ORGANIC LETTERS

2011
Vol. 13, No. 16
4216–4219

Synthesis of Peptides Containing Overlapping Lanthionine Bridges on the Solid Phase: An Analogue of Rings D and E of the Lantibiotic Nisin

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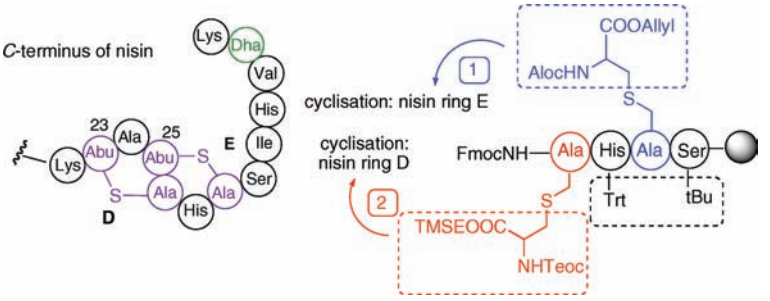
Received June 9, 2011

ABSTRACT



A methodology for the solid-phase synthesis of the overlapping lanthionine bridges found in many lantibiotics has been developed. A novel Teoc/TMSE-protected lanthionine derivative has been synthesized, and this lanthionine, and an Aloc/allyl-protected lanthionine derivative, have been incorporated into a linear peptide using solid-phase peptide synthesis. Selective deprotection of the silyl protecting groups, followed by sequential cyclization, deprotection of the allyl protecting groups, and further cyclization, enabled the regioselective formation of an analogue of rings D and E of nisin.

The rapid rise of antibiotic resistant bacteria makes it imperative that chemical biologists explore the synthesis and mode of action of natural products which exert antibiotic activity through novel pathways. Lantibiotics such as nisin have a unique mode of antibiotic action, which involves binding to lipid II, a key intermediate in the biosynthesis of the cell walls of Gram-positive bacteria.[1,2] They may therefore represent important new leads in the search for new antibacterial agents to treat antibiotic-resistant infections. These peptides have very complex structures, with multiple thioether bridges between side chains, making them a formidable challenge for synthetic chemists.[3,4]

We have previously developed a flexible approach to the solid-phase synthesis of lanthionine-containing peptides, using orthogonally protected lanthionine derivative **1**.[5] This approach has been used by ourselves and others to synthesize fragments and analogues[6−8] of lantibiotics. The

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10.1021/ol201548m
Published on Web 07/18/2011

approach allows both single rings and peptide sequences with multiple thioether bridges in sequence to be prepared, most recently demonstrated by Vederas and co-workers in the total synthesis, on-resin, of lactocin S.[9] However, until now it has not been possible to synthesize lantibiotic sequences having two overlapping thioether bridges using solid-phase techniques. This motif occurs very frequently in lantibiotics, for example in the C-terminus of nisin **2**, rings D and E, which is thought to be the pore-forming region of this lantibiotic.

In this paper, we report the first solid-phase synthesis of a bicyclic peptide containing two overlapping thioether bridges, using a quadruply orthogonal protecting group strategy to prepare an analogue of rings D and E of nisin. We envisaged that the overlapping bridges of rings D and E of nisin could be prepared from a linear resin-bound peptide intermediate **3** (Figure 1). This contains two distinct lanthionine residues with protecting groups orthogonal to each other and also to the transient (Fmoc) and permanent (Boc/tBu) protecting groups used in Fmoc-based solid-phase peptide synthesis. We had already demonstrated that the allyl ester and Aloc protecting groups of **1** could be selectively removed with $Pd(PPh_3)_4$ without loss of either Fmoc or Boc protecting groups.[6,7] Of the many other protecting groups available for amino acids, we selected the β-(trimethylsilyl)ethoxycarbonyl (Teoc)[10] and trimethylsilylethyl (TMSE)[11] groups for the amino and carboxylic acid, respectively, as these silyl-based protecting groups could be easily removed using TBAF under mild conditions at neutral pH.

As a first step, it was necessary to synthesize the (Teoc, TMSE)/Fmoc-protected lanthionine **4**. Trt-D-Ser-OTMSE **5** was prepared from commercially available Boc-D-Ser-(Bzl)-OH and then converted to iodoalanine **6** via a Mitsunobu reaction (Scheme 1).[7] Coupling of **6** to Fmoc-Cys-OTce[12] gave lanthionine **7**, which was converted to the Teoc-protected amine **8** and then successfully deprotected under neutral conditions[13] to afford the desired protected lanthionine **4**.

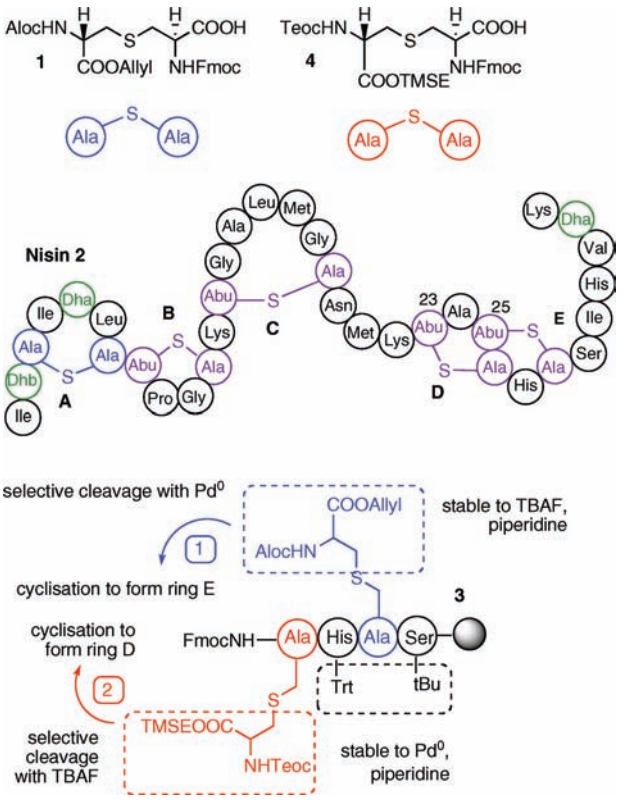
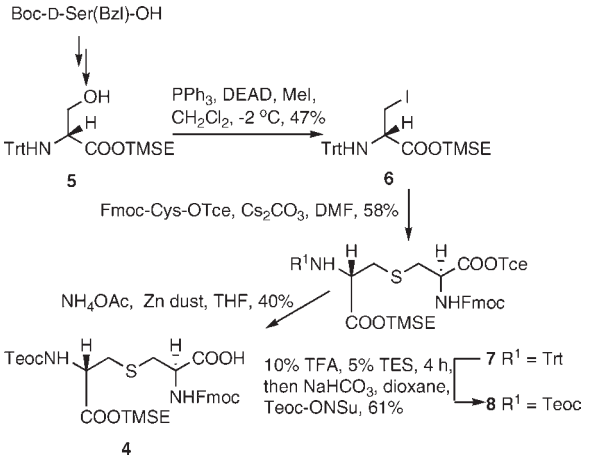


Figure 1. Strategy for synthesizing nisin rings D and E.

Scheme 1. Synthesis of (Teoc, TMSE/Fmoc) Lanthionine **4**



In order to test whether **4** could be used in the solid-phase synthesis of lanthionine-containing thioether-bridged cyclic peptides, we first prepared an analogue of ring D of nisin. The linear resin-bound peptide **9** was first prepared by standard solid-phase peptide synthesis methods, incorporating **4** as the second amino acid, and substituting a Met residue as the third amino acid in place of the other lanthionine (Scheme 2).

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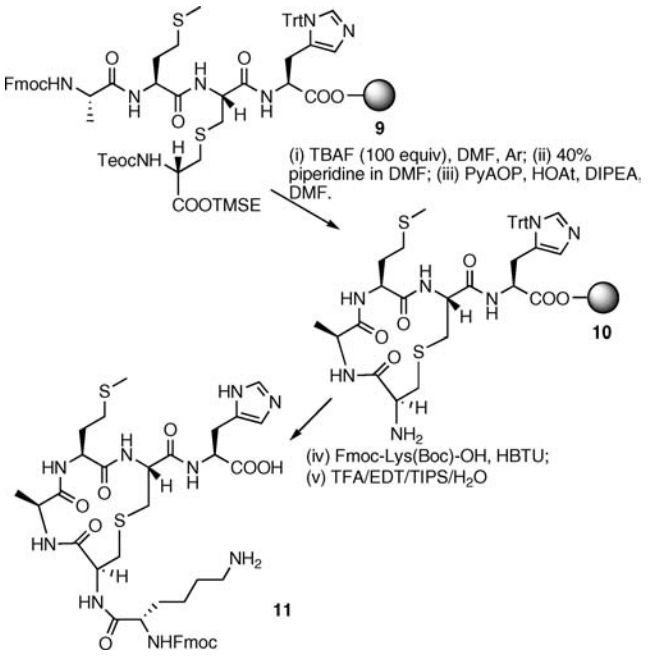
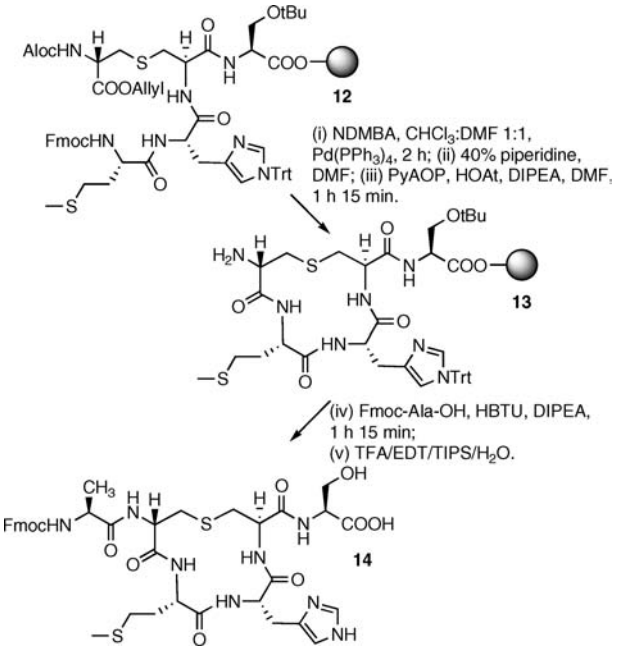
Scheme 2. Synthesis of an Analogue of Ring D of Nisin



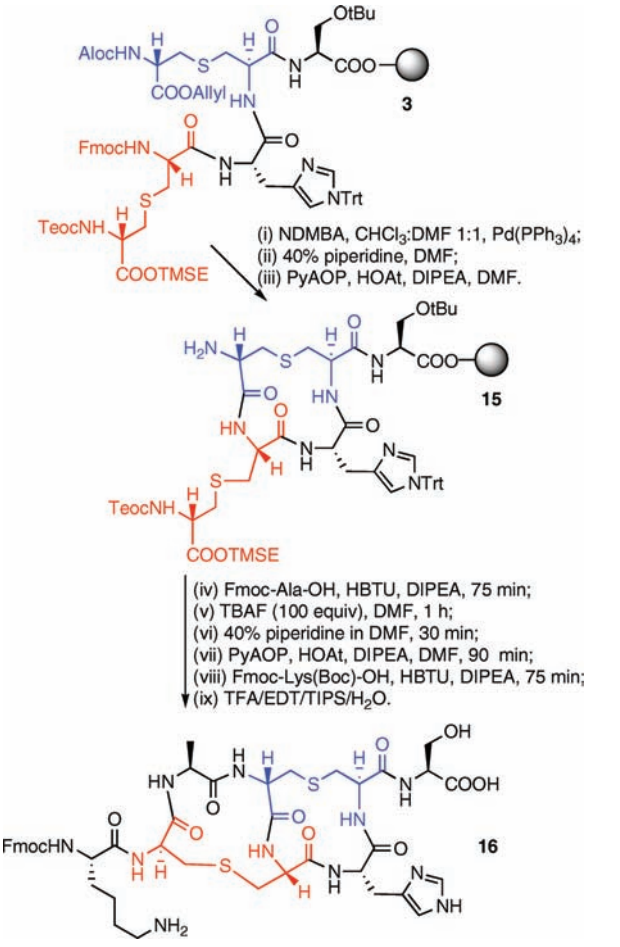
Scheme 3. Synthesis of an Analogue of Ring E of Nisin



Treatment with TBAF successfully removed the Teoc and TMSE groups;[10,11] although the Fmoc group was probably also removed during the reaction,[14] the resin-bound peptide was then treated with piperidine to ensure complete deprotection of the N-terminus. Cyclization on-resin[15] then gave the resin-bound peptide **10**. Finally, Fmoc-Lys(Boc)-OH was added to the free N-terminus and the cyclic peptide **11** cleaved from the resin. Purification by HPLC gave **11**, which was characterized by NMR.

We also used the (Aloc, allyl)/Fmoc-protected lanthionine **1**[7] to prepare an analogue of ring E of nisin. Incorporation of **1** into a linear resin-bound precursor **12** was carried out using standard coupling conditions, again substituting a Met residue for the lanthionine at the fourth amino acid (Scheme 3). Deprotection of the allyl and Aloc groups was carried out with $Ph(PPh_3)_4$ using *N*′,*N*-dimethylbarbituric acid (NDMBA) as an allyl group scavenger.[16] Removal of the Fmoc group was again followed by on-resin cyclization to give **13**; the sequence was then extended with Fmoc-Ala-OH before cleavage from the resin. Purification by reversed-phase HPLC gave **14**, which was also characterized by NMR.

Scheme 4. Synthesis of an Analogue of Rings D and E of Nisin



With the two individual rings successfully synthesized and characterized, we then tackled the synthesis of the bicyclic peptide with two overlapping lanthionine bridges.

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Linear resin-bound peptide **3** was first synthesized, incorporating both of the protected lanthionines **1** and **4** (Scheme 4).

Deprotection of the allyl and Aloc groups proceeded smoothly using the same conditions as before, without removal of the Teoc and TMSE groups. Removal of the Fmoc group from the (Teoc, TMSE/Fmoc) lanthionine residue then allowed ring E to be formed on-resin, giving **15** (Scheme 4). In order to install ring D, chain extension with Fmoc-Ala-OH was then followed by removal of the Teoc and TMSE groups and a second cyclization reaction. Finally, Fmoc-Lys(Boc)-OH was again added to the free N-terminus, and the cyclic peptide **16** was cleaved from the resin. A two-stage purification procedure using C18 SPE columns gave the pure peptide **16**. High-resolution mass spectrometry showed that the desired peptide had been synthesized, with a mass of 1008.37 Da.[17]

In conclusion, we have developed an effective methodology for the solid-phase synthesis of peptides containing overlapping lanthionine bridges. We have demonstrated the applicability of this approach in the solid-phase synthesis of rings D and E of nisin. The D and E segment of nisin has been synthesized previously in solution by Shiba and co-workers using a solution-phase desulfurization approach[18] and has been coupled to other segments of nisin.[19] However the solid-phase synthesis approach described here avoids the pitfalls of segment synthesis[20] and will allow for the rapid preparation of all types of lanthionine containing peptides. This quadruply orthogonal protecting group approach will be a powerful additional tool for the chemical synthesis of highly structurally complex lantibiotics, with the potential to prepare more potent and bioavailable synthetic analogues, unrestricted in amino acid sequence and composition, that could not be accessed via a biosynthetic approach.[21]

Acknowledgment. We thank the Engineering and Physical Sciences Research Council (EPSRC) for an EngD studentship (to B.M.) and Novacta Biosystems Ltd. for financial support. We thank Daniel Nicolau (Department of Chemistry, UCL) for the allyl/Aloc deprotection procedure using NDMBA, Lisa Haigh (Department of Chemistry, UCL) for assistance with the HRMS data, and John Kirkpatrick (Research Department of Structural and Molecular Biology, UCL) for assistance with NMR data.

Supporting Information Available. Experimental procedures and characterization of all new amino acid and lanthionine derivatives and of peptides **11**, **14**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org

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