Pre-organization induced synthesis of a crossed alkene-bridged nisin Z DE-ring mimic by ring-closing metathesis†

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An alkene-bridged mimic of the complex DE-bisthioether-ring system of the antibiotic nisin was prepared in one step from the linear precursor.

Stable, naturally occurring, conformational constraints, such as thioether (sulfide) or disulfide bridges are very important for the three-dimensional shape and thereby biological activity of peptides. In order to investigate whether the repertoire of nature involving these conformational constraints can be extended, we are interested in using among others an alkene bridge as an alternative constraint for bioactive molecules.[1]

The lantibiotic nisin Z produced by a broad range of bacteria *e.g.*, *Bacillus*, *Lactococcus*, *Streptomyces* and *Staphylococcus* species,[2] poses a particular challenge as a target for this aim, since it contains five thioether bridges, two of which form a "knot" comprising the DE-ring system (Fig. 1). It was planned to synthesize ultimately the complete alkene-bridged nisin Z mimic (Fig. 1) starting from the linear fragments constituting the individual ring systems followed by cyclization using ring-closing metathesis (RCM).[3]

In the DE-ring system of nisin the amino acid side chains cross each other. As a consequence, an alkene mimic of this ring system is particularly difficult to synthesize.[4,5] Here we report the first time that RCM has been applied for the synthesis of a crossed alkene bridge for obtaining mimics of the thioether bridges[6] containing lantibiotics.

The most straightforward route towards the crossed alkene-bridged DE mimic is a direct synthesis from the linear peptide RCM-precursor containing the required allylglycine residues. Thus, protected peptide **2** was obtained after solid phase peptide synthesis using Fmoc/^{t}Bu protocols followed by purification in an overall yield of 69% (Scheme 1). This peptide was now treated with 2nd generation Grubbs catalyst. After 2 h a sample was taken from the reaction mixture and the catalyst was immediately removed by filtration over a small silica plug. The remaining reaction mixture was refluxed overnight after addition of more catalyst. First, the reaction intermediates in the sample were analyzed by purification on HPLC and by characterization using LCES-TOF MS/MS. The observed mass in combination with the obtained fragmentation pattern enabled the elucidation of the structure of the formed monocylic intermediates. Theoretically, six monocyclic intermediates could have been formed, however, only four (**3**–**6**) corresponding to the [3,6], [1,4], [1,6] and [4,6] RCM products were found (Scheme 1). Remarkably, the [1,3] RCM-product was not observed, whereas the [4,6] RCM product was. Absence of the [3,4] RCM product might be explained by reluctance of the *trans*-amide bond to assume a cisoid geometry necessary for the eight membered ring of this product. The unique fragmentation pattern of each RCM-product enabled unequivocal

† Electronic supplementary information (ESI) available: Experimental procedures and characterization details for **2**, **7** and **14**. See http://www.rsc.org/suppdata/cc/b4/b415555f/

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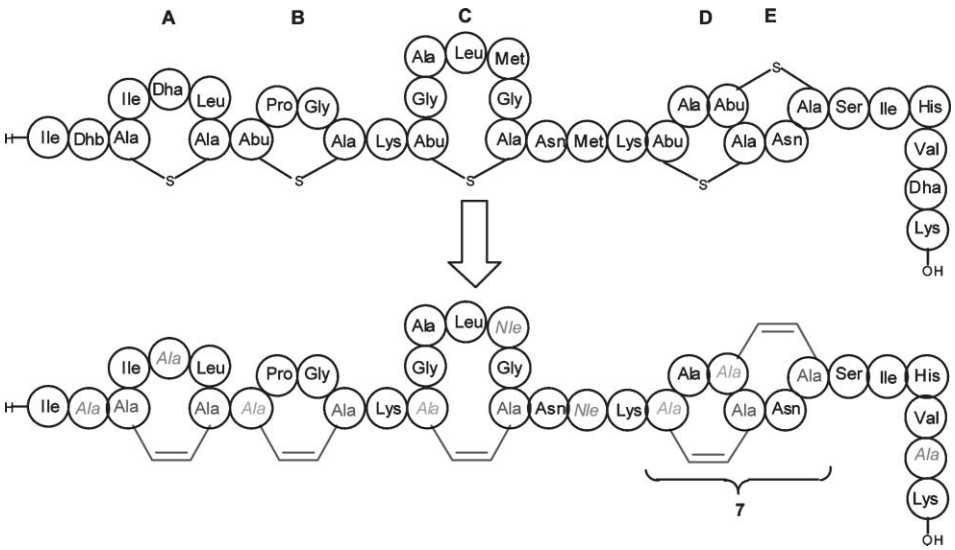
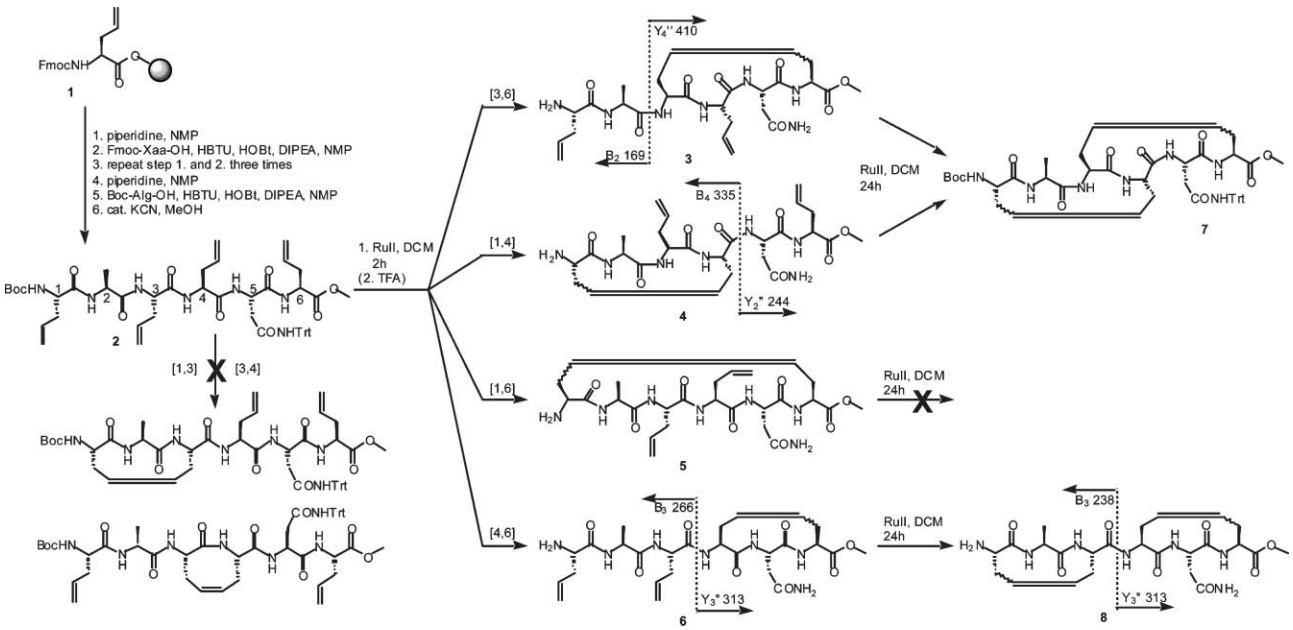
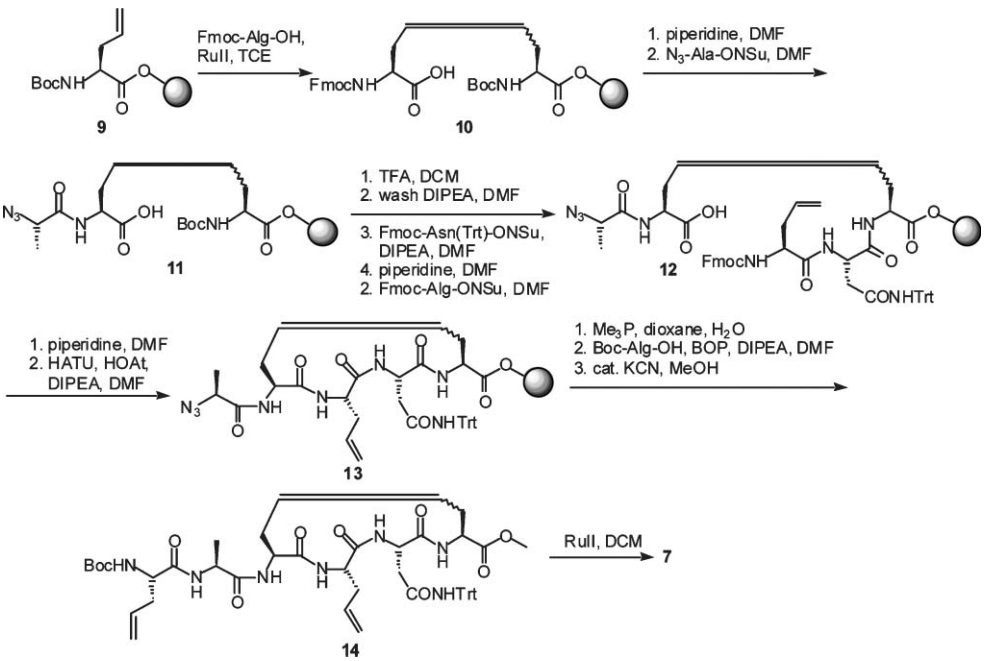


Fig. 1 Nisin Z and its alkene-bridged mimic **7**.



Scheme 1 Possible and observed intermediates in the one step double RCM leading to alkene-bridged mimic **7**.

determination of the position of the cyclic constraint.[1a] The ratio of product formation **3** : **4** : **5** : **6** was found to be *ca.* 1 : 4 : 1 : 2 and thus the reaction mixture contained 63% of the desired intermediates **3** and **4**. A purely statistical distribution—assuming the formation of six possible RCM-products—would only have led to formation of *ca.* 33% of **3** and **4**. Molecular mechanics calculations were in agreement with this preferential formation of **3** and **4**, since the energy of these intermediates was significantly lower.[7] Next, the products obtained after refluxing overnight were isolated and purified. Only two of the three possible bicylic compounds—based on the formed monocyclic compounds in the reaction mixture sample—were observed. Both monocyclic products **3** and **4** cyclized to the desired bicyclic product **7**. Intermediate **6** cyclized to product **8** *i.e.* the [1,3]–[4,6] product. Not unexpectedly (*vide supra*), [1,6] product **5** was not converted to a bicyclic product, since this would require formation of a [3,4] cycle, which is probably difficult (*vide infra*). Thus, the desired bicyclic product was obtained in 72% yield as compared to only 19% of one other bicyclic product (**8**). The preferred formation of monocyclic products **3** and **4** and the ensuing bicyclic product hints at a favorable pre-organization of the linear peptide for formation of the DE-ring alkene mimic, which in view of their ring



Scheme 2 Step-wise synthesis featuring subsequent cross metathesis and ring-closing metathesis to afford alkene-bridged mimic **7**.

size (two 14-membered rings) might be close to an α-helical structure. It is tempting to speculate that this pre-organization might also play a role in construction of the natural DE-ring system in nisin itself.

The structure of the desired mimic was confirmed by independent step-wise synthesis (Scheme 2). Starting from Boc-Alg-Argogel™ (**9**) the alkene bridge of ring E was synthesized by a cross metathesis with Fmoc-Alg-OH thus affording **10**.[8] It was found that protection of the carboxyl moiety was not required. A third orthogonal protecting group was now necessary and after removal of the Fmoc group, N_3-Ala-ONSu was coupled, in which the azide is orthogonal to the Fmoc and Boc-group. Next, acidolysis of the Boc-group in **11** by TFA was followed by coupling of Fmoc-Asn(Trt)-ONSu. After removal of the Fmoc group, Fmoc-Alg-ONSu was coupled to give **12**. Removal of the Fmoc group was followed by lactamization using HATU/HOAt/DIPEA in DMF between residues Alg3 and Alg4 to afford ring E. Reduction of the azide functionality in **13** under Staudinger conditions gave the free amine.[9] Finally, Boc-Alg-OH was coupled and the resulting resin was treated with a catalytic amount of KCN in methanol to give the monocyclic fully protected peptide ester **14** (after purification: 11% overall yield, average of 82% per step). The correct side chain to side chain connectivity of ring E was confirmed by NMR analysis (^{1}H-500 MHz, TOCSY, NOESY and ROESY) and the correct fragmentation pattern was found by mass analysis (LCES-TOF MS/MS). **14** was treated with 2nd generation Grubbs catalyst to give the desired bicyclic peptide **7** in 50% yield. NMR analysis in combination with MS/MS experiments proved that the ring structure was correct and identical with the product obtained by a one step double ring-closing metathesis.

In conclusion, we have prepared an alkene mimic of the challenging nisin DE-ring in a single reaction step involving a double RCM. Preferred formation of the intermediate monocyclic mimic, resulting in formation of the desired bicyclic mimic, may be due to a considerable degree of pre-organization of the linear peptide RCM-precursor.

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