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Synthesis of orthogonally protected lanthionines: a reassessment of the use of alanyl β-cation equivalents

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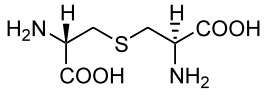
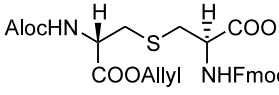
Abstract—Whilst developing a strategy for the solid-phase synthesis of lanthionine-containing peptides, we became aware of some problems with a previously published route for the synthesis of orthogonally-protected lanthionine. We report a structural reassignment of the key iodoalanine intermediate and resulting lanthionine derivatives.

The unusual amino acid lanthionine **1**, a monosulfide analogue of cystine, is the key component of lantibiotic peptides with important antibiotic and other biological properties, such as nisin, duramycin and subtilin.[1] Incorporation of lanthionine into a peptide results in a cyclic structure, bridged by a thioether, which cannot be reductively cleaved, and additionally imparts stability to proteolytic cleavage.[2] It has therefore also been used in the design and synthesis of cyclic analogues of bioactive peptides, for instance analogues of enkephalin[3] and somatostatin.[4] As part of an ongoing programme to develop a new approach for the solid-phase synthesis of cyclic peptides with unnatural side-chain linkages,[5] we are currently investigating the synthesis of mono- and polycyclic lanthionine-bridged peptides.



lanthionine **1**

Our approach required the synthesis of the orthogonally protected lanthionine **2**. A few approaches to the synthesis of lanthionine have been published, involving extrusion of sulfur from cystine,[6] ring-opening of serine β-lactone,[7] Michael addition to dehydroalanine,[8] and approaches based on alanyl β-cation equivalents. We chose to adapt the alanyl β-cation equivalent approach developed by Dugave and Ménez[9] as it was direct, high-yielding and amenable to large-scale synthesis. Moreover, it had none of the drawbacks of regio- or stereoselectivity[6–8] reported with the other strategies. However, during the course of the synthesis, some problems with the original published report became apparent, and we therefore carried out a full investigation of the regio- and stereochemical outcome of the key reaction between the alanyl β-cation equivalent and cysteine. In this paper, we report the results of this investigation, which have led us to a reassessment of the course of this reaction.



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Nucleophilic reaction at the β-position of α-amino acids is known to be most successful when the amino group is trityl-protected; this suppresses competing reactions, particularly elimination, forming dehydroalanine.[10] (*D*)-Serine was therefore converted to the *N*-trityl derivative using a one-pot procedure,[11] and esterified to give **3** (Scheme 1).[12] Following the reported method,[9]

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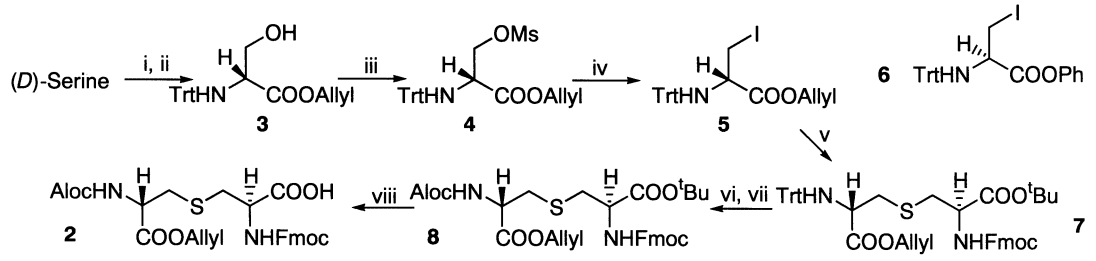
this was then converted to mesylate **4** and reacted with sodium iodide to give iodoalanine **5**. At this point, the presence of two distinct isomeric forms was observed in the ^{1}H NMR spectrum, in a ratio of between 2:1 and 3:2 (dependent on the reaction temperature).[13] Dugave and Ménez had also observed the presence of two isomers in the ^{1}H NMR spectrum when synthesising iodoalanines such as **6**. In the original report, this was attributed to the presence of at least two rotameric forms of the iodoalanines in which the conformations were locked due to the steric bulk of the trityl and iodo moieties.

Again following the published procedure, **5** was reacted with Fmoc-Cys-O^{t}Bu in the presence of Cs_2CO_3 to give lanthionine **7** in excellent yield. Removal of the trityl group and replacement with Aloc to give **8**, followed by removal of the *tert*-butyl ester, appeared to afford **2**, with the desired orthogonal protecting groups for SPPS. Dugave and Ménez had also reported the presence of two rotameric forms for lanthionines formed from iodoalanines such as **6**, and indeed we also observed two clearly distinct sets of ^{1}H NMR signals for **7**, in ratios as high as 4:1. However, we were disturbed to find that, even on removal of the bulky trityl group and its replacement by Aloc, multiple isomers were still observed in the NMR spectrum. Moreover, although these compounds appeared homogeneous by tlc, careful HPLC of **5** and **8** revealed two peaks. Compound **5** was partially resolved by preparative HPLC, and **8** was completely separated to give two distinct isomers (Scheme 1).[14]

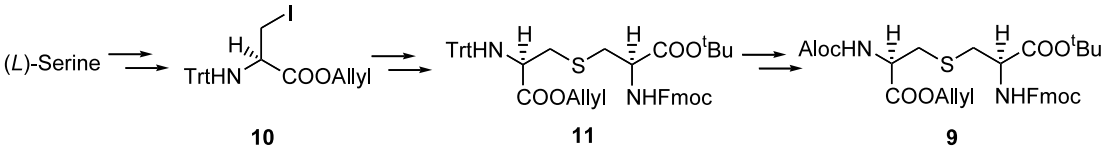
Clearly these results threw doubt on the original assignment of these isomers as the result of restricted rotation. Two other possibilities remained; diastereoisomers resulting from racemisation at the C$^\alpha$-position derived from the serine precursor; or regioisomer formation. Dugave and Ménez had previously demonstrated, via desulfurisation, derivatisation and chiral HPLC measurements of the resulting alanines, that racemisation of this chiral centre does not occur during the coupling of iodoalanines to cysteine.[9] We independently corroborated these observations by the synthesis of the diastereomeric **9** from *L*-serine, via **10** and **11** (Scheme 2).[13] As before, two isomers were observed in the ^{1}H NMR spectra of **10** and **11**, and the two isomers observed for **11** were different from those observed for **7**.[15]

A ^{1}H–^{13}C HSQC spectrum was acquired on iodoalanine **5**, and this revealed that the chemical shift of the α-carbon in the major component was ~30 ppm upfield of that in the minor component (20.1 ppm versus 55.8 ppm), and that of the β-carbon was ~40 ppm downfield (48.2 ppm versus 9.55 ppm). These chemical shifts are only compatible with attachment of iodine at the α-carbon in the major component and at the β-carbon in the minor component (Fig. 1a). This implies that the major component resulting from the reaction of mesylate **4** with NaI is the regioisomer **12**, and the minor component is the desired **5**, not rotamers as previously reported. In order to confirm these observations, we carried out an HMBC experiment on the mixture of lanthionine isomers **7** (Fig. 1b). ^3J Correlations between the β-CH_2 on the Fmoc-protected side of the lanthionine and the α-C on the trityl-protected side and also between the α-C*H* on the Fmoc-protected side of the lanthionine and the β-C on the trityl-protected side were observed for the major isomer. This is only compatible with the formation of the regioisomer **13** from the major component **12**. Finally, the complexity of the spectra suggested that a mixture of two diastereoisomers of **13** is formed, resulting from racemisation of the α-iodo-β-alanine **12**.

In summary, we have demonstrated that the synthesis of lanthionines using *N*-trityl iodoalanine as an alanyl β-cation equivalent is problematic. The major product of this synthetic strategy is the regioisomeric nor-lanthionine **12**, formed from the regioisomeric α-iodo-β-



Scheme 1. *Reagents and conditions*: (i) Me_3SiCl, Et_3N, DCM, Trt–Cl, then MeOH (88%); (ii) Cs_2CO_3, allyl bromide (87%); (iii) MsCl, Et_3N (91%); (iv) NaI, acetone (87%); (v) Fmoc-CysO^{t}Bu, Cs_2CO_3 (90%); (vi) TFA/CH_2Cl_2; (vii) Alloc–Cl, $NaHCO_3$, dioxane/H_2O (87%); (viii) TFA/CH_2Cl_2 (95%).



Scheme 2.

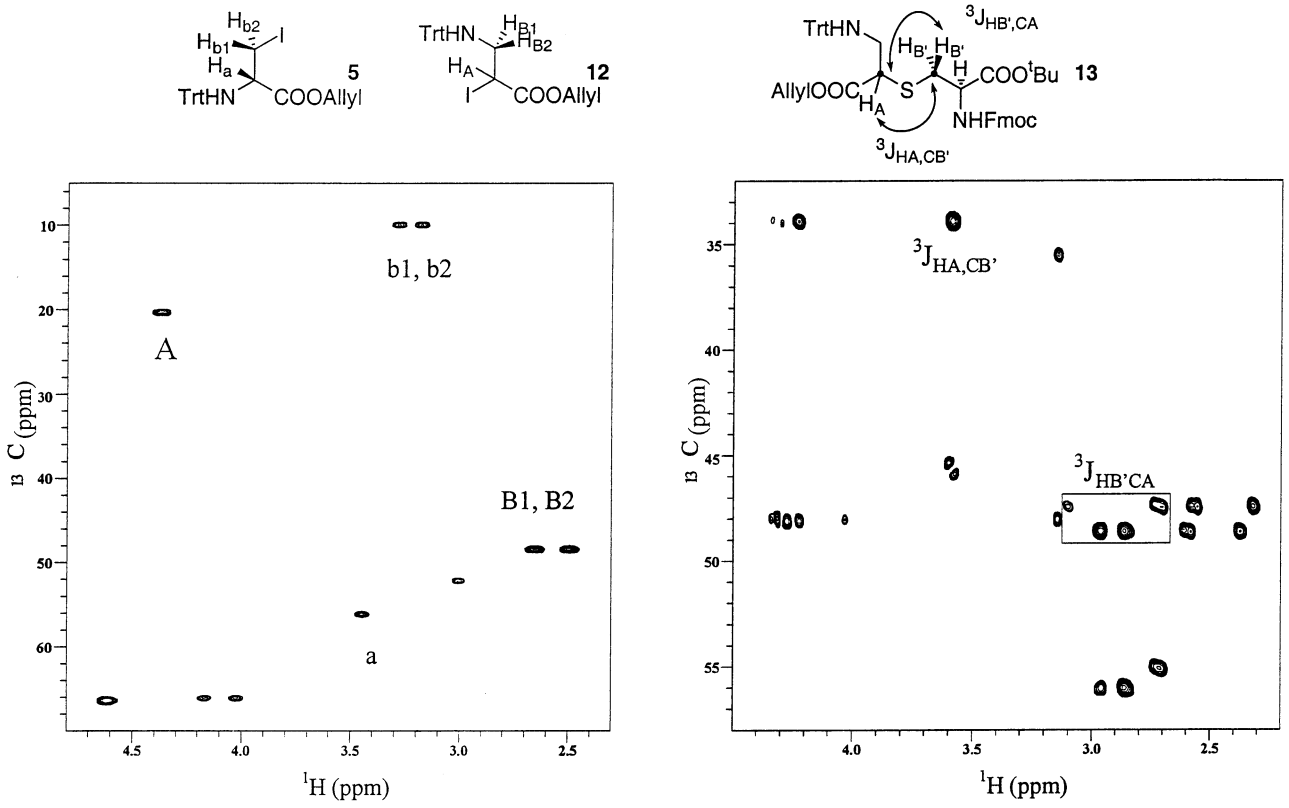


Figure 1. (a) Selected region of the assigned 2D ^1H–^{13}C HSQC spectrum of the mixture of **5** and the regioisomer **12** in $CDCl_3$ recorded on a 600 MHz Varian UNITYplus spectrometer at 283 K. (b) Selected region of the 2D ^1H–^{13}C HMBC spectrum of the mixture of **7** and the regioisomer **13** in CD_3OD recorded on a 500 MHz Varian UNITYplus spectrometer at 283 K. The ^3J correlations across the thioether bridge of the regioisomer **13** are shown.

alanine **12**, and not mixtures of rotameric forms of the desired lanthionine **7** and iodoalanine **5**, respectively, as previously reported.[9] Work is in progress to establish the mechanistic pathway for this rearrangement, and will be published in due course.

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13. In each case, the material was purified by flash column chromatography and, in the case of **5** and its enantiomer, also by recrystallisation. Found for **10**: C, 60.15; H, 4.8; N, 2.7; I, 25.6. $C_{25}H_{24}NO_2I$ requires C, 60.4; H, 4.9; N, 2.8; I, 25.5%. Found for **11**: C, 72.9; H, 6.45; N, 3.6; S, 4.0. $C_{47}H_{48}N_2O_6S$ requires C, 73.4; H, 6.3; N, 3.6; S, 4.2%.
14. Preparative normal phase HPLC separation of **5**: Tech-sil5SILICA column, 22 mm×25 cm, flow rate 15 ml min^{-1}:

gradient 2–5% EtOAc in hexane over 40 min: two partially resolved peaks at 14.5 and 15.5 min corresponding to the two isomers observed by ^{1}H NMR. HPLC separation of **8** was carried out using the same conditions: gradient 17–23% EtOAc in hexane over 45 min: two peaks at 20.4 and 23.6 min were completely separated, corresponding to the two isomers observed by ^{1}H NMR.

15. The chemical shifts of each isomer of the two independently prepared diastereoisomers **11** and **7** are similar, but not identical. Previous work in which the two lanthionine diastereoisomers were unambiguously prepared have also shown the chemical shifts to be similar: Ösapay, G.; Zhu, Q.; Shao, H.; Chadha, R. K.; Goodman, M. *Int. J. Pept. Prot. Res.* **1995**, *46*, 290–301.