative yield.



Scheme 1. Synthesis of orthogonally protected lanthionine.

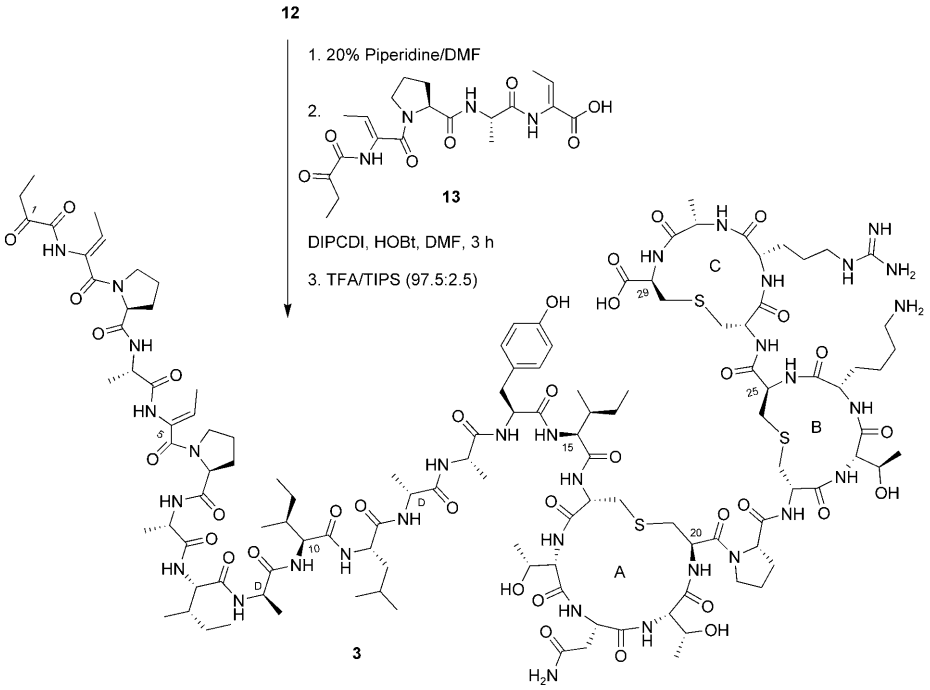
Acid **6** was attached to 2-chlorotrityl chloride resin[14] with a low loading (0.16 mmol g^{-1}) to avoid complications from intermolecular coupling after deprotection of the distal carboxyl and amino groups of the lanthionine residue. The amino acids required for the linear sequence of ring C (**8**) were introduced by Fmoc solid-phase peptide synthesis (SPPS) using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent. Aloc and allyl protecting groups of the lanthionine residue in **8** were removed on-resin[8a] with $[Pd(PPh_3)_4]$ and $PhSiH_3$, followed by removal of the Fmoc group with piperidine. Cyclization to **9** was accomplished by on-resin reaction of the peptide with PyBOP/HOBt (HOBt = 1-hydroxy-1*H*-benzotriazole) for 2 h (Scheme 2).

A small sample of the peptide was cleaved from the solid support using trifluoroacetic acid (TFA) for analysis by electrospray mass spectrometry, which indicated the complete formation of lanthionine ring C in **9**. SPPS was continued for the introduction of the protected amino acids required for the linear portion of ring B. Removal of the Aloc/allyl and Fmoc protecting groups as before, followed by cyclization with



Scheme 2. Solid-phase synthesis of residues (6→29) of Lan-A2. NMM = *N*-methylmorpholine, Boc = *tert*-butoxycarbonyl, Pmc = 2,2,5,7,8-pentamethylchroman-6-ylsulfonyl, Trt = triphenylmethyl.

PyBOP/HOBt (2 × 2.5 h) on the solid support, gave an only partially ring-closed product. The on-resin peptide was reacted for a further 2.5 h to force the reaction to completion to give **10**. After liberation of a sample of the peptide from the solid support with TFA, analysis by MALDI-TOF mass spectrometry showed a major peak corresponding to ring B. In an approach similar to the construction of rings B and C, on-resin extension to the linear precursor of ring A was accomplished by SPPS to introduce amino acid residues 14–21. Removal of the protecting groups led to the unprotected amino and carboxylic acid functionalities required for cyclization. To ensure the reaction was complete, cyclization using PyBOP was done over an extended time period (3 × 2.5 h). A small sample was cleaved from the resin and analyzed by mass spectrometry; this showed the successful formation of the desired tricyclic lanthionine rings in **11**. During the three cyclization steps, none of the interchain dimerized peptide was observed, presumably because of the low resin loading. The unmodified linear region consisting of amino acids 6–15 (including D-Ala at 9 and 12) was introduced by regular Fmoc SPPS to obtain **12**.



Scheme 3. Coupling of residues (1–5) to complete the synthesis of lanthionine analogue of lacticin 3147 A2 (Lan-A2, (**3**)). DIPCDI = diisopropylcarbodiimide.

Residues 1–5 of Lan-A2 (**3**) and lacticin A2 (**2**) consist of two dehydrobutyrine groups and an N-terminal α-ketoamide arising from post-translational modification of L-threonine. The pentapeptide **13** was assembled in solution by using a strategy developed by us[10] that involves coupling of a dehydrodipeptide with a dehydrotripeptide moiety, followed by a transamination to introduce the α-ketoamide. To finish the synthesis of Lan-A2 (**3**), the Fmoc group of residue 6 (proline) in **12** was removed and the coupling of **13** to the amino group of proline was done on-resin using *N*,*N*'-diisopropylcarbodiimide/1-hydroxy-1*H*-benzotriazole (DIPCDI/HOBt; Scheme 3).

The peptide was cleaved from the polymer support with trifluoroacetic acid/triisopropylsilane (TFA/TIPS 97.5:2.5)and purified by reverse-phase HPLC. Analysis by MALDI-TOF mass spectrometry showed a parent signal at 2819.6 Da, which indicates the successful synthesis of the lanthionine analogue of lacticin 3147 A2 in an overall yield of 1.3% (over 22 coupling and 3 cyclization steps, as well as deprotection). This corresponds to an average yield of approximately 84% for each of the 25 sequential coupling/cyclization steps on the solid phase. However, the attachment of the pentapeptide fragment **13** proceeds with lower yields, and it is likely that most other amino acid coupling steps occur in greater than 90–95% yield. To determine the exact mass of **3**, a high-resolution MALDI mass spectrum was obtained using FT-ICR-MALDI mass spectrometry (ICR = ion cyclotron resonance). The structure of **3** was further supported by detailed tandem mass spectrometry studies to confirm the correct sequence as well as the connectivity of the lanthionine rings.

Preliminary biological evaluation of bis(desmethyl) lacticin 3147 A2 (Lan-A2, **3**) was done with the aid of natural lacticin A1 (**1**), against the Gram-positive indicator organism *Lactococcus lactis* subspecies *cremoris* HP. When applied next to natural lacticin A1 (**1**), Lan-A2 (**3**) displayed synergistic antimicrobial activity similar to the natural lacticin A2 (**2**, Figure 3). However, in contrast to natural A2 (**2**), the synthetic bis(desmethyl) analogue **3** did not exhibit any

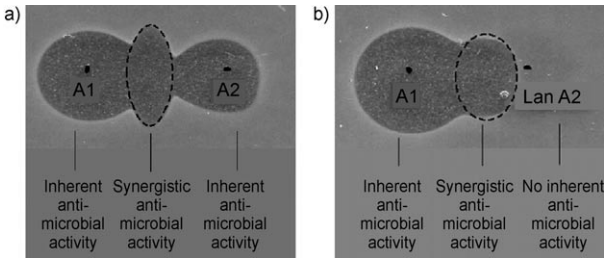


Figure 3. Spot-on-lawn tests for antimicrobial activity showing zones of growth inhibition: a) natural lacticin A1 (**1**) and A2 (**2**) and b) natural lacticin A1 (**1**) and bis(desmethyl) analogue Lan-A2 (**3**).

independent antimicrobial activity at comparable concentrations against the indicator organism. A serial dilution assay performed by mixing Lan-A2 (**3**) with lacticin A1 (**1**) showed that **3** was approximately 100 times less effective than natural

lacticin A2 (**2**) in its ability to exert synergistic activity. However, as the synergistic activity of natural **2** is in the low nanomolar range for many organisms, the desmethyl derivative **3** is still very potent.

Interestingly, peptide **12**, which has an Fmoc group instead of residues 1–5, also exhibited synergistic activity with lacticin A1, albeit at higher concentrations. The synergistic activity of **12** was lost when the Fmoc group on residue 6 was removed. This observation indicates that the Fmoc group can act as a mimic for residues (1→5), probably because of its planarity and hydrophobicity. Further optimization could potentially lead to structurally simpler analogues with fewer amino acids than natural lacticin A2 (**2**).

The present results show that two-peptide lantibiotic lacticin 3147 operates by at least two modes of action. The inherent antimicrobial activity of natural **1** or **2** in the absence of its partner may arise from a mechanism that involves direct binding and sequestering of lipid II, with resulting inhibition of cell wall synthesis.[15] The removal of two methyl groups from the lanthionine rings of the 29-residue peptide **2** effectively eliminates this independent antibiotic action, possibly by drastically decreasing its ability to directly bind to lipid II. However, the established synergistic mechanism which involves pore formation through interaction of **2** (or **3**) with the lacticin A1–lipid II complex[4b] is not as severely affected and continues to function.

In conclusion, the feasibility of solid-supported synthesis of entire lantibiotics has been demonstrated by the chemical synthesis of Lan-A2 (**3**). It has also provided a tool to distinguish the molecular mechanisms responsible for synergistic activity and the inherent independent antimicrobial activity of lacticin 3147. A lead compound **12** for generation of structurally simpler lacticin A2 analogues has also been identified. The current synthetic strategy could be utilized for the preparation of other lantibiotics and their analogues. These would be useful tools for the study of structure–activity relationships, for the confirmation of stereochemistry, and for investigations into their mode of action.

Received: June 18, 2008
Published online: October 20, 2008

Keywords: bacteriocins · lanthionine · lantibiotics · peptides · solid-phase synthesis

[1] a) J. M. Willey, W. van der Donk, *Annu. Rev. Microbiol.* **2007**, *61*, 477–501; b) C. Chatterjee, M. Paul, L. L. Xie, W. A. van der Donk, *Chem. Rev.* **2005**, *105*, 633–683; c) J. I. Nagao, S. M. Asaduzzaman, Y. Aso, K. Okuda, J. Nakayama, K. Sonomoto, *J. Biosci. Bioeng.* **2006**, *102*, 139–149; d) P. D. Cotter, C. Hill, R. P. Ross, *Curr. Protein Pept. Sci.* **2005**, *6*, 61–75; e) O. McAuliffe, R. P. Ross, C. Hill, *FEMS Microbiol. Rev.* **2001**, *25*, 285–308.

[2] a) M. R. Levengood, W. A. van der Donk, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3025–3028; b) M. Paul, G. C. Patton, W. A. van der Donk, *Biochemistry* **2007**, *46*, 6268–6276; c) A. L. McClerren, L. E. Cooper, C. Quan, P. M. Thomas, N. L. Kelleher, W. A. van der Donk, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 17243–17248; d) G. Jung, *Angew. Chem.* **2006**, *118*, 6063–6065; *Angew. Chem. Int. Ed.* **2006**, *45*, 5919–5921; e) P. D. Cotter, P. M. O'Connor, L. A. Draper, E. M. Lawton, L. H. Deegan, C. Hill, R. P. Ross, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18584–18589; f) A. Guder, I. Wiedemann, H. G. Sahl, *Biopolymers* **2000**, *55*, 62–73.

[3] a) S. Garneau, N. I. Martin, J. C. Vederas, *Biochimie* **2002**, *84*, 577–592; b) E. M. Lawton, R. P. Ross, C. Hill, P. D. Cotter, *Mini-Rev. Med. Chem.* **2007**, *7*, 1236–1247.

[4] a) E. Breukink, I. Wiedemann, C. van Kraaij, O. P. Kuipers, H. Sahl, B. de Kruijff, *Science* **1999**, *286*, 2361–2364; b) I. Wiedemann, T. Bottiger, R. R. Bonelli, A. Wiese, S. O. Hagge, T. Gutsmann, U. Seydel, L. Deegan, C. Hill, P. Ross, H. G. Sahl, *Mol. Microbiol.* **2006**, *61*, 285–296; c) E. Breukink, B. de Kruijff, *Nat. Rev. Drug Discovery* **2006**, *5*, 321–332; d) N. I. Martin, E. Breukink, *Future Microbiol.* **2007**, *2*, 513–525; e) J. Parisot, S. Carey, E. Breukink, W. C. Chan, A. Narbad, B. Bonev, *Antimicrob. Agents Chemother.* **2007**, *52*, 612–618; f) L. Smith, H. Hasper, E. Breukink, J. Novak, J. Cerkasov, J. D. Hillman, S. Wilson-Stanford, R. S. Orugunty, *Biochemistry* **2008**, *47*, 3308–3314.

[5] a) N. I. Martin, T. Sprules, M. R. Carpenter, P. D. Cotter, C. Hill, R. P. Ross, J. C. Vederas, *Biochemistry* **2004**, *43*, 3049–3056; b) M. Galvin, C. Hill, R. P. Ross, *Lett. Appl. Microbiol.* **1999**, *28*, 355.

[6] a) P. D. Cotter, L. H. Deegan, E. M. Lawton, L. A. Draper, P. M. O'Connor, C. Hill, R. P. Ross, *Mol. Microbiol.* **2006**, *62*, 735–747; b) R. Rink, J. Wierenga, A. Kuipers, L. D. Kluskens, A. J. M. Driessen. O. P. Kuipers, G. N. Moll, *Appl. Environ. Microbiol.* **2007**, *73*, 5809–5816.

[7] a) M. Paul, W. A. van der Donk, *Mini-Rev. Org. Chem.* **2005**, *2*, 23–37; b) S. L. Cobb, J. C. Vederas, *Org. Biomol. Chem.* **2007**, *5*, 1031–1038; c) R. S. Narayan, M. S. VanNieuwenhze, *Org. Lett.* **2005**, *7*, 2655–2658; d) N. Ghalit, D. T. S. Rijkers, R. M. J. Liskamp, *J. Mol. Catal. A* **2006**, *254*, 68–77; e) M. Matteucci, G. Bhalay, M. Bradley, *Tetrahedron Lett.* **2004**, *45*, 1399–1401.

[8] a) S. Bregant, A. B. Tabor, *J. Org. Chem.* **2005**, *70*, 2430–2438; b) M. F. Mohd Mustapa, R. Harris, N. Bulic-Subanovic, S. L. Elliott, S. Bregant, M. F. A. Groussier, J. Mould, D. Schultz, N. A. L. Chubb, P. R. J. Gaffney, P. C. Driscoll, A. B. Tabor, *J. Org. Chem.* **2003**, *68*, 8185–8192.

[9] K. Fukase, M. Kitazawa, A. Sano, K. Shimbo, S. Horimoto, H. Fujita, A. Kubo, T. Wakamiya, T. Shiba, *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227–2240.

[10] V. R. Pattabiraman, J. L. Stymiest, D. J. Derksen, N. I. Martin, J. C. Vederas, *Org. Lett.* **2007**, *9*, 699–702.

[11] For synthesis of lanthionine and its analogues, see: a) Y. T. Zhu, M. D. Gieselman, H. Zhou, O. Averin, W. A. van der Donk, *Org. Biomol. Chem.* **2003**, *1*, 3304–3315; b) Y. Rew, S. Malkmus, C. Svensson, T. L. Yaksh, N. N. Chung, P. W. Schiller, J. A. Cassel, R. N. DeHaven, M. Goodman, *J. Med. Chem.* **2002**, *45*, 3746–3754; c) V. Swali, M. Matteucci, R. Elliot, M. Bradley, *Tetrahedron* **2002**, *58*, 9101–9109; d) A. K. Galande, A. F. Spatola, *Lett. Pept. Sci.* **2001**, *8*, 247–251; e) L. Yu, Y. H. Lai, J. V. Wade, S. M. Coutts, *Tetrahedron Lett.* **1998**, *39*, 6633–6636; f) S. Burrage, T. Raynham, G. Williams, J. W. Essex, C. Allen, M. Cardno, V. Swali, M. Bradley, *Chem. Eur. J.* **2000**, *6*, 1455–1466; g) C. Dugave, A. Menez, *Tetrahedron: Asymmetry* **1997**, *8*, 1453–1465.

[12] X. M. Zhu, R. R. Schmidt, *Eur. J. Org. Chem.* **2003**, 4069–4072.

[13] For a solid-phase approach, see: J. P. Mayer, J. W. Zhang, S. Groeger, C. F. Lu, M. A. Jarosinski, *J. Pept. Res.* **1998**, *51*, 432–436.

[14] K. Barlos, O. Chatzi, D. Gatos, G. Stavropoulos, *Int. J. Pept. Protein Res.* **1991**, *37*, 513–520.

[15] H. E. Hasper, N. E. Kramer, J. L. Smith, J. D. Hillman, C. Zachariah, O. P. Kuipers, B. de Kruijff, E. Breukink, *Science* **2006**, *313*, 1636–1637.

