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Biomimetic Stereoselective Formation of Methyllanthionine

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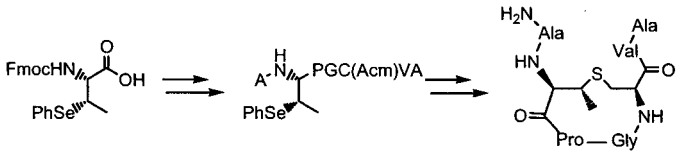
ABSTRACT



Fmoc-(2*R*,3*S*)-3-methyl-*Se*-phenylselenocysteine was used for the synthesis of dehydrobutyrine (Dhb)-containing peptides. Biomimetic cyclization via Michael addition of Cys to a Dhb yielded the B-ring of the lantibiotic subtilin as a single diastereomer. The methyllanthionine product was shown to have the natural configuration by preparation of the authentic stereoisomer. The formation of a single isomer suggests that the prepeptide has a strong intrinsic preference for the stereochemistry observed in lantibiotics.

Lantibiotics are polypeptides with promising biomedical applications against multidrug-resistant pathogens.[1,2] These ribosomally synthesized and posttranslationally modified peptides contain four unique structural motifs (Figure 1),

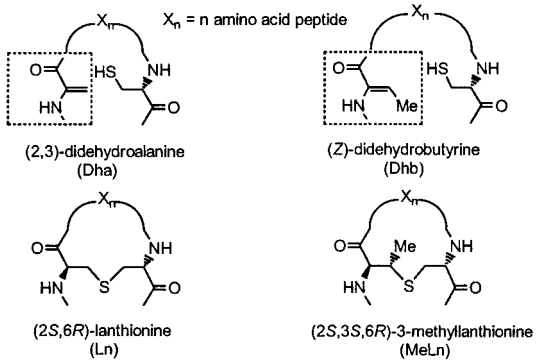
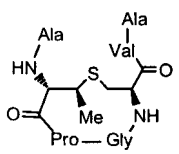


Figure 1.

introduced enzymatically by the LanB and LanC enzymes.[1] The LanB proteins are believed to dehydrate Ser and Thr residues to generate dehydroalanines (Dha) and dehydrobutyrines (Dhb), respectively, whereas LanC proteins are thought to control the subsequent Michael additions of cysteines. Biomimetic studies have shown that short peptides containing Dha and Cys residues undergo stereo- and regioselective cyclizations to give the natural (2*S*,6*R*)-lanthionines (Ln).[3] These studies suggest that the LanB products may have an intrinsic propensity for stereoselective protonation of the enolates formed in the Michael addition to provide the D-configuration at the new stereogenic center. The stereochemistry of methyllanthionine (MeLn) formation adds an additional degree of complexity since it also requires *si*-face selectivity in the addition of Cys to Dhb. We report here the first biomimetic formation of a MeLn.

The synthesis of linear and cyclic lanthionines and methyllanthionines has received much interest, both to understand the properties and biosynthesis of lantibiotics[3,4] and to prepare conformationally restricted peptides.[5] We focused our efforts on the biomimetic formation of the B-ring of subtilin (**1**). This thioether ring is highly conserved in a



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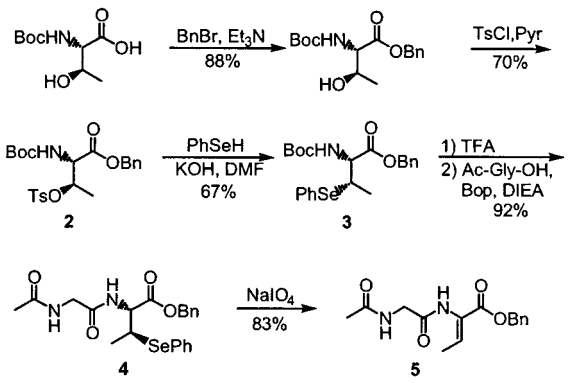
number of type A lantibiotics including nisin,[6] subtilin,[7] epidermin,[8] and gallidermin.[9] Analogues of the B-ring, containing lanthionine instead of methyllanthionine, have been prepared in several laboratories via cyclization of a Cys onto a Dha residue.[3] The authentic B-ring has so far only been prepared using desulfurization of the corresponding cyclic disulfide.[10] Investigation of its formation via a stereoselective Michael addition first required a general and facile route to (*Z*)-dehydrobutyrine.

Because dehydroamino acids are unstable and result in low coupling yields,[11] masked residues are usually employed during peptide synthesis and converted to the dehydroamino acids late in the synthetic route, preferably after global deprotection. Dhb residues have typically been incorporated into peptides via the activation and elimination of threonine derivatives.[12] Such an approach, however, is not compatible if the target contains unmodified Ser and Thr residues. We recently reported a facile, site-specific, and chemoselective method for introduction of Dha residues via the chemoselective oxidative elimination of selenocysteine derivatives.[3b] Given the well-known *syn* stereochemistry of selenoxide elimination,[13] extension of our methodology to the preparation of (*Z*)-Dhb demanded an enantioselective synthesis of (2*R*,3*S*)-3-methyl-*Se*-phenylselenocysteine.

The target compound was synthesized as depicted in Scheme 1. Boc-protected threonine was converted to the

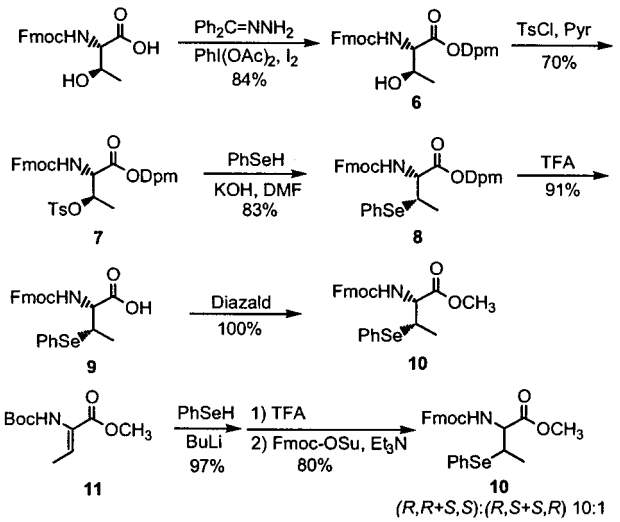
Scheme 1



benzyl ester, and its hydroxyl group was activated with *p*-toluenesulfonyl chloride. Nucleophilic displacement of the tosyl group of **2** with phenylselenolate afforded **3** in 67% yield. Only one diastereomer was detected, suggesting that the product was formed via a clean S_N2 reaction instead of an elimination−addition sequence (vide infra). Dipeptide **4** was synthesized by solution-phase chemistry. As anticipated, upon oxidation only the (*Z*)-isomer of Dhb was observed in the crude reaction mixture. Importantly, no γ-elimination to give allylglycine was detected. The product peptide was isolated in 83% yield after purification by silica gel flash chromatography.[14]

Encouraged by these results we sought to apply the methodology to Fmoc-based solid-phase peptide synthesis (SPPS).[15] Fmoc-(2*R*,3*S*)-3-methyl-*Se*-phenylselenocysteine (**9**) was prepared as illustrated in Scheme 2. Fmoc-threonine

Scheme 2



was protected as the diphenylmethyl (Dpm) ester, and the hydroxyl group was activated as before. Displacement of the

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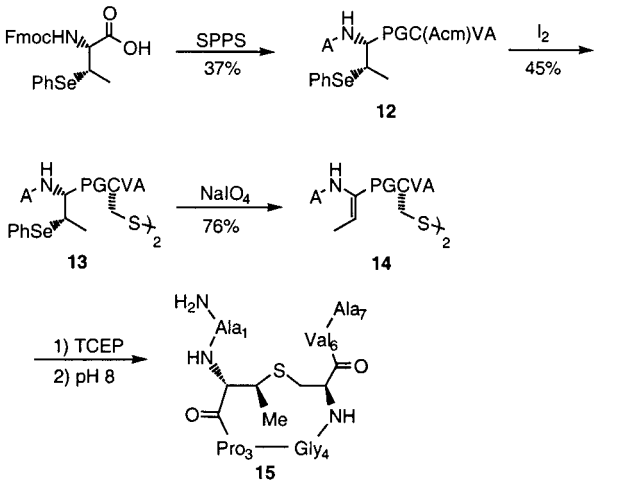
tosyl group with phenyl-selenolate and deprotection of the Dpm ester with TFA afforded the free carboxylic acid **9** needed for SPPS.

To verify the optical purity of **9**, it was converted to the methyl ester. A racemic mixture of diastereomers was also prepared via Michael addition of phenylselenolate to Fmoc-protected dehydrobutyrine methyl ester **11**. The latter reaction afforded *rac*-**10** with a diastereoselectivity of 10:1. The products were analyzed by HPLC on a Whelk-O1 chiral stationary phase.[16] For *rac*-**10** two sets of enantiomers were observed, whereas for the methyl ester obtained by the displacement of the toluenesulfonyloxy group, a single peak was detected,[17] verifying that the nucleophilic displacement occurred without loss of stereochemical purity.[18]

Monomer **9** was used to synthesize the B-ring of subtilin (Scheme 3). Peptide **12** was synthesized on Wang resin[19]

Scheme 3

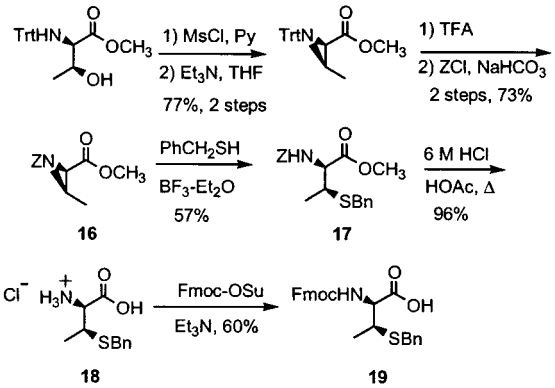


and purified by HPLC after cleavage from the solid support. The acetamidomethyl group was removed oxidatively with I_2 under acidic conditions to provide disulfide **13**, and chemoselective conversion of the phenylselenide to (*Z*)-dehydrobutyrine was achieved by oxidation with $NaIO_4$ in 76% yield after HPLC purification. The biomimetic cyclization of the methyllanthionine ring was achieved by reduction of the disulfide with tris(carboxyethyl)phosphine (TCEP)[20] and subsequent Michael addition at pH 8. This cyclization reaction took place with a rate significantly slower than that previously observed for the formation of the corresponding lanthionine.[3b] The structure of methyllanthionine **15** was confirmed by mass spectrometry and multidimensional NMR spectroscopy. ESI-MS and tandem MS-MS verified the correct molecular weight and indicated a predominant fragment ion at 412.3, corresponding to loss of the C-terminal Ala and Val residues. The ^{1}H NMR spectrum of the product in D_2O displayed two sets of peaks in a ratio of ~3:1. Previous NMR investigations by Goodman and co-workers on the B-ring indicated the presence of two slowly interconverting conformers in a similar ratio,[21] suggesting that the biomimetic cyclization in Scheme 3 provided a single diastereomeric product.

Standard two-dimensional NMR techniques were used to assign the resonances in peptide **15**. Because the peptide in the prior study[21] did not contain the flanking three amino acids present in **15**, the reported assignments could not be used directly to determine whether the cyclization provided the natural stereoisomer. The structural assignments of Goodman and co-workers did allow verification of several characteristic features observed previously. The amide proton of residue 5 occurs upfield of the other NH protons and undergoes very slow solvent exchange in D_2O. These spectral features indicate that this proton is engaged in a rigid hydrogen bond to the carbonyl of residue 2, as expected for a β-turn conformation.

The cyclization in Scheme 3 can give rise to four diastereomeric methyllanthionines: the natural[22] isomer (2*S*,3*S*,6*R*), as well as the 2*R*,3*R*,6*R*, 2*S*,3*R*,6*R*, and 2*R*,3*S*,6*R* diastereomers. To obtain conclusive evidence of the stereochemistry of the single observed product, we performed the reaction in the reverse direction, i.e., the Michael addition of (2*S*,3*S*)-3-methyl cysteine, located at the second residue of the peptide, to a dehydroalanine at the fifth position. This transformation could give rise to two diastereomeric products, (2*S*,3*S*,6*R*)- and (2*S*,3*S*,6*S*)-MeLn, only the former of which can be formed in Scheme 3. (2*R*,3*R*)-1-Benzyloxycarbonyl-3-methyl-2-aziridinecarboxylic acid methyl ester **16** was synthesized from D-threonine in six steps using modifications of existing procedures (Scheme 4).[4a,23] Ring opening of **16**

Scheme 4



with benzyl mercaptan in the presence of $BF_3{\cdot}Et_2O$ afforded **17**. Subsequent acidic hydrolysis yielded the hydrochloride

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(17) See Supplementary Information.

(18) Stereospecific displacements of the *p*-toluenesulfonyloxy group from the β-carbon of amino acids by sulfur and selenium nucleophiles has been previously reported: (a) Wakamiya, T.; Oda, Y.; Fukase, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 536. (b) Morell, J. L.; Fleckenstein, P.; Gross, E. *J. Org. Chem.* **1977**, *42*, 355. (c) Gieselman, M. D.; Xie, L.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 1331 and references therein.

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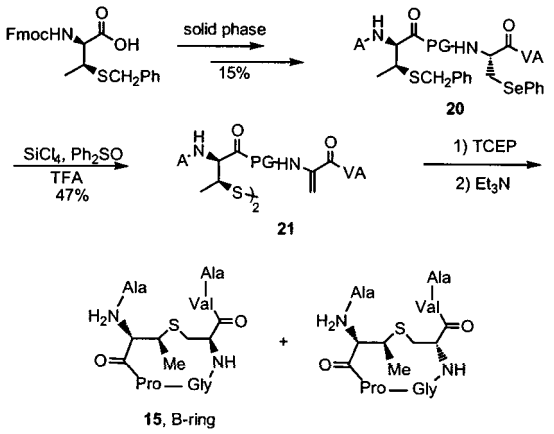
salt of *threo* D-β-methylcysteine, and the final product was obtained after protection of the free amine with Fmoc.

Compound **19** was utilized to synthesize peptide **20** on Wang resin (Scheme 5). Treatment with Ph_2SO and $SiCl_4$

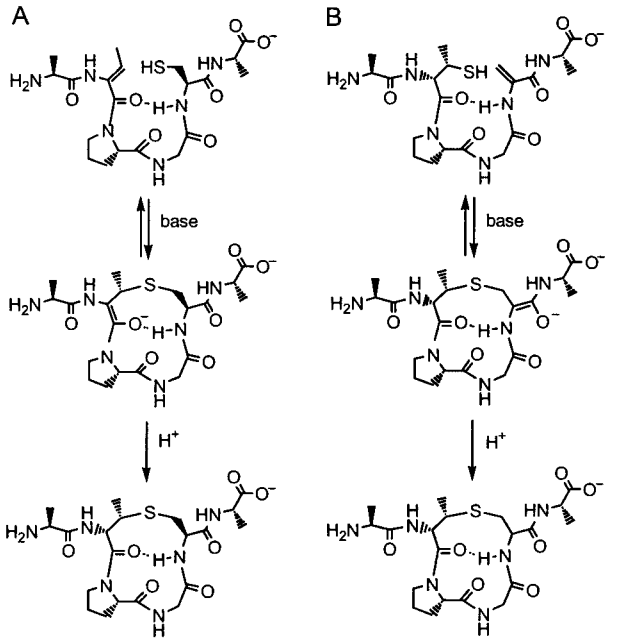
Scheme 5



in TFA[24] resulted in removal of the benzyl group and concomitant elimination of phenylselenide to give peptide **21**, which **21** was reduced with TCEP, followed by adjustment of the pH to 8−9 to initiate cyclization. Unlike previous studies on biomimetic lanthionine formation of four-residue lanthionines,[3,5a] which all resulted in one isomer, two product diastereomers were observed by HPLC in about equal quantities. One of the products was identical to the methyllanthionine obtained in Scheme 3 as evidenced by coelution in HPLC experiments and an identical ^{1}H NMR spectrum. Since the only isomer that can be formed in both cyclizations is the natural diastereomer, methyllanthionine **15** corresponds to the B-ring of subtilin. Thus, this cyclic peptide not only has an intrinsic disposition toward formation of the D-configuration at the α-carbon but also has a propensity toward *si*-face selectivity in the Michael addition at the β-carbon. Tetrameric peptides Xxx-Pro-Gly-Xxx have been shown to populate predominantly β- and γ-turn conformations with the relative preferences determined by the exact sequence.[25] As shown in Scheme 6A, a β-turn will bring the thiol of Cys and the Dhb in relatively close proximity. We have no experimental data that provides information on the reversibility of the initial addition, and consequently at present we cannot conclude whether the single product observed is the result of kinetic preference for *si*-face attack or for protonation of the enolate resulting from *si*-face attack.

The major difference between the cyclization in Scheme 5 and previous reports involves the direction of nucleophilic

Scheme 6



addition by the thiolate, which occurred in the C-to-N direction in prior investigations. Our findings suggest that the formation of essentially a single diastereomer in those reports[3,5a] is due to highly stereoselective protonation of an endocyclic enolate with an adjacent chiral center (Scheme 6A). In contrast, when an exocyclic enolate is formed as in Scheme 6B, the protonation occurs nonselectively. The generality of this observation is currently under investigation as it has interesting implications for the biosynthesis of type B lantibiotics, which contains thioether bridges formed between N-terminally positioned cysteines and C-terminally located Dha and Dhb residues.[1a,26]

In summary, we have developed methodology to install (*Z*)-dehydrobutyrine residues in peptides, and applied this technology to prepare the B-ring of subtilin via a stereoselective biomimetic cyclization.

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Supporting Information Available: Experimental procedures for all transformations that produced previously unknown compounds as well as their full spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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