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PERSPECTIVE

The challenge of the lantibiotics: synthetic approaches to thioether-bridged peptides

Alethea B. Tabor*

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The unique antibacterial properties and structural complexity of the lantibiotics has stimulated considerable interest in the development of methodology to synthesise these peptides. One of the most challenging issues has been the synthesis of polycyclic peptides with multiple thioether bridges between side-chains, which are a characteristic feature of the lantibiotics. In this perspective, the different approaches to this problem, including solution-phase synthesis, solid-phase synthesis, biomimetic approaches and biotransformation strategies, are reviewed, highlighting the advances resulting from each of these approaches.

Introduction

The lantibiotics are an intriguing family of microbially-synthesised peptides with unique structural properties. The characteristic feature of these peptides is that they have one or more thioether bridges, formed by the inclusion of the unusual amino acids lanthionine **1** and methyl lanthionine **2**. Other residues such as dehydroalanine (Dha) **3** and dehydrobutyrine (Dhb) **4**, and bridges formed from other bis-amino acids such as lysinoalanine **5**, are frequently also present.[1,2] Since the isolation[3] and characterisation[4] of the first lantibiotic, nisin (Fig. 1), more than 40 lantibiotic peptides have been discovered. They have generally been classified into two groups, based on their overall structural properties. Type A lantibiotics (exemplified by nisin) have elongated structures with few overlapping thioether bridges, and Type B lantibiotics (exemplified by cinnamycin, Fig. 1) have compact structures with multiple overlapping and nested thioether bridges. More recently, an alternative classification into two groups, class I and class II, has been proposed, based on analysis of the biosynthetic pathways by which these peptides are produced.[5]

The lantibiotics possess a range of antibacterial activities. Nisin, the best studied member of the family, has a dual mode of action on bacterial membranes. The N-terminus (rings A and B) recognise and specifically bind lipid II (a key component of the biosynthesis of bacterial membranes), this is then followed by pore formation by the nisin/lipid II complex in an 8 : 4 ratio, mediated by the C-terminus (rings C, D/E).[6] The structure of the complex between rings A and B and lipid II has been elucidated by NMR,[7] and a second mode of action, involving sequestration of lipid II in the bacterial membrane[2,8] has been proposed for nisin and other related peptides. The continual rise of antibiotic-resistant strains of pathological bacteria has made it very important to investigate naturally-occurring antibiotic molecules which target novel biosynthetic pathways within bacteria, and the lantibiotics may thus represent the basis for a new and more powerful family of antibacterial agents.

The lantibiotics are formed in nature from ribosomally synthesised precursor peptides, which then undergo a series of post-translational modifications to give complex molecular architectures. Extensive research, particularly over the last decade, has resulted in the isolation and sequencing of the gene clusters responsible for the biosynthesis of many lantibiotics. The enzymology and substrate specificity of many of the individual biosynthetic enzymes have been studied. Increased understanding of how the producing organisms make lantibiotics has in turn led to the generation of mutant lantibiotics with the potential for variant biological properties.[2,9–11]

Department of Chemistry, UCL, Christopher Ingold Laboratories, 20 Gordon Street, London, UK, WC1H 0AJ. E-mail: a.b.tabor@ucl.ac.uk; Fax: +44 (0)20 7679 7463; Tel: +44 (0)20 7679 4695

Alethea Tabor

Alethea Tabor was born in 1964 and brought up in London. She obtained her BA (Natural Sciences) (1985) and PhD (1989) from the University of Cambridge, under the supervision of Professor Andrew Holmes FRS. After a postdoctoral position with Professor Stuart Schreiber at Harvard University (1989–1991) she was appointed as a Lecturer at the University of Edinburgh. She moved to UCL in 1995, where she is currently a Reader in Chemical Biology. Her current interests are in the synthesis of complex peptides, including the lantibiotics, and in targeted nanoparticles for the delivery of DNA, siRNA, imaging agents and small molecules.

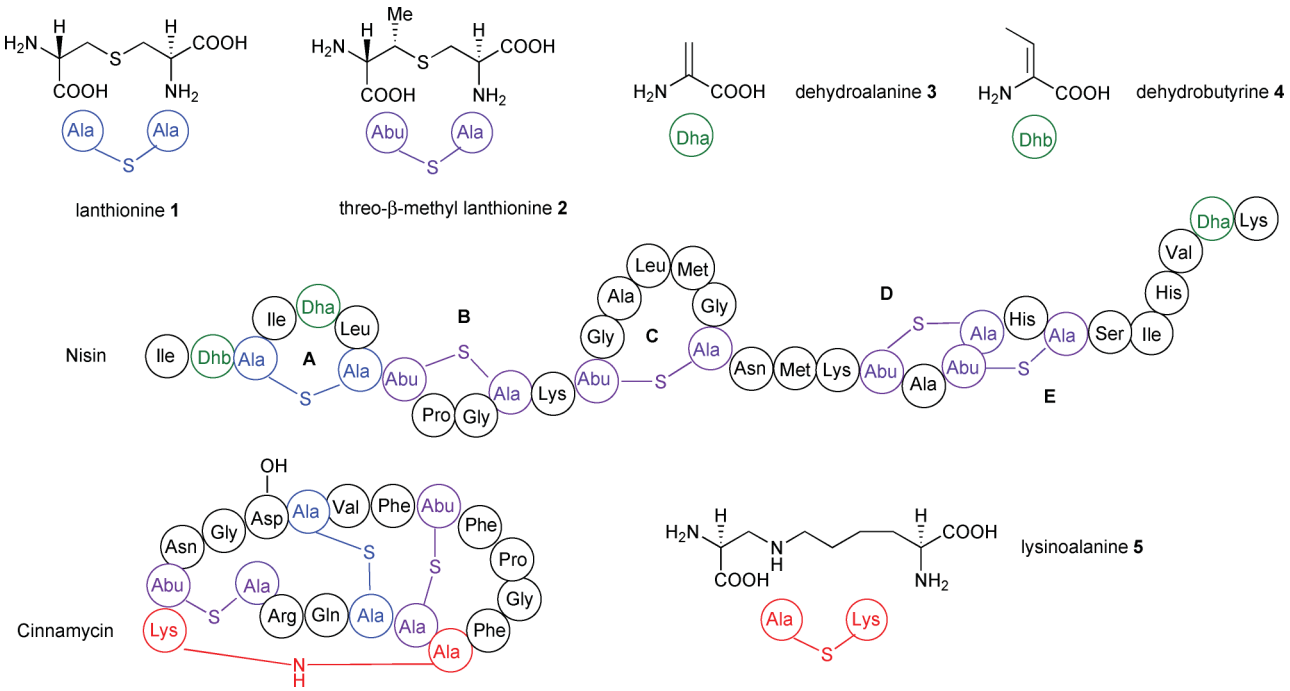


Fig. 1 Lanthionine, methyl lanthionine and some other characteristic features of the lantibiotics.

However, there is still a pressing need to find effective synthetic routes to the lantibiotics. Synthetic approaches hold the promise of rapid incorporation of non-natural amino acids, such as different ring motifs, conformational locks, fluorophores, the use of unnatural linkage chemistry, and the ready production of smaller subunits.

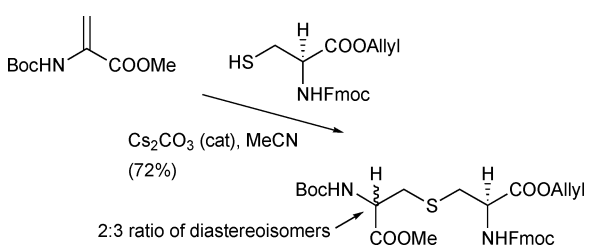
The many unusual structural features of the lantibiotics have posed a formidable synthetic challenge over the last twenty years. Since the first review on this subject appeared in 2005,[12] several important advances in synthetic approaches have been reported. In tackling the challenge of lantibiotic synthesis, there are critical questions that must be addressed. Should the key bis-amino acids, lanthionine and methyl lanthionine, be synthesised first and then incorporated into the peptide, or should the thioether bridge be generated from precursors within a linear peptide? Would the most successful approach be a biomimetic synthesis inspired by the chemistry carried out by the producing organisms, or would it use the enzymes supplied by the producing organisms as reagents, or should the approach be totally synthetic? In this perspective recent developments in this field will be described, highlighting the advances resulting from each of these approaches.

Synthesis of thioether bis-amino acids

For a synthetic approach to the lantibiotics that involves the direct incorporation of lanthionine and methyl lanthionine into the nascent peptide chain, it is first necessary to synthesise the required thioether-bridged bis-amino acids. The synthetic route must deliver the correct enantiomers at the two stereocentres (three stereocentres in the case of methyl lanthionine) and, in addition, must result in bis-amino acids where the two amino and two carboxylate groups are differentially protected, ideally using protecting groups compatible with solution- or solid-phase peptide synthesis techniques.

Michael addition to dehydroalanine residues

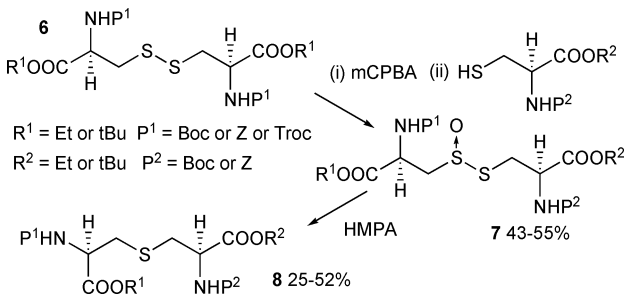
During the post-translational modification of lantibiotic precursor peptides to form lantibiotics, one key step is the formation of the thioether bridge itself. In nature, this is formed by the Michael addition of the thiol side-chain of Cys residues to Dha or Dhb residues elsewhere within the peptide (*vide infra*).[1] The reaction of Cys derivatives with Dha or Dhb could therefore also be a reasonable approach for the synthesis of differentially protected lanthionines and methyl lanthionines. However, Bradley *et al.*[13] have demonstrated that this approach is not stereospecific, and results in a mixture of diastereoisomers being formed at the newly formed chiral centre (Scheme 1). Although, unsurprisingly, the chiral nucleophile does not exert any stereocontrol on this reaction, the diastereoisomers in this case are separable, leading to orthogonally protected lanthionines with suitable protecting groups for SPPS.



Scheme 1 Synthesis of lanthionine by Michael addition.

Lanthionine synthesis *via* desulfurisation

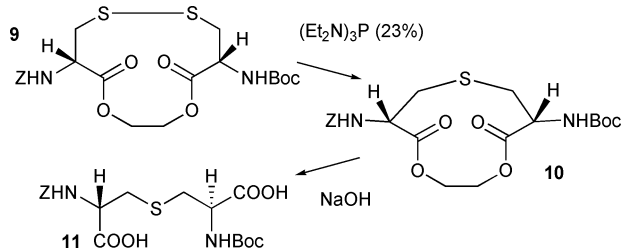
The synthesis of (*R*,*R*)-lanthionine (L-lanthionine) derivatives by desulfurisation of symmetrical (*R*,*R*)-cystine derivatives with aminophosphines was first reported by Harpp and Gleason.[14] The approach was subsequently adapted by Olsen *et al.*[15] to give unsymmetrical lanthionines. Symmetrical cystine derivatives **6** were first oxidised to give thiosulfinates, which were then displaced with differentially protected cysteine derivatives to give the unsymmetrical thiosulfinates **7** (Scheme 2).



Scheme 2 Unsymmetrical lanthionines *via* desulfurisation.

Contraction of these cystines with aminophosphines gave unsymmetrical lanthionines **8** in modest yields: the reversibility of the reactions meant that symmetrical cystine derivatives were also inevitably formed.

Cavalier-Frontin *et al.*[16] also modified this approach to prepare unsymmetrical lanthionine derivatives from cyclic cystine derivatives **9** (Scheme 3) in which the two amino groups are differentially protected. Again, the key desulfurisation reaction proceeded in only moderate yield. Saponification of the glycolic diester linkage of **10** gave an unsymmetrical *meso*-lanthionine **11** in which the amino groups, although not the carboxylic acid groups, are differentially protected.



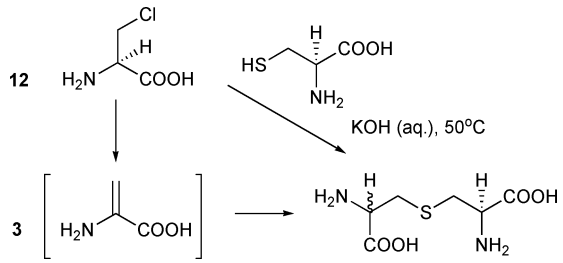
Scheme 3 Unsymmetrical lanthionine derivatives from cyclic cystine derivatives.

This approach has not been subsequently investigated for the preparation of orthogonally protected lanthionines for peptide synthesis, partly because the competing formation of other cystine derivatives[15] and of dehydroalanine derivatives,[13] together with difficulties in purification,[16] all lead to only modest yields of the desired product. In addition, the sulfur extrusion method is not compatible with base-sensitive protecting groups.[17]

Although this is not the route by which lantibiotics are biosynthesised, the formation of lanthionine bridges when proteins with multiple disulfide bridges are treated under strongly basic conditions has also been observed. Spatola *et al.* have comprehensively studied this reaction[18] and have concluded that the mechanism involves elimination to form a Dha residue, followed by loss of a sulfur atom and Michael addition to form the required thioether bridge. The reaction is inherently stereorandom, and therefore could not be a viable route for the synthesis of lantibiotics.

β-Haloalanine and similar synthons

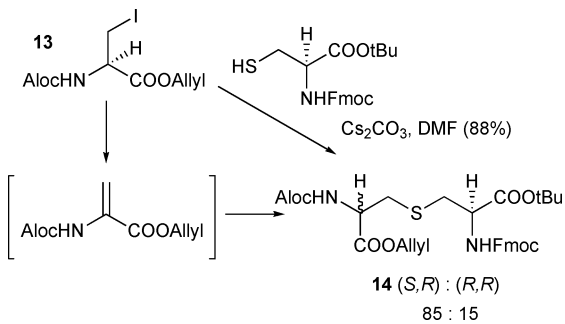
The first reported synthesis of lanthionine[19] involved coupling of β-chloroalanine **12** with cysteine (Scheme 4).



Scheme 4 First reported synthesis of lanthionine.

The strongly basic conditions employed by du Vigneaud and Brown may result in competing conversion of the β-chloroalanine to dehydroalanine **3**, followed by stereorandom Michael addition of cysteine[13] to afford a mixture of diastereoisomers.[20] However, Shiba *et al.* have used this reaction to separately synthesise each of the four stereoisomers of β-methyl lanthionine,[21] apparently without racemisation at the α-centre.

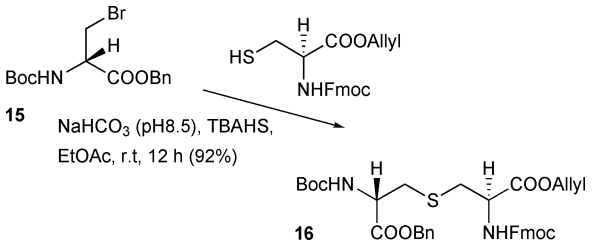
We have shown[22] that carbamate-protected β-iodoalanine derivatives **13** are also prone to competing dehydroalanine formation during the *S*-alkylation of protected cysteines, even employing mild conditions (room temperature using Cs_2CO_3 as the base) under which the Fmoc group is stable (Scheme 5). In this case the diastereoisomers **14** were not separable, and could not be observed by ^{1}H NMR, however the presence of the two diastereoisomers was revealed by ^{13}C NMR and confirmed by independent synthesis of each isomer.



Scheme 5 Formation of dehydroalanines during the synthesis of lanthionine derivatives from carbamate-protected β-iodoalanine.

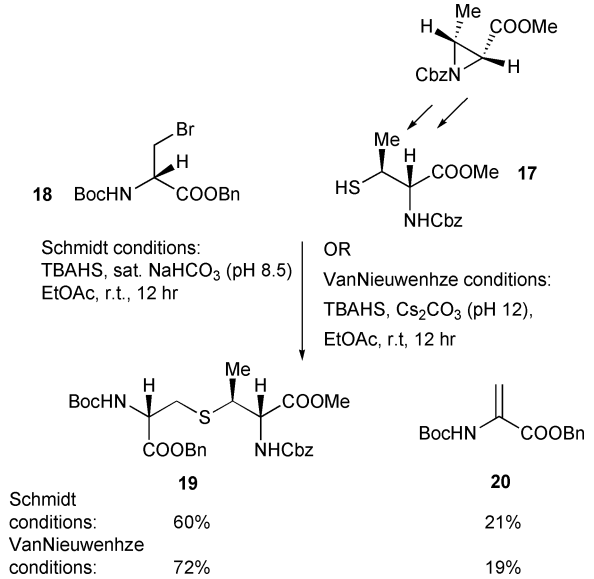
Zhu and Schmidt[23] have circumvented this problem by using β-bromoalanine derivatives **15** as β-alanyl cation equivalents in this reaction, and by the use of the less basic sodium bicarbonate as the base. These carbamate-protected bromoalanine derivatives appear to be sufficiently reactive towards the soft sulfur nucleophile, without being so reactive that elimination to form the dehydroalanine is a significant problem. Moreover, they were able to further tune this reaction by using phase transfer

conditions, with an aqueous solution of $NaHCO_3$ as the base and tetrabutylammonium hydrogensulfate (TBAHS) as the phase transfer catalyst. In this way high yields of single stereoisomers of differentially protected lanthionines **16** could be produced (Scheme 6).



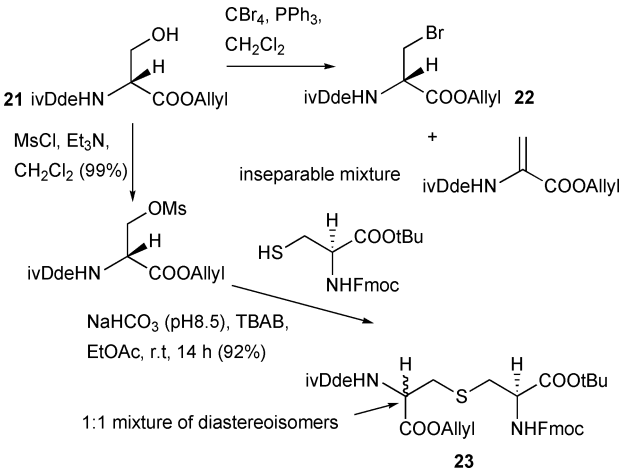
Scheme 6 Zhu and Schmidt synthesis of differentially protected lanthionines.

This method has been further adapted by Narayan and VanNieuwenhze[24] in a synthesis of orthogonally protected β-methyl lanthionine (Scheme 7). Protected methyl cysteine derivatives **17** were first prepared *via* regioselective ring-opening of aziridines. These were then *S*-alkylated with similar carbamate-protected bromoalanine derivatives **18** to afford methyl lanthionine derivatives **19**. However, the reduced nucleophilicity of the more sterically challenged methyl cysteine meant that the competing elimination reaction was again observed. Even after optimisation of the conditions, including the use of aqueous Cs_2CO_3, significant amounts of the unwanted dehydroalanine byproduct **20** were produced, although Michael addition of methyl cysteine to form mixtures of diastereoisomers was not observed.



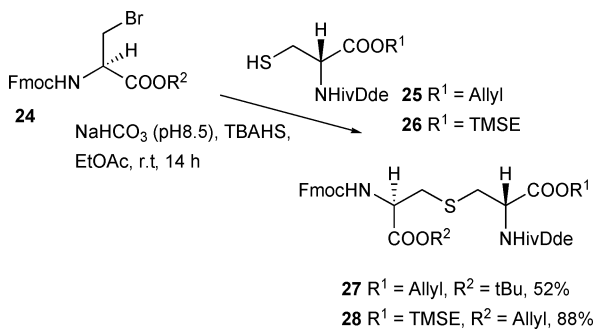
Scheme 7 Narayan and VanNieuwenhze synthesis of orthogonally protected β-methyl lanthionine.

The Schmidt approach, although successful with robust carbamate and ester protecting groups, cannot be easily generalised to more sensitive protecting groups routinely used in peptide synthesis. Martin[25] has recently disclosed a synthesis of tetra-orthogonally protected (2*S*,6*R*) lanthionines bearing the ivDde protecting group (Scheme 8). Attempted bromination of



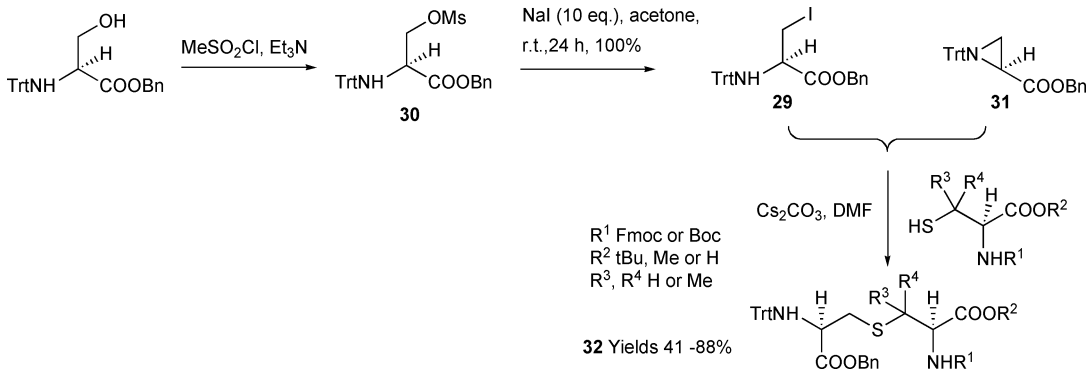
Scheme 8 Attempted synthesis of (ivDde, allyl)/(Fmoc, tBu) lanthionine.

ivDdeSer-OAllyl **21** to give β-bromoalanine **22** was unsuccessful, as extensive competing elimination occurred. Predictably, when **21** was converted to the mesylate and this was used to *S*-alkylate Fmoc-Cys-OtBu directly, extensive racemisation at the C_2-position of lanthionine **23** was observed. Deuterium incorporation experiments confirmed that this was likely to arise through an elimination followed by Michael addition. However, inversion of this strategy, such that Fmoc-protected β-bromoalanine **24** was reacted with ivDde-Cys derivatives **25** or **26** under Schmidt conditions, gave the desired single lanthionine diastereoisomers **27** or **28**, the structure and chiral integrity of which was confirmed by ^{13}C NMR. It is notable that a low yield of **27** was achieved with ivDde-Cys-OAllyl **25** as the nucleophile, due to the instability of this Cys derivative, whereas much better yields were obtained with ivDde-Cys-OTMSE **26** (Scheme 9).



Scheme 9 Martin's synthesis of ivDde-protected lanthionine derivatives.

The tendency of carbamate-protected serine and β-halogenoalanine derivatives towards elimination, giving the corresponding dehydroalanine, are well-documented. Trityl protection of the amino group of serine has been developed as a general solution to this problem.[26] Dugave and Ménez developed a concise and high-yielding route to lanthionine, methyl lanthionine and β,β-dimethyl lanthionine from the reaction of trityl-protected iodoalanine **29** with cysteine, methyl cysteine and penicillamine derivatives in the presence of Cs_2CO_3 (Scheme 10).[27] The iodoalanine **29** was prepared from the corresponding mesylate **30** by treatment with NaI, with aziridine **31** being formed as a byproduct. For both the iodoalanine **29**, and the resulting lanthionine



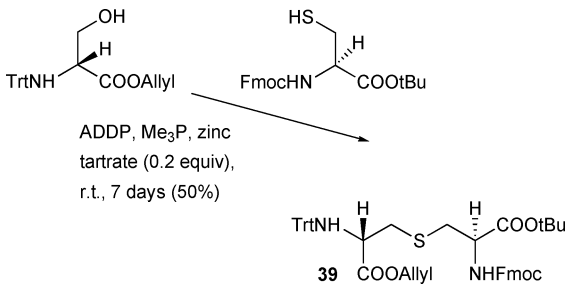
Scheme 10 Dugave and Ménez' synthesis of orthogonally protected lanthionine derivatives.

derivatives **32**, doubling of peaks in the proton NMR spectra was observed. These doubled peaks were attributed to the presence of two rotamers. This approach has also been used by other groups to prepare orthogonally protected lanthionine derivatives.[28,29]

However, we subsequently investigated the regio- and stereochemical outcome of the formation and reaction of iodoalanine **33** with cysteine derivatives using VT, HSQC and HMBC NMR experiments. We demonstrated that during the formation of the iodoalanine from mesylate **34**, a rearrangement takes place, *via* aziridine **35**, to give the α-iodo β-alanine **36** as the major product, as a mixture of enantiomers, rather than exclusively the β-iodo α-alanine **33** as a mixture of rotamers, as previously suggested.[27,28] The preferential α-attack is probably due to the increased positive charge in the presence of the ester. Subsequent reaction with cysteine derivatives and Cs_2CO_3 gave both diastereoisomers of the unwanted nor-lanthionine **37**, as an inseparable mixture with the correct lanthionine regioisomer **38** (Scheme 11).

As an alternative, we have developed two approaches to lanthionine using Mitsunobu chemistry. Aliphatic thiols are generally not sufficiently acidic to react with alcohols under the standard Mitsunobu conditions of Ph_3P and DEAD. However, we were able to use the more reactive combination of ADDP and Me_3P, combined with catalytic zinc tartrate, to access protected lanthionine **39** directly from Fmoc-Cys-OtBu and Trt-Ser-OAllyl (Scheme 12) in moderate yield.[30,31]

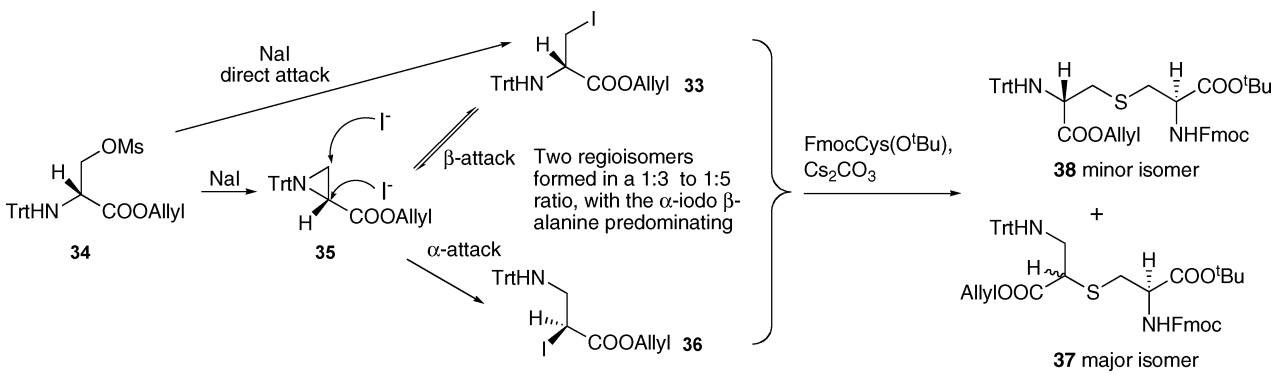
In the second approach, iodoalanine **33** was synthesised directly from Trt-Ser-OAllyl: using Mitsunobu conditions at low temperature prevented formation of the unwanted α-iodo β-alanine



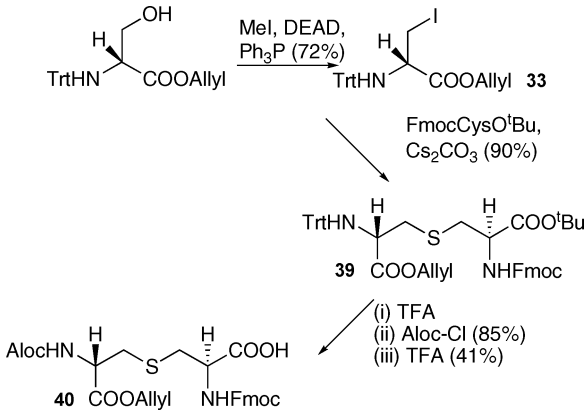
Scheme 12 Synthesis of lanthionine *via* Mitsunobu reaction.

regioisomer, although trace amounts of the aziridine byproduct are still formed.[22] Reaction with Fmoc-Cys-OtBu in the presence of Cs_2CO_3 afforded lanthionine **39**. This was then converted to protected lanthionine derivative **40**, suitable for use in the solid-phase synthesis of lantibiotics (Scheme 13).

Although the reaction of β-haloalanine derivatives with cysteine nucleophiles has generally proven to be a realistic approach for the synthesis of differentially protected lanthionines, this approach does not work with all combinations of protecting groups, electrophilic and nucleophilic component. In addition to the competing reactions of elimination and/or rearrangement outlined, this strategy is also quite sensitive to steric factors. As described above, the change in steric bulk between cysteine and methyl cysteine results in a very significant drop in both yield and selectivity when methyl lanthionine, rather than lanthionine, is being synthesised.[24]



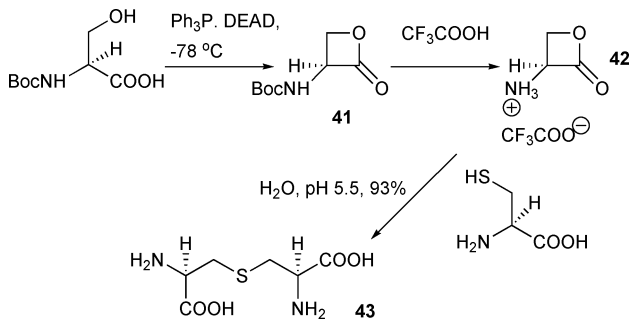
Scheme 11 Rearrangement of trityl iodoalanine derivatives *via* aziridine formation.



Scheme 13 Bregant and Tabor's synthesis of orthogonally protected lanthionine.

Ring-opening of lactones, aziridines and sulfamidates

An alternative approach to lanthionine, using serine β-lactone as a starting point, was first developed by Vederas *et al.*[32] Modified Mitsunobu conditions were first used to generate lactone **41** from Boc-(*S*)-serine, which was then deprotected to give **42**. Although **42** is readily hydrolysed, it could be easily ring-opened with (*R*)-cysteine at pH 5.5 to give the unprotected (*R*,*R*)-lanthionine **43** in excellent yield (Scheme 14).

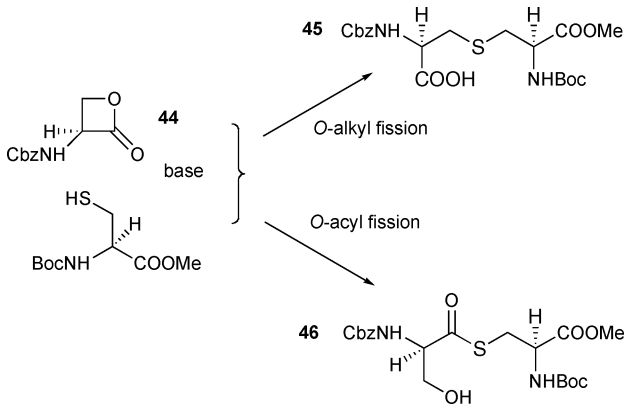


Scheme 14 Ring-opening of the Vederas lactone by cysteine.

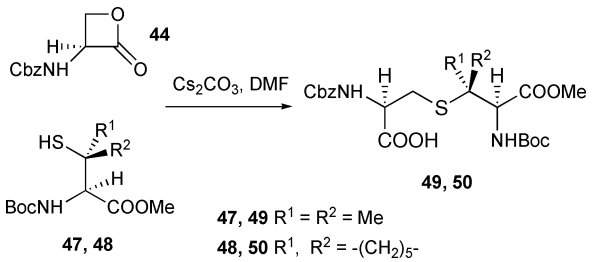
This approach was subsequently developed by Goodman *et al.* for the synthesis of differentially protected lanthionine derivatives.[33] Ring-opening of the *N*-Cbz-protected (*S*)-serine β-lactone **44** with Boc-(*S*)-Cys-OMe, in organic solvents in the presence of base, gave the desired protected lanthionine **45**. However, competing *O*-acyl fission was also observed; indeed with many combinations of base, polar organic or aqueous solutions, the unwanted thioester **46** was the only product formed (Scheme 15). The best yields (50%) of the desired lanthionine **45** were obtained with Cs_2CO_3 as the base and DMF as the solvent.

With more hindered nucleophiles, such as the penicillamine (**47**) and β,β-pentamethylenecysteine (**48**) derivatives, however, almost exclusive *O*-alkyl fission was observed, giving the desired conformationally locked lanthionines **49** and **50** with only traces of the unwanted thioester (Scheme 16).[33]

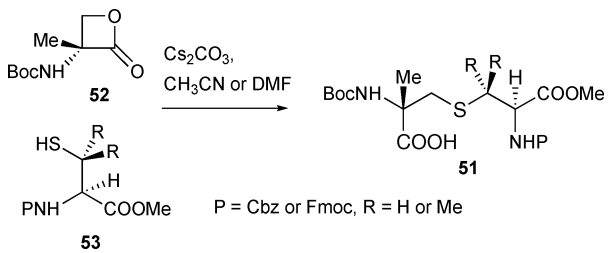
The serine β-lactone approach is clearly very useful when preparing lanthionine analogues that are sterically hindered at the α-position. Smith and Goodman[34] were able to prepare α-methyl lanthionines **51** by ring-opening the α-methyl analogue **52** of the Vederas lactone with protected Cys derivatives **53** (Scheme 17).



Scheme 15 *O*-alkyl *versus* *O*-acyl fission in the ring-opening of serine β-lactones.



Scheme 16 Goodman *et al.* synthesis of sterically hindered lanthionine derivatives.

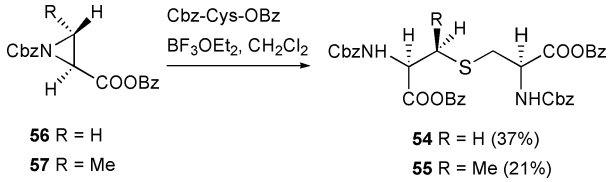


Scheme 17 Synthesis of α-methyl analogues of lanthionine.

Minor amounts (0–24%) of the thioester byproduct were formed. In this case the alternative β-haloalanine approach, *via* S_N2 displacement of the appropriate β-iodo-α-methyl alanine, failed. Ring-opening of the Vederas lactone can also lead to orthogonally protected lanthionine derivatives that are suitable for the on-resin synthesis of lanthionine-bridged peptides using the PCOR method (*vide infra*). However, it has not generally proved possible to adapt this approach to deliver the more delicate protecting groups required for Fmoc-based SPPS: Bradley and co-workers[13] have reported that the ring-opening of serine β-lactones with Fmoc-Cys-OAllyl results only in decomposition of the starting materials. It is also notable that this approach has not yet been reported for the synthesis of any of the diastereoisomers of methyl lanthionine **2**, either by ring-opening of serine β-lactones such as **43** or **44** by methyl cysteine, or by ring-opening of threonine β-lactones.[35] In both cases one can speculate that the additional steric hindrance at the β-position may well result in an unacceptable amount of competing *O*-acyl fission.

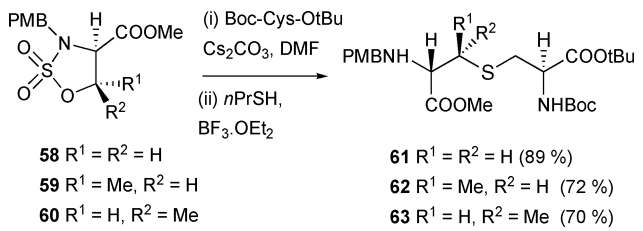
Little attention has so far been given to the synthetic possibilities afforded by ring-opening of aziridinecarboxylic acids. Nakajima

et al. reported the synthesis of lanthionine (**54**) and methyl lanthionine (**55**) derivatives by the $BF_3.OEt_2$-catalysed ring-opening of aziridines derived from serine (**56**) or threonine (**57**) (Scheme 18).[36] The poor yields have not encouraged subsequent investigations of this reaction.



Scheme 18 Synthesis of lanthionine derivatives by ring-opening of aziridines.

A third ring-opening approach to the synthesis of protected lanthionine and methyl lanthionine was recently reported by the Vederas group.[37] In this work cyclic sulfamidates derived from (*R*)- or (*S*)-serine (**58**), threonine (**59**) or *allo*-threonine (**60**) were ring-opened with Boc-Cys-OtBu in the presence of Cs_2CO_3 to give differentially protected (2*R*,6*S*) lanthionine, *meso*-lanthionine (**61**), (2*S*,3*R*,6*R*) methyl lanthionine (**62**) and (2*S*,3*S*,6*R*) methyl lanthionine (**63**) derivatives respectively (Scheme 19). The choice of orthogonal protecting groups was dictated by the preparative conditions: allyl and Aloc groups were not compatible with the oxidising conditions required to form the sulfamidates, and the Fmoc group of Fmoc-Cys-OtBu was removed during the ring-opening reaction. However, under these ring-opening conditions, competing elimination reactions were not observed.

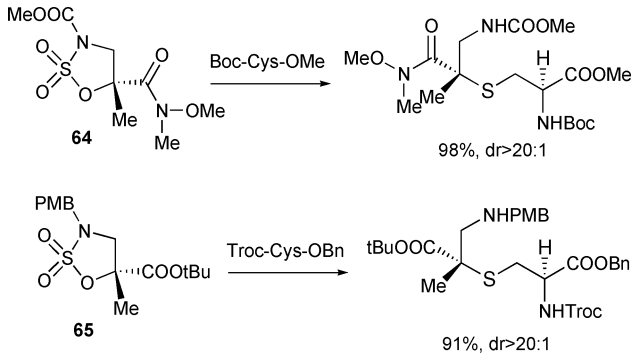


Scheme 19 Vederas *et al.* synthesis of orthogonally protected methyl lanthionine derivatives.

A similar approach has also been used by Peregrina *et al.* to access α-methyl nor-lanthionine derivatives.[38] The regioisomeric sulfamidates were prepared from α-methylisoserine, and ring-opening reactions at the quaternary centre were carried out. A variety of differentially protected cysteine and sulfamidates were explored (Scheme 20). Excellent yields were obtained with sulfamidates **64** and Boc-Cys-OtBu, and also with **65** and Troc-Cys-OBn. However, all attempts to use allyl esters on either the sulfamidate or cysteine components gave complex mixtures of products.

Synthesis of thioether-bridged peptides: lantibiotics and other analogues

As the preceding discussion makes clear, it is by no means straightforward to synthesise lanthionine, methyl lanthionine, and analogues, with the amino and carboxylic acid moieties differentiated and with complete control of the regio- and stereoselectivity. Similar reactions have also been used in the synthesis



Scheme 20 Synthesis of α-methyl nor-lanthionine derivatives from sulfamidates.

of lanthionine-bridged peptides from linear precursors. In some cases, performing the crucial C–S bond-forming reactions between side-chains of a linear peptide ameliorates the problems of regio- and stereoselectivity encountered when using isolated amino acid precursors. However, other issues, particularly of connectivity, may become problematic when constructing the cyclic peptide framework from a linear precursor.

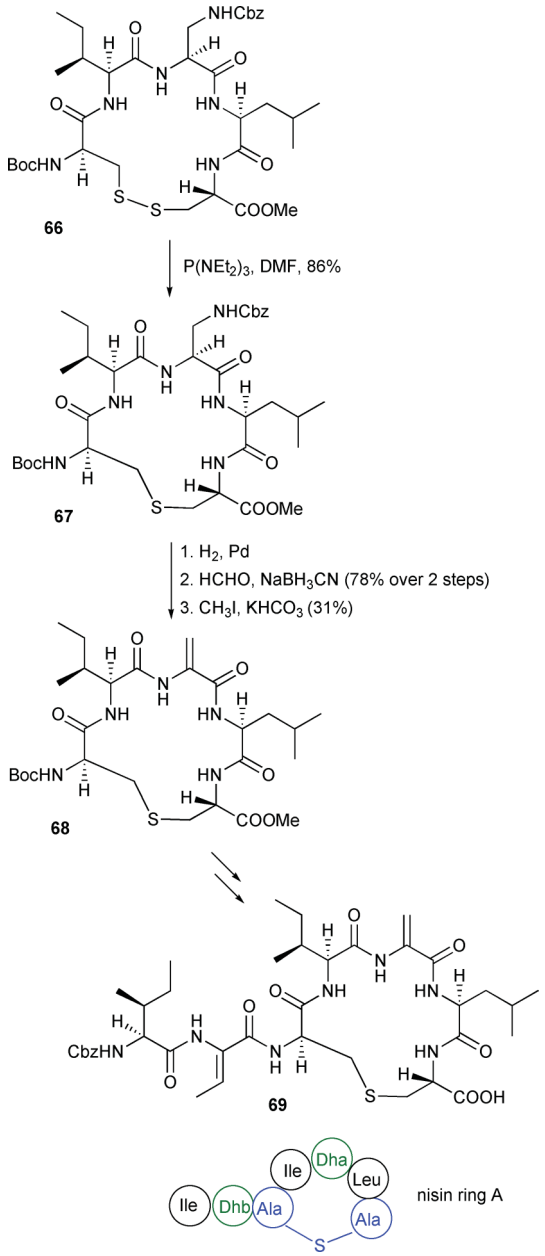
Total synthesis of nisin *via* desulfurisation

The first total synthesis of a lantibiotic, nisin, was achieved by Shiba *et al.* in 1988.[39] The key breakthrough that enabled this synthesis was their demonstration that the desulfurisation approach to lanthionine of Harpp and Gleeson[14] could be used for the desulfurisation of cystine-bridged cyclic peptides. For example, they were able to make ring A of nisin from the cyclic precursor **66** by treatment with $P(NEt_2)_3$ to give cyclic peptide **67** (Scheme 21).[40]

Subsequent removal of the side-chain protecting group of the diaminopropionic acid (Dpr) residue by hydrogenation, in the presence of the thioether, proceeds in a remarkably high yield. This is then followed by *N*-methylation and elimination to install the Dha residue, giving **68**. This was later extended by the addition of Ile and Thr residues, followed by elimination with EDC/$CuCl_2$ to give the Dhb residue, to give the N-terminal sequence **69** of nisin.[41]

The synthesis of ring B of nisin by desulfurisation[42] was potentially more challenging, as in this case a methyl lanthionine bridge must be created. The precursor peptide, **70**, with a disulfide bridge between *threo*-3-methyl cysteine (MeCys) and cysteine, was again treated with $P(NEt_2)_3$ to effect the desulfurisation. Only one diastereoisomer, **71**, containing the desired *threo*-β-methyl lanthionine, was formed (Scheme 22). This suggests that the thiolate anion of *threo*-3-methyl cysteine is more stable than the thiolate anion of cysteine and is preferentially formed, and that therefore the S_N2 reaction takes place exclusively at the C–S bond of the cysteine residue. This intramolecular reaction avoids many of the problems of disulfide exchange encountered during the desulfurisation of isolated cystine analogues.[14] Similar chemistry was used to prepare ring C of nisin, which also includes a MeLan residue.[43]

The most difficult challenge of the nisin synthesis, however, is the regioselective formation of the overlapping D and E rings at the C-terminus. This was achieved by the regioselective formation of two MeCys-Cys bridges to give peptide **72** (Scheme 23),



Scheme 21 Synthesis of ring A of nisin by desulfurisation.

followed by simultaneous desulfurisation to give the two MeLan bridges of **73**.[44] The double desulfurisation reaction proceeded smoothly in reasonable yield without rearrangement of the rings or oligomerisation being observed. By contrast, stepwise cystine bridge formation and desulfurisation, forming first the E then the D rings, proceeded in lower yield with multiple oligomeric byproducts. With all of the lanthionine-bridged rings successfully synthesised, segment coupling of each section, and the linear C-terminal sequence, followed by removal of the remaining protecting groups, gave nisin itself.[41]

Biomimetic synthesis of lantibiotics and other thioether-bridged peptides

The biosynthetic route by which lantibiotics are formed has been extensively studied over the past decade, and the details are now well-understood.[2] As an example, the posttranslational maturation pathway by which nisin is formed is shown in Scheme 24. A prepeptide, NisA (**74**), is first ribosomally synthesised: this consists of a sequence of 34 amino acids that will eventually form the structure of nisin, together with an N-terminal leader peptide sequence. This is then modified by a dehydratase enzyme, NisB, which converts all of the Ser and Thr residues to Dha and Dhb respectively, giving **75**. A cyclase enzyme, NisC, then catalyses the Michael addition of the Cys-SH groups to selected Dha and Dha residues, giving **76**, from which the leader sequence is then cleaved with NisP to give nisin itself. This sequence of dehydration, Michael addition and leader peptide removal appears to be common to the biosynthesis of all classes of lantibiotics so far studied.

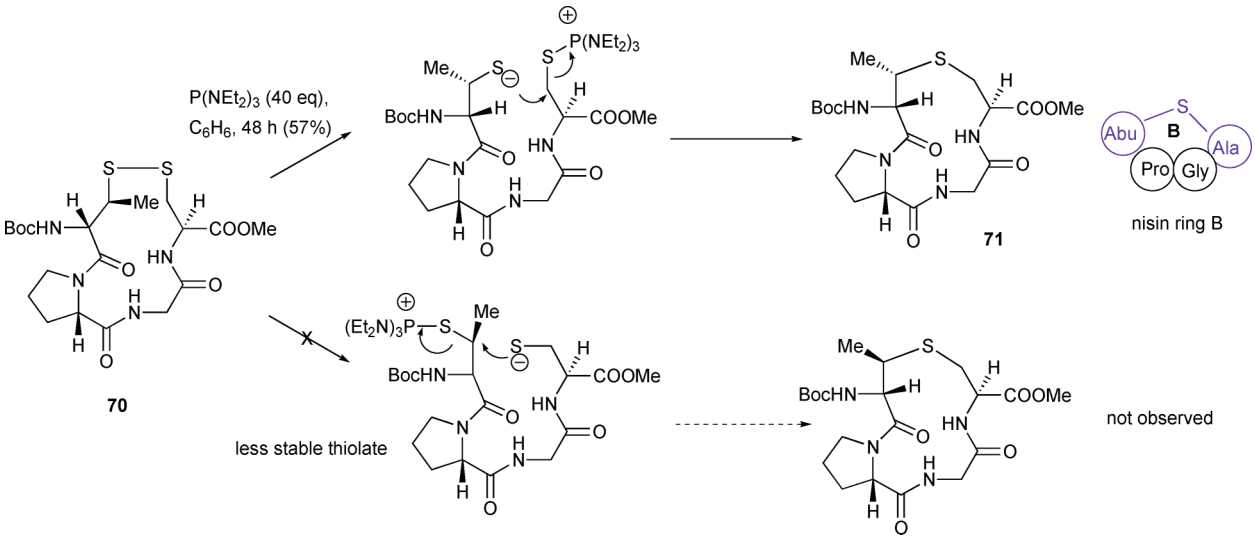
Whilst class I lantibiotics, such as nisin, all use two enzymes for the ring formation step (a dehydratase enzyme, LanB, followed by a cyclase enzyme, LanC), in class II lantibiotics such as lacticin 481, a single modification enzyme, LanM, is used to carry out both of these processing steps.

Is the cyclase enzyme really necessary? The Michael addition of thiols to α,β-unsaturated amino acids, particularly to Dha, is a well-precedented, facile process which takes place in mildly basic conditions.[45] Although, as discussed earlier, the reaction of isolated Dha with Cys derivatives is not stereospecific, it would be reasonable to ask whether linear peptide precursors such as **75** are pre-organised in such a way that, even without the cyclase enzyme present, only one stereo- and regiochemical outcome of the sequence of Michael addition reactions is possible.

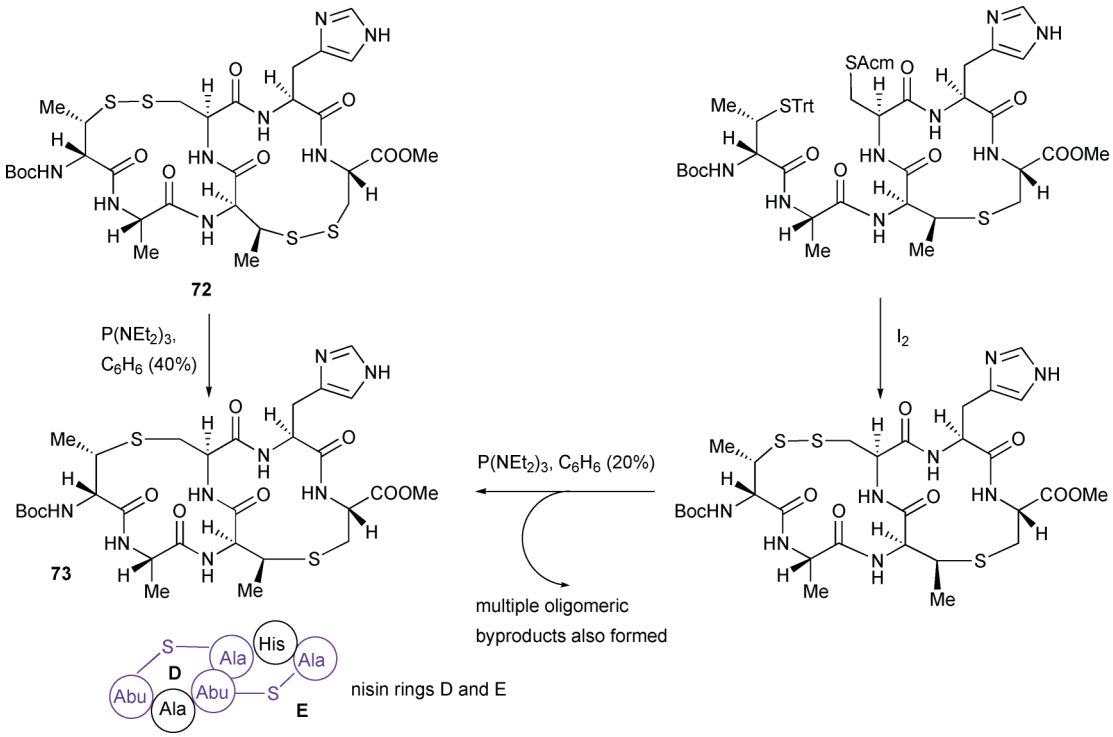
A number of groups have explored this hypothesis, as a potential biomimetic route to lantibiotics and other thioether-bridged peptides. Goodman *et al.* were the first to report a biomimetic approach for the synthesis of lanthionine-bridged enkephalin analogues (Scheme 25).[46] Pentameric peptides with a Dha residue such as **77** were synthesised on a MBHA resin using Boc chemistry. The Fm protecting group on the side-chain of the Cys residue was then deprotected using piperidine; under these mildly basic conditions the peptide cyclised to give lanthionine-bridged enkephalin analogues such as **78**, with exclusively the (*R*)-stereochemistry being formed at the α-position.

The excellent diastereoselectivity of this reaction was originally attributed to the steric hindrance provided by the adjacent solid-support. However, Toogood subsequently investigated the biomimetic cyclisation in solution of peptide **79** to give an analogue of ring B of epidermin (Scheme 26).[47] Again, a single diastereomeric product, **80**, with the naturally occurring stereochemistry at the newly formed α-position, was formed. Interestingly, NMR studies showed that at room temperature, **80** existed as two slowly interconverting conformers.[48]

The Pro-Gly sequence found in ring B of the related epidermin, nisin and subtilin clearly promotes a β-turn conformation, which not only facilitates cyclisation but also appears to pre-organise the linear peptide for diastereoselective Michael addition of the Cys-SH to the Dha residue. Bradley and co-workers have demonstrated that this reaction proceeds diastereoselectively and in high yield to form the lanthionine analogues of ring B of subtilin,[49] ring E of subtilin,[49] and ring B of nisin.[50] In order to facilitate this biomimetic approach, Bradley *et al.* have developed a mild, selective oxidation procedure, in which *S*-methyl cysteine residues undergo an oxidation/elimination sequence to give Dha residues,



Scheme 22 Shiba *et al.* synthesis of ring B of nisin by desulfurisation to give a methyl lanthionine bridge.

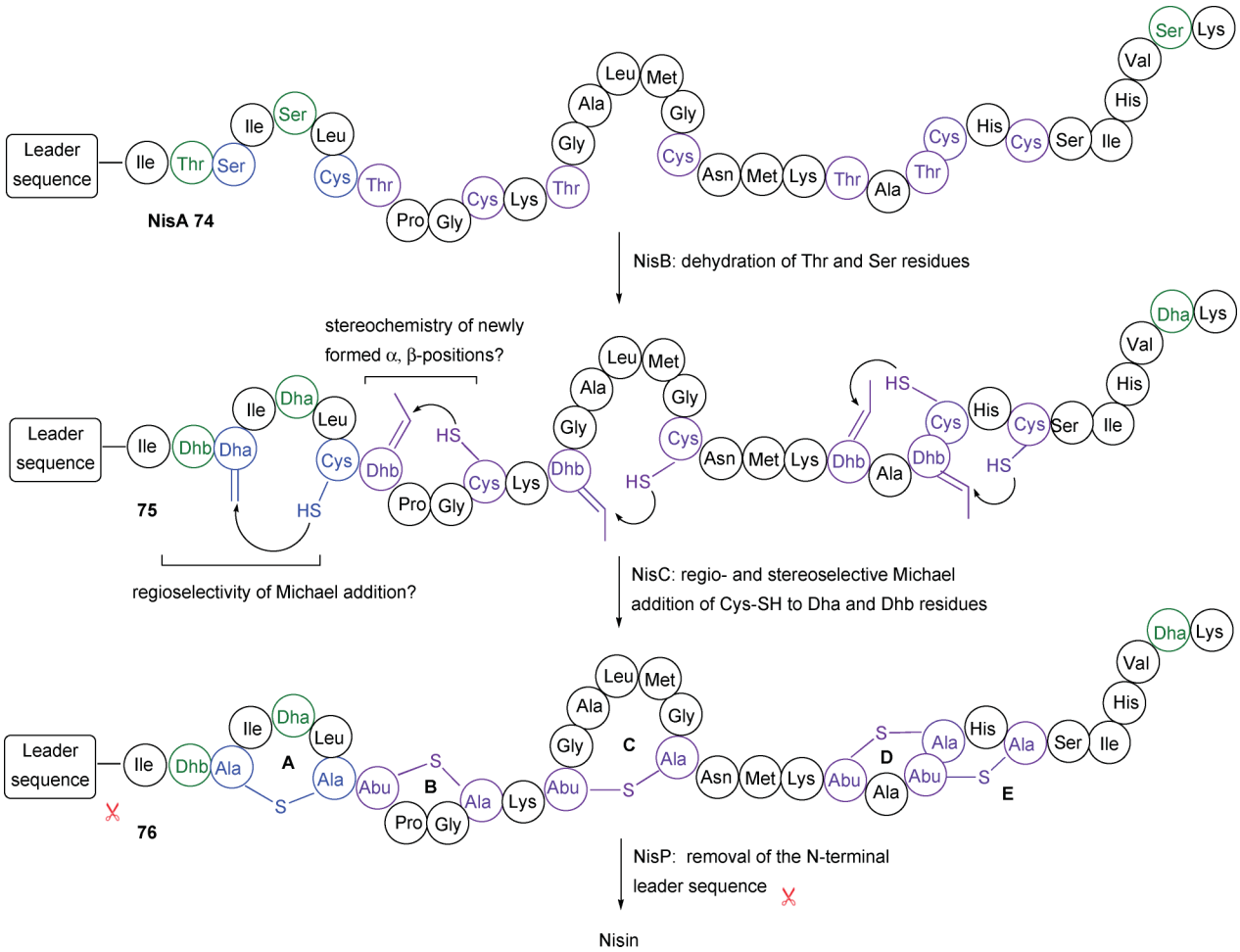


Scheme 23 Double desulfurisation to give rings D and E of nisin.

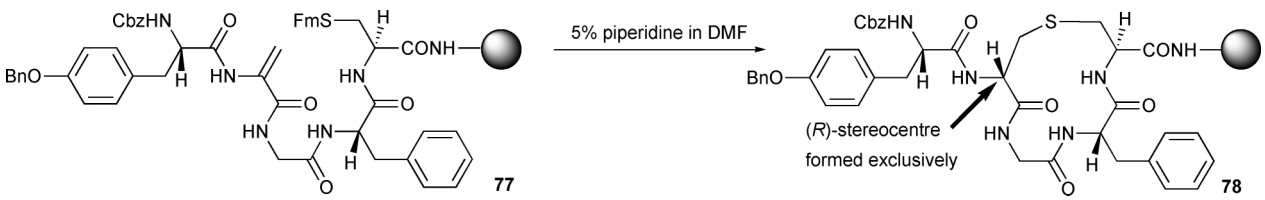
in the presence of *S*-trityl protected cysteine residues. Thus, for the synthesis of ring B of nisin,[50] linear resin-bound peptide **81** was transformed to give **82**, whilst the other, *S*-trityl protected cysteine residue remained intact. Subsequent resin cleavage and trityl deprotection with TFA gave the linear precursor **83**, which was cyclised with mild base to give the desired cyclic peptide **84** (Scheme 27).

Van der Donk *et al.* have also studied this biomimetic cyclisation. The unnatural amino acid (Se)-phenylselenocysteine (Sec(Ph)) was first synthesised as a suitably protected derivative, (*R*)-Fmoc-Sec(Ph) and used in the solid-phase synthesis of linear peptides which also contained a Cys(Trt) residue. Chemoselective oxidation/elimination of the Sec(Ph) residue with $NaIO_4$ then afforded the required Dha residue. Subsequent deprotection of the Cys(Trt) residue, followed by treatment with base, gave lanthionine-bridged peptides.[51]

This approach was then extended to give ring B of subtilin, with the bridge formed from a methyl lanthionine residue, as in the naturally occurring peptide (Scheme 28).[52] The key linear peptide was prepared from **85**, with the *Z*-Dhb residue formed *via* chemoselective oxidation of 3-methyl-(Se)-phenylselenocysteine, followed by elimination. Subsequent disulfide cleavage revealed **86**, which underwent cyclisation at pH 8 to give a single diastereoisomer of the peptide **88** in 30% yield. This was identified as having the



Scheme 24 Posttranslational modifications of NisA to give nisin.



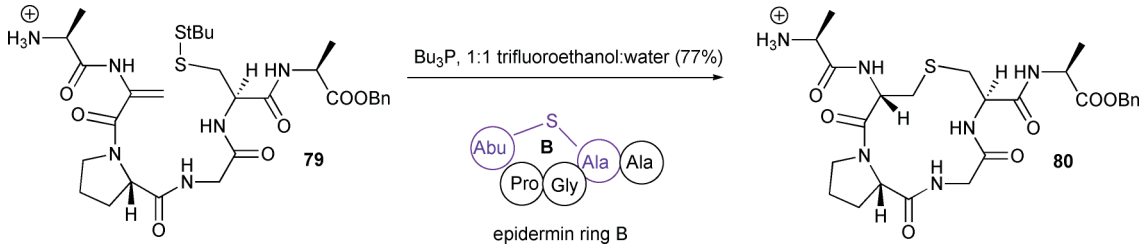
Scheme 25 Goodman *et al.* biomimetic synthesis of a lanthionine-bridged enkephalin analogue.

naturally occurring stereochemistry of the B ring of subtilin. The extra steric hindrance from the methyl group on the Dhb residue resulted in a considerably slower rate of reaction.

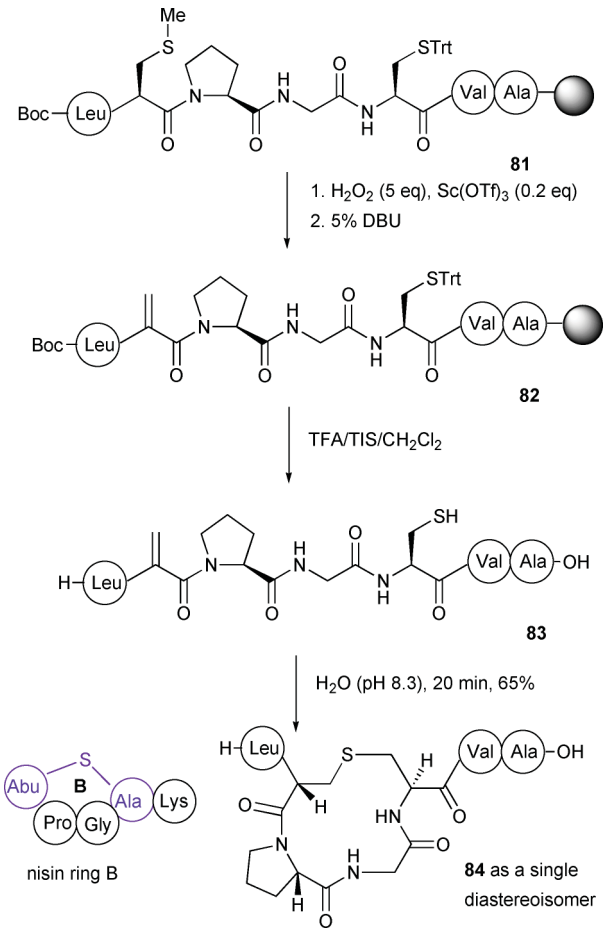
The directionality of the Michael addition is crucial. When the positions of the thiol and the Michael acceptor were reversed (Scheme 29) the cyclisation of a methyl cysteine side-chain to a Dha residue in peptide **89** gave **88** as a 1 : 1 mixture of diastereoisomers. This was attributed to non-selective protonation of the intermediate exocyclic enolate **90**, compared with the selective protonation of the intermediate endocyclic enolate **87** (Scheme 28).

Perhaps unsurprisingly, the excellent diastereoselectivities and regioselectivities observed for these relatively small cyclic peptides are not replicated for larger or more complicated systems. Bradley *et al.* investigated the cyclisation of the precursor peptide **91** to give ring A of subtilin.[49] In order to synthesise ring A from this precursor, the Cys-SH must distinguish between two Dha residues at positions 2 and 4 (Scheme 30). In this reaction, cyclisation took place exclusively at position 2, leading to the regioselective formation of ring A of subtilin **92** with the correct topology. However, the newly formed chiral centre was formed as a mixture of diastereoisomers, in a 3 : 1 ratio.

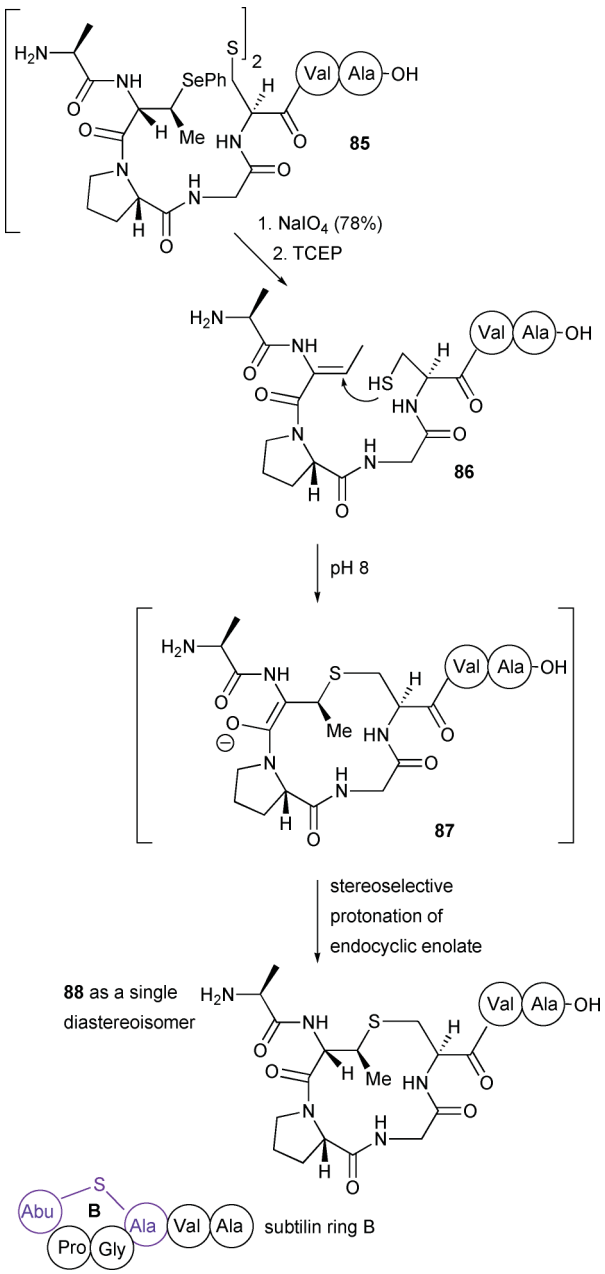
So far, only one attempt has been made to synthesise two lanthionine rings simultaneously *via* a biomimetic route. To establish whether the N-terminus of nisin, rings A and B, could be formed in this way, van der Donk *et al.* prepared precursor peptide **93** using selective oxidation/elimination of Sec(Ph) and MeSec(Ph) to give the required Dha and Dhb residues (Scheme 31).[53] Cleavage of the Cys-S–S-Cys bond with TCEP revealed two Cys-SH nucleophiles and four dehydro residues. For ring B of nisin to be formed



Scheme 26 Biomimetic synthesis of ring B of epidermin.



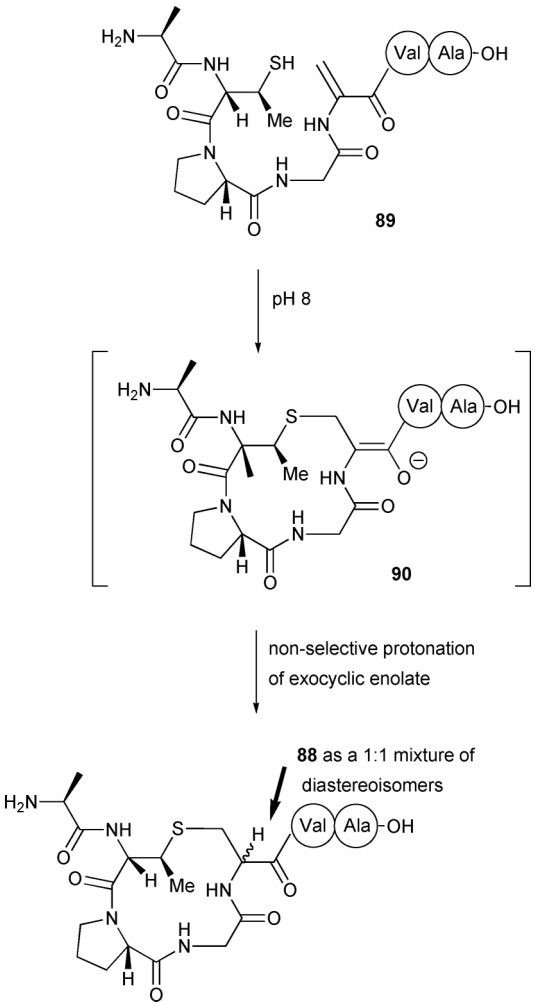
Scheme 27 Bradley *et al.* synthesis of nisin ring B.



Scheme 28 Biomimetic cyclisation to give subtilin ring B.

regioselectively, Cys11 would have to react with Dhb8, and for ring A to form, Cys7 and Dha3 would have to react. In fact, the alternative ring structure of **94** is formed instead, in which the Cys-SH have exclusively added to the more reactive Dha residues. (The stereochemistry at the newly formed chiral centres was not determined.) Clearly, for complex polycyclic lantibiotic structures, the cyclase enzymes are vitally important for controlling the regio- and stereoselectivities of the thia-Michael addition reactions. In the absence of such enzymatic control, the faster cyclisation rate for Dha residues appears to determine the regioselectivity of cyclisation reactions where two or more pathways are possible.

Finally, when linear peptide sequences which do not correspond to known lantibiotic sequences are cyclised, there appears to be no conformational bias favouring one diastereoisomer rather than another. Galante and Spatola[54] have studied the transformation of cyclic peptides with (*i*, *i* + 3) cystine bridges to lanthionine-bridged peptides *via* a base-assisted reaction in water. They determined that this reaction proceeded *via* an initial elimination to give a Dha residue, followed by thia-Michael addition. Regardless of the



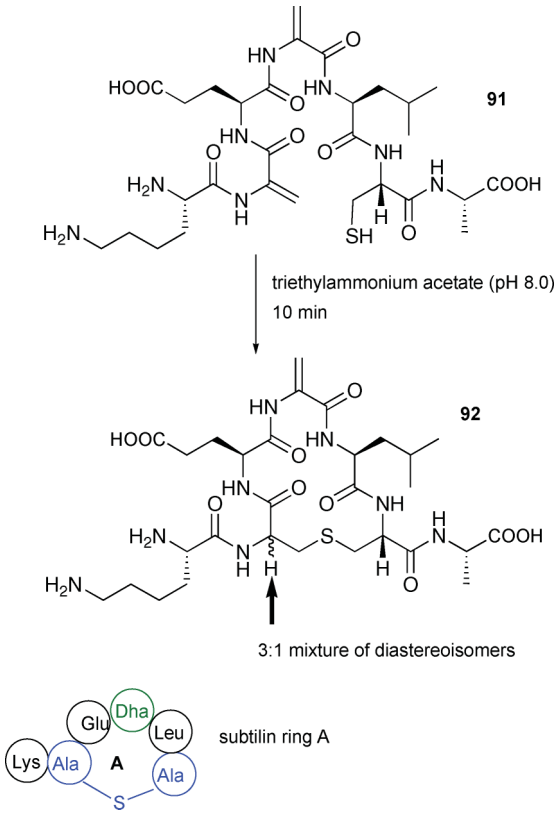
Scheme 29 Diastereoselectivity of the Michael reaction is controlled by the directionality of cyclisation.

underlying peptide sequence, mixtures of diastereoisomers were always formed in this reaction; however, the ease of cyclisation did depend on the degree of pre-organisation of the linear peptide, with sequences containing D-amino acids cyclising most readily. However, when a lanthionine-bridged dipeptide was formed using similar chemistry, good stereoselectivities were observed.[55]

Biotransformation approach to the synthesis of lanthionine-bridged peptides

In parallel with the development of synthetic routes to lantibiotics, a number of groups have used protein engineering approaches to generate mutant lantibiotics in whole cells. A detailed discussion of these strategies is outside the scope of this review, and the reader is referred to several in-depth articles which have recently been published in this area.[10,11,56–59]

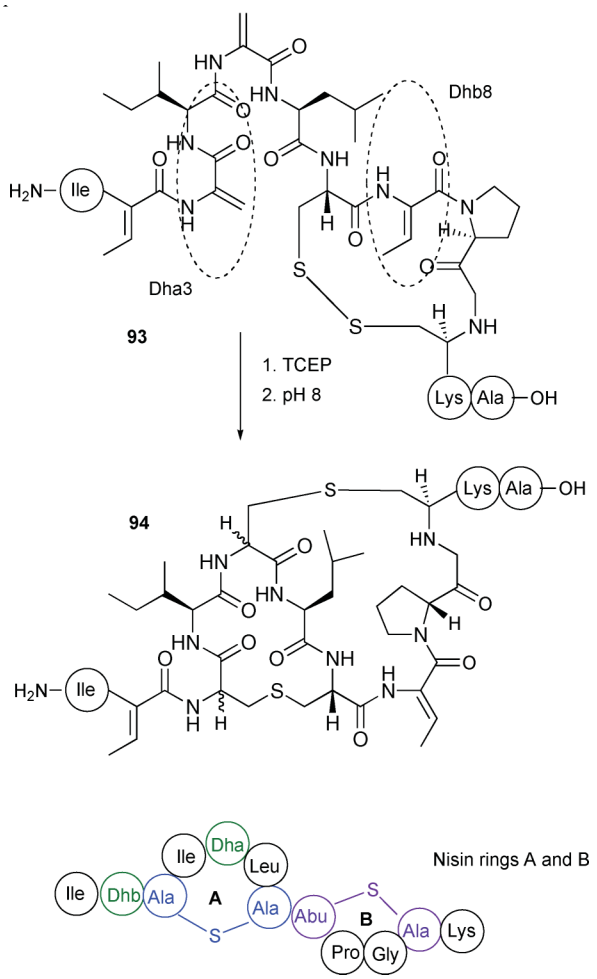
A biotransformation approach, in which the enzymes responsible for the dehydration and/or cyclisation steps in lantibiotic biosynthesis are isolated and used to generate lanthionine bridges in non-natural peptide substrates would be a very powerful way to generate novel lantibiotics and other conformationally constrained biologically active peptides. In order for this strategy to be successful, two key questions must be addressed: whether



Scheme 30 Biomimetic approach to subtilin ring C.

the processing enzymes will tolerate a range of peptide substrates; and what features of the peptide substrates are required for successful dehydration and cyclisation. Van der Donk *et al.* have carried out extensive studies on the biosynthesis of lacticin 481.[60] This class II lantibiotic uses a single modification enzyme, LctM, to dehydrate Ser and Thr residues and carry out the subsequent cyclisation reaction. This group has demonstrated that the N-terminal leader sequence of the substrate peptide is not absolutely required for processing by LctM; however, it is necessary for the correct directional processivity of lacticin 481-like peptides, and it enhances the dehydration activity of the enzyme.[61] It was then demonstrated that mutants of the precursor peptide, LctA, in which the leader sequence remained unaltered and the substrate sequence was altered with single point mutations, truncated analogues and unrelated sequences, could in general be successfully dehydrated by LctM, with thioether rings being formed where appropriately positioned Cys residues were available.[62] This showed that LctM has a fairly broad substrate specificity, with the requirements that the leader sequence should be present and the Ser or Thr residues should be at a minimum distance from the leader sequence.

With these results in hand, van der Donk *et al.* then investigated the processing of semisynthetic peptides with non-native amino acids and analogues in the substrate sequence. These were prepared in two ways: by using intein technology to express the leader sequence as the C-terminal thioester, followed by native chemical ligation to the substrate sequence;[62–64] or by preparing the leader sequence as the C-terminal but-3-ynylamide and using click chemistry to ligate this to an azide-modified substrate sequence[65–67] (Fig. 2). In this way they were able to demonstrate that LctM



Scheme 31 Attempted biomimetic synthesis of rings A and B of nisin.

can tolerate a wide range of thiol substrates. Ring formation was observed when D-Cys, L- or D-homocysteine, or β^3(*R*)- or (*S*)-homocysteine were substituted for Cys in the substrate sequence,[62,63] although (2*R*,3*R*)-methyl cysteine was not accepted as a substrate.[63] The enzyme was less tolerant of variants in residues to be dehydrated; sequences where allo-Thr or longer alkyl analogues of Thr were substituted for Ser residues were not accepted as substrates.[64]

LctM also displays an impressive ability to accept sequences containing non-proteinogenic amino acids as substrates, allowing a wide range of lacticin analogues to be prepared by *in vitro* mutasynthesis (IVM). Unnatural α-amino acids such as propargyl alanine, norvaline (Nva), norleucine (Nle), 4-cyano-2-aminobutyric acid (Cba), aminocyclopropanoic acid (Acpc) and naphthylalanine (Nal), and β-amino acids such as β-Ala and β^3-Arg, are all tolerated at a range of positions in the substrate sequence.[65,66] At certain points in the sequence, D-amino acids and *N*-methyl amino acids (Sar, *N*-Nle) were also accepted, but not when these residues were in close proximity to the Ser or Thr residues that are dehydrated prior to thioether bridge formation.[65,66] Following processing of each peptide with LctM, it was then possible to remove the leader sequence (and the 1,2,3-triazole linker, as appropriate) by cleavage with the endoproteinase LysC[67] or trypsin[67] (Fig. 2).

Finally, van der Donk *et al.* were able to demonstrate that this versatile enzyme could be used for the synthesis of a wide range of unrelated bioactive peptides with thioether bridges as conformational constraints. Thioether bridged analogues of enkephalin (Scheme 32) and contryphan were prepared *via* cyclisation of semisynthetic leader-substrate peptides with LctM, followed by enzymatic removal of the leader sequence.[67]

A second example of the use of a LanM enzyme for biotransformation of unnatural substrates was recently reported by Sonomoto *et al.* The modification enzyme, NukM, which carries out the dehydration and cyclisation steps in the biosynthesis of the class II lantibiotic nukasin ISK-1 was co-expressed in *E. coli* with a His-tagged nukasin ISK-1 prepeptide, NukA.[68] Different sequence variants of NukA were then co-expressed and processed with NukM, giving variant nukasin ISK-1 peptides, including one in which an extra thioether bridge had been introduced.[69]

The biotransformation of unnatural substrates to produce novel thioether-bridged peptides can also be carried out by class I biosynthetic enzymes. Moll *et al.* have comprehensively studied the nisin biosynthesis pathway with a view to using the nisin processing enzymes for biotransformations. Initial comparison *in silico* of all known lantibiotic sequences did not reveal any strongly preferred sequence motifs, although the sequences flanking the Ser and thr residues that are dehydrated are more frequently hydrophobic, non-aromatic amino acids.[70] Moreover, within the nisin prepeptide itself there seems to be no strong preference for particular flanking sequences, indicating that the dehydratase enzyme, NisB, does not have a strict substrate specificity. This group demonstrated that NisB could dehydrate nonlantibiotic sequences, such as enkephalin, angiotensin and vasopressin, if they were fused to the leader peptide sequence, and that the resulting dehydropeptides could be transported out of the cell by NisT.[71] When the leader-substrate fusion sequences were co-expressed with a plasmid containing the *nisBTC* genes, cyclisation of Cys residues to the dehydroalanine residues was also observed.[72] More detailed studies, comparing peptides processed by cells containing *nisBTC* with cells containing *nisBT*, showed that cyclisation will take place spontaneously in substrate peptides containing dehydroalanine residues without NisC being necessary.[73] This approach was recently used to synthesise a thioether-bridged analogue of angiotensin(1–7) (Scheme 33).[74] In this work, a Factor Xa cleavage site sequence was also added to the substrate peptide, enabling the angiotensin analogue to be released by trypsin digestion. In these studies, the stereochemistry at the newly formed C^{α} position was not determined, but was assumed to be the (*S*)-configuration as in the naturally occurring lantibiotics. Whilst synthetic studies suggest that this is a reasonable assumption for sequences that are similar to those seen in the naturally occurring lantibiotics,[49,50] as discussed above, spontaneous cyclisation of lantibiotic-like peptides,[49] and unrelated sequences,[54] does not always generate a single diastereoisomer.

However, when substrate peptides containing dehydrobutyrine residues were generated, NisC was required for cyclisation, reflecting the lower reactivity of dehydrobutyrine also observed in synthetic studies.[52,53] Whilst certain sequences (*e.g.* leader sequence-Ala-Dhb-Val-Trp-Cys-Glu) were not recognised as substrates by NisC and could not be cyclised, when appropriate substrate sequences were designed, NisC was able to form novel peptides

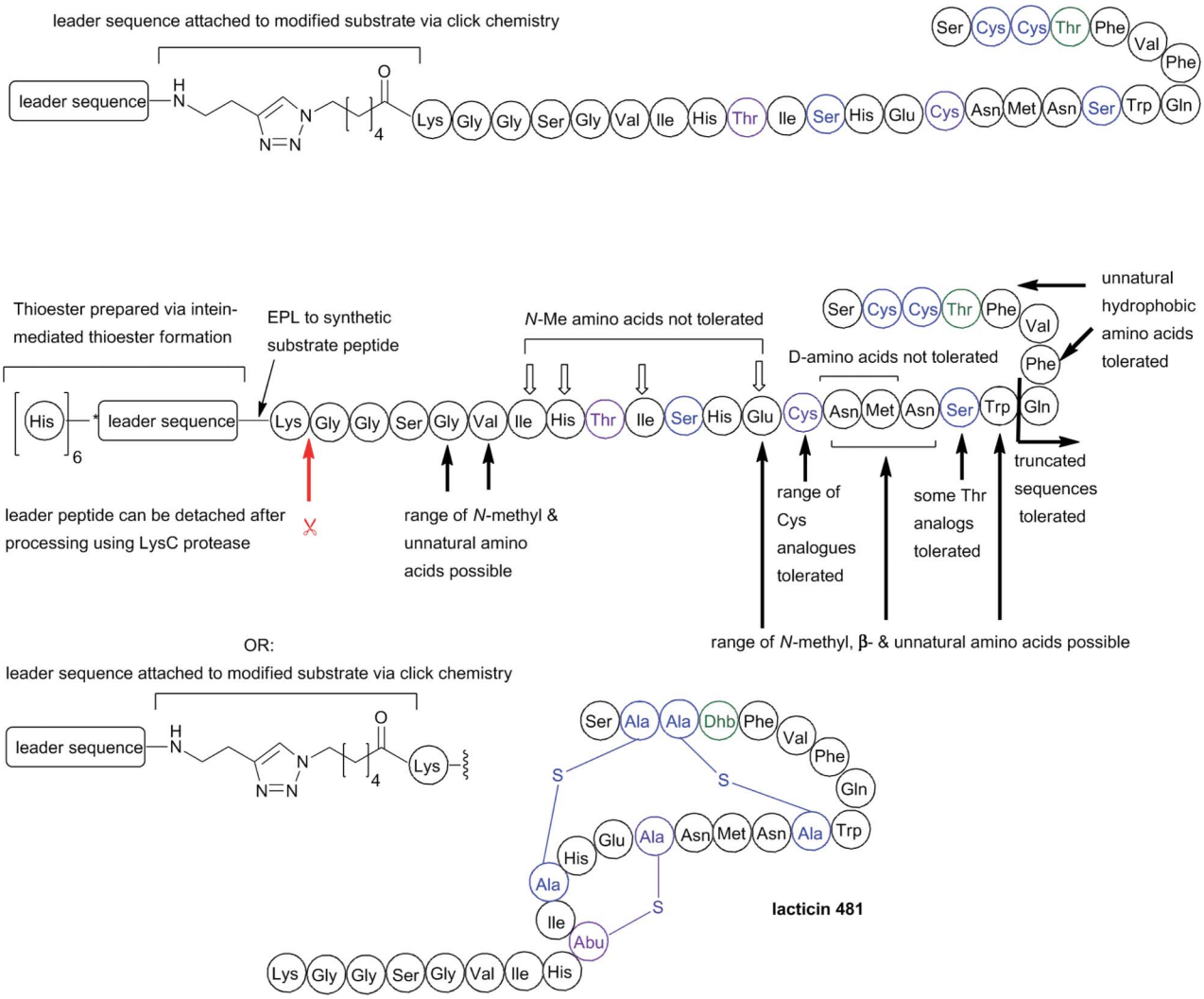
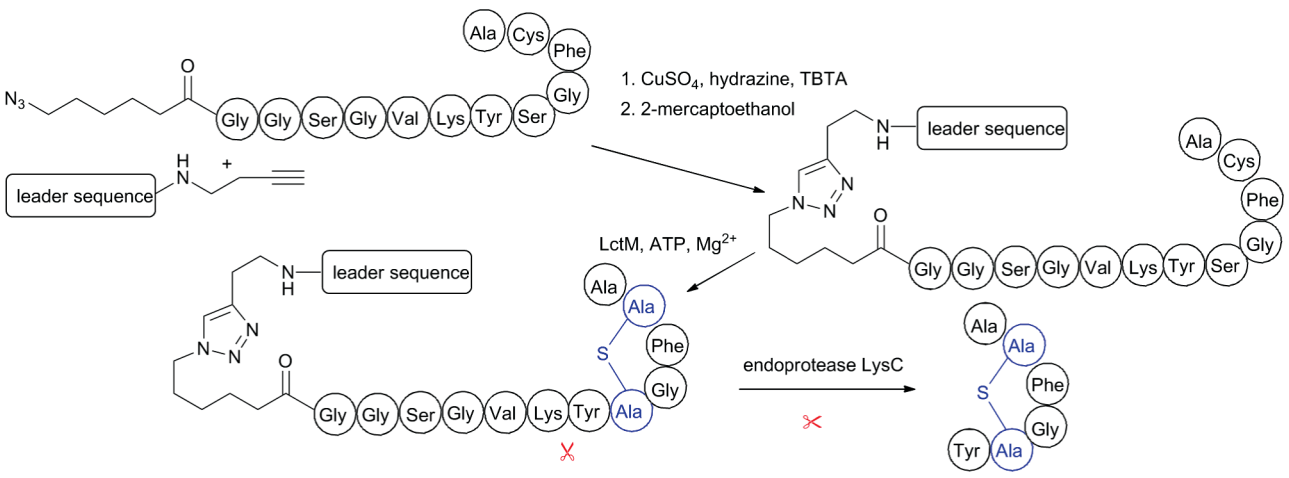
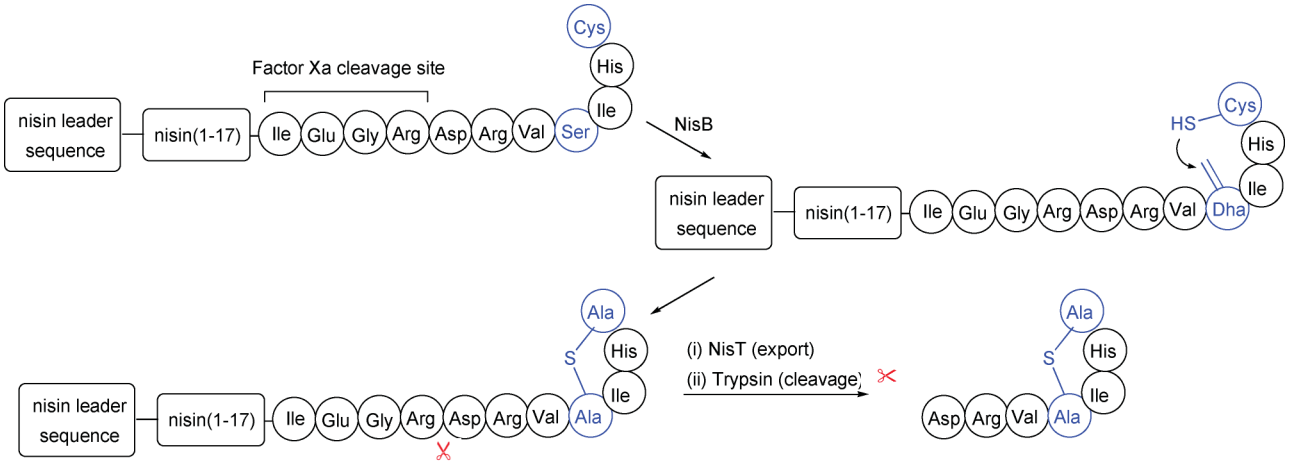


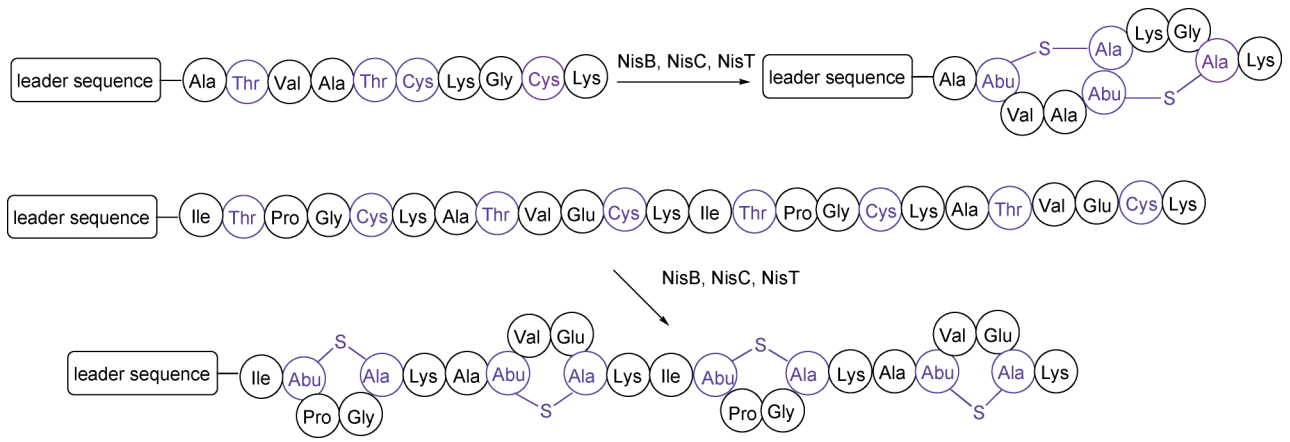
Fig. 2 Summary of amino acid substitutions in semisynthetic LctA peptides, prepared by EPL, that are accepted by LctM as substrates.



Scheme 32 Van der Donk *et al.* synthesis of an enkephalin analogue *via* biotransformation with LctM.



Scheme 33 Synthesis of a thioether bridged analogue of angiotensin(1–7) *via* biotransformation with NisB.



Scheme 34 NisC can catalyse cyclisation of designed nonlantibiotic peptides.

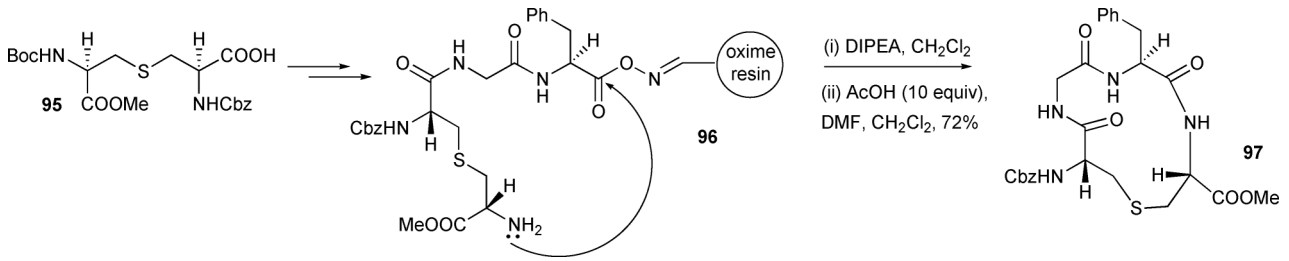
with overlapping thioether bridges, and multiple thioether bridges (Scheme 34).[73]

Kuipers *et al.* have recently extended this work further, using the nisin biosynthesis enzymes NisB, NisC and NisT to produce an unrelated two-component lantibiotic, pneumococcin A1 (PneA1) and pneumococcin A2 (PneA2).[75] Plasmids encoding for two fusion peptides, one with the nisin leader sequence fused to the PneA1 sequence, and one with the nisin leader sequence fused to the PneA2 sequence, were constructed, and the peptides co-expressed with a plasmid containing the *nisBTC* genes. Despite the fact that the pneumococcins are almost certainly class II lantibiotics, and would therefore normally have a different leader peptide sequence and be processed by a LanM enzyme, both peptides were processed and secreted, with two thioether bridges being formed in each peptide. The leader sequences were subsequently removed by trypsin or leucine aminopeptidase treatment, giving mixtures of peptides with significant antibacterial activity. Although analysis of the peptide mixtures indicated that post-translational modification was not complete for all peptides, these results strongly suggest that the lantibiotic processing enzymes will be powerful tools for generating unusual antimicrobial agents. Three reviews have recently appeared in which the biotransformation approach to antimicrobial peptides is discussed in depth.[76,77,78]

A different biotransformational approach to the synthesis of methyl lanthionine-containing peptides has recently been disclosed by Suga *et al.*[79] Reprogramming of the genetic code was first undertaken in order to introduce vinylglycine (Vgl) residues into peptide sequences, replacing the Thr residues that would be converted to Dhb residues during lanthionine bridge biosynthesis. After ribosomal synthesis and isolation of the Vgl-containing peptides, thermal isomerisation of the double bond converted the Vgl residues to Dhb, with spontaneous cyclisation of Cys-SH to form the desired lanthionine bridge. This approach was used to synthesise ring B, and ring C, of nisin. The stereochemistry at the newly formed C^{α} position was not determined; for the ring B analogue it was assumed that the naturally occurring diastereoisomer was formed, in line with previous studies.[52]

Solid-phase peptide synthesis using orthogonally protected bis-amino acids

Using a solid-phase peptide synthesis approach to access the lantibiotics and synthetic analogues would be complementary to the approaches already described. There would also be several possible advantages to such a strategy, including the ability to incorporate unusual amino acids which might not be accepted by the



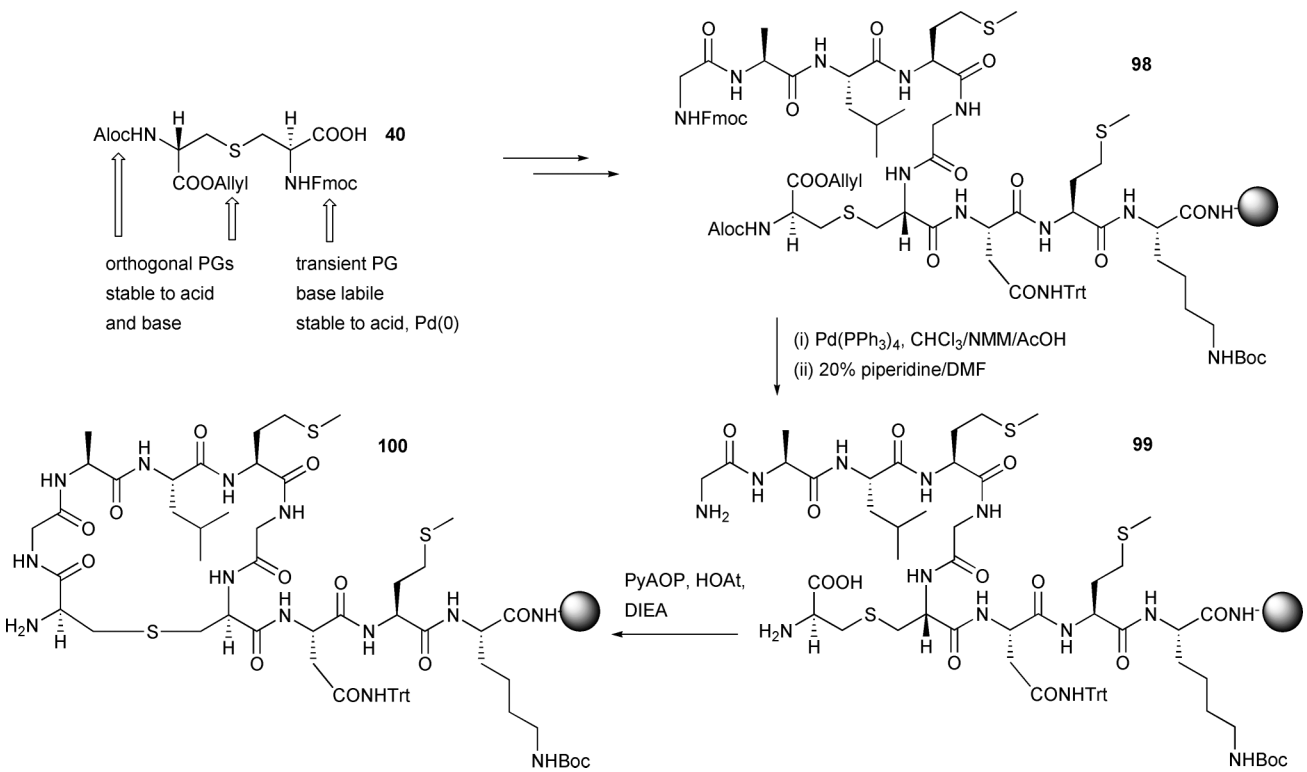
Scheme 35 Lanthionine-containing peptides *via* PCOR.

biosynthetic enzymes, whilst avoiding the issues associated with segment synthesis.[80] If pre-formed lanthionine building blocks with appropriate protecting groups are used, the problems of regio- and stereo-selectivity encountered in the biomimetic approach can also be avoided. The advantages of using differentially protected lanthionine residues had previously been demonstrated by Zervas *et al.*[81] and Goodman *et al.*[82] in solution-phase syntheses of thioether-bridged peptides.

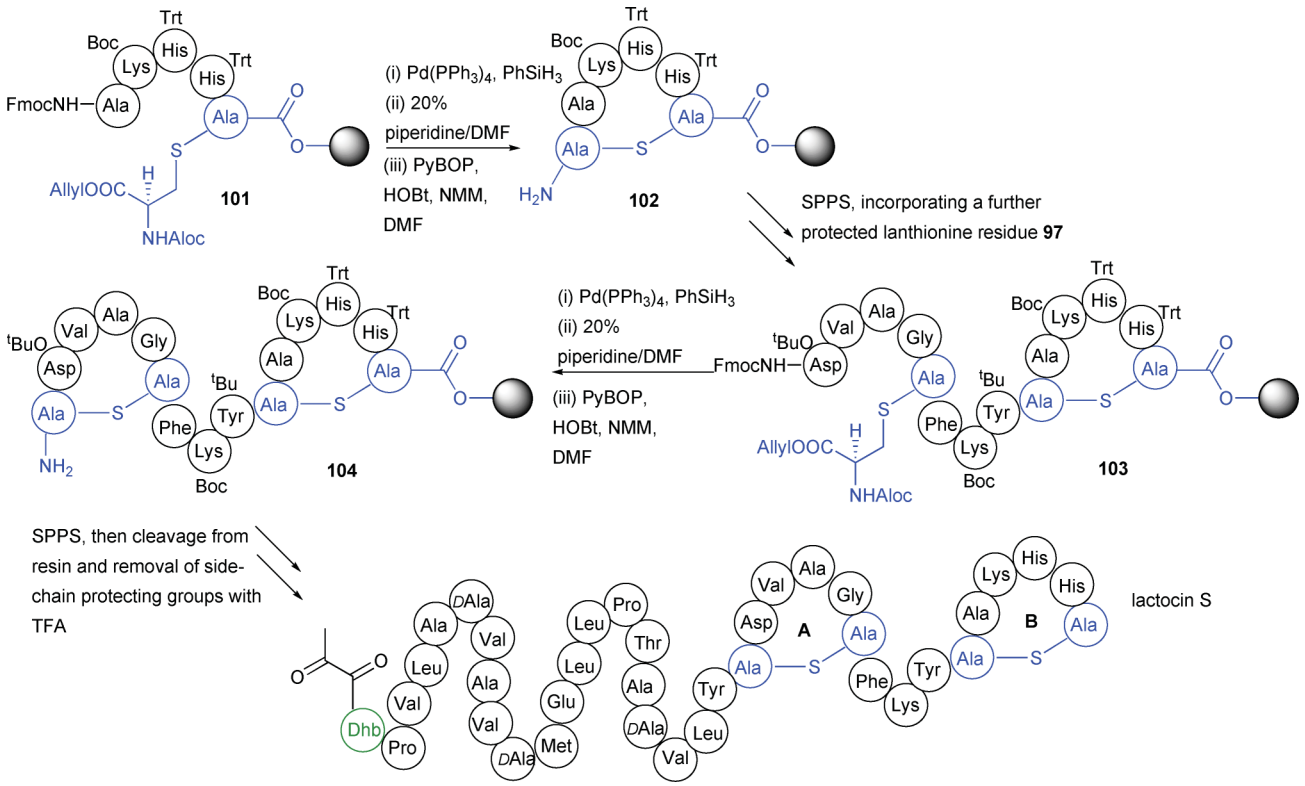
The first successful approach to the solid-phase synthesis of lanthionine-containing peptides was developed by Ösapay and Goodman, as an extension of their Peptide Cyclisation on an Oxime Resin (PCOR) method for the synthesis of cyclic peptides. Orthogonally protected lanthionine **95**, in which the two $-NH_2$ and –COOH groups are differentiated, was prepared from serine β-lactone as discussed above. This was then incorporated into a linear precursor peptide, on an oxime resin, using standard Boc chemistry (Scheme 35).[83] After removal of the N-terminal Boc group to give **96**, neutralisation with DIPEA is then followed by simultaneous peptide cyclisation and acetic acid-catalysed cleavage from the oxime resin to give the desired cyclic peptide **97**.

Several analogues of biologically active peptides were prepared using this approach, including analogues of the enkephalins[83] and somatostatin,[17,83] and also model conformationally constrained peptides with lanthionine[84] and β,β-dimethylcyclolanthionine[85] residues.

Whilst the PCOR approach is powerful, regio- and stereoselective, and gives high yields of lanthionine-bridged peptides, it can only be used to prepare cyclic peptides with a single lanthionine bridge. Moreover, the process of cyclising the peptide results in cleavage from the resin, and if extension of the peptide at the N-terminus was required, this would therefore have to be carried out subsequently in solution. We have developed methodology using a different combination of orthogonal protecting groups in which this problem is circumvented. Lanthionine derivative **40** (Scheme 13) also has the two $-NH_2$ and –COOH groups differentiated, with the amino group of one of the amino acid moieties Fmoc-protected, and the other amino acid moiety protected with allyl and Aloc groups. This can also be incorporated into a linear peptide precursor such as **98** (Scheme 36) using conventional Fmoc-based solid-phase synthesis. In order to form



Scheme 36 Synthesis of an analogue of nisin ring C on-resin.



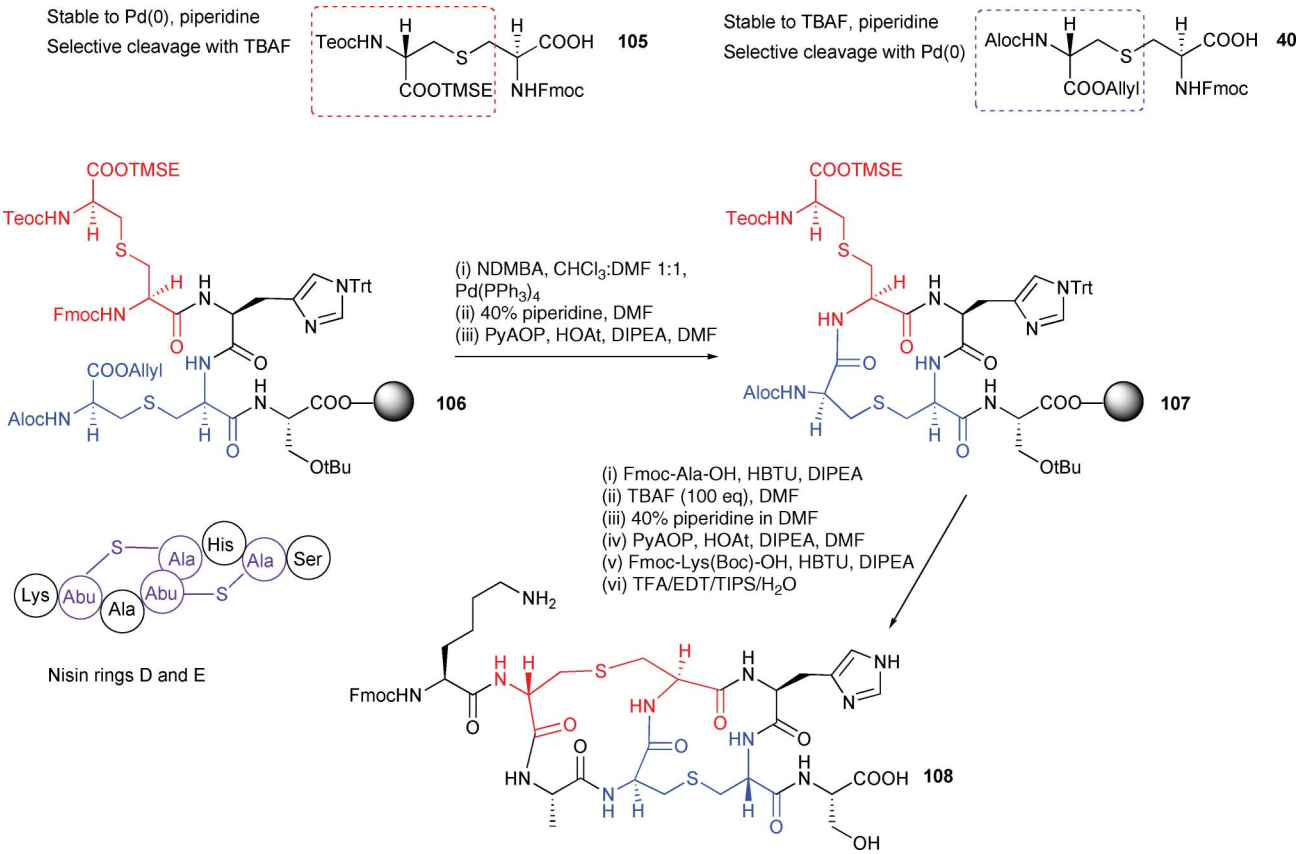
Scheme 37 Vederas *et al.* synthesis of lactocin S on-resin.

the lanthionine bridge, chemoselective deprotection of the allyl and Aloc groups, followed by removal of the Fmoc group, gives precursor **99**. This is then cyclised on-resin to give **100**, in which the lanthionine bridge has been installed with complete regio- and stereo-selectivity. We have used this approach to synthesise a lanthionine-bridged analogue of ring C of nisin[22] and also a nor-lanthionine-bridged analogue of ring C of nisin.[86]

One crucial advantage of this strategy is that the cyclisation step results in a resin-bound peptide, such as **100**, with a free N-terminus for subsequent chain extension. This in turn allows the solid-phase synthesis of lantibiotic structures with several lanthionine rings installed sequentially. Vederas *et al.* have used this approach for the on-resin synthesis of an analogue of lacticin 3147 A2[87] (containing three lanthionine bridges in sequence) and, most recently, in the first solid-phase synthesis of the naturally occurring sequence of a lantibiotic, lactocin S (Scheme 37).[88]

In this latter synthesis, lanthionine derivative **40**, synthesised *via* the approach of Zhu and Schmidt,[23] was attached directly onto 2-chlorotrityl chloride polystyrene resin, and linear peptide precursor **101** synthesised *via* standard Fmoc SPPS. Selective removal of the allyl and Aloc groups, followed by removal of the Fmoc group and on-resin cyclisation with PyBOP gave ring A of lactocin S (**102**). Chain extension at the resulting $-NH_2$ by standard Fmoc SPPS, including a second protected lanthionine **40**, gave **103**. A second sequence of selective removal of the allyl and Aloc groups, removal of the Fmoc group and on-resin cyclisation with PyBOP resulted in the formation of ring B of lactocin S (**104**). Further SPPS, cleavage from the resin and deprotection of the (acid-labile) protecting groups on the side-chains of the other amino acids gave the required lactocin S, in an excellent yield of 10% over 71 steps.

Many lantibiotics, such as nisin, actagardine, lacticin 481 and cinnamycin, have two or more lanthionine bridges which overlap. These structures can also be tackled *via* SPPS, if a suitably protected lanthionine with a different set of orthogonal protecting groups is available. We have recently synthesised the protected lanthionine **105**,[89] with the amino group of one of the amino acid moieties Fmoc-protected, and the other amino acid moiety protected with trimethylsilylethyl (TMSE) and Teoc groups. These silyl protecting groups may be removed under neutral conditions with TBAF, but should be stable to Pd(0) and mild base; hence they are mutually orthogonal to the protecting groups used on lanthionine derivative **40**, as well as to the transient (Fmoc) protecting group used in linear SPPS. Incorporation of both **40** and **105** into a linear peptide would enable the (Teoc, TMSE) and (Aloc, allyl) protecting groups to be chemoselectively removed at different points in the synthesis, allowing peptides with overlapping lanthionine bridges to be prepared (Scheme 38). We have successfully used **40** and **105** in the solid-phase synthesis of an analogue of the overlapping rings D and E of nisin.[89] Linear precursor **106**, which includes both derivatives, was synthesised on-resin by standard methods. Selective removal of the (Aloc, allyl) protecting groups with Pd(0), followed by Fmoc deprotection and cyclisation, delivered **107** in which ring E was formed regioselectively. Subsequent chain extension was followed by selective removal of the (Teoc, TMSE) protecting groups with TBAF; Fmoc deprotection and cyclisation to form ring D, followed by resin cleavage, then afforded **108** (Scheme 38).



Scheme 38 Quadruply-orthogonal protecting group strategy for the on-resin synthesis of overlapping lanthionine rings.

Carba-, oxa- and cystathionine analogues

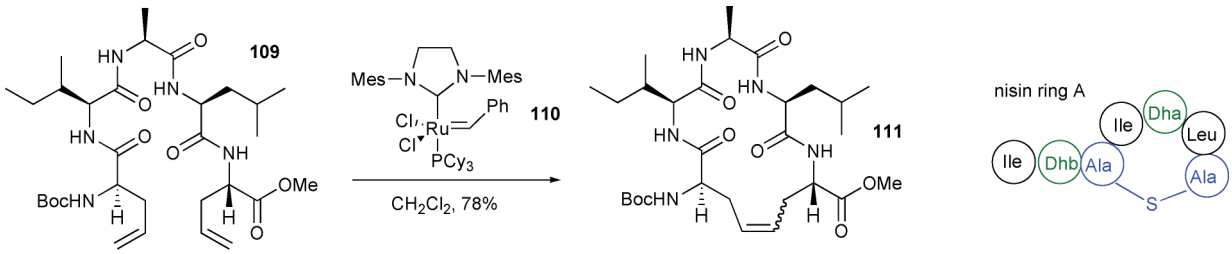
One clear advantage of using a synthetic approach to the lantibiotics is the possibility of preparing analogues with some or all of the lanthionine bridges replaced with other, unnatural, side-chain cross-links. Such cross-links may have additional metabolic stability, compared to the lanthionine linkage which is prone to oxidation, and may also enable the three-dimensional structure of the lantibiotic rings to be fine-tuned. Carbocyclic linkages, prepared by ring-closing metathesis (RCM) of amino acids with unsaturated side-chains, have been explored as conformational constraints for many biologically active peptides.

In particular, alkene-bridged cyclic peptides have been previously demonstrated to be good mimics of Cys-Cys disulfide bridges in other cyclic peptides.[90] Replacement of the lanthionine bridge by such a carbocyclic bridge would be of interest, as this would result in a ring-expanded analogue (with one more atom in the ring) with slightly different conformational preferences.

Liskamp *et al.* have extensively explored the replacement of lanthionine bridges in nisin with alkene and other carbocyclic linkages, using a RCM approach. For example, the linear precursor **109**, incorporating two allyl glycine (Alg) residues, was synthesised and then cyclised using the second-generation Grubbs catalyst **110** to give the alkene-bridged peptide **111**, an analogue of ring A of nisin (Scheme 39).[91] This approach was used to give analogues of ring B (**112**) and ring C, in all cases as mixtures of *cis*- and *trans*-alkenes (Fig. 3).[91] Using a similar approach, ring-closing alkyne metathesis (RCAM) on linear peptides incorporating (*S*)-2-amino-4-hexynoic acid (Bug) residues we used to prepare alkyne analogues of ring C (**113**), ring A and ring B.[92] Coupling of these fragments was used to prepare analogues of rings A and B, and also analogues of rings A, B and C, such as **114**, in which the lanthionine and methyl lanthionine bridges of the wild-type sequence are all replaced by alkene bridges (Fig. 3).[93]

The complete set of alkene and alkyne-bridged analogues of rings A and B, and also analogues of rings A, B and C, were prepared, and also a set of analogues in which the alkene moieties were reduced to give alkane-bridged analogues, and the binding of these analogues to lipid II was measured. All of the carbocyclic analogues were less active than the wild-type sequences; however, the alkane-bridged analogues were, in general, the most active.[93]

Liskamp *et al.* were also able to prepare alkene-bridged analogues of the overlapping rings D and E of nisin.[94,95] A stepwise approach, in which the alkene linkage for ring E was formed first, followed by installation of the two other Alg residues and cyclisation, gave **115** in good yield. Intriguingly, when a linear precursor **116**, with four Alg groups, was treated with **110**, the predominant products were the monocyclic [1,4]-bridged peptide **117** and the monocyclic [3,5]-bridged peptide **118** (approximately 60% of the products from this reaction) (Scheme 40). A second cyclisation reaction with **110** converted both **117** and **118** to the desired **115** in 72% yield. Other monocyclic intermediates were produced in low yield, with some being unable to cyclise further. Clearly, the linear precursor peptide must be largely pre-organised in a conformation favourable for cyclisation to give the overlapping bridge connectivity observed in nisin rings D and E.



Scheme 39 Preparation of carbocyclic analogues of lantibiotics by RCM.

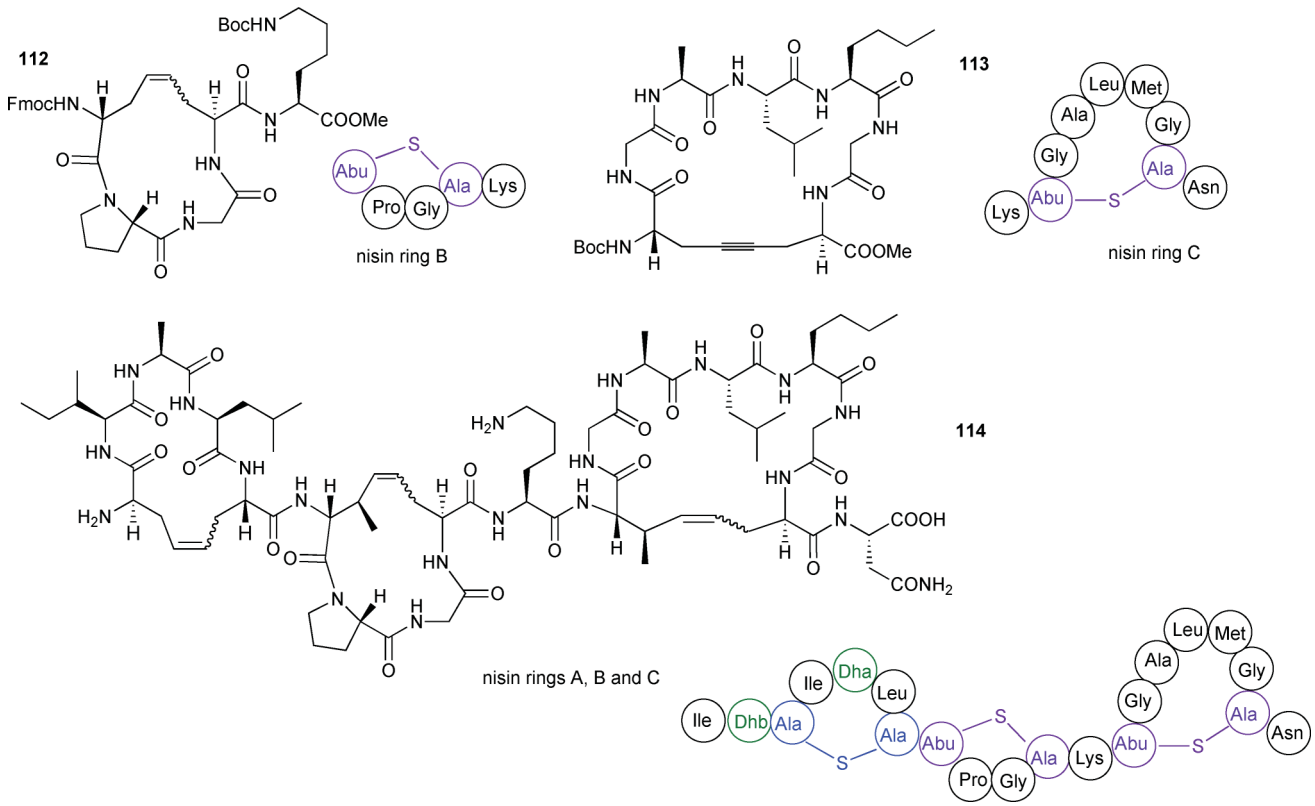


Fig. 3 Alkene- and alkyne-bridged analogues of the N-terminal sequence of nisin.

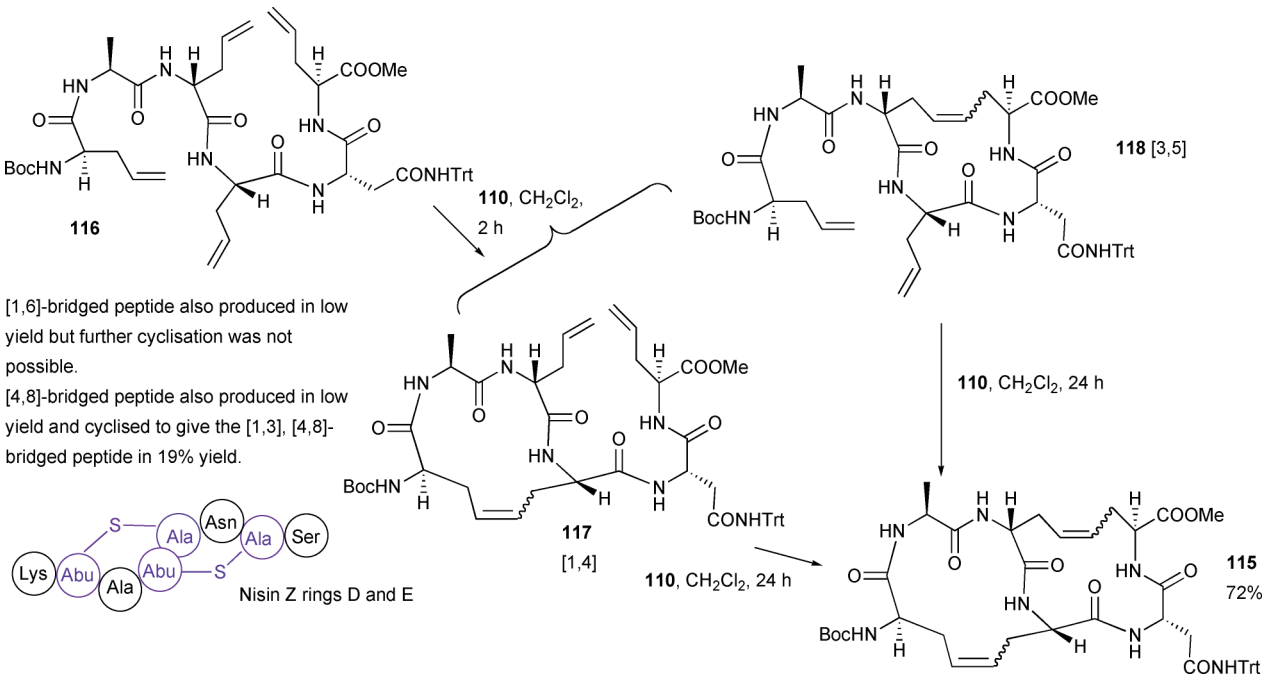
Vederas *et al.* have also used RCM to prepare alkene-bridged, ring-expanded analogues of the lacticin 3147 A2 peptide.[96] In contrast to the approach used by Liskamp *et al.*, the majority of this peptide was synthesised on-resin; the linear precursor for each ring was synthesised, cyclised with the second generation Grubbs catalyst **110**, and then further amino acids added on-resin. Residues 1–5 of lacticin 3147 A2, which contain two Dhb residues, were finally added as a single segment.

Vederas *et al.* have also used SPPS to prepare another analogue of lacticin 3147 A2, in which the sulfur atoms of the lanthionine bridges are replaced by oxygen.[97] In this work, tetra-orthogonally protected oxa-DAP, and 3-methyl oxa-DAP (**119**) analogues were first synthesised.[98] As before, these protected bis-amino acids could be incorporated into linear peptides by standard SPPS. For instance, **119** was attached directly to a low-loading Wang resin and the linear precursor **120** synthesised by standard methods. Selective removal of the allyl ester and Fmoc groups, followed by on-resin cyclisation, gave the cyclic resin-bind peptide **121** (Scheme 41). The pNZ group could then be removed under reductive conditions to allow the next 3-methyl oxa-DAP residue to be installed. Successive cycles of linear peptide synthesis, selective deprotection and cyclisation afforded oxa-lacticin 3147 A2.

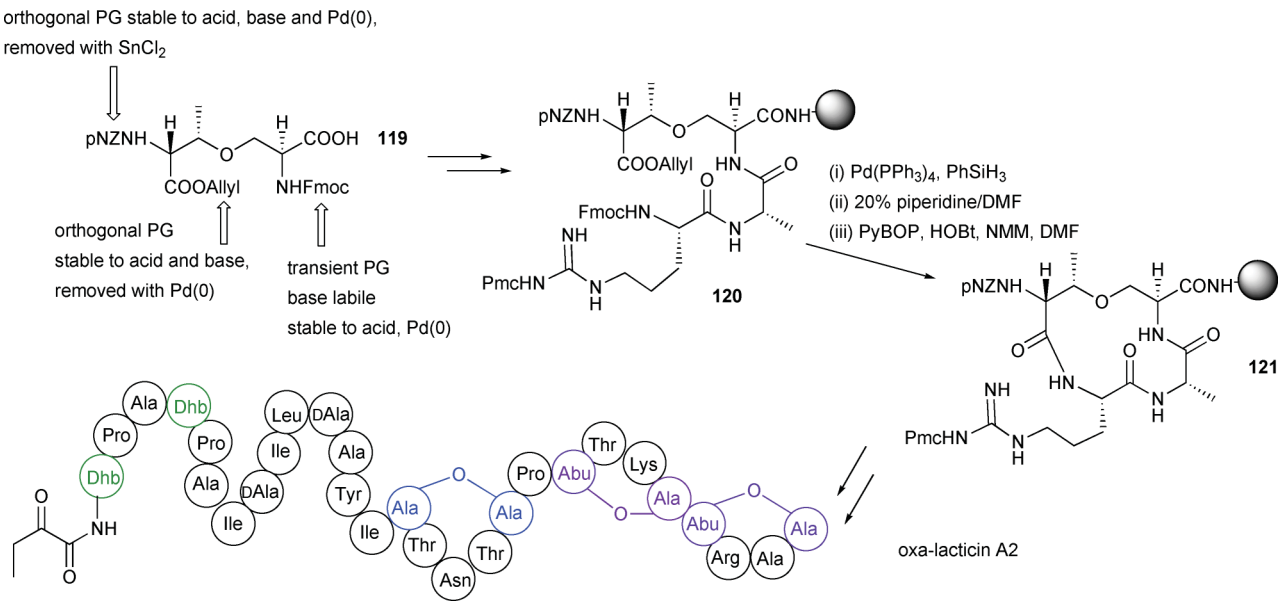
The orthogonally protected bis-amino acid approach could also be used to prepare bridged analogues with different lengths of alkane linkers,[99,100] although such analogues of lantibiotics have not yet been prepared.[101] Similarly, although a number of groups have synthesised cystathione-bridged analogues of disulfide-bridged cyclic peptides,[102–104] again this approach has not yet been applied to the lantibiotics.

Other unusual amino acids

After lanthionine and methyl lanthionine, the unusual amino acids most frequently encountered in the lantibiotics are the dehydro residues Dha and Dhb. The synthesis and conformational properties of dehydroamino acids have been the subject of much research interest, and have been recently reviewed in detail.[105,106]



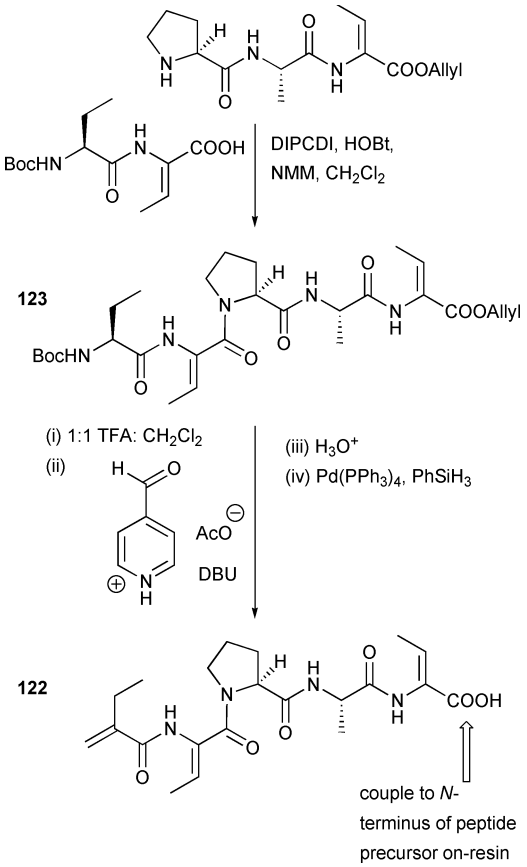
Scheme 40 Liskamp *et al.* synthesis of alkene-bridged analogues of nisin rings D and E.



Scheme 41 Synthesis of oxa-lacticin 3147 A2 on-resin using quadruply orthogonal protecting group strategy.

However, not all methods for the synthesis of dehydroamino acids are appropriate for the preparation of lantibiotics; for example, many oxidising agents will also oxidise thioethers, and strong bases will also lead to cleavage of the thioether bridge. In their total synthesis of nisin,[40,41] Shiba *et al.* installed these residues, either by treating Thr and Ser residues with EDC/CuI, as described earlier (Scheme 21), or by selective methylation of the side-chain of Dpr, followed by mild base elimination. Conversely, Bradley *et al.*, and Van der Donk *et al.*, have approached this problem by developing chemoselective oxidation methods for S-methyl cysteine[50] (Scheme 27) or Sec(Ph) (Scheme 28) residues,[51] followed again by mild base-catalysed elimination. In lactocin S and lacticin 3147 A2 the dehydro amino acids, and an α-ketoamide residue, are conveniently situated at the N-terminus of these peptides. Hence, in the total syntheses of analogues of lacticin 3147 A2[87,96,97] Vederas *et al.* were able to prepare the N-terminal segment **122** separately (Scheme 42), *via* solution-phase synthesis of **123** followed by transamination to give the α-ketoamide,[96] and couple this to the rest of the peptide on-resin. A similar approach was followed for the total synthesis of lactocin S.[88]

Few of the other unusual amino acids that are unique to lantibiotics have so far been investigated. We have recently reported the synthesis of orthogonally protected lysinoalanine,



Scheme 42 Synthesis of the N-terminal fragment of lacticin 3147 A2.

a key component of cinnamycin which forms one of the bridging constraints, although this has not yet been incorporated into cyclic peptides.[107] Synthetic strategies capable of delivering the characteristic C-terminal modifications found in epidermin, mersacidin and other lantibiotics, *S*-aminovinyl-D-cysteine (AviCys) and *S*-aminovinyl-3-methyl-D-cysteine (AviMeCys),[108] have not yet been reported.

Conclusions

In the last two decades, considerable progress has been made in addressing the many synthetic challenges presented by the lantibiotics. Synthesising lanthionine itself presents many challenges, with issues of differentiation of the two amino and carboxylic acid groups, and problems of regio- and stereo-selectivity in forming the thioether bond itself, however several reliable approaches are now available that will delivery differentially protected lanthionine.

As far as the synthesis of lantibiotics themselves are concerned, of the approaches that have so far been explored, the solid-phase peptide synthesis approach with orthogonally protected lanthionine residues, and the biotransformation approach using the biosynthetic machinery of the producing enzymes, currently hold the most promise. Both approaches are clearly capable of producing peptides with single lanthionine bridges, or with two or more in series, or overlapping, and can also be used to access analogues with unnatural amino acids. The "best" approach will be different for each target; a solid-phase peptide synthesis approach may well be best for shorter fragments, analogues with many unnatural amino acids, or for ring structures dissimilar to naturally occurring lantibiotics, whereas a biotransformation approach will give a very rapid approach to full-length lantibiotics and analogues which are similar in structure and topography.[109]

In addition to the potential benefits to exploring the synthesis of lantibiotics, and the promise that this research could lead to the development of a new generation of antibacterial agents, there is now considerable interest in synthesising thioether-bridged analogues of other biologically active peptides,[46,76,77,78,83,110,111] as well as lipopeptides containing lanthionine.[112] Many of these analogues have superior biological activities to their linear counterparts, as well as being metabolically stable, and the chemistry developed for the synthesis of lantibiotics will be very useful for tackling these cyclic peptides.

Note added in Proof

Just before publication of this perspective, Vederas *et al.* reported the chemical synthesis of both components of lacticin 3147 on the solid phase.[113] In this work, they have carried out for the first time a synthesis of the correct, naturally occurring methyl lanthionine diastereoisomer *via* regioselective ring opening of the *p*-nitrobenzyloxycarbonyl (*p*-NZ)-protected aziridine formed from D-Thr, with Fmoc-Cys-OH. This led to MeLan derivatives with orthogonal protecting groups suitable for on-resin peptide synthesis, selective deprotection and cyclisation. The overlapping MeLan bridges of the C and D rings of lacticin 3147 were installed using a quadruply orthogonal protecting group strategy, using (Aloc, allyl)/Fmoc-protected MeLan and (*p*-NZ, *p*-nitrobenzyl)/Fmoc-protected MeLan. The second of these MeLan moieties was selectively deprotected using $SnCl_2$, prior to Fmoc deprotection and cyclisation. This elegant synthesis solves many of the remaining issues with solid-phase synthesis of lantibiotics, and illustrates again the power of using orthogonally protected lanthionine moieties to access these challenging peptides.

Abbreviations

Acpc	aminocyclopropanoic acid
ADDP	1,1′-(azodicarbonyl)dipiperidine
Alg	allyl glycine
AviCys	*S*-aminovinyl-D-cysteine
AviMeCys	*S*-aminovinyl-3-methyl-D-cysteine
Bug	(*S*)-2-amino-4-hexynoic acid
Cba	4-cyano-2-aminobutyric acid
DAP	diaminopimelic acid
DEAD	diethylazodicarboxylate
Dha	dehydroalanine
Dhb	dehydrobutyrine
DIPCDI	diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
EDC	Fm, fluorenylmethyl
HOBt	1-hydroxybenzotriazole
IVM	*in vitro* mutasynthesis
MBHA	methylbenzhydryl
MeSec(Ph)	3-methyl-(Se)-phenylselenocysteine
Nle	norleucine
N-Nle	*N*-butyl glycine

NMM	*N*-methyl morpholine
Nva	norvaline
pNZ	*p*-nitrobenzyloxycarbonyl
PCOR	Peptide Cyclisation on an Oxime Resin
PyBOP	benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
RCAM	ring-closing alkyne metathesis
RCM	ring closing metathesis
Sar	sarcosine (*N*-methyl glycine)
Sec(Ph)	(Se)-phenylselenocysteine
SPPS	solid phase peptide synthesis
TBAB	tetrabutylammonium bromide
TBAHS	tetrabutylammonium hydrogensulfate
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
TCEP	tris(2-carboxyethyl)phosphine hydrochloride
Teoc	β-(trimethylsilyl)ethoxycarbonyl
TMSE	trimethylsilylethyl
Troc	2,2,2-trichloroethoxycarbonyl
Vgl	vinylglycine

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