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Solid Supported Chemical Syntheses of Both Components of the Lantibiotic Lacticin 3147

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Supporting Information

ABSTRACT: Lantibiotics are antimicrobial peptides produced by bacteria. Some are employed for food preservation, whereas others have therapeutic potential due to their activity against organisms resistant to current antibiotics. They are ribosomally synthesized and posttranslationally modified by dehydration of serine and threonine residues followed by attack of thiols of cysteines to form monosulfide lanthionine and methyllanthionine rings, respectively. Chemical synthesis of peptide analogues is a powerful method to verify stereochemistry and access structure−activity relationships. However, solid supported synthesis of lantibiotics has been difficult due to problems in generating lanthionines and methyllanthionines with orthogonal protection and good stereochemical control. We report the solid-phase syntheses of both peptides of a two-component lantibiotic, lacticin 3147. Both successive and interlocking ring systems were synthesized on-resin, thereby providing a general methodology for this family of natural products.

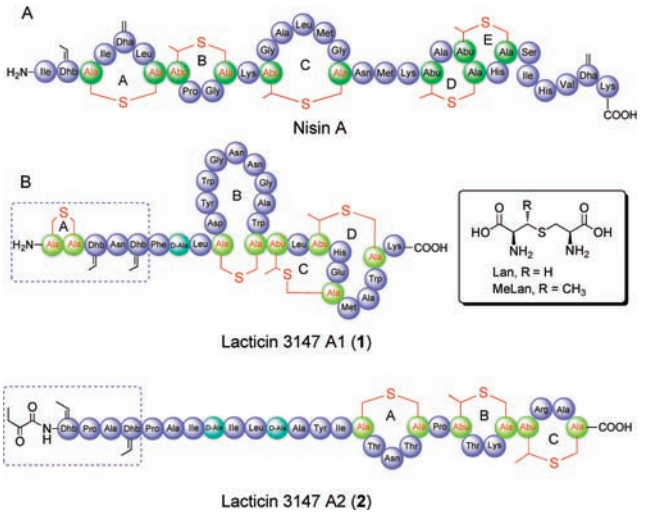

Figure 1. (A) Nisin A. (B) Lacticin 3147 A1 (**1**) and A2 (**2**) peptides. The blue rectangles indicate the pentapeptide fragments that were synthesized in solution phase (vide infra).

Bacteriocins are antimicrobial peptides that are ribosomally synthesized and exported by bacteria to eliminate competing microorganisms.[1,2] Some are unmodified peptides (typically 25 to 60 residues), but the others, such as the lantibiotics (lanthionine-containing antibiotics), undergo extensive post-translational modifications such as dehydration of serine and threonine residues and monosulfide ring formation via Michael addition of cysteine thiols on the resulting dehydroamino acid moieties.[3] This can result in formation of lanthionine (Lan) or methyllanthionine (MeLan) residues that can be either non-overlapping (successive) or interlocking on the peptide chain. The dehydroalanine units derived from serine can also be reduced to D-alanine residues in the peptide.[4] Nisin A, first discovered in 1928, has been widely used in preservation of dairy products for almost 50 years without significant development of resistance.[5] It contains both successive rings (A, B, C) and interlocking ones (D, E) (Figure 1A). Its potent broad spectrum activity (often low nanomolar) against Gram-positive bacteria is due to binding of the peptidoglycan precursor lipid II and membrane pore formation.[6,7] However, it is generally not suitable for meat preservation or as a therapeutic agent because it is unstable at neutral or basic pH and reacts readily with nucleophiles such as water or thiols.[8] Efforts to find more stable compounds led to the isolation of two component lantibiotics such as lacticin 3147 (Figure 1B)[9] and the alkaline stable haloduracins.[10] As these peptides are gene-encoded, mutation of the structural genes of lantibiotics enables the rapid generation of analogues for structure−activity relationship (SAR) studies.[11−14] An alternative approach to analogue generation, which has provided fascinating insights into enzyme mechanism, has relied on reconstitution of the biosynthetic enzymes and in vitro mutasynthesis.[15,16]

Nevertheless, there are limitations to bioengineering. The structures of the modified precursor peptides may be incompatible with the enzymatic machinery for posttranslational modification. As a result, production of the target peptide may be reduced or even abolished. Even if the targeted compound is formed in substantial quantity in bacterial hosts, lack of activity can make detection and isolation of the product difficult. In principle, total chemical synthesis of lantibiotics offers an alternative approach that can allow greater structural variation for SAR investigations and production in sufficient quantities for clinical studies. In the 1980s, Shiba and co-workers completed the first solution-phase synthesis of a natural lantibiotic, nisin A.[17] This work was a monumental achievement, but chemical synthesis of lantibiotics has proven difficult, and a variety of elegant approaches have not yet led to total syntheses of such compounds.[18−21] Major obstacles include generation of protected Lan and MeLan with good stereochemical purity bearing masking

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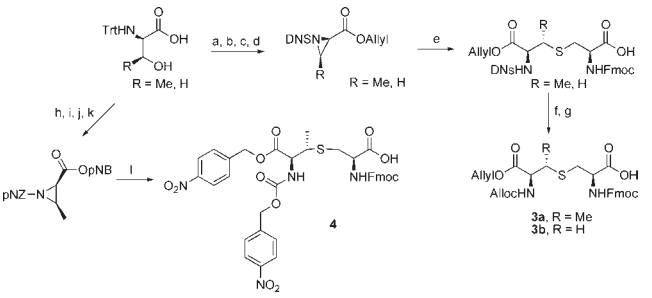
groups that are compatible with standard peptide synthesis and that can be selectively removed to allow macrocyclic ring formation. Our group has reported the solid-supported peptide syntheses (SPPS) and activity of analogues of lacticin 3147 A2 peptide wherein the sulfur is replaced with oxygen[22] or two carbons[23] and also where MeLan bridges are replaced with Lan residues.[24] The only natural lantibiotic to be synthesized using SPPS is lactocin S, a single component antimicrobial that has no MeLan bridges or interlocking rings in its structure.[25] In the present study, we describe facile solid supported syntheses of both the A1 and A2 peptides (Figure 1B, compounds **1** and **2**, respectively) of lacticin 3147, a two-component lantibiotic. These synergistic peptides and their analogues have considerable therapeutic potential as they are stable at physiological pH. Lacticin 3147 is active against *Mycobacterium tuberculosis*[26] and a variety of pathogens resistant to current antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[27] It has been incorporated into a teat seal product for prevention and treatment of mastitis in cows[28] and is being investigated for use in oral hygiene products.[29]

Previous studies directed to chemical synthesis of lantibiotics mentioned above offered a number of approaches to the preparation of orthogonally protected lanthionines, but we chose aziridine ring-opening[22] by cysteine derivatives as a rapid method that provided very good stereochemical control (Scheme 1, see Supporting Information for details). This approach is effective for rapid generation of stereochemically pure Lan and MeLan derivatives bearing distal protecting groups such as allyl and alloc, as well as *p*-nitrobenzyloxycarbonyl (*p*-NZ) and *p*-nitrobenzyl (*p*-NB) that can be selectively removed on-resin by palladium or by tin chloride reduction, respectively. This allows construction of interlocking rings (see below). For the synthesis of the lacticin 3147 A1 peptides, the aziridine opening afforded the necessary derivatives of Lan **3b** and MeLan **3a** and **4**. The absolute stereochemistries of these compounds as well as of the Lan and MeLan residues in **1** and **2** were confirmed by deprotection/hydrolysis, conversion to volatile derivatives (dimethyl esters with bis-*N*-pentafluoropropanoyl groups) and chiral gas chromatography–mass spectrometric (GC–MS) analyses.[30] Other isomers of Lan and MeLan were prepared as standards. After the absolute configurations of the two peptides were confirmed to be as initially proposed,[9] we commenced the synthesis of lacticin 3147 A2 (**2**).

Scheme 1. Synthesis of Protected Lan and MeLan Derivatives 3a, 3b and 4[a]

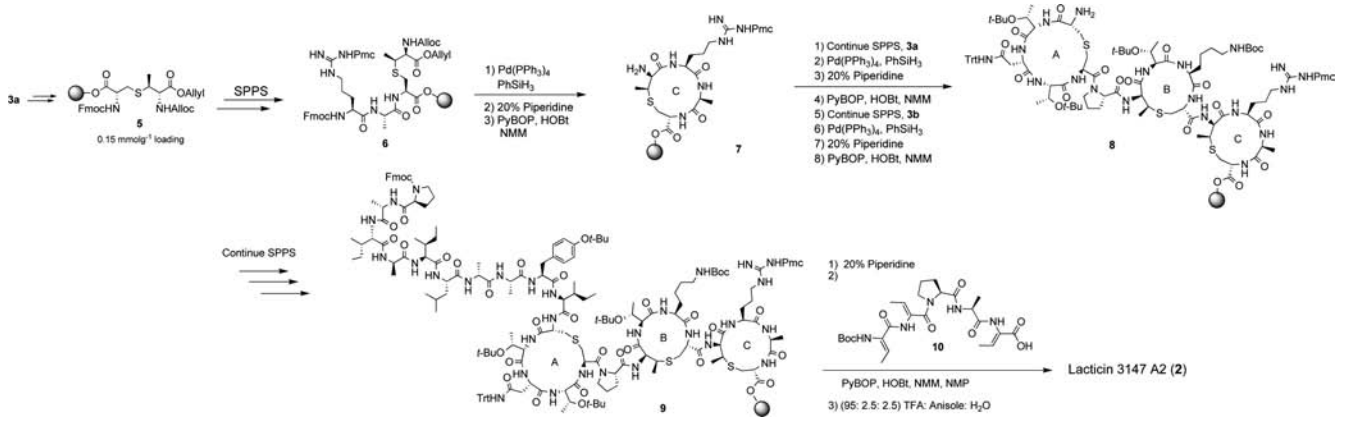


[a] Reagents and conditions: (a) allyl chloride, $NaHCO_3$, quantitative. (b) MsCl, Et_3N, 0 °C. (c) 3:3:1 TFA/DCM/MeOH, 0 °C. (d) DNs-Cl, Na_2CO_3, 61% over three steps. (e) Fmoc-Cys-OH, $BF_3 \cdot Et_2O$, 0 °C, 40%. (f) Thioglycolic acid, DIPEA. (g) Alloc chloride, DIPEA, 44%. (h) *p*-NBOH, *p*-TsOH. (i) TrtCl, Et_3N, 60% over two steps. (j) MsCl, Et_3N, reflux. (k) 3:3:1 TFA/DCM/MeOH, 0 °C, *p*-NZCl, Na_2CO_3, 60%. (l) Fmoc-Cys-OH, $BF_3 \cdot Et_2O$, 46%.

The protected MeLan **3a** was first loaded onto 2-chlorotrityl polystyrene resin with the low substitution of 0.15 mmol·g^{-1} to give **5**, which is crucial to avoid intermolecular coupling during on-resin ring formation (Scheme 2). Then, alanine and protected arginine were introduced using standard Fmoc-SPPS methodology to give the linear tripeptide **6**. The allyl and alloc groups of the MeLan residue in **6** were removed by $Pd(PPh_3)_4$ and $PhSiH_3$, followed by removal of the Fmoc with 20% piperidine in DMF. Compound **7** was then obtained by cyclization using PyBOP/HOBt. Subsequently, rings B and A were constructed in a similar fashion to give tricyclic product **8**. An additional ten residues from isoleucine to proline (residues 15 to 6) were then installed. The *N*-terminal pentapeptide **10** was synthesized in solution due to its heavily modified nature. Coupling of **10** to **9** on-resin was followed by acidic cleavage from the resin with concomitant removal of all acid labile protecting groups. The liberation of the N-terminus as a primary enamine results in spontaneous hydrolysis to the desired α-keto amide to provide the lacticin 3147 A2 peptide **2**.

The synthesis of the A1 peptide **1** began with the loading of Fmoc-Lys(Boc)-OH onto Wang resin (0.15 mmol·g^{-1}), followed by standard coupling of orthogonally protected MeLan **3a** to give **11** (Scheme 3). The next six residues were

Scheme 2. Solid Phase Synthesis of Lacticin 3147 A2 (2)



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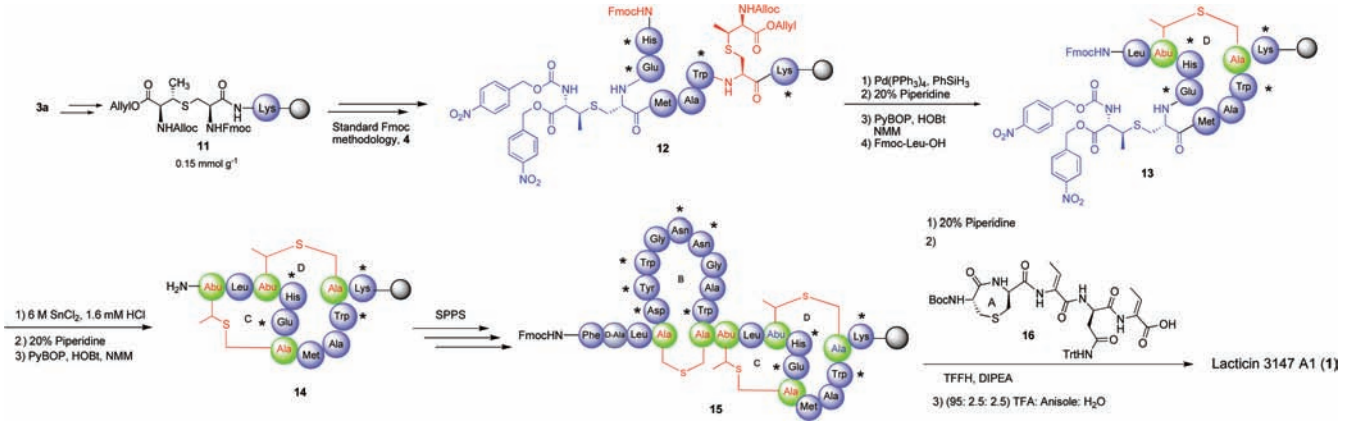
Scheme 3. Solid Phase Synthesis of Lacticin 3147 A1 (1)[a]



[a] The resin-bound peptides were protected on side chains at asterisk sites. The free peptide was not side-chain-protected.

then coupled on-resin sequentially, including the orthogonally protected MeLan **4** to give linear peptide **12**. After deprotection of allyl and alloc groups on the first of the MeLan residues using $Pd(PPh_3)_4$ and $PhSiH_3$, ring D in **13** was synthesized through on-resin amide formation in the presence of PyBOP and HOBt. The following residue (Leu) was then installed. Next, the *p*-NZ and *p*-NB groups in the second MeLan moiety were removed simultaneously using $SnCl_2$. Subsequent Fmoc-deprotection and cyclization furnished the interlocking rings C and D in **14**. SPPS synthesis was continued with initial attachment of protected Lan **3b.** This was followed by linking 9 more residues needed for ring B and then Pd deprotection of the distal Lan allyl and alloc groups as before. Cyclization of the large ring B proceeded smoothly, and attachment of three more residues gave the tricyclic peptide **15**. The N-terminal pentapeptide **16** containing ring A was synthesized in solution because of its extensive and densely packed modifications (see Supporting Information). Coupling between fragments **15** and **16** on-resin was followed by acidic cleavage with concomitant removal of all acid labile protecting groups to yield the A1 peptide **1** of lacticin 3147.

After purification by reverse-phase HPLC, analysis of synthetic A1 **1** and A2 **2** by MALDI-TOF MS showed peaks at 3305.3 and 2847.4 Da $(M + H)^+$, respectively, which are identical to those of the natural products. Further MS/MS studies confirmed the correct sequence. The overall yields of A1 and A2 were 1.4% and 1.0%, respectively. Each synthesis encompassed more than fifty steps, and therefore the yield of each coupling and deprotection is greater than 95%. Each synthetic peptide was tested in conjunction with its synergistic partner of natural lacticin 3147 against the Gram-positive indicator organism *Lactococcus lactis* subspecies *cremoris* HP. As expected, synthetic **1** and **2** showed biological activity indistinguishable from the corresponding natural peptide isolated from the producer organism, *L. lactis* subspecies *lactis* DPC 3147 (Figure 2). In each case, both **1** and **2** show inherent antimicrobial activity, but there is a strong synergism as indicated by the enhanced inhibition zone in the area between the application of two peptides. Our earlier studies with oxa- and bis des-methyl analogues of **2** had shown that the antimicrobial activity of this two peptide system is exquisitely sensitive to small alterations in lanthionine ring geometry. For example, replacement of sulfur by oxygen in **2** gives an analogue that retains independent

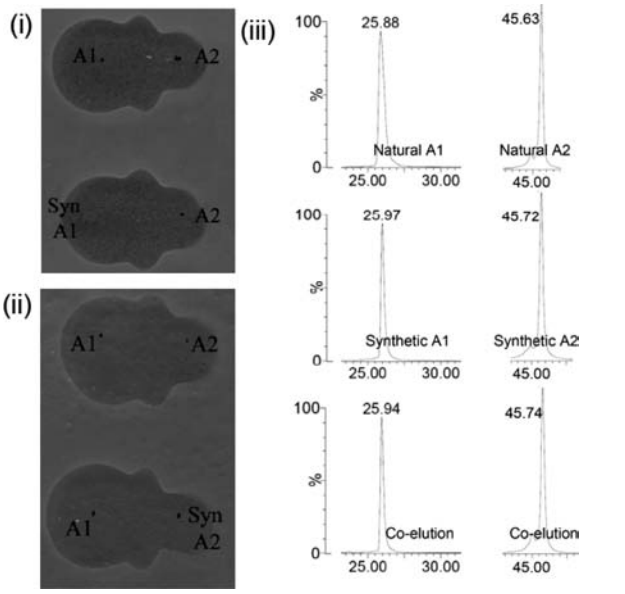


Figure 2. Spot-on-lawn tests for antimicrobial activity showing zone of growth inhibition against *L. lactis* subspecies *cremoris* HP. The activities of synthetic lacticin 3147 A1 and synthetic 3147 A2 were compared to the corresponding natural peptide, shown in (i) and (ii) respectively. LC results are shown in (iii).

antimicrobial activity against Gram-positive bacteria but lacks the synergistic activity with the natural A1 peptide **1**. Interestingly, the analogue of **2** wherein MeLan bridges are replaced with Lan (bis-desmethyl) shows the converse effect: it has no inherent activity but displays synergistic activity with **1**. These effects as well as potent activity of the parent system that we have now synthesized can be explained through dual modes of action, namely, inhibition of peptidoglycan biosynthesis through binding of lipid II by each individual component and subsequent pore formation by the ternary complex consisting of lipid II and both peptides.[31]

The present synthesis of both components of a two-peptide lantibiotic on solid support using standard Fmoc methodology serves as a template for production of Lan and MeLan containing peptides having either sequential or interlocking rings. This rapid

and semiautomated methodology will facilitate the development of high-throughput SAR studies of lantibiotics in cases that are not readily accessible by enzymatic transformation either in bacteria or in vitro.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, NMR and GC−MS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

Abu-S-Ala, β-methyllanthionine; Ala-S-Ala, lanthionine; Boc, butyloxycarbonyl; Dhb, dehydrobutyric acid; DIPEA, *N*,*N*-diisopropylethylamine; DNs-Cl, 2,4-dinitrobenzenesulfonyl chloride; Fmoc, fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; NMP, 1-methylpyrrolidinone; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; *p*-NB, *p*-nitrobenzyl*p*-NZ, *p*-nitrobenzyloxycarbonyl; PyBOP, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate; SPPS, solid phase peptide synthesis; TFA, trifluoroacetic acid; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; Trt, trityl

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