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Synthesis of the Lantibiotic Lactocin S Using Peptide Cyclizations on Solid Phase

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Peptides with multiple sulfur-containing rings occur throughout nature in plants, animals, and bacteria.[1] Lantibiotics are lanthionine-containing peptides produced by bacteria that exhibit potent antimicrobial activity against a broad range of Gram-positive organisms.[2,3] These peptides and the organisms that generate them are already used to preserve food (e.g., nisin A) and have great potential for treatment of antibiotic-resistant infections. The monosulfide lanthionine rings form by enzyme-catalyzed dehydrations of serine or threonine residues followed by intramolecular Michael addition of cysteine thiols to generate (in the cases examined) a D stereocenter.[4] However, the stereochemistry of the lanthionines has been confirmed only in a small number of peptides.[5−8] Isolation of lantibiotics from natural sources is often challenging, as the producer organisms may make small amounts of the potent peptides. Synthesis provides an alternative avenue for studying lantibiotics. To date, the pioneering solution-phase synthesis of nisin A by Shiba and co-workers is the only chemical synthesis of a natural lantibiotic.[9,10] Multiple groups have tackled the synthetic challenges of producing lantibiotics and their analogues.[11−18] We now report the total synthesis of lactocin S (**1**), a 37-residue lantibiotic discovered in *Lactobacillus sakei* by Nes and co-workers.[19−21] This represents the first solid-supported synthesis of a natural lantibiotic and also confirms the stereochemistry of the lanthionine rings (Figure 1).

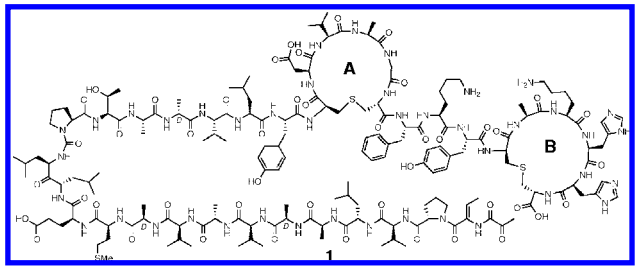
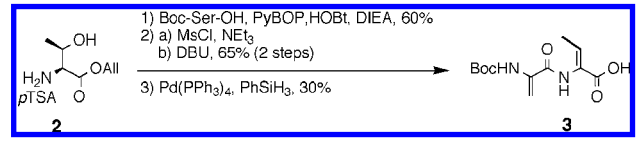


Figure 1. Confirmed structure of lactocin S (**1**).

The synthetic approach to **1** involved the synthesis of residues 3−37 on a solid support and residues 1 and 2 (e.g., **3**) in solution followed by coupling of the two portions on resin. Acidic cleavage from the resin with concomitant removal of protecting groups was envisioned to give an N-terminal primary enamine that should spontaneously hydrolyze to the desired pyruvamide moiety. This methodology avoids the difficult coupling of a fragment containing an α-ketoamide.[15] The N-terminal dipeptide was readily made by coupling L-threonine allyl ester (**2**) with Boc-L-serine (Scheme 1). Mesylation followed by elimination introduced the desired dehydroalanine and dehydrobutyrine functionalities. Finally, removal of the allyl group provided **3** ready for coupling.

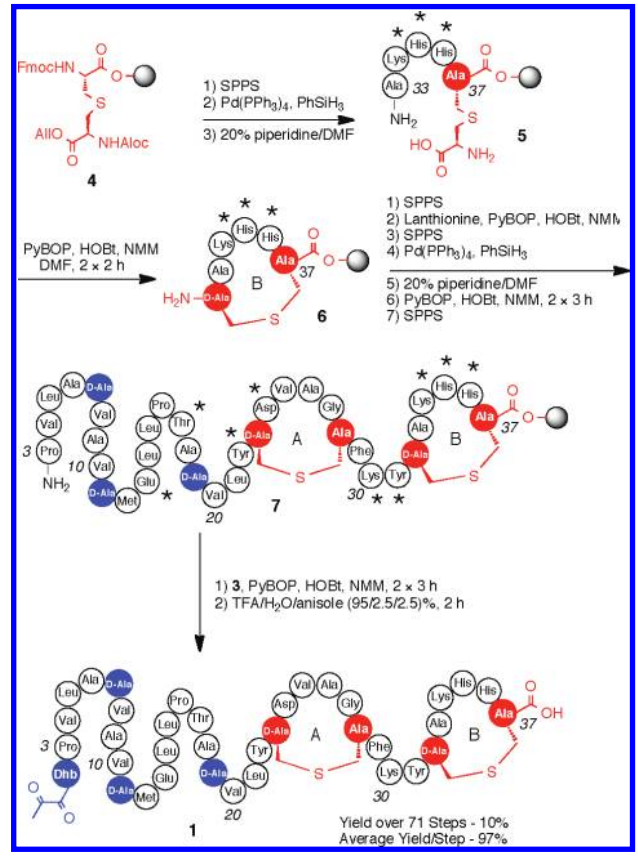
The resin-bound synthesis of lactocin S commenced with the loading of a protected DL-lanthionine residue onto 2-chlorotrityl chloride polystyrene resin (**4**) (Scheme 2). The protected lanthionine

Scheme 1. Synthesis of the Protected N-Terminal Dipeptide Fragment of Lactocin S



was obtained according to a literature procedure.[11,12,22] The amino acids of ring B were then introduced via standard Fmoc solid-phase peptide synthesis (SPPS) using the coupling reagents PyBOP and HOBt. Following removal of the allyl and Aloc protecting groups

Scheme 2. Solid-Phase Synthesis of Lactocin S[a]



[a] The resin-bound peptides were protected on side chains at asterisk sites with acid-removable groups as follows: His(*N*-trityl); Lys(*N*-Boc); Tyr(*O*-t-Bu); Asp(*O*-t-Bu); Thr(*O*-t-Bu); Glu(*O*-t-Bu). The free peptide was not side-chain-protected. Red, lanthionines; blue, uncommon residues.

and the N-terminal Fmoc of Ala33, **5** was converted to ring B (**6**) by treatment with PyBOP and HOBt. The amino acids between rings A and B were introduced by SPPS, and then a second protected lanthionine (Fmoc, Aloc, allyl as in **4**) was incorporated

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via its free carboxyl. After removal of the Fmoc and addition of the amino acids within ring A, removal of allyl and Aloc with Pd as before permitted cyclization to form ring A. The peptide was then further elongated on resin to include amino acid residues 22 to 3, affording **7** (shown after removal of the N-terminal Fmoc protecting group). Fragmental coupling between **3** and **7** on resin introduced the last two residues.

The synthesis of **1** was completed by cleavage of the peptide from the resin using a 95:2.5:2.5 trifluoroacetic acid/anisole/water cocktail. At this point, all of the protecting groups were removed, including the N-terminal Boc group. The resulting enamine functionality tautomerized to the iminium ion, which was hydrolyzed under the cleavage conditions to give the desired N-terminal α-ketoamide functionality. The synthetic lactocin S (**1**) was purified using reversed-phase HPLC. FTICR-ESI-MS analysis revealed a signal at *m/z* 941.4767 ($[M + 4H]^{4+}$) representing the desired lactocin S with a mass of 3761.9 Da. The correct peptide sequence was confirmed by tandem MS/MS analysis.

To compare the synthetic compound to natural **1**, the lantibiotic was isolated from *L. sakei* L45 using a procedure adapted from that published by Nes and co-workers.[20] Both pure peptides displayed the same HPLC behavior and mass spectra. The biological activities of the synthetic **1** and the natural peptide were analyzed using spot-on-lawn assays. The two peptides inhibited the growth of *Pediococcus acidilactici* Pac 1.0 comparably. As expected, neither peptide showed antibacterial activity against *L. sakei* L45, the producer organism. To further confirm the stereochemistry of the lanthionine residues in **1**, both the synthetic and natural peptides were fully hydrolyzed with 6 M HCl and derivatized as their pentafluoropropanamide methyl esters for chiral GC−MS analysis. The hydrolysate derivatives were analyzed by GC−MS alone and also with the addition of synthetic standards derived from each possible stereochemistry of lanthionine (DD, DL, LL).[5] Our results support the original proposal that **1** contains two DL-lanthionines.

In summary, the synthesis of lactocin S (**1**) was completed in an overall purified yield of 10% for 71 steps (including all deprotections and couplings) based on the initial lanthionine bound to the chlorotrityl resin. This corresponds to an average yield of 97% per step, although it should be noted that most of the couplings used a protected amino acid monomer in considerable excess. Although solid-supported syntheses of two analogues of a lantibiotic (lacticin 3147 A2 peptide) have been reported recently by us,[15,22] this work represents the first such preparation of a natural lantibiotic. Comparison of the synthetic lactocin S to the natural lantibiotic isolated from *L. sakei* L45 confirmed the structure of lactocin S, including the stereochemistry of the lanthionine residues, which had previously been assigned by analogy to that found in a small number of other lantibiotics. There is currently great interest in modifying lantibiotics to improve their activity, solubility, and stability under physiological conditions.[23] Site-specific mutagenesis of the structural gene is a rapid and effective approach, but it can interfere with correct processing of the peptide and may lead to unexpected products. Generation of analogues by *in vitro* mutasynthesis using biosynthetic enzymes on precursors having unusual amino acids is a very powerful approach used successfully to make lacticin 481 derivatives.[24] Both of these methods require isolation and structure elucidation of the resulting active compounds to confirm their identities. Chemical synthesis on solid supports provides a complementary platform for making new, chemically diverse lantibiotics. A solution for the challenge of making interlocking rings, such as found in nisin A or lacticin 481, by total synthesis on a solid support remains to be reported.

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Supporting Information Available: Experimental procedures and characterization of novel compounds, procedure for obtaining natural lactocin S, biological testing results, and chiral GC−MS results. This material is available free of charge via the Internet at http://pubs.acs.org.

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