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Multiple On-Resin Olefin Metathesis to Form Ring-Expanded Analogues of the Lantibiotic Peptide, Lacticin 3147 A2

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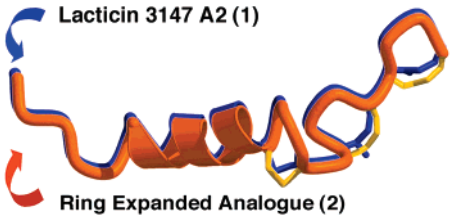
ABSTRACT



Chemical synthesis of lantibiotic analogues wherein monosulfide bridges are replaced with other groups can shed light on structure–activity relationships and generate variants that are resistant to aerobic oxidation and have better metabolic stability. This work describes the first complete synthesis of a carbocyclic lantibiotic analogue 2, using sequential on-resin ring-closing olefin metathesis and solution-phase peptide synthesis. The methodology described should find wide application for the preparation of rigidified peptidomimetics containing multiple carbocyclic rings.

The dramatic increase of pathogenic bacteria resistant to traditional antibiotics has encouraged investigation of lantibiotics as agents effective at nanomolar concentrations against most Gram-positive organisms.[1] Lantibiotics (e.g., nisin A, lacticin 3147) are ribosomally synthesized bacterial peptides (bacteriocins) that contain lanthionine and β-methyllanthionine rings as a result of post-translational modification (Figure 1).[2] Nisin A, which is approved for use in food, is the only lantibiotic to have been chemically synthesized.[3] A number of groups have been interested in chemical synthesis of lantibiotics[4] as well as possible replacement of disulfide or monosulfide bridges in biologically active peptides.[5] Such replacements not only shed light on structure−activity relationships but also can generate variants that display better metabolic stability.[5b,6] In this regard, formation

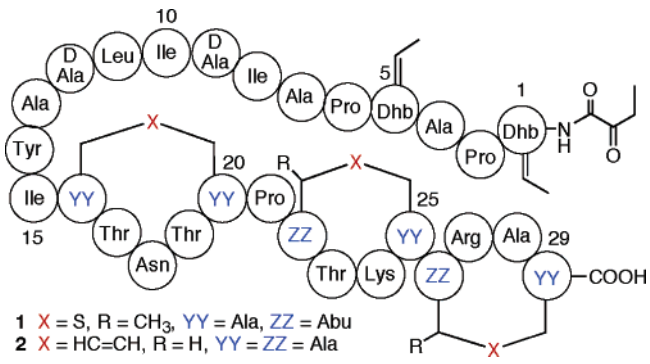


Figure 1. Lacticin 3147 A2 (**1**) and a larger ring analogue (**2**).

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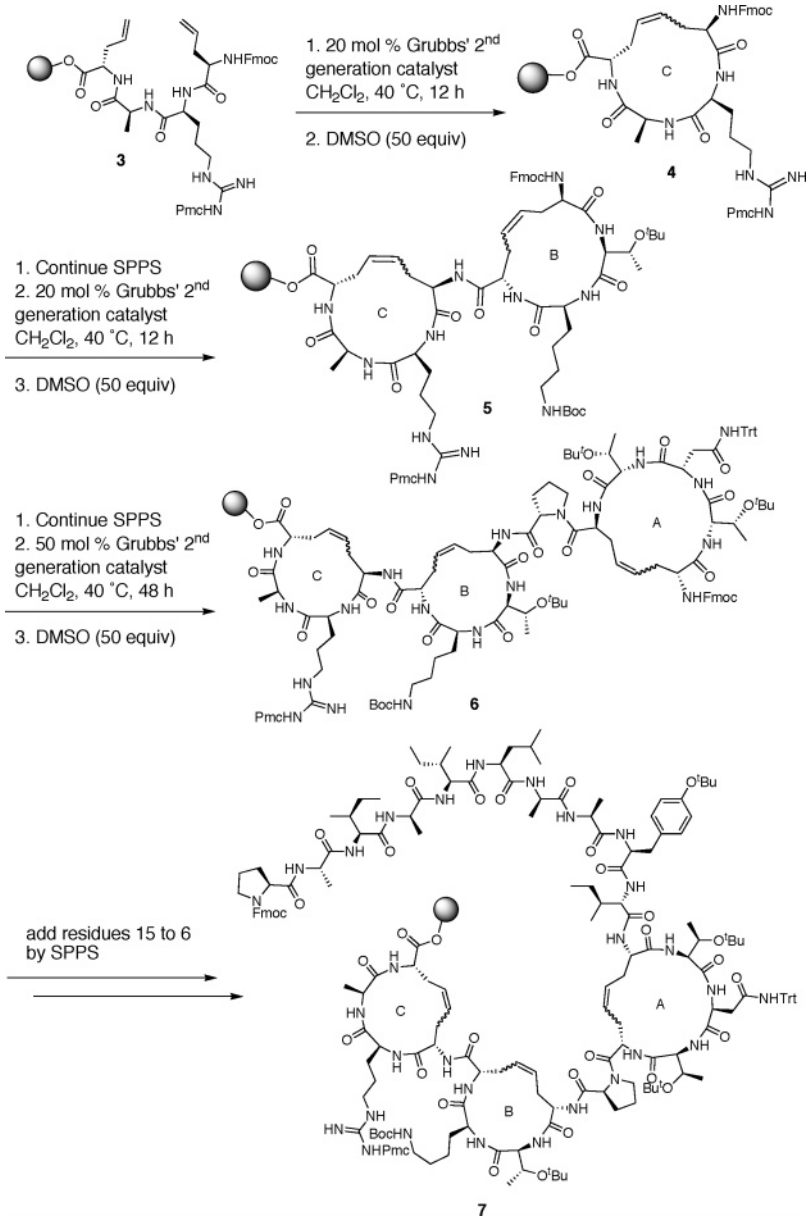
Scheme 1. Sequential On-Resin Ring-Closing Metathesis



of carbocyclic fragments of lantibiotics using ring-closing metathesis (RCM) has recently been reported by the Liskamp and co-workers.[5a] We now describe the preparation of **2**, which represents the first complete synthesis of a carbocyclic lantibiotic analogue, using sequential on-resin RCM to form three consecutive rings with a high degree of control. This work also provides methodology to prepare the unusual N-terminal fragment of lacticin 3147 A2 (**1**).

To initiate the synthesis, Fmoc-L-allylglycine was attached to Wang resin with a loading of 0.8 mmol/g. Solid-phase peptide synthesis (SPPS) then employed standard Fmoc methodology to generate linear precursor **3**, which was treated with 20 mol % of Grubb's second-generation catalyst in CH_2Cl_2 for 12 h at 40 °C (Scheme 1). Cleavage of a sample and analysis (MS) showed that the first carbocycle (ring C) formed cleanly on-resin to give **4** (cis/trans mixture). After treatment of the resin with DMSO to remove any ruthenium byproducts,[5b] SPPS was continued to introduce amino acids required for formation of the B ring. RCM as before furnished the bicyclic compound **5** as the only detectable product based on MS analysis of the peptide resulting from acidic cleavage (TFA) from the resin with concomitant deprotection. SPPS was continued to give the precursor to **6**, but the last cyclization required an increase of catalyst loading to 50 mol % and of the reaction time to 48 h. Cleavage of the peptide using TFA and MS analysis showed the desired product peak as the only major product.

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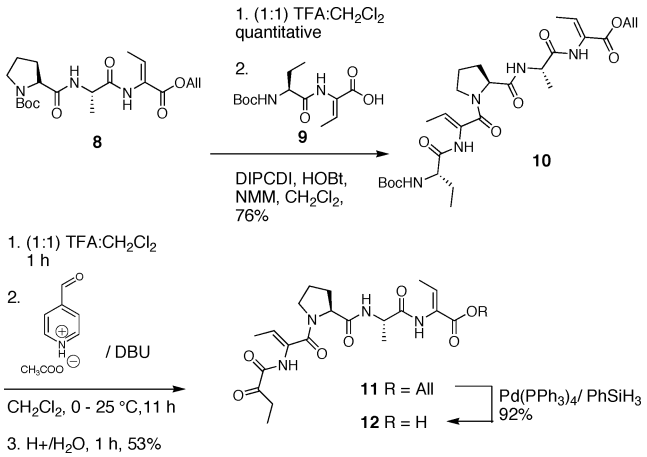
To exclude the possibility of any scrambling of carbon bridges by ring-opening metathesis and subsequent ring closure, LC-MS/MS analysis was done on the compound obtained by cleavage and deprotection of tricyclic derivative **6**. This confirmed the absence of crossover products. Thus, this method is likely to be generally useful for construction of multiple carbocyclic ring peptides on solid support. Attempted reduction of olefins on resin-bound **6** using diimide and Wilkinson's catalyst gave only the Fmoc-deprotected **6** and none of the saturated tricycle.

Continuation of SPPS introduced residues (10→15). As the polymer support turned dark due to repeated exposure to the RCM catalyst, colorimetric Kaiser tests[7] to monitor the peptide coupling could no longer be used. Consequently, each Fmoc-deprotection step was monitored using UV at 301 nm[8] and the completion of acylation steps was determined by mass spectrometry. To avoid aggregation caused by nine hydrophobic side chains in the section of residues 6−15, *N*-methylpyrrolidinone (NMP)[9] was used as the solvent to successfully yield **7**.

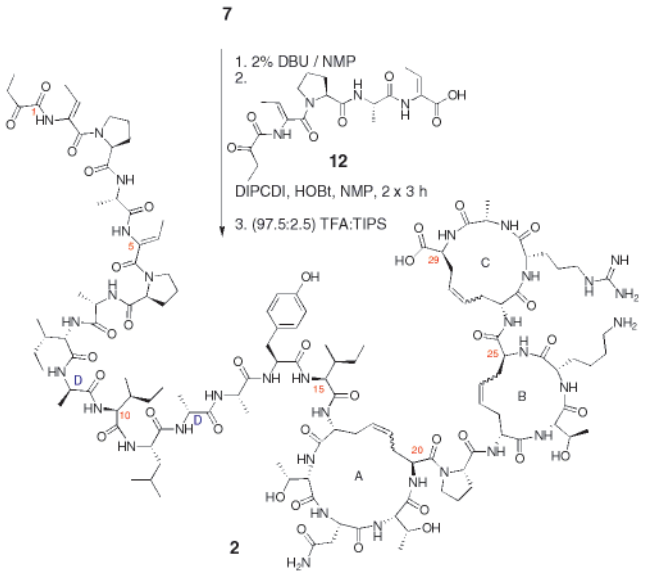
Residues 1−5 of lacticin A2 (**1**) contain two dehydrobutyrine (Dhb) residues and an N-terminal α-ketoamide as a result of enzymatic post-translational modification.[2,6] Although methods to prepare dehydrobutyrines on solid support have been reported,[10] we chose solution-phase synthesis of the pentapeptide containing these unusual residues (Scheme 2). This allows facile control of scale as well as use of this fragment in other syntheses of **1** and its analogues. The dehydroamino acid moieties in both **8** and **9** were introduced at the dipeptide stage by treating the corresponding threonine derivatives with MsCl/DBU[11] to obtain the dehydrobutyrine dipeptides. In both instances, this elimination reaction gave only the *Z* stereoisomer, as confirmed by NOE experiments. DIPCDI/HOBt proved to be the best reagents for the difficult coupling of the relatively unreactive carboxyl group of dehydrodipeptide **9** with the secondary amino group of proline obtained after deprotection of **8**. The coupling proceeded to give **10** in 76% isolated yield. The N-terminal amino group of **10** was liberated with TFA and then treated with 4-pyridinecarboxaldehyde acetate salt and DBU.[12] Aqueous acidic workup led to transamination with hydrolysis akin to that catalyzed by pyridoxal phosphate[13] and produced peptide **11** having the desired α-ketoamide with the dehydrobutyrines intact. The *O*-allyl protecting group could be removed with $Pd(PPh_3)_4$ to yield **12** as a single stereoisomer.

Scheme 2. Solution Synthesis of the Unusual N-Terminus Sequence



To complete the synthesis of **2**, the pentapeptide fragment **12** was coupled to the N-terminus of peptide **7** on solid support (proline, residue 6). This difficult coupling was again done in the presence of DIPCDI/HOBt (Scheme 3). Cleavage of **2** from the resin was achieved using (97.5:2.5) TFA/TIPS. Water was omitted from the cleavage cocktail, as the dehydro residues are susceptible to Michael addition by water under strongly acidic conditions.[14] Purification of **2** by reverse-phase HPLC, followed by analysis using MALDI-TOF MS, revealed a major peak at 2801.6 Da, corresponding to the

Scheme 3. Completion of the Synthesis of the Larger Ring Analogue **2**



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mass of the desired larger ring analogue **2** (overall yield = 0.5% for 25 coupling steps and 3 RCM reactions). A detailed MS/MS analysis was performed on **2**, to confirm the correct sequence as well as the connectivity of the carbocyclic rings. Compound **2** is a mixture of cis/trans stereoisomers at the macrocyclic alkene sites, but it was tested as such to determine whether antimicrobial activity was maintained.

Preliminary biological evaluation of this larger ring analogue revealed **2** was lacking antimicrobial activity at concentrations comparable to the natural lacticin A2 (**1**) in the presence of the lacticin A1 peptide.[6] This suggests that the larger rings do not allow a conformation required by the active natural peptide for its binding to its target, the lacticin A1 complex with lipid II.[15] In this case, the replacement of sulfur with two carbon atoms is deleterious, whereas such replacement of disulfides in peptides can maintain high levels of biological activity.[5b,e]

In conclusion, the method reported for the synthesis of sequential carbocyclic rings by RCM on solid support should find wide application for the preparation of rigidified peptidomimetics containing multiple carbocyclic rings. The chemistry described for the synthesis of the N-terminus of **2**, containing multiple dehydroamino acid residues and α-ketoamide (i.e., **12**), is highly amenable for scale-up and is being applied toward the synthesis of **1** and other peptide analogues for SAR studies on lantibiotics.

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Supporting Information Available: Experimental procedure and spectral data for all compounds synthesized. This material is available free of charge via the Internet at http://pubs.acs.org.

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