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Synthesis and Biological Activity of Oxa-Lacticin A2, a Lantibiotic Analogue with Sulfur Replaced by Oxygen

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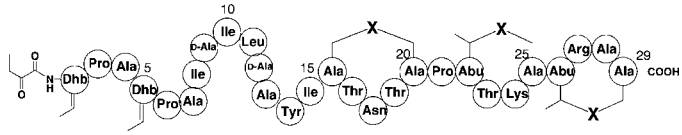
ABSTRACT



2 X = S, Natural lacticin 3147 A2

3 X = O, Oxa-lacticin 3147 A2

An oxidatively stable analogue 3 of lacticin 3147 A2 (2), wherein the sulfur atoms are replaced with oxygens, was synthesized using solution phase peptide synthesis and sequential on-resin cyclizations. Biological evaluation suggests that oxa-lacticin A2 (3) retains independent antimicrobial activity against Gram-positive bacteria but lacks the synergistic activity with natural lacticin A1 that is characteristic of the native lacticin A2 peptide.

Lantibiotics[1] (e.g., nisin A, lacticin 3147) are bacterially produced antimicrobial peptides (bacteriocins) that preserve food and have potential as drugs due to their potent activity against a wide range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE). Lantibiotics occur in dairy products as well as other foods and are often consumed as part of a human diet. They contain the unusual amino acids, *meso*-lanthionine and (2*S*,3*S*,6*R*)-3-methyllanthionine, as a result of post-translational modification. The two-component lantibiotic, lacticin 3147, consists of peptides A1 (**1**) and A2 (**2**) that display not only individual antimicrobial activity but also synergistic activity in nanomolar concentration (Figure 1).[2] It is postulated that the individual activity results from direct binding of lacticin A1 or A2 peptides to the peptidoglycan biosynthesis prescursor, lipid II, thereby inhibiting the formation of the bacterial cell wall.[3] The synergistic activity is attributed to pore formation in the cell membrane facilitated by a trimeric complex between A1-lipid II and A2 peptide.[4] The dual functionality of these peptides makes lacticin 3147 several orders of magnitude more potent than most traditional antibiotics such as penicillin.[2a] The lack of toxicity of many lantibiotics to mammals and their ability to avoid development of significant resistance in pathogenic bacteria make them attractive targets for development as antibiotics. However, the sulfur atoms in lanthionine rings are vulnerable to oxidation to sulfoxides, which renders many lantibiotics inactive.[2a,5] To address this problem and to examine structure−activity

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relationships, a number of research groups have examined the chemical synthesis of lantibiotic analogues and fragments.[6] Only one total synthesis of a natural lantibiotic (nisin A) has been reported using solution phase methods.[7] Two solid-supported syntheses of analogues of lantibiotics have been recently described by us.[6a,8]

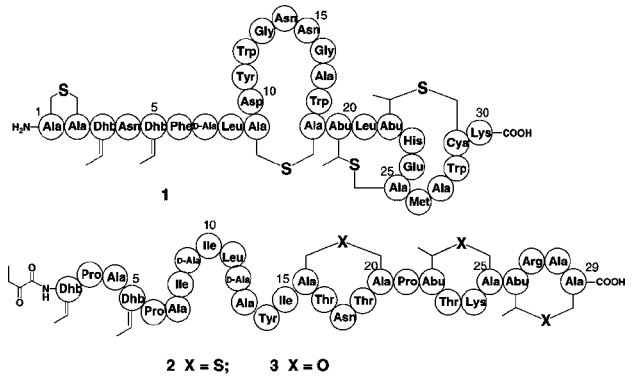


Figure 1. Lacticin 3147 component A1 (**1**) and A2 (**2**) and oxygen analogue of A2 (**3**).

We now report the solid phase synthesis and biological evaluation of oxa-lacticin A2 (**3**), an analogue of A2 (**2**) wherein all sulfur atoms in the lanthionine and methyllanthionine rings are replaced with oxygen atoms. Although sulfur and oxygen have different single bond lengths to carbon (1.80 vs 1.43 Å), the more acute bond angle of C−S−C compared to C−O−C bonds (101.4° vs 111.4°) compensates to make the overall length of *meso*-lanthionine and its oxygen analogue, *meso*-3-(oxa)diaminopimelic acid (*meso*-oxa-DAP), very similar (ca. 6% difference).[9]

Our synthetic strategy involved solid phase peptide synthesis, followed by fragment coupling between a tricyclic peptide on-resin and an N-terminal pentapeptide (1−5) prepared in solution (Figure 2). The on-resin formation of the "oxa-lanthionine" rings could be done via an intramolecular cyclization using orthogonally protected *meso*-oxa-DAP (or 3-methyl-oxa-DAP) precursors.[8]

The tetra-orthogonally protected *meso*-oxa-DAP (**4a**) and its 3-methyl derivative (**4b**) ((2*R*,3*S*,6*S*)-isomer) were prepared using an aziridine ring-opening methodology previously reported by our group.[10] The *tert*-butyl groups were selectively removed under acidic conditions to give **5a** and **5b**, respectively (Scheme 1).

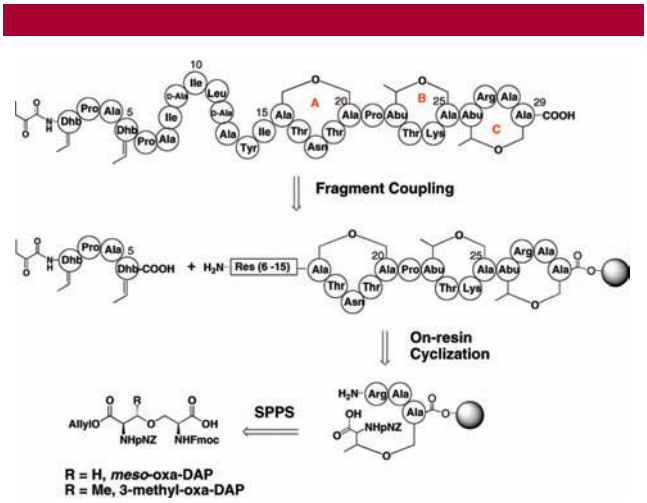
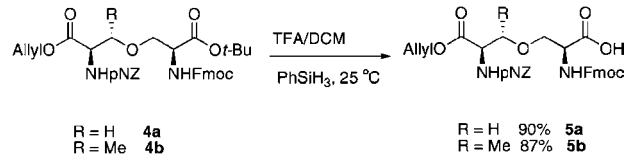


Figure 2. Retrosynthetic analysis of **3**, oxa-lacticin 3147 A2.

Scheme 1. Synthesis of oxa-DAP and 3-Methyl-oxa-DAP Precursors for SPPS



To initiate Fmoc solid-phase peptide synthesis (SPPS), the protected (2*R*,3*S*,6*S*)-3-methyl-oxa-DAP derivative **5b** was attached to Wang resin with a low substitution of 0.1 mmol/g. The low loading is crucial to avoid interstrand cross-linking during on-resin cyclization of the deprotected oxa-DAP (or 3-methyl-oxa-DAP) residue.[11] Standard Fmoc SPPS furnished the linear sequence of ring C (**7**) using PyBOP as the coupling reagent. The selective removal of the allyl group with $Pd(PPh_3)_4$ and $PhSiH_3$ followed by Fmoc deprotection with 20% piperidine afforded the precursor for on-resin cyclization. The cyclization proceeded readily using PyBOP/HOBt in 2 h (Scheme 2). A small sample of the resin was treated with TFA/H_2O/TIPS (95:2.5:2.5) to cleave the peptide from it.

Analysis of this peptide by mass spectrometry demonstrated that ring C of **8** was formed without any observable dimer. The pNZ group was then removed under reductive conditions with $SnCl_2$ (3 × 45 min) in the presence of a low concentration of acid (1.6 mM HCl/dioxane).[12] Completion of the reaction was ascertained by MALDI-TOF MS

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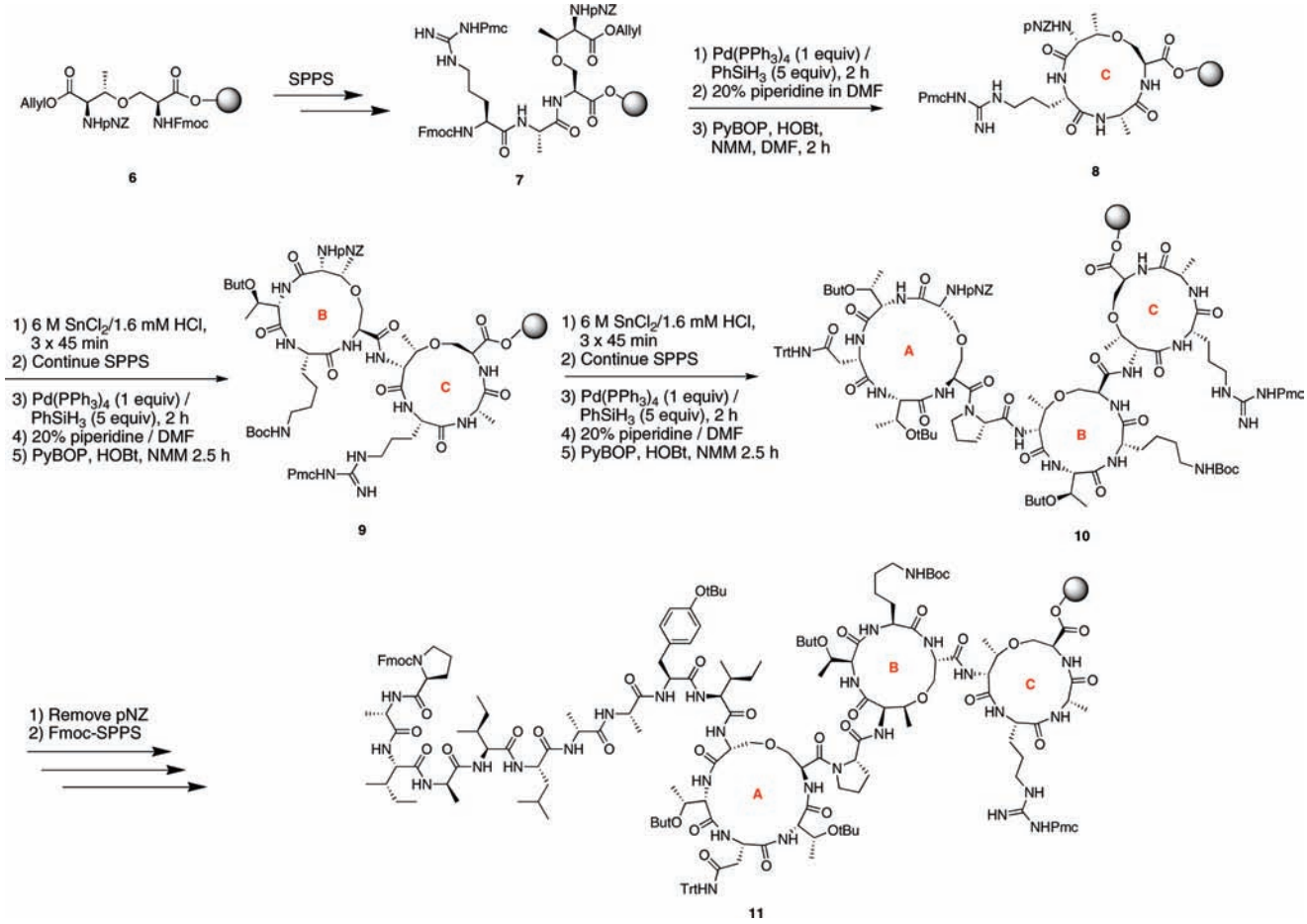
(11) Interstrand cross-linking and dimer formation is observed when higher loading resin is used. We believe the pNZ urethane adjacent to the activated carboxyl suppresses epimerization during macrolactamization.[12]

Scheme 2. Solid Phase Synthesis of Residues (6−29) of Oxa-lacticin 3147 A2



analysis after treatment of a small portion of the resin with TFA to liberate the peptide.

Fmoc-SPPS was continued to introduce the required amino acids for the formation of ring B. After removal of the allyl and Fmoc groups as before, the cyclization was allowed to proceed on-resin with PyBOP/HOBt for 2.5 h. Cleavage of a small portion of the peptide as before followed by MALDI-TOF mass spectrometry showed a major peak corresponding to the bicyclic product **9**. After removal of the pNZ group with $SnCl_2$, the linear precursor to ring A was prepared by SPPS in a manner similar to the formation of rings C and B. Removal of the allyl and Fmoc protecting groups and on-resin cyclization gave the tricylic compound **10**. Mass spectrometric analysis of a small fraction of the peptide cleaved from resin confirmed the generation of tricyclic **10** as the major product. More importantly, as before, no dimerized or oligomerized peptides were observed. After removal of the pNZ group, residues 6−15 were introduced using standard Fmoc SPPS to furnish **11**.

Completion of the synthesis was done by on-resin coupling of the pentapeptide **12**, which was obtained by a method previously reported by us.[6] This difficult fragment coupling between a proline and dehydroamino acid was sluggish and required 7 h for completion. To avoid aggregation of the long hydrophobic peptide chain, *N*-methylpyrrolidinone (NMP) was used as the solvent for the coupling (Scheme 3).[13]

The final deprotection and cleavage of oxa-lacticin (**3**) from the resin was effected with (97.5:2.5) TFA/TIPS. Purification by reverse-phase HPLC and analysis by MALDI-TOF MS showed a clean peak at 2799.2 Da (M + H) corresponding to the mass of the desired oxa-lacticin A2. Detailed MS/MS studies confirmed the correct sequence and connection of the ether rings in **3**. The overall yield was 0.3% for ca. 53 steps (22 deprotections and couplings, 3 macrocyclizations, 3 pNZ removals, and 3 allyl deprotections). This corresponded to just under 90% per step, but as the attachment of **12** proceeded in low yield, it is likely that each deprotection/coupling was >95%.

Preliminary biological evaluation of oxa-lacticin A2 (**3**) was done in conjunction with natural lacticin A1 (**1**) against a panel of Gram-positive bacteria.[2a] Oxa-lacticin A2 (**3**) exhibited only independent antibacterial activity (*L. lactis* subsp. *cremoris* HP and *Leuconostoc mesenteroides* Y105)

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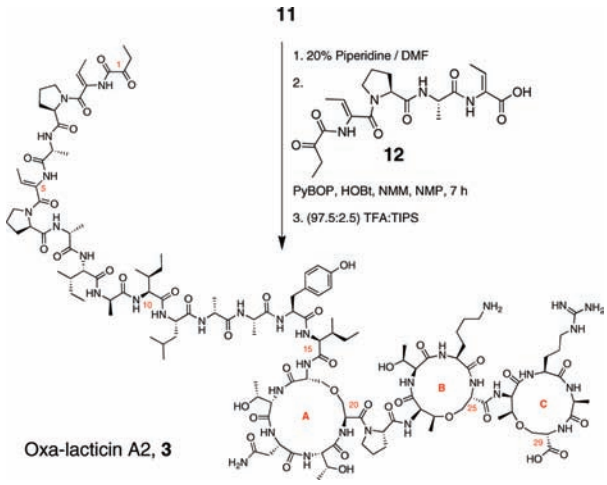
Scheme 3. Fragment Coupling of Residues (1−5) to Complete the Synthesis of Oxa-lacticin 3147 A2



or no biological activity [five other Gram-positive bacteria] (Figure 3a). When compared to natural A2, oxa-A2 showed a reduced spectrum of activity. A serial dilution assay of oxa-A2 (**3**) and natural A2 (**2**) indicated that the inherent antimicrobial activity of the oxygen analogue was approximately 20 times less than natural lacticin A2. Interestingly, oxa-A2 (**3**) showed no synergistic antimicrobial activity with natural A1 (**1**) against any of the organisms tested (Figure 3b). In contrast, our previous work with bis(desmethyl) lacticin A2,[8a] which has the two β-methyllanthionine bridges replaced by lanthionines, demonstrated that the desmethyl analogue bearing sulfurs has very little independent activity but shows a strong synergistic effect with natural A1 (**1**).

These results support the hypothesis that the two-peptide lantibiotic lacticin 3147 has at least two modes of action.[8a] In the natural system, synergistic activity results from pore formation in the cell membrane via the interaction of the A2 peptide with the lacticin A1−lipid II complex.[4] The distance between carbons 2 and 6 in lanthionine is about 0.7 Å longer than the corresponding distance in oxa-DAP. Thus, the sulfur-containing rings in natural **2** will be slightly larger than those in the unnatural oxygen derivative **3**. The loss of synergistic activity for oxa analogue **3** presumably results from poor recognition of the A1−lipid II complex. This may be due to an additive effect of the three modification sites or perhaps to differences in the size of sulfur vs oxygen (van der Waals radius 1.80 vs 1.52 Å) or their electrostatic properties (Pauling scale electronegativity 2.58 vs 3.44). However, the small conformational changes may be tolerated, for the direct binding to lipid II, possibly by the B/C ring portion of the lantibiotic analogue.[1c,3,4] This would result in inhibition of cell wall biosynthesis and the observed antimicrobial activity for the lone peptide. The methyl groups on carbons adjacent to sulfur in **2** are essential for the independent activity,[8a] and this may also be true for **3**.

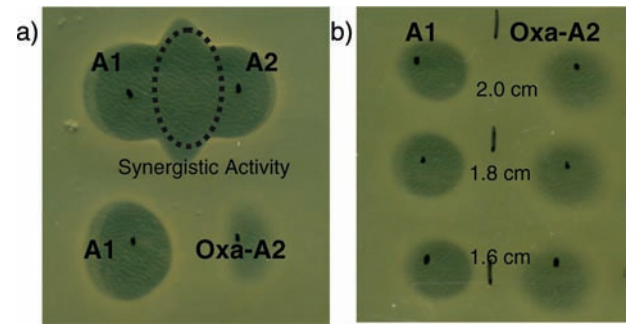


Figure 3. Spot-on-lawn tests for antimicrobial activity showing zone of growth inhibition against *L. lactis* subsp. *cremoris* HP: (a) the synergistic and inherent activity of natural A1 (**1**) and A2 (**2**) [top]; the inherent independent activity of natural A1 (**1**) and oxa-A2 (**3**) [bottom]; (b) the activity of A1(**1**) and oxa-A2 (**3**) at differing distances (no synergism).

In conclusion, multiple on-resin cyclizations using triorthogonally protected *meso*-oxa-DAP and 3-methyl-oxa-DAP strategy together with fragment coupling were successfully utilized for the chemical synthesis of oxa-lacticin A2. These unusual oxygen bridged bisamino acids could serve as oxidatively stable conformational constraints in medically relevant peptides. The biological evaluation of oxa-lacticin A2 supported the proposal of two modes of action for the two component peptides, lacticin 3147 A1 and A2.

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Supporting Information Available: Experimental procedures and spectral data for all compounds synthesized, HPLC trace, and MS/MS analysis as well as biological testing assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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