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Synthesis of cyclic peptides containing nor-lanthionine bridges via a triply-orthogonal protecting group strategy

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Abstract—We report a new approach to the on-resin synthesis of cyclic peptides containing unnatural thioether side-chain bridges. An orthogonally protected nor-lanthionine was incorporated in a linear precursor peptide via stepwise solid-phase synthesis. This was followed by double allyl deprotection, cyclisation using PyAOP and subsequent chain-extension to give an analogue of ring C of nisin.

The synthesis of peptides incorporating unnatural side-chain to side-chain linkages has received considerable attention recently, in part due to the metabolic stability and unusual conformational constraints that may be achieved using such an approach. The cyclisation of peptides via thioether linkages has been of special interest, as such peptides are effective mimics of cystine-linked structures. Such structures are found naturally, in the lantibiotic peptides, which contain multiple lanthionine and methyllanthionine moieties;[1] lanthionine[2,3] and analogues[4] have also been incorporated into medicinally relevant peptides as conformational constraints. The only reported total synthesis of a naturally occurring polycyclic lantibiotic, nisin, was achieved in solution by Wakamiya and Shiba,[5] in which the lanthionine bridges in each peptide fragment were formed by sulfur extrusion from cystine, followed by fragment condensation. This approach, however, has not been optimised for solid-phase synthesis. Two important solid-phase methods have recently been reported for the synthesis of shorter lanthionine-containing peptides. The PCOR (peptide cyclisation on an oxime resin) method involves the synthesis of a linear peptide containing an orthogonally protected lanthionine residue: head-to-tail cyclisation, with concomitant cleavage of the cyclic peptide from the resin, affords the lanthionine-bridged peptide.[2,6] However, it would be difficult to generalise this approach for the synthesis of polycyclic lanthionine-containing peptides. In the second, biomimetic, approach, a linear peptide containing a cysteine and a dehydroalanine residue is synthesised and the lanthionine bridge formed by intramolecular 1,4-addition of the cysteine thiol group to the dehydroalanine.[7,8] Complete diastereoselectivity was generally, although not always, observed in the 1,4-addition step; however, in the absence of an enzyme active site controlling this cyclisation, it would be difficult to predict the stereochemical course of this reaction for any given peptide. We have been developing a new approach for the solid-phase synthesis of cyclic peptides with unnatural side-chain linkages, based on a triply-orthogonal protecting group strategy,[9] and in this paper we report its application to the synthesis of a nor-lanthionine analogue of ring C of nisin.

In the preceding paper,[10] we reported our attempt to synthesise the differentially-protected lanthionine **1**, which gave, via an unexpected rearrangement, predominantly the nor-lanthionine regioisomer **2**. Although this material was not useful for the synthesis of lanthionine-containing peptides, we were interested in incorporating it into cyclic peptides. We envisaged this as a further proof of principle of our methodology, and

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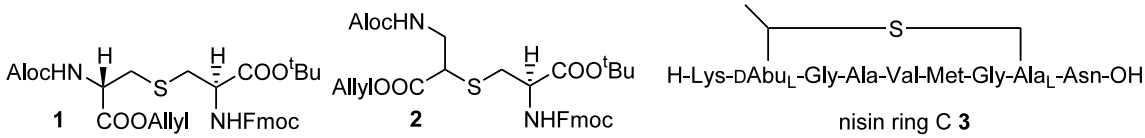
also as a route to cyclic peptides with novel, non-cleavable side-chain linkages which might result in unusual peptide conformations. We therefore undertook the synthesis of an analogue of ring C of nisin **3**, in which the methyllanthionine bridge was replaced by **2**.



The peptide synthesis was carried out in a Merrifield bubbler, utilising the strategy that we have previously reported.[9] Thus, NovaSyn® TGT resin, pre-loaded with Asn(Trt), was used to synthesise the linear peptide **4** via standard Fmoc deprotection, amino acid coupling and capping steps (Scheme 1). Deprotection of the allyl/Aloc groups,[11] followed by removal of the N-terminal Fmoc and then on-resin cyclisation using PyAOP, generated the cyclic peptide **5**. The final Lys residue was then attached, the peptide cleaved from the resin and the side-chain protecting groups removed to give the crude peptide. Purification of this material by preparative HPLC[12] revealed *two* peptides, **6a** and **6b**, of the desired mass in a 3:1 ratio and a combined yield of 55%.

Full assignment of the ^{1}H TOCSY and ^{1}H–^{13}C HSQC spectra for both peptides clearly showed that each had the desired cyclic peptide structure and sequence. HMBC experiments on both of these peptides also confirmed that, in each case, a nor-lanthionine bridge had been formed, with ^3J correlations between the β-CH_2 and α-C, and between β-C and α-C*H* as indicated (Fig. 1).

However, the variation in chemical shifts between the spectra indicated that the two peptides must be diastereoisomeric. This is consistent with the observation that nor-lanthionine **2** was also prepared as two diastereoisomers using the route reported.[10,13] A full structural and conformational analysis of these unusual cyclic peptides is underway, and will be published shortly.

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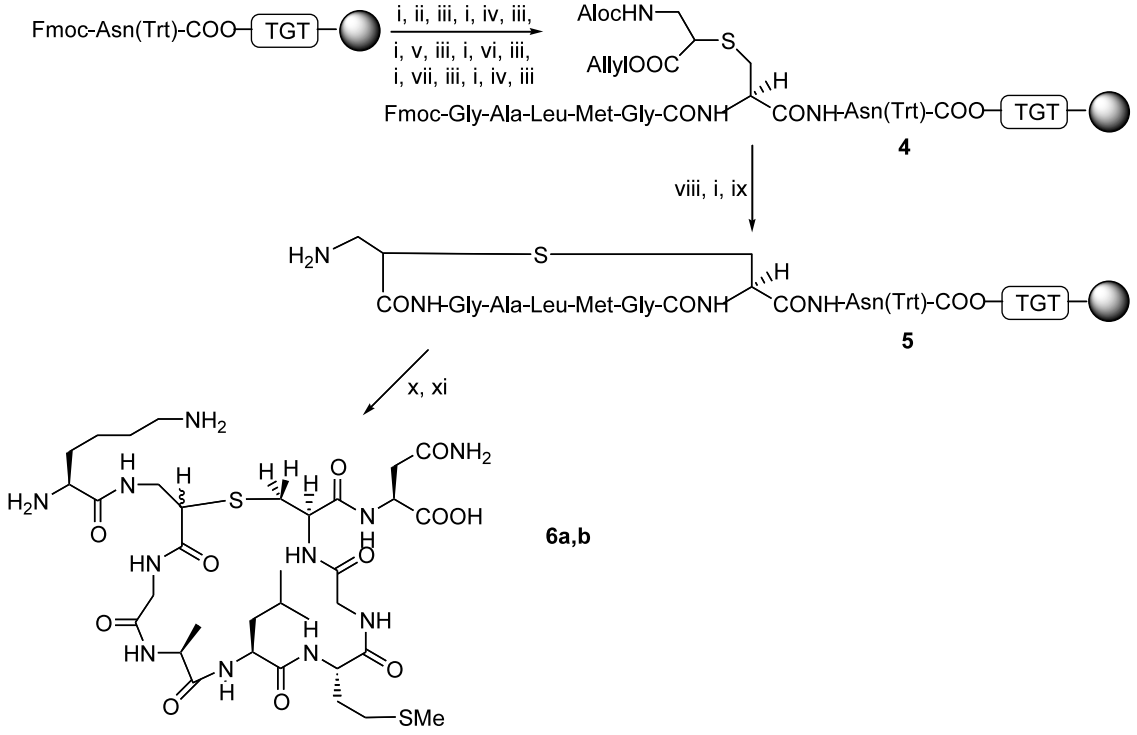

Scheme 1. *Reagents and conditions*: (i) 20% piperidine; (ii) **2**, DIC, HOBt; (iii) Ac_2O, DIEA, HOBt; (iv) Fmoc-Gly, DIC, HOBt; (v) Fmoc-Met, DIC, HOBt; (vi) Fmoc-Leu, DIC, HOBt; (vii) Fmoc-Ala, DIC, HOBt; (viii) $Pd(PPh_3)_4$, $CHCl_3$/AcOH/NMM; (ix) PyAOP, HOAt, DIEA; (x) Fmoc-Lys, DIC, HOBt, (xi) TFA, H_2O, Et_3SiH.

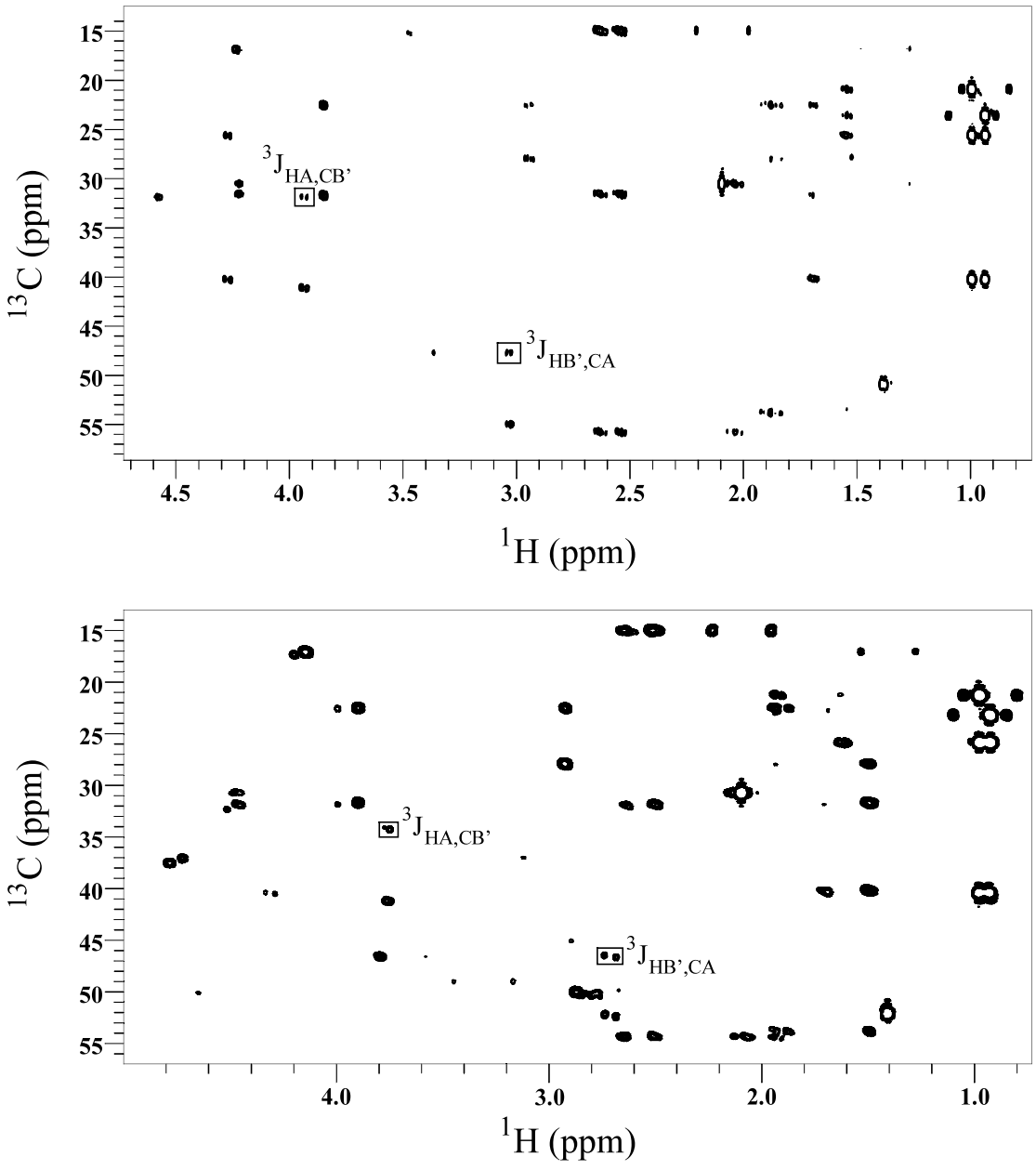

Figure 1. Selected region of the 2D ^{1}H–^{13}C HMBC spectra of (a) **6a** and (b) **6b** in CD_3OD, recorded on a 600 and 500 MHz Varian UNITYplus spectrometer, respectively, at 283 K. In each case the ^{3}J correlations across the thioether bridge are shown.

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12. Preparative reverse-phase HPLC separation of **6a**,**b**: Vydac 216TP C_{18} column, 22×250 mm, flow rate 15 ml min^{-1}: gradient 15–18% MeCN in H_2O in 30 min (0.1% TFA) over 30 min. Retention time for **6a**: 8.9 min; for **6b**: 11.7 min. Electrospray mass spectrum: 884.4 $[M+Na]^+$ (10%), 862.4 $[M]^+$ (100%), 442.7 $[(M+Na)/2]^+$ (25%) 431.9 $[(M+2)/2]^+$.
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