JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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The Synthesis of Active and Stable Diaminopimelate Analogues of the Lantibiotic Peptide Lactocin S

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Supporting Information

ABSTRACT: Lantibiotic peptides are potent antimicrobial compounds produced by Gram-positive bacteria. They can be used in food preservation, and some also show potential for clinical applications. Unfortunately, some of these peptides can be susceptible to inactivation by oxidation of the sulfur-containing amino acid lanthionine, limiting their use. Here we describe the synthesis and testing of diaminopimelate analogues of the lantibiotic lactocin S. These analogues were designed to improve the oxidative stability of the peptide by replacing the sulfur in lanthionine with a methylene unit. Lanthionine was systematically replaced with diaminopimelate during solid-phase peptide synthesis to produce several analogues. One analogue, A-DAP lactocin S, was found to retain full biological activity in addition to displaying increased stability. This is the first time a synthetic lanthionine ring analogue of a lantibiotic has retained natural activity levels. This methodology is potentially very promising for use in producing more stable, medically relevant lantibiotics.

Lantibiotics are a class of ribosomally synthesized, highly post-translationally modified peptides produced by bacteria as a form of chemical warfare against competing microorganisms.[1−5] These compounds show potent activity against a wide range of Gram-positive bacteria, making them a promising weapon against bacterial infections in mammals. They are already extensively utilized in the preservation of food from pathogens.[6] Lantibiotics are characterized by the presence of their namesake amino acids, lanthionine (Lan) and methyllanthionine (MeLan). These structural units are derived from serine and threonine residues that are enzymatically dehydrated and then attacked in a Michael fashion by cysteine.[2,7,8] Many lantibiotics kill bacteria in two main ways: they inhibit the synthesis of the cell wall, and they aggregate with lipid II to form pores in the cell membrane.[8−11] Some of these peptides are able to act in both modes, making it much harder for bacteria to develop resistance to them.

Lantibiotics are extremely potent antibacterial compounds, but some are stable only at acidic pH, and many are prone to inactivation by air oxidation to sulfoxides.[12,13] This limits their use in the preservation of food or in the clinic. As a result, attempts are ongoing in the scientific community to discover and design peptides that are more stable and equally potent in comparison with their natural counterparts. Bioengineering of the structural genes for lantibiotics has been used to produce many compounds.[14] However, alteration of the cyclic lanthionine residues is problematic, as they are the result of post-translational enzymatic processing. Chemical synthesis offers an attractive alternative for the production of cyclic lanthionine analogues at these positions. Several groups have developed such methodology,[15−23] and indeed, several full-length ring analogues have been synthesized.[24−26]

Lactocin S (**1**) is a lantibiotic peptide produced by a strain of *Lactobacillus sakei* that was first isolated by Nes and co-workers from fermented sausage.[27−29] This 37 amino acid peptide (Figure 1) is generated in very small quantities by the producer

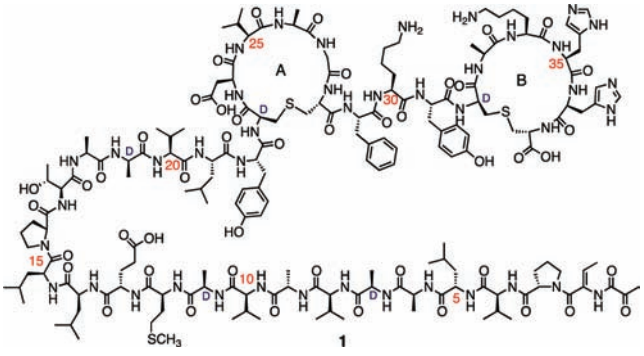


Figure 1. Structure of the lantibiotic lactocin S (**1**).

organism. This low production along with oxidative instability has limited the investigation of clinical and food-oriented applications for the compound. Previously we completed the total chemical synthesis of **1**.[13] The stereochemical configuration of the two Lan residues was determined by chiral GC−MS studies after hydrolytic degradation of the lantibiotic, and this result confirmed the proposed chemical structure of **1**.

We now report the syntheses of three diaminopimelate (DAP)-containing analogues of lactocin S, compounds **2**, **3**, and **4**, wherein the Lan residues in each ring (**A** and **B**) are systematically replaced with DAP (Figure 2). These compounds, which have sulfur replaced by a methylene carbon, retain significant antimicrobial activity. A-DAP lactocin S (**2**) shows potency comparable to that of natural **1** and enhanced stability. These analogues are the first DAP derivatives of a lantibiotic to be reported and demonstrate that such ring alterations can yield fully active final compounds.

Received: November 25, 2011
Published: January 24, 2012

   dx.doi.org/10.1021/ja211088m | *J. Am. Chem.Soc.* 2012, 134, 2008−2011

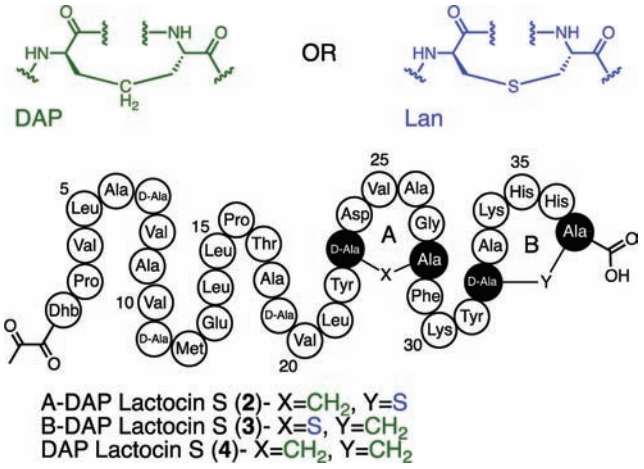
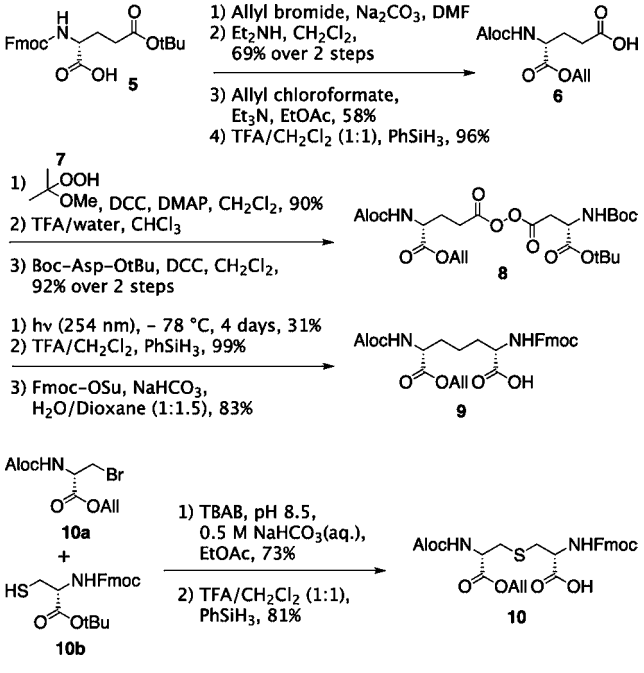


Figure 2. Analogues **2**–**4** of lactocin S wherein sulfur is replaced by CH_2.

Our synthetic approach utilized amino acid building blocks made in solution to complete a solid-supported synthesis for each peptide. Orthogonally protected DAP (ortho-DAP) (**9**) was produced using procedures from our earlier work (Scheme 1).[30] This solution synthesis was adapted to allow the incorporation of the allyl (All) and allyloxycarbamate (Aloc) protecting groups necessary for the ring formation strategy used during the solid-phase peptide synthesis (SPPS).

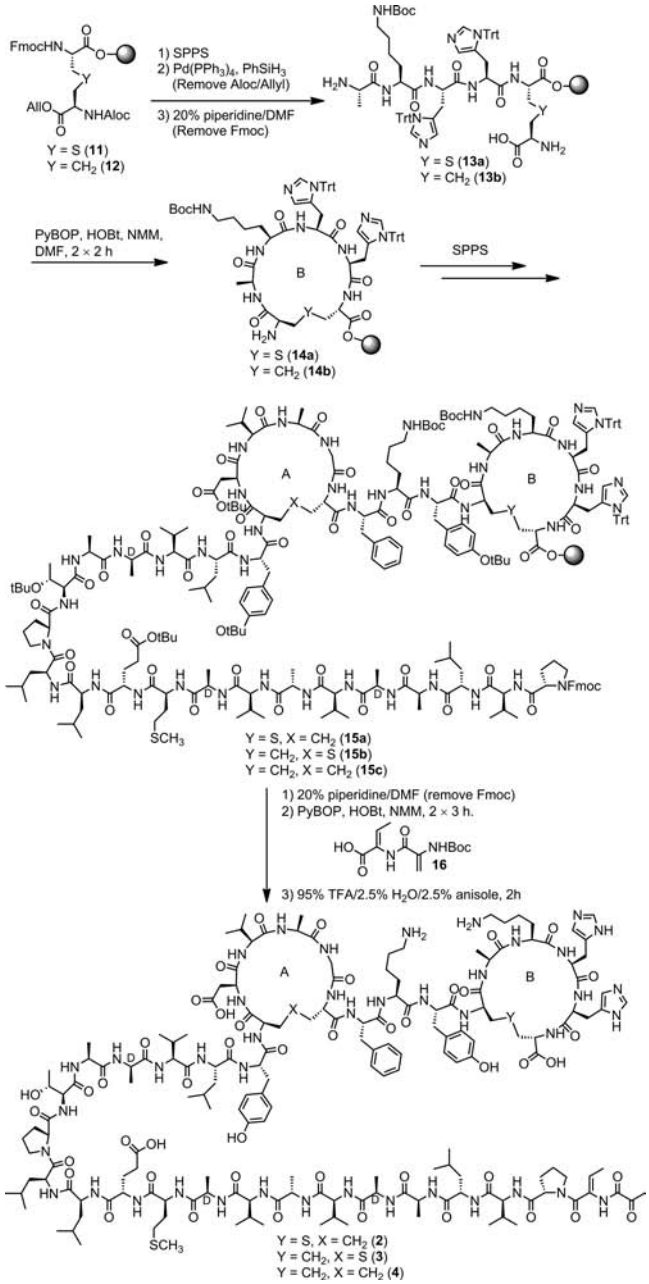
Scheme 1. Synthesis of Orthogonally Protected DAP (9)



Briefly, Fmoc-D-Glu(OtBu)-OH (**5**) was treated with allyl bromide to give an allyl ester. Next, the Fmoc group was removed under basic conditions, and allyl chloroformate was used to protect the resultant free amine as a carbamate. The γ-acid was then deprotected with trifluoroacetic acid (TFA) to give **6**, which was coupled with hydroperoxide **7** [see the Supporting Information (SI) for preparation details] to give a perester. Generation of a peracid using TFA/water was followed by coupling to Boc-L-Asp-OtBu with 1,3-dicyclohexylcarbodiimide (DCC). Diacyl peroxide **8** was irradiated with UV light (254 nm) at low temperature to generate a DAP derivative. Subsequent removal of the Boc and tBu protecting groups with TFA followed by protection of the free amine with Fmoc-OSu yielded **9**.

Ortho-Lan (**10**) was made using a literature procedure.[25,31,32] For each peptide, the SPPS was initiated by coupling **10** or **9** onto 2-Cl-trityl-Cl resin to give **11** or **12**, respectively. The amino acids of the B ring were then introduced by following the standard Fmoc SPPS procedure using benzotriazole-1-yl-oxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) and 1-hydroxybenzotriazole (HOBt) as coupling agents (Scheme 2). To form the B ring, the Fmoc and allyl/Aloc protecting groups were removed with piperidine and $Pd(PPh_3)_4$, respectively, to yield **13a** or **13b**. The N-terminal amine was then coupled with the free acid moiety of Lan or

Scheme 2. SPPS of DAP-Containing Analogues 2–4



dx.doi.org/10.1021/ja211088m | J. Am. Chem.Soc. 2012, 134, 2008–2011

DAP using PyBOP and HOBt to give the cyclic product **14a** or **14b** in which the amine of Lan or DAP is the new N-terminus of the peptide. SPPS was used to incorporate the amino acids between the rings, and then the second ortho-Lan or ortho-DAP was installed. Once the amino acids in the A ring were added, this ring was cyclized by the same method as above.

SPPS was used to introduce residues 22 to 3, yielding individual samples of the three peptides **15a−c** bound on resin. The dipeptide precursor **16** for the last two residues was made in solution using a literature procedure[13] and then coupled to the three peptides **15a−c** while they were still immobilized on the resin. The full-length peptides were cleaved from the resin, and all of the protecting groups were simultaneously removed by treatment with 95:2.5:2.5 TFA/anisole/water. The N-terminal α-ketoamide functionality was formed under these acidic conditions by tautomerization of the deprotected enamine to an iminium ion that was subsequently hydrolyzed.[13] Each of the peptides **2−4** was purified by reversed-phase HPLC and then analyzed by Fourier transform ion cyclotron resonance electrospray ionization mass spectrometry (FTICR-ESI-MS) (see the SI). Additionally, the sequences of the peptides were confirmed by MS/MS analysis. The peptides were obtained pure in overall yields of 1.6−2.5% over 71 steps (deprotections and couplings), corresponding to ∼95% yield per reaction.

The antibacterial activities of the analogues were compared to that of lactocin S (**1**) using a spot-on-lawn serial dilution assay. The compounds were tested against *Pediococcus acidilactici* Pac 1.0 (Figure 3), *Pediococcus pentosaceus* FBB63, *Lactobacillus acidophilus* 4356, *Lactobacillus bulgaricus* 11842, *L. sakei* L45, and *Listeria monocytogenes* Scott A. The activity profiles of the analogues closely mirrored that seen for **1**. Both *P. acidilactici* and *L. bulgaricus* were sensitive to the peptides, whereas the remaining organisms showed no growth inhibition at the concentrations tested. It is interesting to note that the producing organism *L. sakei* appeared to have retained immunity to all of the analogues. Although B-DAP (**3**) and DAP (**4**) were less active than **1**, A-DAP (**2**) retained the full activity of **1**. To the best of our knowledge, all previous attempts to alter the ring structure of lantibiotics via synthesis have resulted in peptides with reduced activity.[24−26] This result represents the first time synthetic alteration of the rings of a lantibiotic has resulted in full retention of activity.

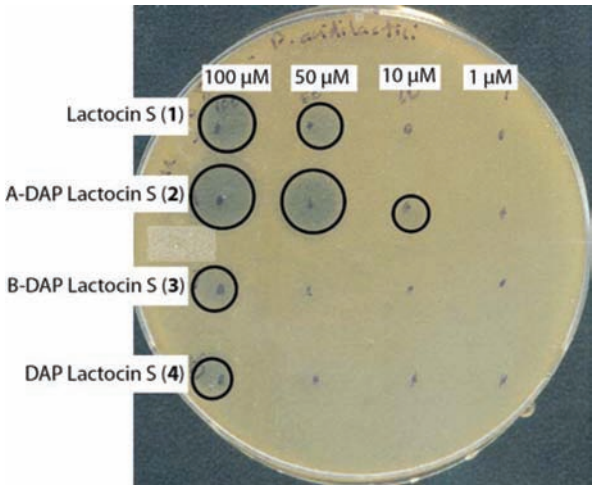


Figure 3. Comparison of the activities of **1−4** against *P. acidilactici* Pac 1.0. Dark circular areas indicate zones of clearing where the bacterial growth was inhibited by the presence of the peptide.

To investigate whether **2** is also more stable toward oxidation, solutions of **1** and **2** were placed under an atmosphere of oxygen for up to 6 h. Activity testing found that **1** was no longer active after 6 h, whereas **2** retained its activity, albeit at a somewhat reduced level (zone of clearing decreased by 50%). The reduction in activity seen for **2** is likely due to oxidation of the B-ring lanthionine to the corresponding sulfoxide as well as oxidation of the sulfur of the methionine at position 12. The marked increase in oxidative stability of **2** relative to **1** supports the assertion that DAP is a very good replacement for Lan and should be considered in other lantibiotics with high clinical relevance, such as lacticin 3147. In previous studies using the lacticin 3147 A2 peptide, replacement of the sulfurs in Lan and MeLan with two carbon linkers abolished the antimicrobial activity.[24] Corresponding replacement of sulfur by oxygen reduced the inherent antimicrobial activity of the peptide and abolished its ability to act synergistically with its A1 partner lantibiotic.[26] Conversely, removal of two methyl groups from the natural A2 peptide (replacement of MeLan by Lan) eliminated the inherent antimicrobial activity but resulted in retention of the synergistic activity with the A1 peptide.[25] In light of these results, the full retention of the activity by **2** along with the observed increased oxidative stability represents an important step toward the goal of developing highly active and stable lantibiotic peptides for applications in food preservation and the clinic.

Our study suggests that the sulfur in the B ring of lactocin S (**1**) is more important for activity, whereas that in the A ring may be replaced by methylene. From an examination of the structure of the two rings, it is obvious that their electrostatic environments are very different. At acidic pH, the A ring has no charged residues, whereas the B ring has three. Although little about the mode of action of **1** has been reported, a positively charged C-terminal ring may allow the peptide to interact with the negatively charged lipids on the outside of bacterial cells. This could facilitate its insertion into the membrane to form pores, as seen for the lantibiotic nisin A.[5,10] This interaction would likely be affected by small conformational changes in the B ring when DAP replaces Lan. Conversely when the A ring contains DAP, the required structural changes may have little or no effect on the activity. Further structure−activity studies of the B ring, focusing on the charged residues, could improve our understanding of the mode of action of this lantibiotic.

In summary, we have developed a methodology for the synthesis of orthogonally protected DAP for use in Fmoc SPPS. This has been used to produce three DAP analogues of lactocin S (**1**). One of these is a fully active synthetic analogue of a lantibiotic with enhanced stability toward oxidation. Our findings indicate that DAP can be a very useful and versatile replacement for Lan. Further investigation of DAP incorporation into lantibiotics may prove to be extremely fruitful, especially in peptides of high clinical relevance such as lacticin 3147 and nisin.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

dx.doi.org/10.1021/ja211088m | *J. Am. Chem.Soc.* 2012, 134, 2008−2011

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chair in Bioorganic and Medicinal Chemistry for financial support. We are grateful to Bela Reiz and Randy Whittal (University of Alberta) for assistance with mass spectrometric analysis

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