Chemical and Enzymatic Synthesis of Lanthionines

M. Paul and W.A. van der Donk*

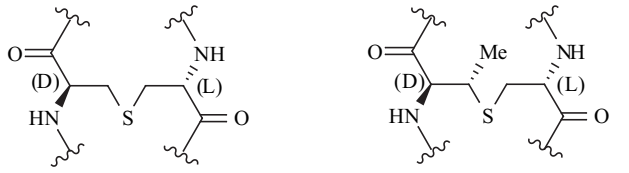
Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Ave, Urbana, Illinois 61801, USA

Abstract: Lantibiotics are peptide-derived antimicrobial agents that are ribosomally synthesized and post-translationally modified to their biologically active forms. The post-translational modifications involve the formation of dehydroalanine and dehydrobutyrine residues and the subsequent attack of cysteines within the peptide onto the dehydro amino acids. This generates the so-called lanthionine and methyllanthionine thioethers that have given lantibiotics their name. One family member, nisin, has attracted much attention recently due to its novel mechanism of action including specific binding to the bacterial cell wall precursor lipid II, followed by membrane permeabilization. Nisin has been commercially used as a food preservative, while other lantibiotics show promising activity against bacterial infections. This mini-review focuses on the recent developments in the characterization of these enzymes as well as the progress in chemical synthesis of lanthionine containing peptides.

Keywords: Lanthionine, lantibiotics, nisin, lacticin, dehydroalanine, dehydrobutyrine.

I. INTRODUCTION

The unusual amino acid lanthionine (Ln) consists of two alanine residues crosslinked on their β-carbons by a thioether linkage (3,3'-thiodialanine) (Fig. **1**). These compounds are present in a variety of materials including the so-called lantibiotics [1], a class of promising gene encoded peptide antibiotics, in bacterial cell walls [2], and in proteins processed in food applications [3]. Lantibiotics are produced by a large number of gram-positive bacteria and contain the *meso*-diastereomer of lanthionine within cyclic peptides as well as a methyl substituted derivative ((2*S*,3*S*,6*R*)-3-methyllanthionine, MeLn, Fig. **1**). Nisin (Fig. **2**), the most studied lantibiotic, is produced by *Lactococcus lactis* and



meso-lanthionine (Ln) (2*S*,3*S*,6*R*)-3-methyllanthionine (MeLn)

Fig. (1). The structures of lanthionine and methyllanthionine as found in lantibiotics.

has been used as a food preservative for over forty years in more than eighty countries without substantial development of bacterial resistance [4]. Discovered in 1928 [5,6], one year prior to penicillin [7], the compound is one of the oldest known antibacterial agents but its structure was not determined until elegant studies by Gross and Morell in 1971 [8]. Nisin is active at low concentrations (MIC ~ 3 nM) against many strains of gram-positive bacteria including multi-drug resistant strains and the food pathogen *Clostridium botulinum*. At present more than 30 different lantibiotics are known with varying structure, size, and mode of action. One other member, subtilin, is depicted in Fig. **2**. It is produced by *Bacillus subtilis* and has significant structural similarity to nisin.

The unique structural features of lantibiotics are installed by way of an interesting biosynthetic strategy (Fig. **3**). They are initially produced as ribosomally synthesized prepeptides, containing an *N*-terminal leader sequence and a *C*-terminal structural region. A post-translational modification process [9] then involves site-specific dehydration of threonine and serine residues in the structural region to provide 2,3-didehydrobutyrine (Dhb) and 2,3-didehydroalanine (Dha) residues, respectively. Subsequently, cyclase enzymes catalyze the regio- and stereospecific Michael addition of nearby cysteine residues onto the Dha and Dhb residues to form lanthionine and methyllanthionine rings, respectively. In some lantibiotics other post-translational modifications take place that are beyond the scope of this mini-review [1]. Finally, the leader peptides are proteolytically cleaved to provide active, mature lantibiotics [10].

Various synthetic methods have been developed to prepare cyclic and acyclic lanthionines for studies of lantibiotics, as stable non-reducable analogs of disulfide bridges, or for introduction of conformational rigidity and proteolytic stability in peptidomimetics. This review will discuss several strategies for chemical lanthionine synthesis as well as recent exciting developments in enzymatic production of these structures.

II. EARLY LANTHIONINE SYNTHESES

In 1941, Horn and coworkers reported the first isolation of β-amino-β-carboxyethyl sulfide (**1**) from the treatment of wool with sodium carbonate and called the compound lanthionine (Latin, *lana* = wool) [11]. Further structural characterization was performed by Brown and du Vigneaud, who sought to chemically synthesize the compound from L-

*Address correspondence to this author at the Department of Chemistry, University of Illinois at Urbana-Champaign, 600 South Mathews Ave, Urbana, Illinois 61801, USA; E-mail: vddonk@uiuc.edu

1570-193X/05 $50.00+.00

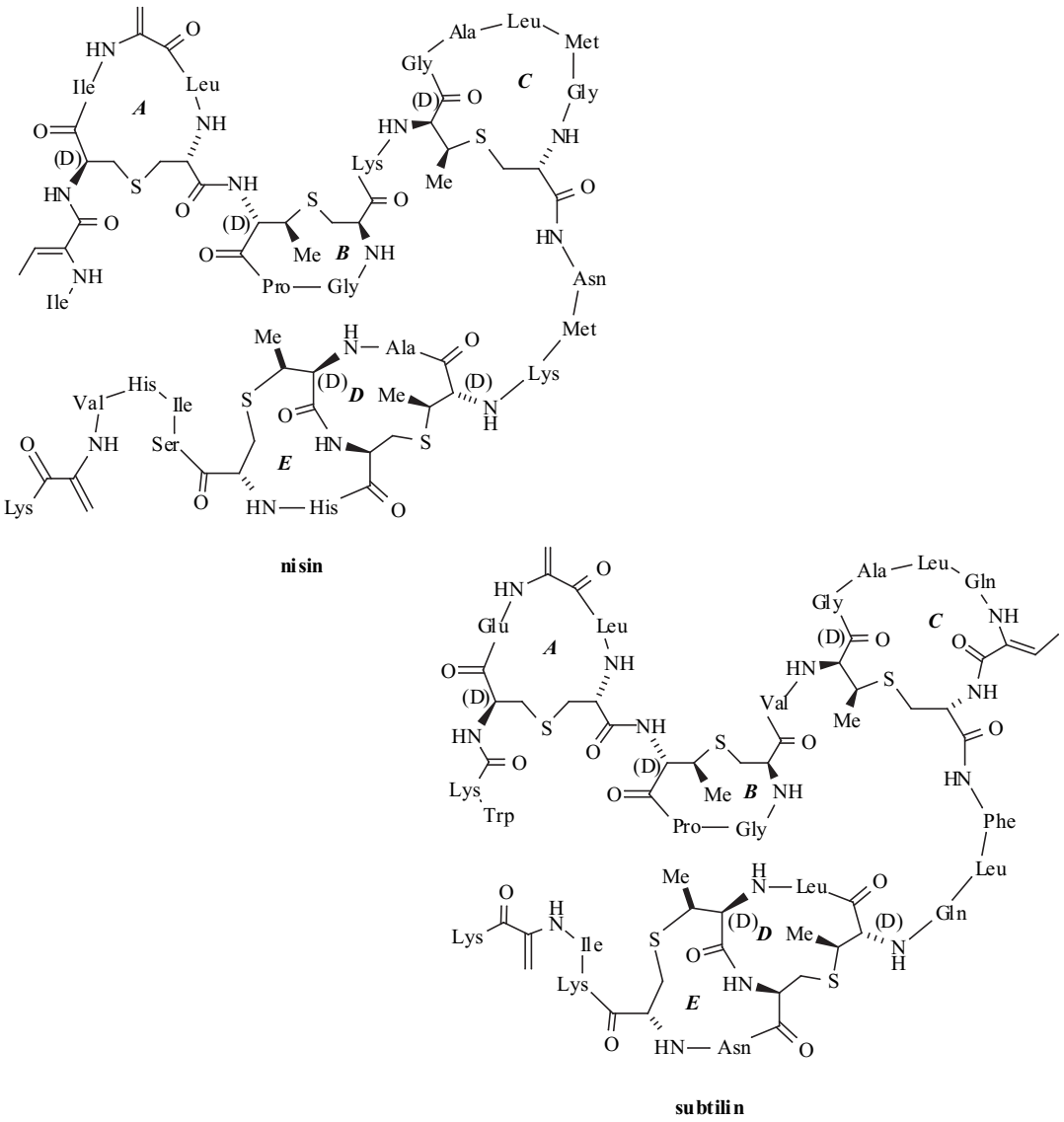
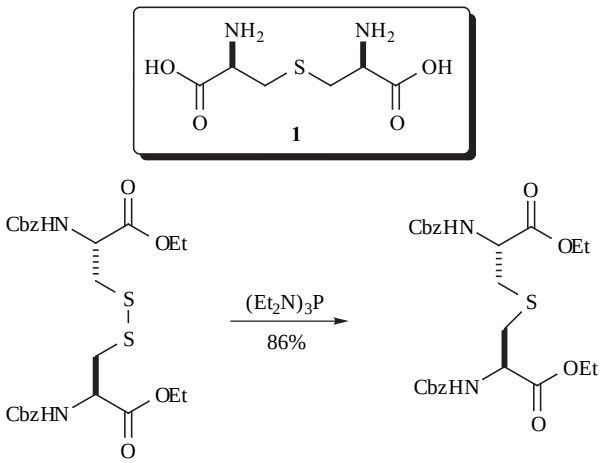


Fig. (2). The structures of two lantibiotics, nisin and subtilin, that have been targets of synthetic studies.

cysteine and methyl DL-α-amino-β-chloropropionate [12]. Comparison of the chemical and structural properties of the resulting lanthionines with D,L- and L,L-configuration to those of the previously isolated compound from wool indicated that the lanthionine structure obtained by Horn had the *meso* configuration, consistent with the observed absence of optical rotation [11]. In their original work, Horn and coworkers also reported stereoisomers of *meso* lanthionine in base-treated wool. Their origin is most likely cystines, which have been shown to form lanthionines under basic conditions [13]. A novel stereoselective synthesis of lanthionines was later published by Harpp and Gleason [14]. In this work, L,L-lanthionine derivatives were synthesized by desulfurization of cystines using tris(diethylamino) phosphine (Scheme **1**). Although this method has been used successfully in the total synthesis of nisin (*vide infra*) [15], it is often limited to the use of symmetrical disulfides since the phosphine catalyzes an equilibration when unsymmetrical disulfides are used, resulting in various possible products [16]. Moreover, yields are very sensitive to protecting groups and reaction conditions [15-18] and the strategy is not amenable to solid phase peptide synthesis (SPPS) [19].



Scheme 1.

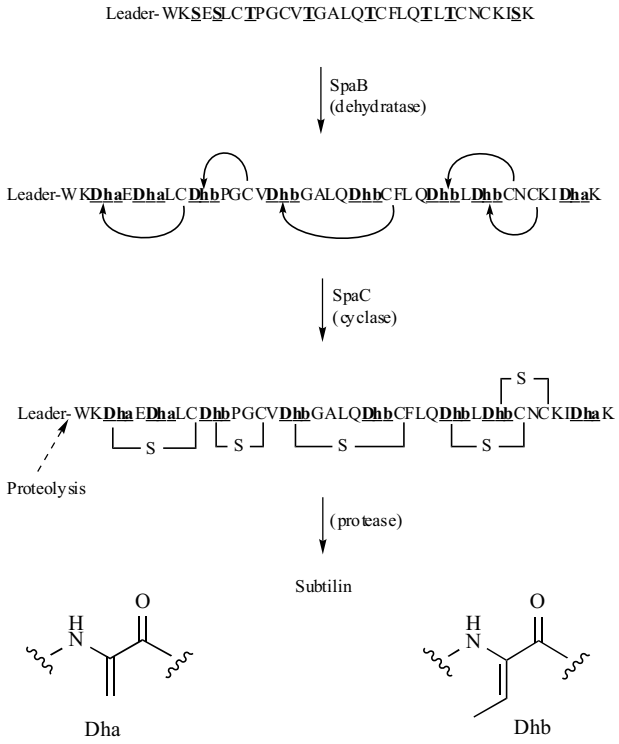
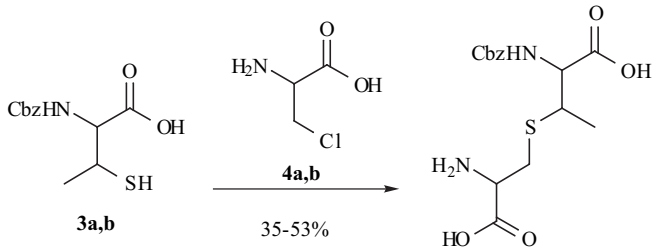


Fig. (3). Representative example of the post-translational maturation process of lantibiotics. The prepeptide for subtilin biosynthesis is ribosomally synthesized, followed by SpaB catalyzed dehydration of a subset of Ser and Thr residues. SpaC catalyzes the conjugate addition of Cys residues in a regio- and stereospecific manner to the Dha and Dhb residues to generate five cyclic thioethers, one lanthionine and four methyllanthionines. In the final step towards mature subtilin, the leader peptide that has not been modified is proteolytically removed. The sequence of this leader peptide is MSKFDDFDLDVVKVSKQDSKITPQ.

III. SYNTHESIS OF LINEAR LANTHIONINES

In 1983, Nakajima and coworkers reported the syntheses of both *meso* and L,L-lanthionine *via* the reaction of L-cysteine derivatives with aziridine **2a** or its enantiomer, prepared from L– and D–serine, respectively (Scheme **2**) [20]. A similar strategy with the methyl substituted aziridine **2b** afforded the corresponding methyllanthionines, albeit in low yield. Wakamiya and coworkers subsequently described the preparation of four diastereomers of methyllanthionine by reacting protected derivatives of *(2S,3S)*- and (2*S*,3*R*)-3-methyl-D-cysteine (**3a,b**) with either D- or L-β-chloroalanine (**4a,b**) (Scheme **3**) [21]. The displacement was shown to take place without loss of the stereochemical integrity at the α-carbon of the chloroalanine precluding involvement of an elimination-addition pathway. These compounds (**5a-d**) were utilized as authentic samples for determination of the configuration of methyllanthionines in several lantibiotics.



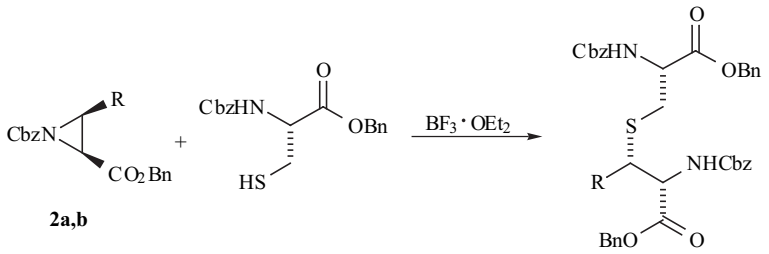
3a: 2*S*,3*S*
3b: 2*S*,3*R*

4a: L-isomer
4b: D-isomer

5a: 2*S*,3*S*,6*R*
5b: 2*S*,3*S*,6*S*
5c: 2*S*,3*R*,6*R*
5d: 2*S*,3*R*,6*S*

Scheme 3.

Efficient use of lanthionines in peptide synthesis requires discrimination of the two amino and two acid functionalities. Consequently, several recent studies have focused on preparation of orthogonally protected



2a: R = H, 37%
2b: R = CH_3, 22%

Scheme 2.

lanthionines using similar alanyl β-cation equivalents as discussed above. A number of challenges have been uncovered in these studies including the prevention of elimination-addition pathways given the higher propensity for elimination in these protected synthons. Such a mechanism would generate dehydroalanine intermediates, which would lead to the production of epimers by non-stereospecific Michael type addition. Dugave and Ménez surveyed a series of electrophiles including the mesylate of serine and iodoalanine, with the latter showing the best results (Scheme **4**) [22]. Trityl protection of the amino group was chosen as it suppresses elimination of the leaving group [23]. Using their optimized conditions, the preparation of linear orthogonally protected lanthionines was reported with good yields (81-88%) and stereoselectivity. In addition to lanthionine production, aziridine formation was also observed as a side process. Tabor and coworkers recently called into question the assignment of the products produced via this method [24]. These authors showed that under the published reaction conditions for the synthesis of iodoalanine, the major product is the protected α-iodo-β-alanine **6** (Fig. **4**), presumably formed via an aziridine intermediate. It was suggested that the presence of two different constitutional isomers of iodoalanine went unnoticed in the previous work because the signals of **6** in the ^{1}H NMR spectrum may have been erroneously attributed to a rotamer of the desired protected iodoalanine [22]. Reaction of the mixture of constitutional isomers of protected iodoalanine produced the desired lanthionine **7** as the minor product and nor-lanthionine **8** as the major product (Fig. **4**). Bradley and coworkers also utilized the Dugave method of thioalkylation of iodoalanine in the synthesis of an orthogonally protected lanthionine [18]. In this study the Garegg procedure (PPh_3, imidazole, I_2) [25] was used for the preparation of iodoalanine derivatives and the presence of two sets of NMR signals that did not coalesce in variable temperature experiments was reported. This might also suggest that two isomers of iodoalanine were formed. However, in recent work by Goodman and coworkers [26], it was shown that *N*-Trt-3-iodoalanine benzyl ester prepared using the Garegg procedure gave a product of significantly higher optical purity than the original Dugave report [27] ($[\alpha]^{25}_D$ of +14.7º *vs* + 5º). Hence, it appears that iodoalanine may be a suitable synthon as long as care is taken to avoid generation of its isomer **6**. Bradley and coworkers used their iodoalanine derivatives in the thioalkylation with Fmoc-protected cysteine **9** resulting in the orthogonally protected *meso* lanthionine **10**. ^{1}H NMR analysis after conversion to the corresponding Mosher amide demonstrated >95% stereochemical purity suggesting that the iodoalanine in this study also did not contain any **6**. Lanthionine **10** was further elaborated for use on solid phase by judicious choice of protecting group manipulations (Scheme **5**).

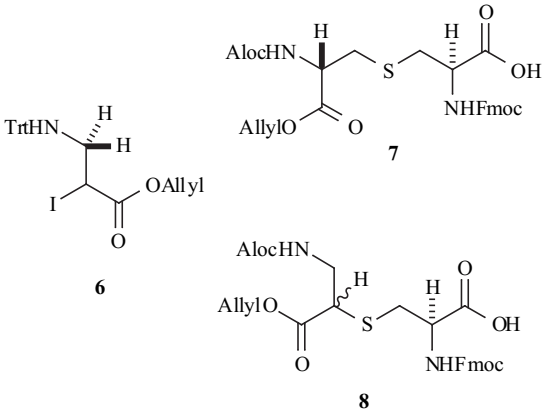
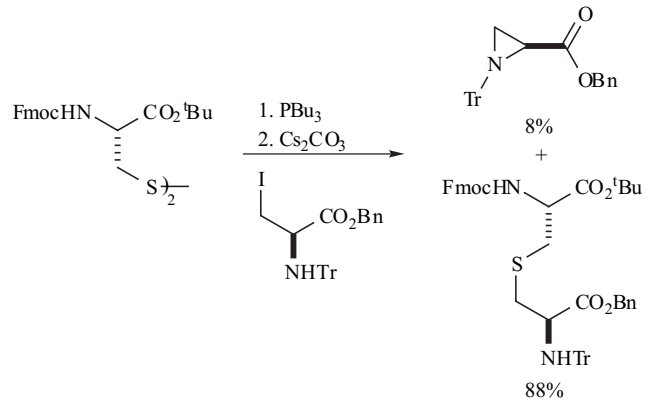
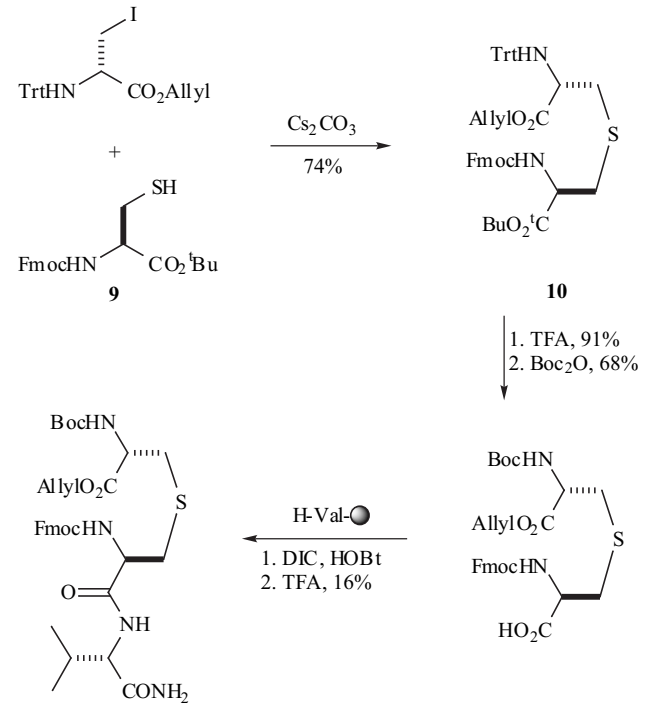


Fig. (4). 2-Iodo-β-alanine (**6**) is produced as major product in addition to the desired 3-iodo-α-alanine in the reaction of mesylated serine with sodium iodide. Reaction of this mixture of isomers with cysteine derivatives produced the desired lanthionine (**7**) as the minor and norlanthionine (**8**) as the major product [24,28].



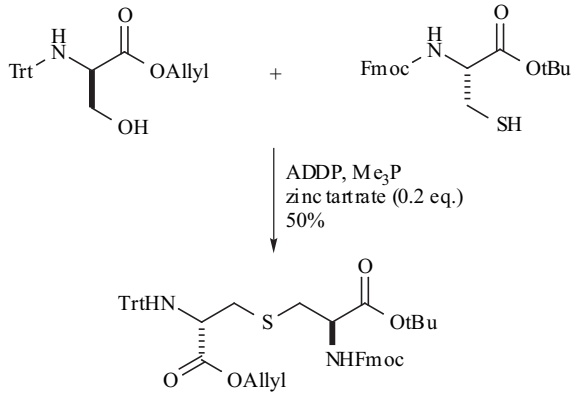
Scheme 4.



Scheme 5.

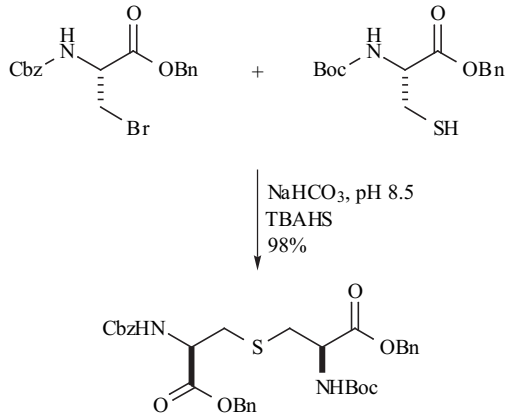
In order to overcome the problems with using iodoalanine, Tabor's laboratory developed an alternative synthetic route to generate lanthionines using Mitsunobu type chemistry [28]. Trityl-protected D-serine allyl ester was reacted with Fmoc-protected cysteine t-butyl ester in the presence of (azodicarbonyl)dipiperidine (ADDP) and trimethylphosphine (Scheme **6**). The use of catalytic zinc tartrate was required to increase the typically low reactivity

of aliphatic thiols in Mitsunobu chemistry resulting in the desired *meso* lanthionine **11** in 50% yield as a single stereoisomer. A drawback of this approach lies in the long reaction times that are required (7 d, 25 ºC). More recently, Zhu and Schmidt reported the reaction of various protected β-bromoalanine derivatives with Boc-cysteine-OBn (Scheme **7**) [29]. Tetrabutylammonium hydrogensulfate (TBAHS) was used as phase transfer catalyst in a biphasic system at pH 8.5 that tolerated a variety of protecting groups. Several diastereomers of lanthionine were prepared without the observation of aziridine or norlanthionine side products or epimerization.



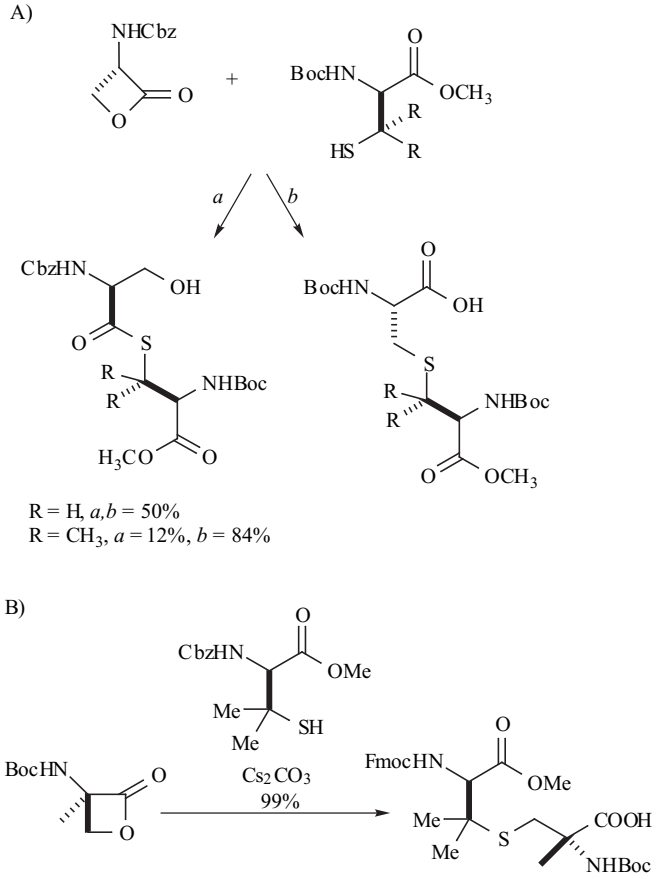
11

Scheme 6.



Scheme 7.

In addition to halogenated alanines, the Vederas β-lactone has also been applied as electrophile for lanthionine synthesis. Whereas the use of protected cysteine derivatives gives a mixture of products resulting from attack at the carbonyl and β-methylene providing thioester and lanthionine products, respectively (Scheme **8A**), the use of cysteine derivatives carrying substituents on the β-carbon resulted in a switch towards *O*-alkyl fission [30]. Hence, 3,3-dimethyllanthionines were obtained in good to excellent yields. This methodology was extended, more recently, to α-methyl lanthionines *via* the use of α-methyl-D-serine-β-lactone (Scheme **8B**) [31]. As discussed in section V, such lanthionine analogs may find use in peptidomimetics. Finally, a biomimetic approach has been used for the preparation of linear lanthionines. Unlike the studies on cyclic derivatives discussed below, Michael-type addition of cysteine derivatives to protected dehydroalanines occurred non selectively producing a mixtures of diastereomers [19].



Scheme 8.

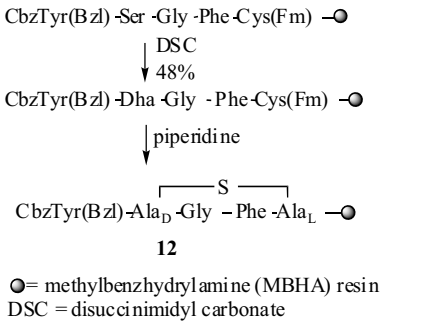
IV. SYNTHESIS OF CYCLIC LANTHIONINES

Cyclic lanthionine-containing peptides have been synthesized with the aim of understanding the biosynthesis of lantibiotics and to make smaller fragments to investigate their mode of action. Furthermore, cyclic lanthionine containing peptides have found use as mimics of natural products that contain disulfide bridges or to limit the conformational flexibility of bioactive compounds. The synthetic strategies for the preparation of these metabolically more stable mimics as well as fragments of lantibiotics have varied from a biomimetic approach to on-bead solid phase methodologies.

IV.1. Biomimetic Michael-type Additions

The cyclic thioether analog **12** of enkephalin was accessed by base promoted intramolecular stereoselective Michael addition of a Cys to a Dha contained within an immobilized pentamer peptide (Scheme **9**) [32]. After cleavage from the resin, only one diastereomer was detected and assigned the *meso* configuration by amino acid analysis and comparison with authentic samples prepared by the sulfur extrusion method discussed above. At the time, this was the first example of a stereoselective protonation of the enolate intermediate formed during the conjugate addition, but this has now proven a common observation, especially in cyclic lanthionines containing two additional amino acids

in the ring. In a recent mechanistic study, Zhu *et al.* demonstrated that this preference has a kinetic rather than thermodynamic origin [33]. Toogood observed similarly high stereoselectivity in the biomimetic synthesis of a lanthionine analog **13** of the methyllanthionine in ring B of the lantibiotic epidermin (Fig. **5**) [34], and Bradley and coworkers reported high selectivities for the lanthionine analogs of the subtilin B- and E-ring [35]. In both studies, extensive NMR studies were performed that resulted in assignment of the *meso* stereochemistry to the products. The precursor peptides in both cases were prepared by a fragment condensation approach, in which the Dha-containing peptide and the Cys-containing peptide were prepared individually. In the latter work, the Dha residue was installed by the use of *S*-methylcysteine during peptide synthesis, followed by oxidation and pyrolitic elimination. Very recently, improved conditions were reported for this route that allowed the solid phase preparation of peptides containing both cysteines and dehydro amino acids thereby obviating the need for fragment condensation (Scheme **10**) [36]. In this permutation, the cysteines are protected with a triphenylmethyl group which facilitates chemoselective oxidation of the *S*-methylcysteine with H_2O_2/$Sc(OTf)_3$ followed by DBU catalyzed elimination of the *S*-methylcysteine sulfoxide. This improvement allowed the biomimetic synthesis of an analog (**14**, Scheme **10**) of the B-ring of nisin on a solid support [37]. Similar chemoselectivity had been described previously by Okeley *et al.* who used *N*-Fmoc-*Se*-phenyl selenocysteine **15** as a masked dehydroalanine equivalent [38]. This approach proved amenable to solid phase synthesis of the cyclization precursors for the B- and E-rings of subtilin since the mild oxidation conditions were compatible with trityl and disulfide protection of the cysteine residues. As in the studies of the Toogood and Bradley groups, the Michael type addition stereoselectively afforded cyclic peptides **16** and **17** containing a *meso* lanthionine residue (Scheme **11**). This approach was extended to the synthesis of Dhb-containing peptides through the use of Fmoc-(2*R*,3*S*)-3-methyl-*Se*-phenylselenocysteine (**18**), which allowed the first test of the stereochemistry of biomimetic formation of cyclic methyllanthionines (Scheme **12**) [39]. Through independent synthesis of the natural stereoisomer, it was shown that this Michael addition also occurred with very high selectivity in favor of the naturally occurring diastereomer.



Scheme 9.

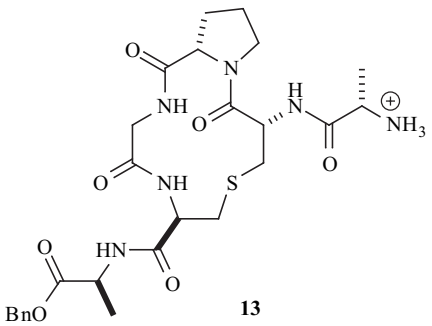
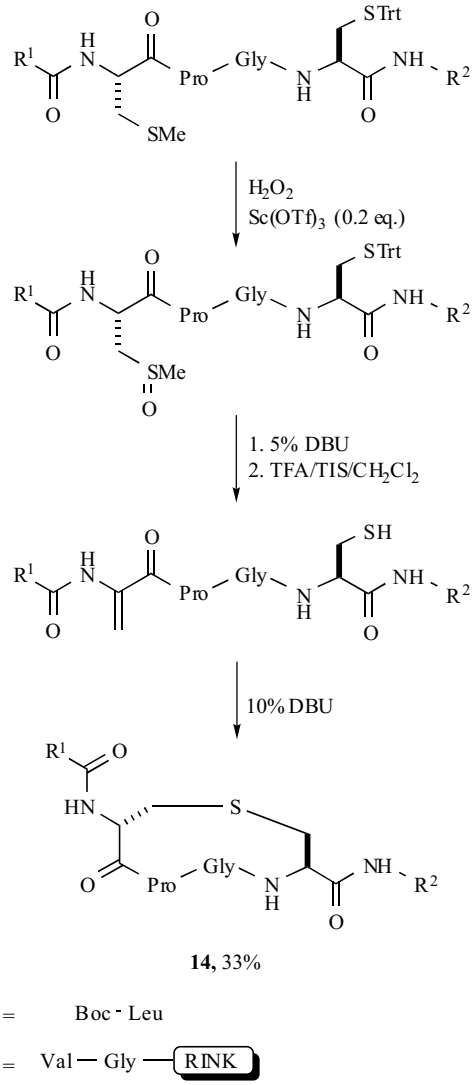
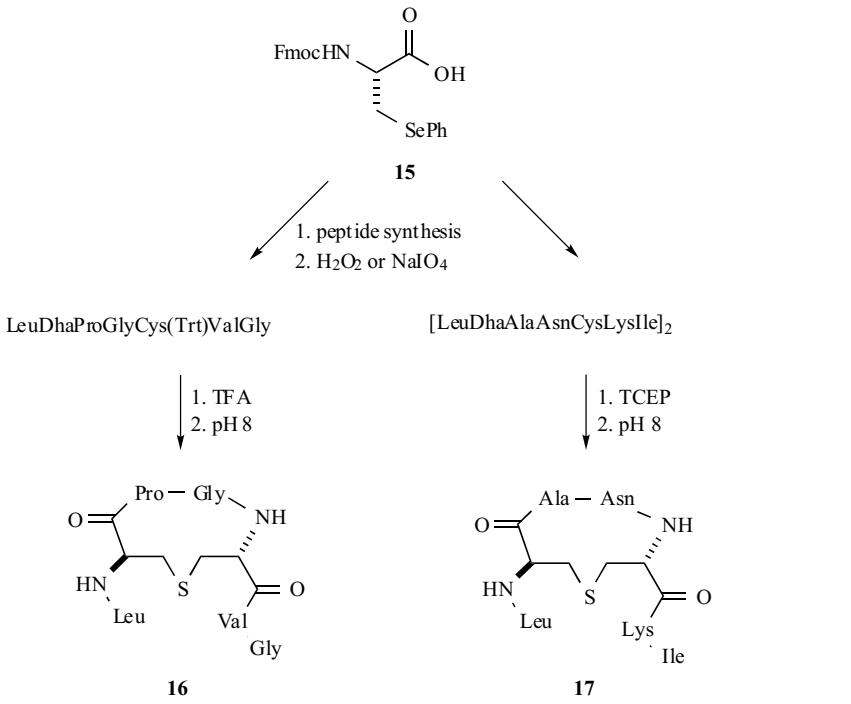


Fig. (5). Lanthionine analog of the methyllanthionine in the epidermin B-ring.

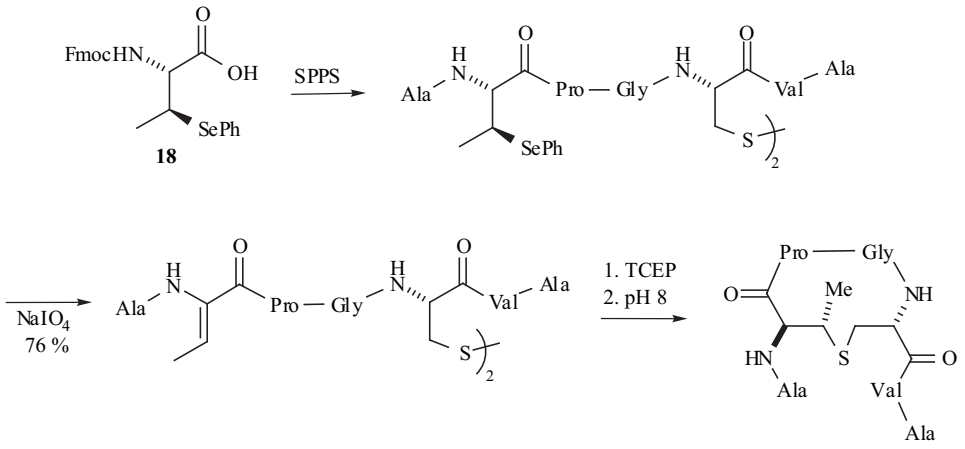


Scheme 10.

During the proposed biosynthesis of lantibiotics, the cellular machinery must not only control the stereochemistry of the cyclization reactions, but also the regiochemistry (Fig. **3**). As described above, the stereochemical outcomes of biomimetic cyclizations have been in accord with the stereochemistry found in lantibiotics questioning the need for enzyme catalysis. Two studies have interrogated the regioselectivity of biomimetic ring formation. Bradley and coworkers investigated the biomimetic formation of the A ring of nisin (**19**, Scheme **13**). The biosynthetic precursor contains two dehydrated amino acids, which were installed by the *S*-methylcysteine methodology discussed above. Deprotection of the cysteine nucleophile followed by



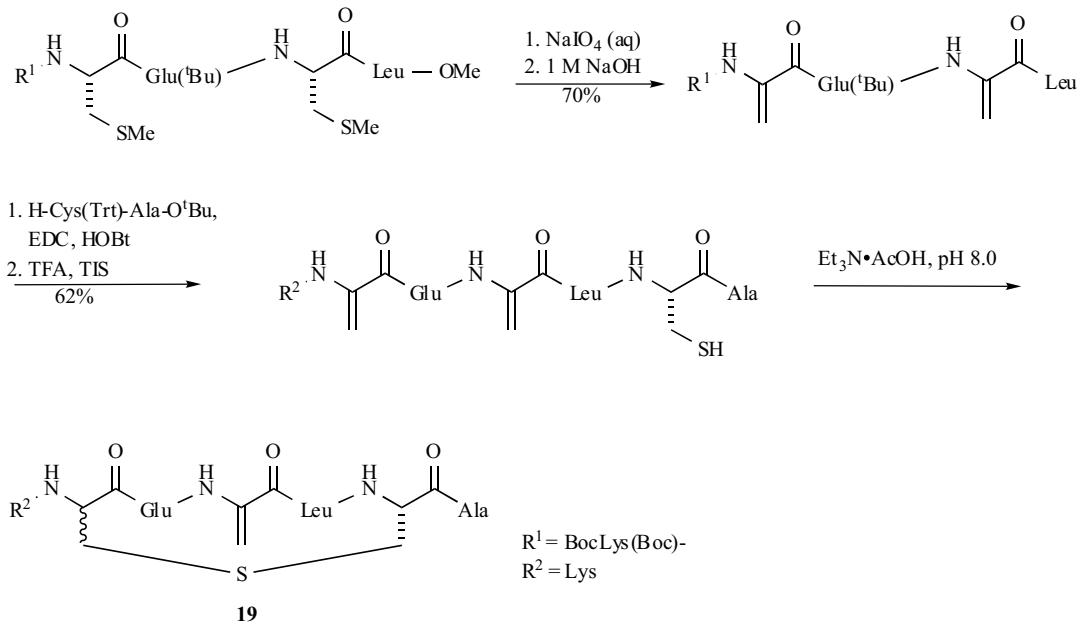
Scheme 11.



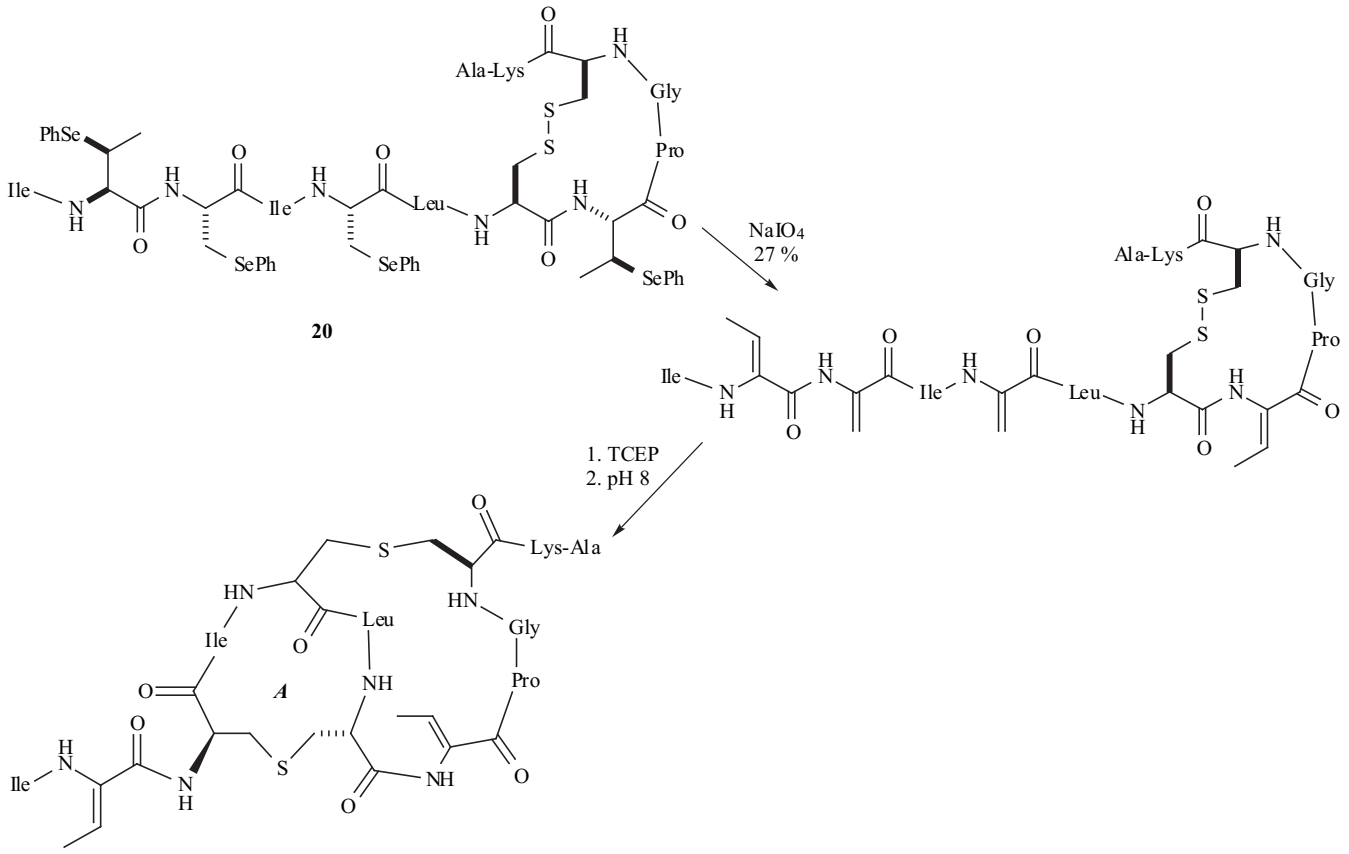
Scheme 12.

Michael addition led to two products in a 3:1 ratio. After purification, NMR analysis revealed that these compounds both contained the connectivity of the natural subtilin A ring, but with different stereochemistry at the newly formed stereocenter. Hence, whereas the correct regiochemistry was observed, the protonation of the enolate intermediate is less selective in this example than that observed for rings containing four amino acids (*vide supra*). Zhu *et al.* recently addressed the chemoselectivity of the intramolecular Michael addition of the precursor peptide to the A- and B-rings of nisin [33]. Peptide **20** was prepared by SPPS and the two Dha and two Dhb residues were oxidatively unmasked (Scheme **14**). The intramolecular disulfide was reduced, and upon addition of base cyclization took place. ^{1}H NMR analysis revealed that the product did not consist of the A and B rings of nisin. Instead, the resonances of the vinyl protons of the two dehydrobutyrines were still present in the product, whereas the signals for the vinyl protons of the two Dha residues were absent. Hence, the much faster cyclization rate for lanthionines compared to methyllanthionines [39] prevents the biomimetic cyclization in which one Ln (A-ring) and one MeLn (B-ring) would have been formed (see nisin structure, Fig. **2**). Based on the result of Bradley in Scheme **13**, the products of cyclization of **20** were assigned as depicted in Scheme **14**. The important conclusion from these studies is that lantibiotic biosynthesis requires enzymatic control over the chemoselectivity and/or processivity of the cyclization reactions.

Another activation/elimination/Michael addition sequence has been employed to generate conformationally restricted methyllanthionines. Botta and coworkers have demonstrated that cyclic methyllanthionine dipeptides can be constructed through activation of threonine as a mesylate followed by treatment with $LiAlH(OMe)_3$ to form a mixture of two dehydrobutyrine isomers (Scheme **15**) [40]. Deprotection of an acetyl-protected cysteine initiated a Michael addition providing a 2:1 ratio of two diastereomers **21a** and **21b**.



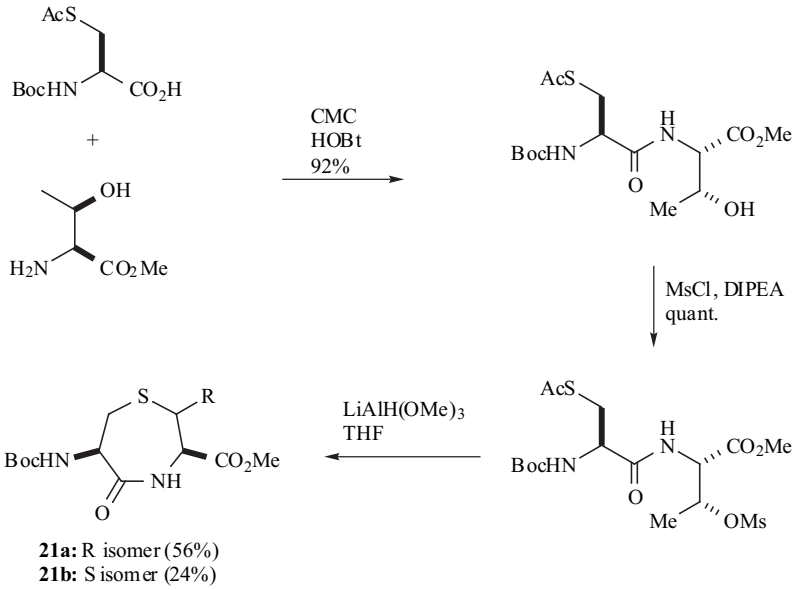
Scheme 13.



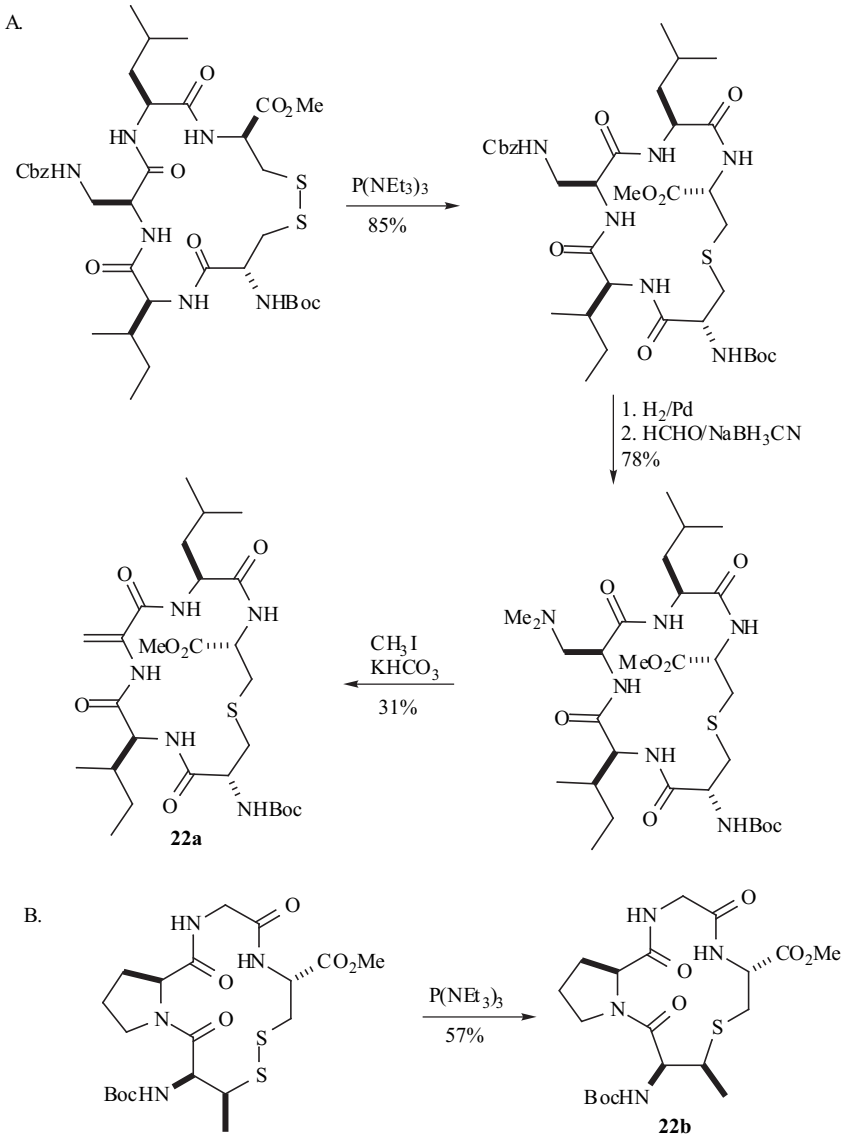
Scheme 14.

IV.2. Sulfur Extrusion: Total Synthesis of Nisin

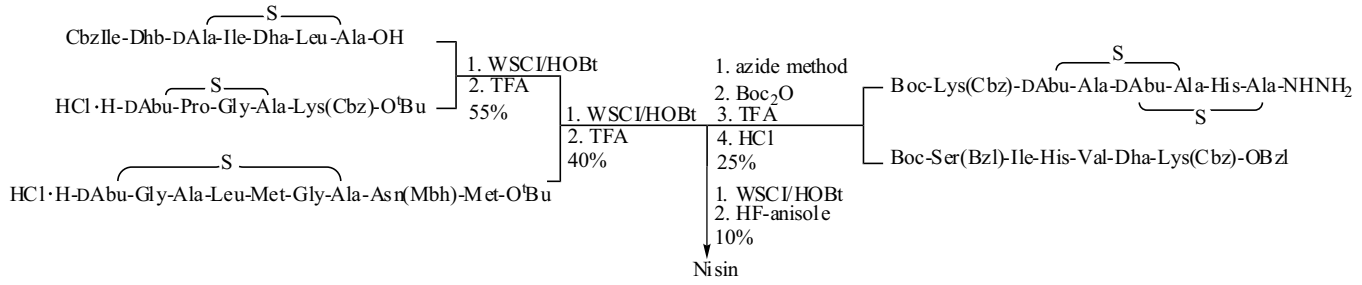
The studies on regio- and chemoselectivity of intramolecular Michael reactions indicate that the full length lantibiotics likely cannot be prepared via a true biomimetic sequence. Indeed, the only total synthesis of a lantibiotic to date was accomplished by Wakamiya and Shiba and coworkers using a fragment condensation approach [41]. In a seminal series of papers, the A, B, and C-rings as well as the fused D- and E-rings of nisin were prepared individually from the corresponding disulfide analogs using the sulfur extrusion method of Harpp and Gleason as shown for the A- and B-ring (**22a,b**, Scheme **16**) [15,17,42,43]. This regioselective procedure maintains the stereochemical configuration of the β-carbon of the 3-methyl-D-cysteine, affording the methyllanthionine ring with the correct



Scheme 15.



Scheme 16.



Scheme 17.

stereochemical configuration (Scheme **16B**) [15]. All segments were prepared by Boc-based solution phase peptide synthesis, and assembled to generate the nisin molecule (Scheme **17**).

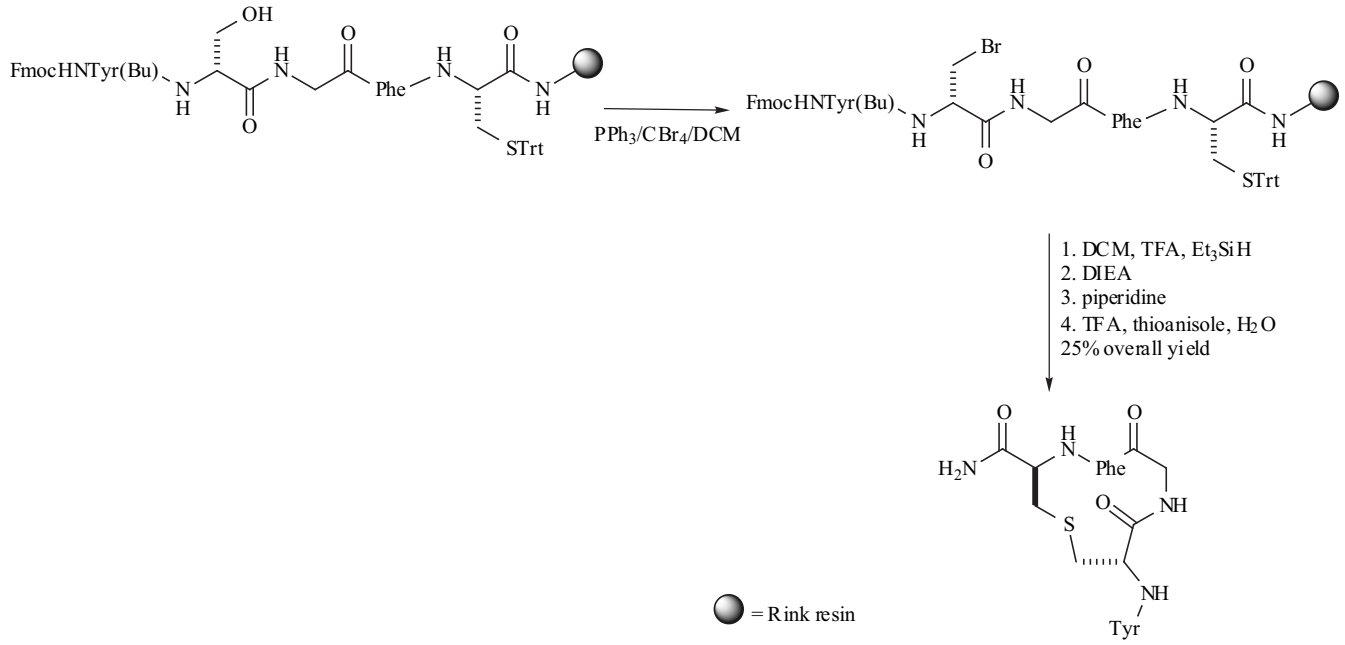
IV.3. On Resin Lanthionine Formation Using Halogenated Alanines

In analogy to the synthetic strategies for linear lanthionines, halogenated alanines or activated serines have been used as electrophiles for intramolecular reaction with cysteines. Mayer and coworkers used on-resin cyclization by converting serines into bromoalanines with subsequent deprotection of the cysteine (Scheme **18**) [44]. Interestingly, when the products starting with D- and L-bromoalanine were compared, they were identical. This suggests that the reaction proceeds through an elimination-Michael addition sequence with the latter being stereoselective, similar to what was observed in other syntheses of cyclic lanthionines by conjugate addition of cysteines to dehydroalanines (*vide supra*).

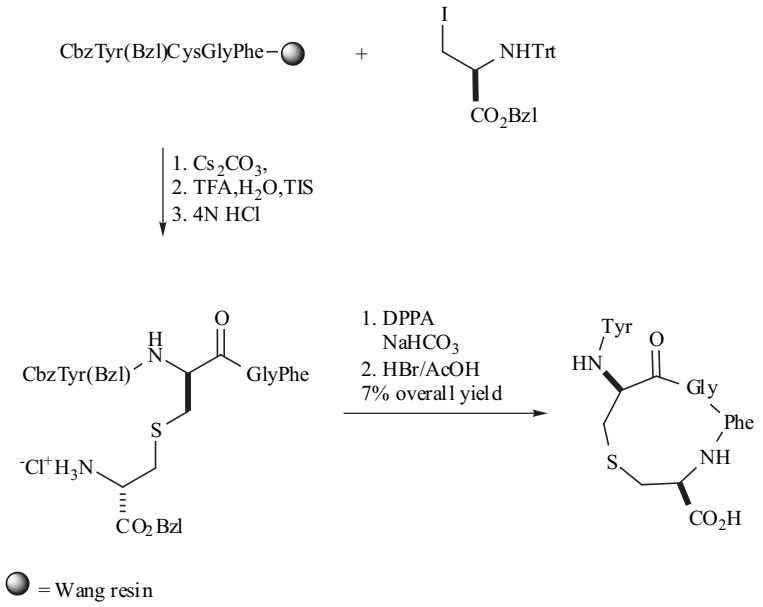
Goodman and coworkers utilized the Dugave approach for cyclic lanthionine generation [26]. A tetrapeptide was synthesized using Fmoc chemistry, followed by alkylation of the cysteine thiol with *N*-trityl-iodoalanine benzyl ester and subsequent cleavage from the resin (Scheme **19**). The cyclization of the lanthionine was achieved in solution by intramolecular amide bond formation. Although the use of iodoalanine has been prone to side reactions as discussed above, the desired enkephalin analog was successfully synthesized with no reports of epimerization or norlanthionine formation.

IV.4. Peptide Cyclization on Oxime Resin (PCOR)

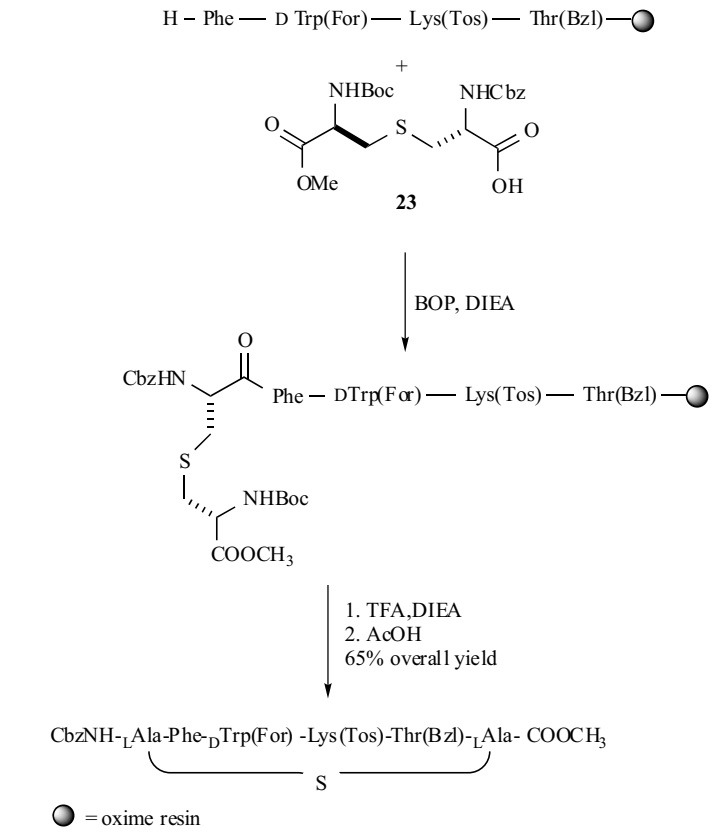
An interesting cyclization method was developed by Goodman and Ösapay in which one of the amino functionalities of orthogonally protected lanthionines is incorporated at the N-terminus of a growing peptide chain linked to Kaiser's oxime resin (Scheme **20**) [45,46]. After removal of the protecting group of the second amine, an intramolecular reaction takes place with the oxime ester to generate the cyclic lanthionine in high yields. In this way, thioether analogs of somatostatin, a key regulatory hormone containing a cyclic cystine were prepared [47]. Previous work had shown that sandostatin, a synthetic analog of somatostatin, possessed higher biological activity than the natural compounds but conformational analysis suggested that the disulfide did not sufficiently restrict the backbone in the proposed bioactive conformation. By replacing this



Scheme 18.



Scheme 19.

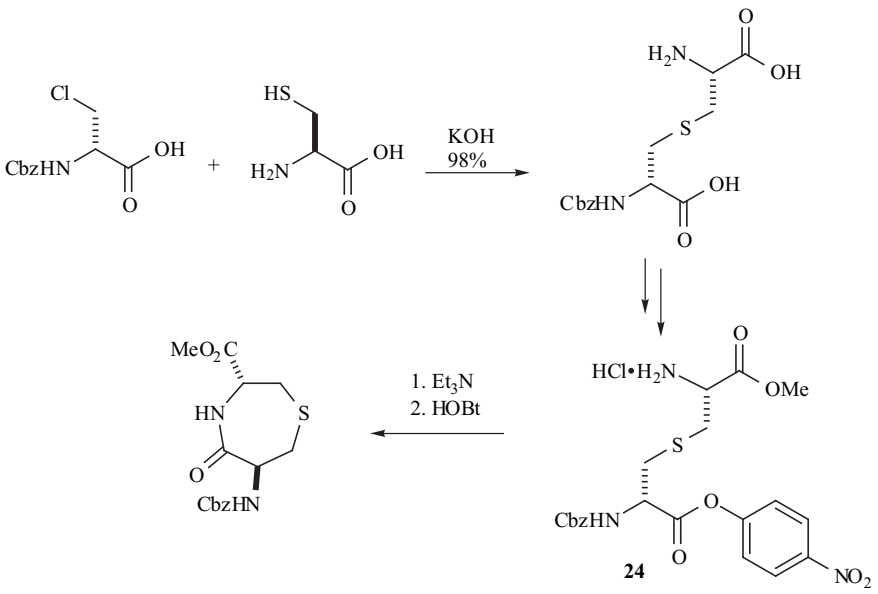


Scheme 20.

disulfide with a lanthionine, Goodman and coworkers conferred additional rigidity to the molecule resulting in a high stability toward enzymatic degradation and a prolonged half-life.

IV.5. Cyclization Through Amide Bond Formation

Perhaps the most straightforward route to lanthionines involves cyclization through amide bond formation. This method requires the relatively labor intensive synthesis of complex orthogonally protected precursors, but like the PCOR method it has the advantage of completely stereodefined products. The first use of this approach was reported by Photaki and coworkers, for the synthesis of cyclic dipeptide lanthionines [48]. The thioether moiety was installed by the displacement of chloride from 3-chloroalanine with cysteine, followed by protecting group manipulation to access lanthionine **24** (Scheme **21**). Amide

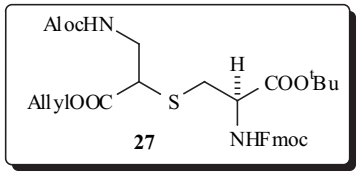


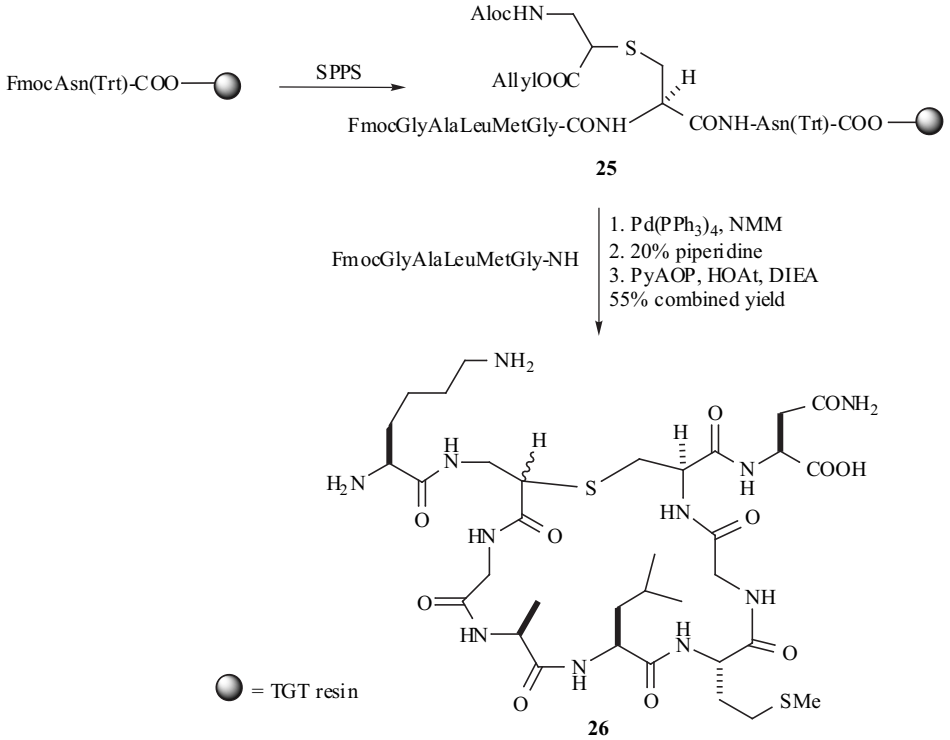
Scheme 21.

bond formation with HOBt afforded the desired compound. Lanthionines have also been prepared using this type of strategy as conformationally constrained ligands of integrins, important mediators of cell adhesion [49]. In this work, a convergent solution phase synthesis featured three distinct segments, joined in a final amide bond forming step to yield the desired product.

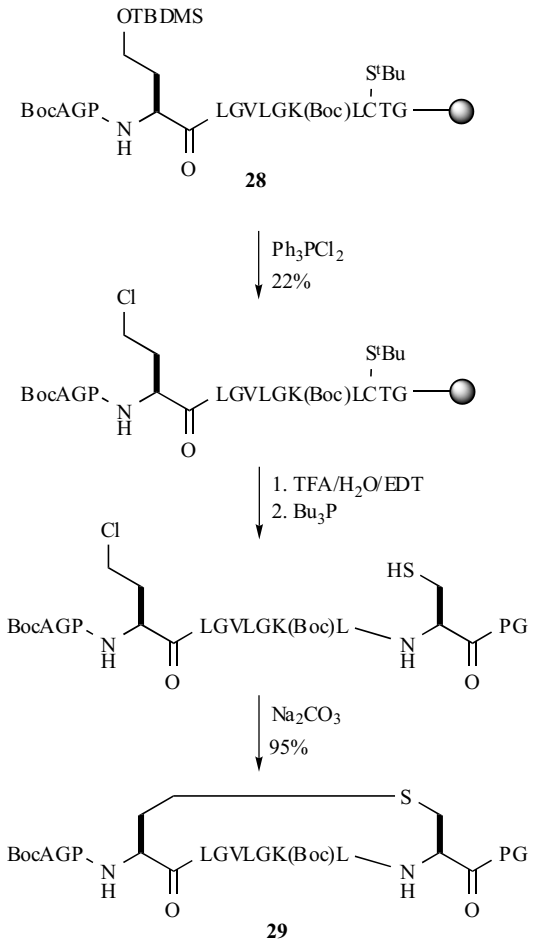
V. SYNTHESIS OF LANTHIONINE ANALOGS

Tabor and coworkers have generated the norlanthionine analog (**26**) of the C-ring of nisin [50,51]. The *tert*-butyl ester of a diastereomeric mixture of norlanthionine **27** was used in a linear solid phase synthesis to prepare the triply protected heptapeptide **25** (Scheme **22**). Following allyl and Fmoc deprotection, intramolecular amide bond formation was carried out in good yield on the resin.

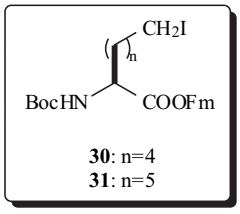




Scheme 22.



Scheme 23.

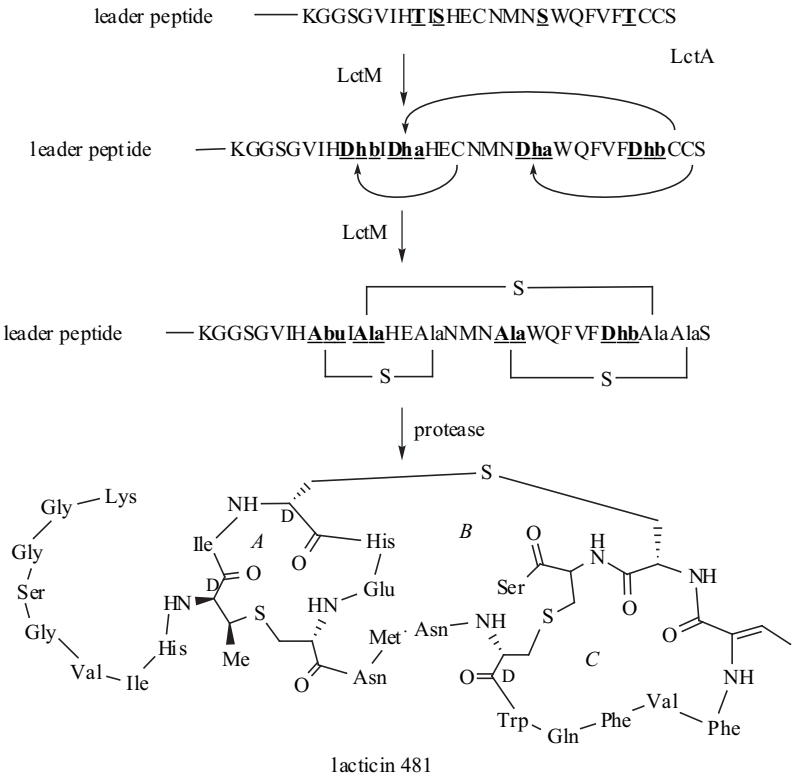


A novel homolanthionine, another non-cleavable disulfide analog, was reported by Yu *et al.* [52]. The *tert*-butyldimethylsilyl (TBDMS) ether of homoserine was incorporated into a 14-mer peptide (**28**) (Scheme **23**) and converted to the alkylchloride with triphenylphosphine dichloride. Cleavage of the peptide from the resin and removal of the t-butyl protecting group from the cysteine led to cyclization resulting in an efficient synthesis of a single diastereomer of the desired homolanthionine-containing peptide **29**. Very recently, Grieco and coworkers were able to affect on-resin cyclization of homologated lanthionines with varying ring sizes using iodinated amino acids **30** and **31** [53]. These compounds were irradiated in the presence of a resin bound peptide containing a deprotected cysteine, generating the homologated lanthionines of varying carbon chain length. Cyclization was achieved by treatment with piperidine and HBTU/DIEA. In a different modification of lanthionines, Vederas and coworkers prepared the corresponding sulfoxides and sulfones [54]. They recognized the structural similarity between *meso*-lanthionine and *meso*-diaminopimelic acid (DAP), an essential component in bacterial cell walls, and showed that several stereochemically pure lanthionine sulfoxides, prepared according to the Brown and du Vigneaud procedure [12], are competitive inhibitors of DAP decarboxylase.

VI. ENZYMATIC LANTHIONINE SYNTHESIS

As a result of their interesting structures, mode of biosynthesis, and biological activities, the lantibiotics family of peptide antibiotics have garnered great interests from synthetic chemists, biochemists, and microbiologists [1]. Nisin and many other lantibiotics owe their bactericidal activity to the depolarization of energized cell membranes through pore-formation. Nisin is effective at concentrations that are several orders of magnitude lower than many other pore forming antibacterial agents and is significantly more potent toward Gram-positive intact cells than liposomes. This unusual profile was recently explained by the observation that nisin initially associates with membrane bound lipid II, an intermediate in cell wall biosynthesis that is also the target of vancomycin [55-57]. This suggests that nisin, as well as other members of the lantibiotic family [58,59] may be applicable as broad-range antibiotics in the same manner as vancomycin and has generated interest in exploiting efficient biosynthetic methods to generate non-natural variants that may have higher stability and better activity than the natural counterparts.

Despite many efforts in several laboratories, *in* vitro reconstitution of lantibiotic biosynthesis has remained elusive until very recently. Two different types of systems have been identified through sequencing of the biosynthetic gene clusters. One group of microorganisms utilizes two different dedicated enzymes for the dehydration (LanB proteins, Fig. **3**) and cyclization steps (LanC enzymes). The other group uses a single enzyme (LanM) for both reactions (*e.g.* Scheme **24**). These latter proteins have sequence homology at their C-terminus with the LanC enzymes but no homology exists with the LanB proteins. Recently, van der Donk and coworkers have reported the first successful *in vitro* synthesis of a lantibiotic, lacticin 481 produced by *Lactoccocus lactis* [9]. The bifunctional enzyme LctM was recombinantly expressed and purified, and upon addition to its natural peptide substrate LctA lacticin 481 was produced (Scheme **24**). Variants of LctA in which serines, threonines or cysteines were mutated were accepted as alternate substrates by the synthetase resulting in lacticin 481 analogs showing great promise for lantibiotic engineering. Furthermore, LctM converted truncated substrates to products containing single lanthionine rings (*e.g.* Fig. **6**) illustrating the vast possibilities of utilizing the biosynthetic machinery of lantibiotic producing organisms in order to generate new and possibly more active compounds. ATP and Mg^{2+} were required in order to carry out these post-translational modifications, although at present, the exact role of the cofactor is unknown. It may activate the serines and threonines for elimination by phosphorylation, or it may provide the energy for peptide translocation during the series of dehydration and cyclization reactions. Other studies suggest the enzyme contains a Zn^{2+}, which may act as electrophilic catalyst during the Michael cyclization [60]. Future investigations will be required to understand the molecular logic underlying the exquisite control of chemo-,



Scheme 24.

stereo- and regiochemistry displayed by this remarkable biosynthetic machine.

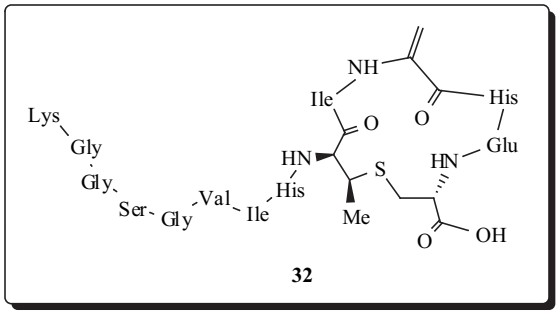


Fig. (6). The LctM enzyme accepts modified substrates to produce individual lanthionine rings such as **32**.

VII. CONCLUSIONS

A number of different methods have been developed for lanthionine synthesis since the discovery of cyclic thioether containing peptides over fifty years ago. The sulfur extrusion method is currently the only approach that has been applied to the total synthesis of a lantibiotic. Despite this success, we and others have experienced low yields and a narrow window of successful conditions for this methodology [16,18,19]. With the development of increasingly better methods to prepare orthogonally protected lanthionines, the ring closure by amide bond formation with its defined stereochemical outcome is becoming the method of choice. The PCOR method has the same advantages, although, so far it has only been applied to N-terminal lanthionines and its use for the preparation of cyclic lanthionines in the interior of longer peptides is unexplored. For certain cyclic lanthionines the biomimetic approach is well suited. The most recent methodology from the Bradley and van der Donk laboratories allows installation of the dehydroamino acids in a chemoselective fashion that is compatible with most amino acids used in SPPS. For those sequences, in which the biomimetic Michael-type addition is highly stereoselective, such as cyclic lanthionines in which two additional amino acids are present within the ring, a single diastereomer is typically obtained. However, this method has proven less successful when multiple dehydro amino acids are present in the peptide. The use of halogenated alanines as electrophiles has met varying levels of success. Some studies report clean S_N2 displacement whereas other laboratories have observed either elimination-addition processes or aziridine formation.

In early 2004, some 15 years after the first sequencing of lantibiotic gene clusters, the first *in vitro* activity of a lantibiotic synthetase has been achieved. The enzyme involved in the biosynthesis of lacticin 481 shows low substrate specificity, a promising finding for future preparation of analogs through variation of the substrate structure. It is hoped that the lessons learned from this system will also allow future establishment of *in vitro* systems for the lantibiotics produced by LanB/C enzymes.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (GM58822).

REFERENCES

[1] Sahl, H.-G.; Bierbaum, G. *Annu. Rev. Microbiol.* **1998**, *52*, 41-79.
[2] Satyanarayana, S.; Grossert, J. S.; Lee, S. F.; White, R. L. *Amino Acids* **2001**, *21*, 221-235.

[3] Friedman, M. *J. Agric. Food Chem.* **1999**, *47*, 1295-1319.
[4] Delves-Broughton, J.; Blackburn, P.; Evans, R. J.; Hugenholtz, J. *Antonie van Leeuwenhoek* **1996**, *69*, 193-202.
[5] Rogers, L. A. *J. Bacteriol.* **1928**, *16*, 321-325.
[6] Rogers, L. O.; Whittier, E. O. *J. Bacteriol.* **1928**, *16*, 211-229.
[7] Fleming, A. *Brit. J. Exper. Path.* **1929**, *10*, 226-236.
[8] Gross, E.; Morell, J. L. *J. Am. Chem. Soc.* **1971**, *93*, 4634-4635.
[9] Xie, L.; Miller, L. M.; Chatterjee, C.; Averin, O.; Kelleher, N. L.; van der Donk, W. A. *Science* **2004**, *303*, 669-681.
[10] van der Meer, J. R.; Polman, J.; Beerthuyzen, M. M.; Siezen, R. J.; Kuipers, O. P.; de Vos, W. M. *J. Bacteriol.* **1993**, *175*, 2578-2588.
[11] Horn, M. J.; Jones, D. B.; Ringel, S. J. *J. Biol. Chem.* **1941**, *138*, 141-149.
[12] Brown, G. B.; du Vigneaud, V. *J. Biol. Chem.* **1941**, *140*, 767-771.
[13] Galande, A. K.; Trent, J. O.; Spatola, A. F. *Biopolymers* **2003**, *71*, 534-551.
[14] Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1971**, *36*, 73-80.
[15] Fukase, K.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2505-2508.
[16] Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. *Tetrahedron: Asymmetry* **1992**, *3*, 85-94.
[17] Fukase, K.; Kitazawa, M.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1838-1840.
[18] Swali, V.; Matteucci, M.; Elliot, R.; Bradley, M. *Tetrahedron* **2002**, *58*, 9101-9109.
[19] Probert, J. M.; Rennex, D.; Bradley, M. *Tetrahedron Lett.* **1996**, *37*, 1101-1104.
[20] Nakajima, K.; Oda, H.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 520-522.
[21] Wakamiya, T.; Oda, Y.; Fukase, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 536-539.
[22] Dugave, C.; Ménez, A. *Tetrahedron: Asymmetry* **1997**, *8*, 1452-1465.
[23] Cherney, R. J.; Wang, L. *J. Org. Chem.* **1996**, *61*, 2544-2546.
[24] Mustapa, M. F. M.; Harris, R.; Mould, J.; Chubb, N. A. L.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *Tetrahedron Lett.* **2002**, *43*, 8359-8362.
[25] Garegg, P. J.; Samuelsson, B. *J. Chem. Soc. Perkin Trans. 1* **1980**, 2866-2869.
[26] Rew, Y.; Malkmus, S.; Svensson, C.; Yaksh, T. L.; Chung, N. N.; Schiller, P. W.; Cassel, J. A.; DeHaven, R. N.; Goodman, M. *J. Med. Chem.* **2002**, *45*, 3746-3754.
[27] Dugave, C.; Ménez, A. *J. Org. Chem.* **1996**, *61*, 6067-6070.
[28] Mustapa, M. F. M.; Harris, R.; Bulic-Subanovic, N.; Elliott, S. L.; Bregant, S.; Groussier, M. F. A.; Mould, J.; Schultz, D.; Chubb, N. A. L.; Gaffney, P. R. J.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8185-8192.
[29] Zhu, X.; Richard R, S. *Eur. J. Org. Chem.* **2003**, *2003*, 4069-4072.
[30] Shao, H.; Wang, S. H. H.; Lee, C.-W.; Ösapay, G.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 2956-2957.
[31] Smith, N. D.; Goodman, M. *Org. Lett.* **2003**, *5*, 1035-1037.
[32] Polinsky, A.; Cooney, M. G.; Toy-Palmer, A.; Ösapay, G.; Goodman, M. *J. Med. Chem.* **1992**, *35*, 4185-4194.
[33] Zhu, Y.; Gieselman, M.; Zhou, H.; Averin, O.; van der Donk, W. A. *Org. Biomol. Chem.* **2003**, *1*, 3304-3315.
[34] Toogood, P. L. *Tetrahedron Lett.* **1993**, *34*, 7833-7836.
[35] Burrage, S.; Raynham, T.; Williams, G.; Essex, J. W.; Allen, C.; Cardno, M.; Swali, V.; Bradley, M. *Chem. Eur. J.* **2000**, *6*, 1455-1466.
[36] Matteucci, M.; Bhalay, G.; Bradley, M. *Org. Lett.* **2003**, *5*, 235-237.
[37] Matteucci, M.; Bhalay, G.; Bradley, M. *Tetrahedron Lett.* **2004**, *45*, 1399-1401.
[38] Okeley, N. M.; Zhu, Y.; van der Donk, W. A. *Org. Lett.* **2000**, *2*, 3603-3606.
[39] Zhou, H.; van der Donk, W. A. *Org. Lett.* **2002**, *4*, 1335-1338.
[40] Crescenza, A.; Botta, M.; Corelli, F.; Santini, A.; Tafi, A. *J. Org. Chem.* **1999**, *64*, 3019-3025.
[41] Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 2227-2240.
[42] Wakamiya, T.; Shimbo, K.; Sano, A.; Fukase, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2044-2049.
[43] Fukase, K.; Oda, Y.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1758-1763.
[44] Mayer, J. P.; Zhang, J.; Groeger, S.; Liu, C.-f.; Jaronsinski, M. A. *J. Peptide Res.* **1998**, *51*, 432-436.
[45] Ösapay, G.; Goodman, M. *J. Chem. Soc. Chem. Commun.* **1993**, 1955-1600.
[46] Ösapay, G.; Zhu, Q.; Shao, H.; Chadha, R. K.; Goodman, M. *Int. J. Pept. Prot. Res.* **1995**, *46*, 290-301.
[47] Ösapay, G.; Prokai, L.; Kim, H.-S.; Medzihradszky, K. F.; Coy, D. H.; Liapakis, G.; Reisine, T.; Melacini, G.; Zhu, Q.; Wang, S. H. H.; Mattern, R.-H.; Goodman, M. *J. Med. Chem.* **1997**, *40*, 2241-2251.
[48] Photaki, I.; Samouilidis, I.; Caranikas, S.; Zervas, L. *J. Chem. Soc. Perkin Trans. 1* **1979**, 2599-2605.
[49] Li, H.; Jiang, X.; Goodman, M. *J. Peptide Sci.* **2001**, *7*, 82-91.
[50] Mustapa, M. F. M.; Harris, R.; Mould, J.; Chubb, N. A. L.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *Tetrahedron Lett.* **2002**, *43*, 8363-8366.
[51] Mustapa, M. F. M.; Harris, R.; Esposito, D.; Chubb, N. A. L.; Mould, J.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8193-8198.
[52] Yu, L.; Lai, Y.; Wade, J. V.; Coutts, S. M. *Tetrahedron Lett.* **1998**, *39*, 6633-6636.
[53] Campiglia, P.; Gomez-Monterrey, I.; Longobardo, L.; Lama, T.; Novellino, E.; Grieco, P. *Tetrahedron Lett.* **2004**, *45*, 1453-1456.
[54] Kelland, J. G.; Arnold, L. D.; Palcic, M. M.; Pickard, M. A.; Vederas, J. C. *J. Biol. Chem.* **1986**, *261*, 13216-13223.
[55] Breukink, E.; Wiedemann, I.; van Kraaij, C.; Kuipers, O. P.; Sahl, H.; de Kruijff, B. *Science* **1999**, *286*, 2361-2364.
[56] Wiedemann, I.; Breukink, E.; van Kraaij, C.; Kuipers, O. P.; Bierbaum, G.; de Kruijff, B.; Sahl, H. G. *J. Biol. Chem.* **2001**, *276*, 1772-1779.
[57] Breukink, E.; van Heusden, H. E.; Vollmerhaus, P. J.; Swiezewska, E.; Brunner, L.; Walker, S.; Heck, A. J.; de Kruijff, B. *J. Biol. Chem.* **2003**, *278*, 19898-19903.
[58] Brötz, H.; Bierbaum, G.; Reynolds, P. E.; Sahl, H. G. *Eur. J. Biochem.* **1997**, *246*, 193-199.
[59] Hsu, S. T.; Breukink, E.; Bierbaum, G.; Sahl, H. G.; de Kruijff, B.; Kaptein, R.; van Nuland, N. A.; Bonvin, A. M. *J. Biol. Chem.* **2003**, *278*, 13110-13117.
[60] Okeley, N. M.; Paul, M.; Stasser, J. P.; Blackburn, N.; van der Donk, W. A. *Biochemistry* **2003**, *42*, 13613-13624.

Received: 14 February, 2004 **Accepted: 30 April, 2004**