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Biomimetic studies on the mechanism of stereoselective lanthionine formation †

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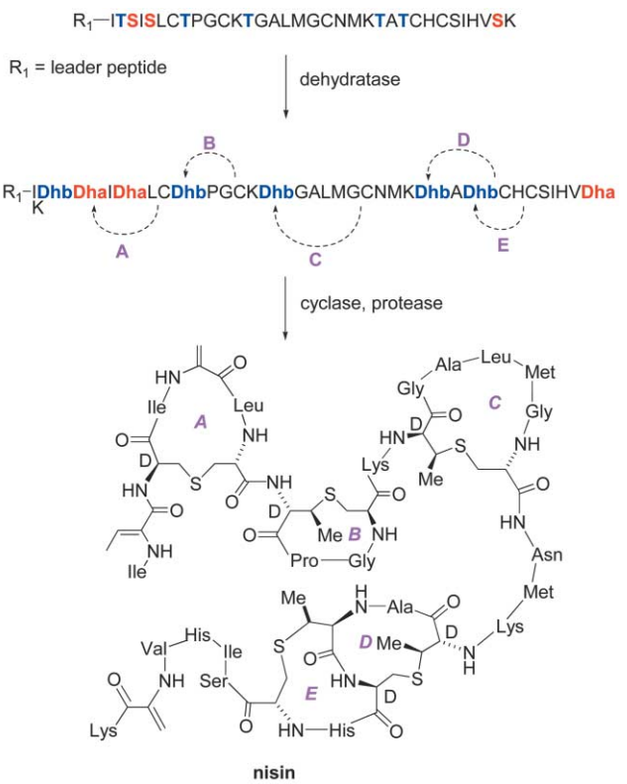
Received 12th May 2003, Accepted 15th August 2003
First published as an Advance Article on the web 10th September 2003

Selenocysteine derivatives are useful precursors for the synthesis of peptide conjugates and selenopeptides. Several diastereomers of Fmoc-3-methyl-*Se*-phenylselenocysteine (FmocMeSec(Ph)) were prepared and used in solid phase peptide synthesis (SPPS). Once incorporated into peptides, the phenylselenide functionality provides a useful handle for the site and stereospecific introduction of *E*- or *Z*-dehydrobutyrine residues into peptide chains *via* oxidative elimination. The oxidation conditions are mild, can be performed on a solid support, and tolerate functionalities commonly found in peptides, including variously protected cysteine residues. Dehydropeptides containing unprotected cysteine residues undergo intramolecular stereoselective conjugate addition to afford cyclic lanthionines and methyllanthionines, which have the same stereochemistry as found in lantibiotics, a family of ribosomally synthesized and post-translationally modified peptide antibiotics. The observed stereoselectivity is shown to originate from a kinetic rather than a thermodynamic preference.

Introduction

Lantibiotics are a class of post-translationally modified peptides that belong to the family of bacteriocins. They possess high antimicrobial activity against Gram-positive bacteria and have been used for a variety of applications including food preservation.[1–3] Among the family members is the promising antibacterial agent nisin that interacts with lipid II,[4] the physiological target of vancomycin. The proposed biosynthetic pathway for lantibiotics is shown for nisin in Scheme 1. The precursor peptides that are the substrates for post-translational modification consist of an *N*-terminal leader sequence that remains unchanged during maturation (R_1, Scheme 1) and a *C*-terminal propeptide that undergoes site-specific dehydration of serine and threonine residues catalyzed by a putative dehydratase to give dehydroalanine (Dha) and *Z*-dehydrobutyrine (Dhb) residues, respectively.[1,5–8] Subsequent Michael additions of cysteines to the Dha and Dhb residues catalyzed by a putative cyclase in a regio- and stereoselective manner produce thioethers with the D-configuration at the newly created stereogenic centers. The lantibiotics derive their name from these cyclic structures, which are called lanthionines when originating from Ser (*e.g.* ring A in nisin) and methyllanthionines when formed from Thr (*e.g.* rings B–E, Scheme 1). In the final step of maturation, the leader sequence is removed by LanP-catalyzed proteolysis and the lantibiotic is released.[9,10]

We have been interested in devising chemical methods for the incorporation of 1,2-didehydroamino acids into peptides through the use of selenocysteine derivatives[11] and have investigated the stereochemistry of non-enzymatic intramolecular Michael additions that produce lanthionines and methyllanthionines.[12,13] These studies as well as those in other laboratories[14–16] have shown that biomimetic intramolecular conjugate additions produce cyclic thioethers with the same stereochemistry as that found in lantibiotics. It is unclear at present whether the origin of this selectivity is thermodynamic or kinetic, and only the regiochemistry of the formation of the A-ring of nisin has been investigated.[15]



Scheme 1

Our previous studies have shown that cyclization of peptide **1** containing a *Z*-Dhb residue stereoselectively produces methyllanthionine **2** (Scheme 2),[13] the B-ring found in subtilin, a close relative of nisin. In the ^{1}H NMR spectrum of **2**, the amide proton of residue 5 occurs upfield of the other NH protons and undergoes very slow solvent exchange in D_2O, indicating that this proton is engaged in a rigid hydrogen bond, presumably to the carbonyl of residue 2 in a β-turn conformation (Scheme 2).[17] During the transition state of the conjugate addition leading to the enolate intermediate, this hydrogen bond may increase in strength and lower the energy barrier of the reaction. It is tempting to speculate that the β-turn

† Electronic supplementary information (ESI) available: separation of the diastereomers of **5**; cleavage of peptides from resins; COSY NMR spectrum of the product obtained from cyclization of both *E*-**1** and **19**. See http://www.rsc.org/suppdata/ob/b3/b304945k/

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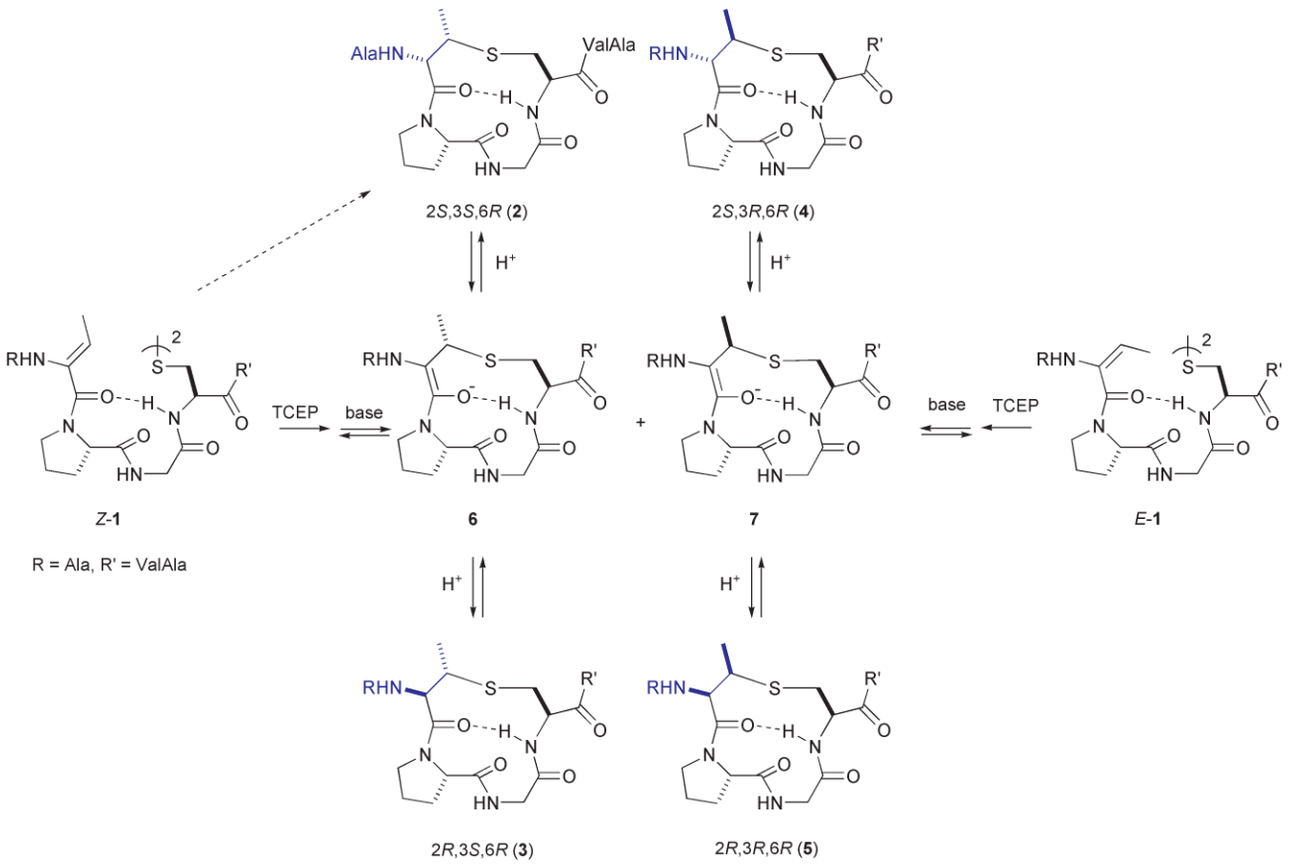


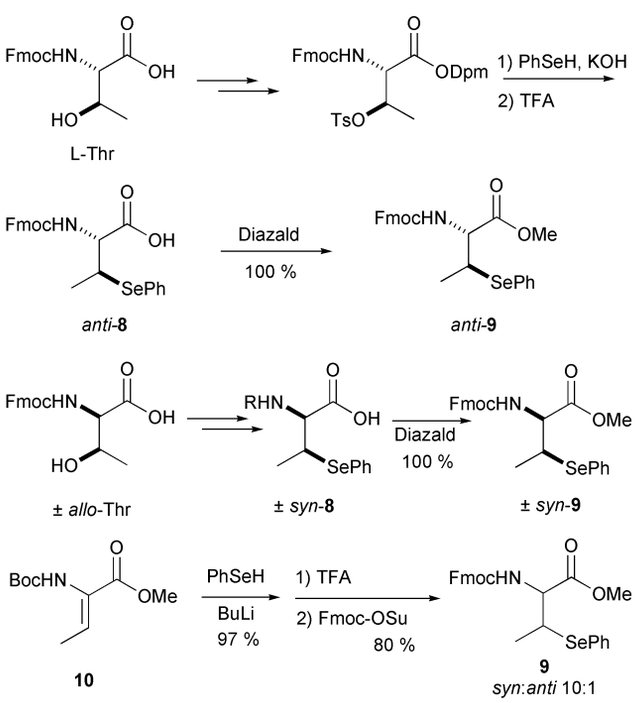
Scheme 2

conformation is also responsible for the intrinsic propensity to form just one of four possible diastereomers, but other explanations are possible. For instance, the observed high stereoselectivity could have a thermodynamic rather than a kinetic origin if the Michael addition is reversible under the reaction conditions. In that scenario, reduction of the disulfides of *Z*-**1** and its geometric isomer *E*-**1** would generate thiolate isomers that would equilibrate with the same pair of enolates and/or methyllanthionine products (Scheme 2). Hence, both reactions should provide identical products. In this report, we present new methodology to prepare *E*-Dhb residues within peptides to provide insights into this question. Along the way, we also explored the use of solid phase reaction conditions for our oxidative elimination methodology to install dehydro amino acids and investigated the regiochemistry of the formation of the A and B rings of nisin.

Results and discussion

Synthesis of selenocysteine derivatives

In order to test the model in Scheme 2, an efficient synthesis of *E*-Dhb-containing peptides was required. We previously reported the preparation of Fmoc-(2*R*,3*S*)-3-methyl-*Se*-phenylselenocysteine (*anti*-**8**)[13] for use in solid phase peptide synthesis (SPPS).[18] Our design to utilize selenocysteine derivatives for the preparation of stereodefined dehydrobutyrine containing peptides rested on the supposition that selenides could be selectively oxidized in the presence of unprotected functionalities in peptides. Given the known *syn*-selectivity of the oxidative elimination,[19,20] we reasoned that the *Z*- or *E*-isomers of dehydrobutyrine could be stereospecifically accessed from *anti*-FmocMeSec(Ph) or *syn*-FmocMeSec(Ph), respectively. To test this strategy, racemic *syn*-**8** was accessed from ±-*allo*-threonine in analogous manner to the preparation of *anti*-**8**[13] as shown in Scheme 3. For the purpose of verifying clean inversion at C3 during the displacement of the sulfonate, *anti*-**8** and *syn*-**8** were transformed to their methyl esters *anti*- and *syn*-**9**. A racemic mixture of all four possible diastereomers of **9** was also prepared *via* Michael addition of phenylselenolate to Boc-protected dehydrobutyrine methyl ester **10**. The latter reaction afforded *rac*-**9** with a diastereoselectivity of 10 : 1 in favor of the *syn*-stereoisomers. The product mixture could be separated into four near baseline-resolved peaks by HPLC on a Whelk-O1 chiral stationary phase[21] (ESI †). The first two eluting



Scheme 3

Table 1 Oxidation of Sec(Ph) and MeSec(Ph) containing peptides[a]

Entry	Peptide	Dehydropeptide[b]	Solvent	Yield (%)[c]
1	[Boc-Sec(Ph)Cys-OMe]$_2$	[Boc-DhaCys-OMe]$_2$	MeOH	75
2	Fmoc-Cys(SEt)Sec(Ph)-ODpm	Fmoc-Cys(SEt)Dha-ODpm	CH_2Cl_2/MeOH	70
3	Ac-Gly-(2*R*,3*S*)MeSec(Ph)-OBn	Ac-Gly-(*Z*)-Dhb-OBn	CH_2Cl_2/MeOH	83
4	Ac-Gly-*syn*-MeSec(Ph)-OBn	Ac-Gly-(*E*)-Dhb-OBn	CH_2Cl_2/MeOH	79
5	[A-(2*R*,3*S*)-MeSec(Ph)-PGCVA]$_2$[d]	[A-(*Z*)-Dhb-PGCVA]$_2$	H_2O/MeCN	76
6	[A-*syn*-MeSec(Ph)-PGCVA]$_2$[d]	[A-(*E*)-Dhb-PGCVA]$_2$	H_2O/MeCN	68

[a] Dha = dehydroalanine, Dhb = dehydrobutyrine, Sec(Ph) = (*Se*)-phenylselenocysteine. [b] Conditions for entries 1-4: aq $NaIO_4$ (4 equiv.) was added at 25 °C to solutions of the peptides (final peptide concentrations 7-30 mM). For entries 5 and 6, the final concentrations of the peptides were 3 mM. [c] Yields are for HPLC purified products. [d] The symmetrical disulfide was formed by oxidation with I_2 of the purified peptide containing Acm-protected Cys.

compounds showed identical retention times as ±-*syn*-**9** prepared from *allo*-Thr according to Scheme 3. The last isomer to elute was identified as (2*R*,3*S*)-**9** (*anti*-**9**) by coinjection with the material prepared from L-Thr. This latter compound displayed a single peak verifying that the nucleophilic displacement of the *p*-toluenesulfonate in Scheme 3 occurred without loss of stereochemical purity provided the stoichiometry of the reagents and the order of addition are carefully controlled (see experimental).

Preparation of dehydropeptides

We have recently shown that the oxidative elimination is compatible with oxidation sensitive amino acids, such as methionine, trityl or disulfide protected cysteines, and tryptophan unprotected at the indole moiety.[12] Synthetic trityl protected cysteine residues can be chemoselectively oxidized with I_2 to the corresponding symmetrical disulfides without modification of the selenide. Conversely, the phenylselenide can be oxidatively eliminated with $NaIO_4$ without effecting trityl or disulfide protected cysteines (*e.g.* Table 1, entries 1 and 2). The ability to selectively oxidize cysteines to disulfides or selenocysteines to dehydroalanines proved extremely useful for the preparation of lanthionines (*vide infra*). As envisioned, oxidative elimination of (2*R*,3*S*)-MeSec(Ph) produced only the *Z*-isomer of dehydrobutyrine (entry 3). This outcome could either reflect the preferential elimination of the more acidic α-proton over the γ-methyl protons, or alternatively it could be the result of initial elimination of a γ-proton to provide vinylglycine, which subsequently rearranged to the thermodynamically more stable *Z*-dehydrobutyrine. This latter possibility was ruled out by the formation of *E*-dehydrobutyrine when the experiment was performed with a peptide containing *syn*-MeSec(Ph) (entry 4).

The versatility of this chemoselective and site-specific methodology is demonstrated with longer peptides prepared by SPPS. As with cysteine derivatives, caution must be taken to avoid racemization during coupling of the Sec-derivatives in SPPS. This was achieved using DIC/HOBt as coupling reagents thereby avoiding the use of tertiary amine bases.[22] Furthermore, higher yields of purified peptides were obtained when deprotection protocols with piperidine were kept short. With longer exposure times, elimination of phenylselenide occurs to some extent as evidenced by detection of β-piperidylalanine containing peptides (M-71) in the crude product after cleavage from the resin. With these precautions, our yields of purified Sec containing peptides were comparable to those obtained for other peptides of similar length prepared in our laboratory. The oxidations of the purified peptides in entries 5 and 6 proceeded slowly as monitored by RP-HPLC, with MS analysis indicating a rapid initial oxidation to the selenoxide and a slow subsequent elimination (5 hours). Typical functionalities found in peptides such as amines, amides, guanidines, alcohols, and acids do not interfere when left unprotected during the oxidation.[12] However, when an unprotected *N*-terminal Ser or Thr is present, H_2O_2 must be used as oxidant since periodate would oxidize these amino acids.[23]

Preparation and use of dehydropeptides on solid phase

In principle, both oxidative elimination to form dehydropeptides and subsequent Michael addition should be amenable to solid phase techniques. The feasibility of this approach has been explored using a model tripeptide, Ac-Ala-Sec(Ph)-Ala, bound to Wang resin. Both the oxidative elimination and subsequent Michael addition of an external nucleophile were monitored by on-resin magic angle spinning (MAS) NMR spectroscopy[24,25] with spin echo enhancement.[26,27] Diagnostic peaks were observed in the starting material at 8.25 ppm corresponding to three overlapping amide protons, and at 3.27 ppm associated with the methylene protons of Sec(Ph) (Fig. 1A). Upon treatment with 5 equivalents of H_2O_2 in DMF, the three amide protons resolved into three distinct resonances at 9.21, 8.84, and 8.42 ppm (Fig. 1B). In addition, the methylene peak at 3.27 ppm disappeared and a new resonance at 6.40 ppm due to one of the vinyl protons of the newly formed dehydroalanine appeared. 2-Mercaptoethylamine was then used as a nucleophile to add to the dehydropeptide on the resin (Fig. 1C). During this reaction, the three amide proton resonances were converted into two sets of diastereomeric amide proton signals (8.67, 8.58, 8.41, and 8.36 ppm). Furthermore, the vinyl proton of dehydroalanine disappeared, and a new group of peaks between 3.00–3.40 ppm emerged that corresponded to the newly generated methylene protons of the amino acid.

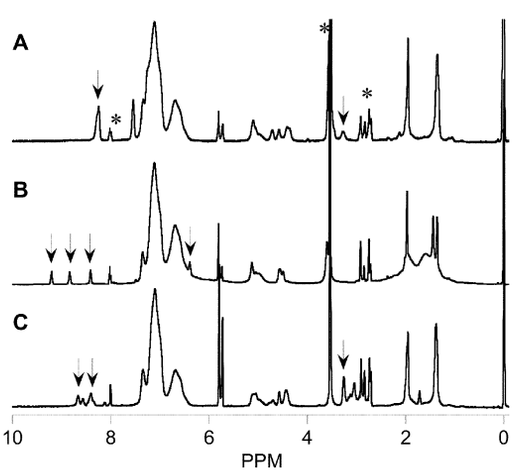


Fig. 1 MAS spin echo enhanced NMR spectroscopy of (A) Ac-Ala-Sec(Ph)-Ala bound to Wang resin, (B) after treatment for 45 min with H_2O_2, and (C) after addition of 2-mercaptoethylamine. Arrows indicate characteristic resonances discussed in the text.

In addition to NMR spectroscopy, the Michael addition reaction was also monitored by quantitative ninhydrin test of the free amine group that is added during the transformation.[15,28] Fig. 2A shows the increase in absorbance at 570 nm corresponding to the ninhydrin product *versus* time. Also depicted is the time dependent increase in the integration of the resonance at 3.26 ppm of the product. Non-linear regression

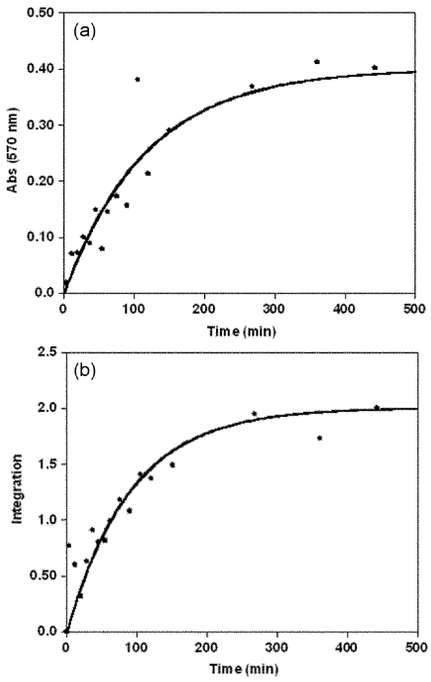
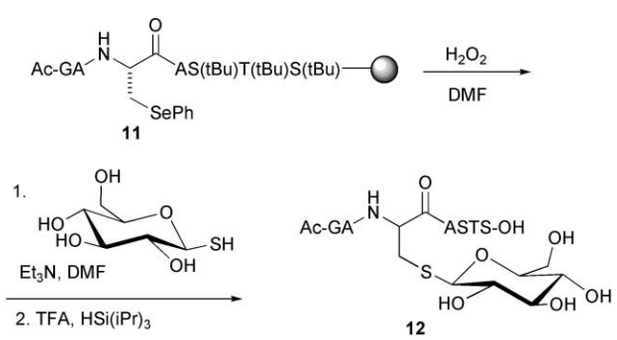


Fig. 2 Kinetics of Michael addition of 2-mercaptoethylamine to Ala-Dha-Ala on Wang resin determined by quantitative ninhydrin test (A) and ^{1}H-MAS-NMR spectroscopy (B).

analysis of the data using a single exponential equation provided rate constants of $(8.5 \pm 1.0) \times 10^{-3}$ min^{-1} (Fig. 2A) and $(11.0 \pm 1.0) \times 10^{-3}$ min^{-1} (Fig. 2B). In both cases, the graphs indicate that the Michael addition appears to be complete after 6 h. Thus, phenylselenocysteines can be transformed into dehydroalanines under mild oxidizing conditions on the solid phase, and the product can be used for subsequent Michael addition. To evaluate the generality of these results, a solid phase conjugate ligation was used to synthesize glycopeptide **12** (Scheme 4). Oxidative elimination of resin-bound peptide **11**, subsequent Michael ligation and deprotection and cleavage from the resin provided glycoconjugate **12** after HPLC purification in 32–45% overall yield based on resin loading.[29] This represents a significant improvement compared to performing the oxidation and Michael addition in solution,[30] in part as a result of decreasing the number of HPLC purifications from three to one.

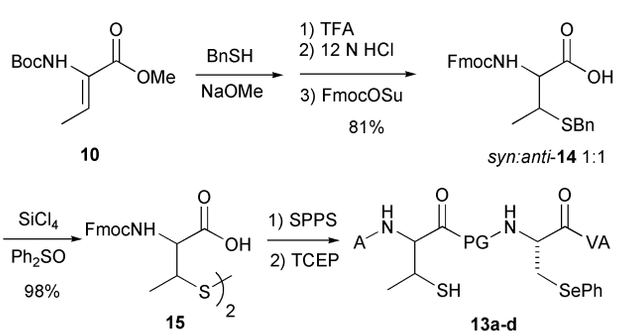


Scheme 4

Mechanistic studies on the biomimetic formation of methyllanthionines

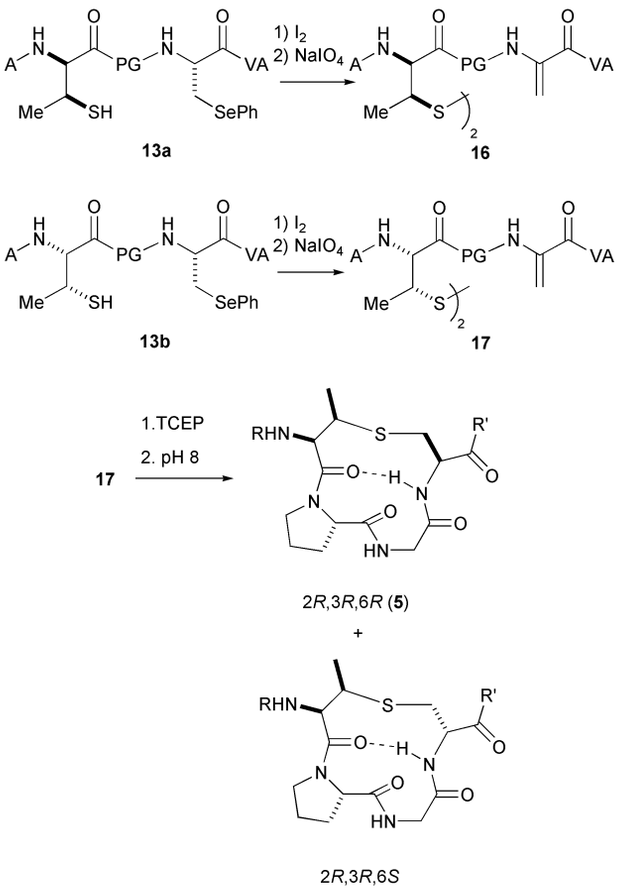
The synthesis of *E*-**1** was accomplished by incorporation of the precursor *syn*-**8** into the peptide by SPPS and subsequent oxidation with $NaIO_4$. The *E*-stereochemistry of the Dhb generated was confirmed by ^{1}H NMR spectroscopy. The *Z*-isomer of peptide **1** was prepared as previously described.[13] If the cyclization reaction were under thermodynamic control, then upon reduction of the disulfides of *Z*-**1** and *E*-**1** both thiolate isomers would equilibrate with the same pair of enolates and/or methyllanthionine products (Scheme 2), and both reactions should provide identical products. Similar to our previous report on the cyclization of *Z*-**1**,[13] the reaction with *E*-**1** indeed produced one major peak when analyzed by HPLC. However, the ^{1}H NMR spectrum of the product indicated that it consisted of a different MeLn isomer than was obtained with *Z*-**1**. This rules out a thermodynamic origin of the observed stereoselectivity. The product formed with *Z*-**1** therefore originates from preferential *Si*-face attack on the *Z*-Dhb residue to provide **6**, followed by protonation of the enolate from the least hindered side to give **2** (Scheme 2). On the other hand, the Michael addition with *E*-**1** must provide **7** since otherwise the same final product should have been obtained. Hence, both Michael additions involve preferential attack of the thiolate on the bottom face (as drawn in Scheme 2) of either geometric isomer of dehydrobutyrine.

In analogy to **6**, we anticipated that the protonation of enolate **7** in the cyclization of *E*-**1** would occur *anti* to the methyl group on the β-carbon to produce (2*R*,3*R*,6*R*)-3-methyllanthionine **5**. Authentic methyllanthionines **4** and **5** were required to confirm this hypothesis. It was envisioned that these targets could be obtained by performing the Michael reaction in the reverse direction with stereodefined 3-methylcysteine residues at the second position of the peptide and a dehydroalanine at the fifth position. Such transformations could give rise to two diastereomeric methyllanthionine products with either the L or D-configuration at the newly formed α-stereocenter of the fifth residue, only the former of which can be formed according to Scheme 2. Four diastereomeric peptides **13a–d** were prepared as shown in Scheme 5. Conjugate addition of benzylthiol to Boc-protected *Z*-dehydrobutyrine methyl ester **10** followed by protecting group exchange produced a 1 : 1 mixture of diastereomers **14** that were debenzylated and converted to the corresponding disulfides **15**. The mixture of diastereomers of **15** was used in SPPS to produce four diastereomeric peptides **13** that were separated by HPLC and oxidized to the corresponding Dha-containing disulfides (Scheme 6). One of the diastereomers was identified as **16** by comparison of its NMR spectrum with authentic material prepared in our previous work.[13] A second diastereomer was assigned structure **17** by use of (±)-*syn*-**14**, which resulted in a mixture of peptides **16** and **17**. Reduction of the disulfide of dehydropeptide **17** followed by increasing the pH of the reaction mixture led to the formation of two methyllanthionine diastereomers, neither of which coeluted with the product formed from peptide *E*-**1**. Hence, contrary to our expectation, (2*R*,3*R*,6*R*)-3-methyllanthionine **5** was not produced in the cyclization of *E*-**1**.

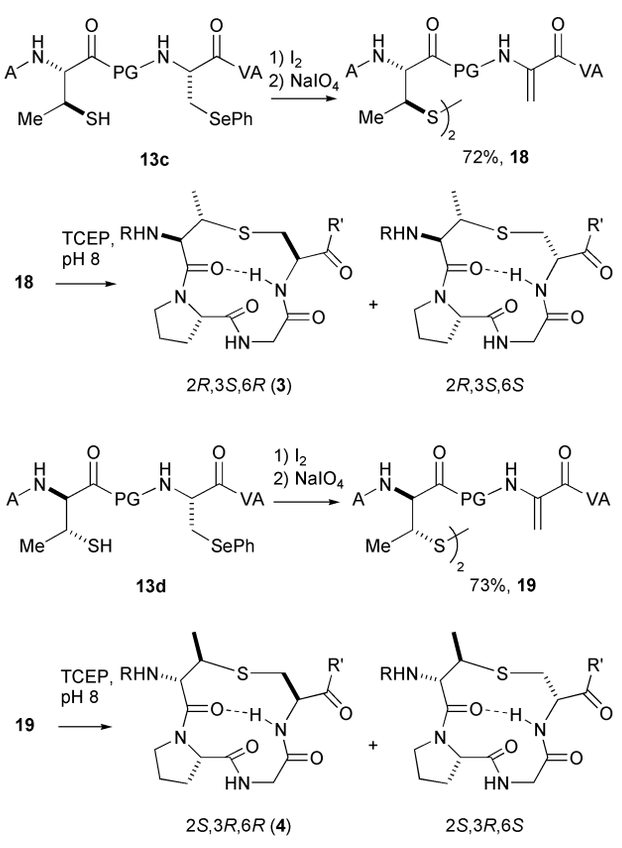


Scheme 5

The two remaining diastereomers of **13** were therefore also oxidized to the corresponding Dha-containing disulfides (Scheme 7). Both peptides were subjected to the conditions for cyclization resulting in a ~3 : 1 mixture of methyllanthionine diastereomers for both reactions. HPLC analysis showed that in one reaction neither product corresponded to the methyl-



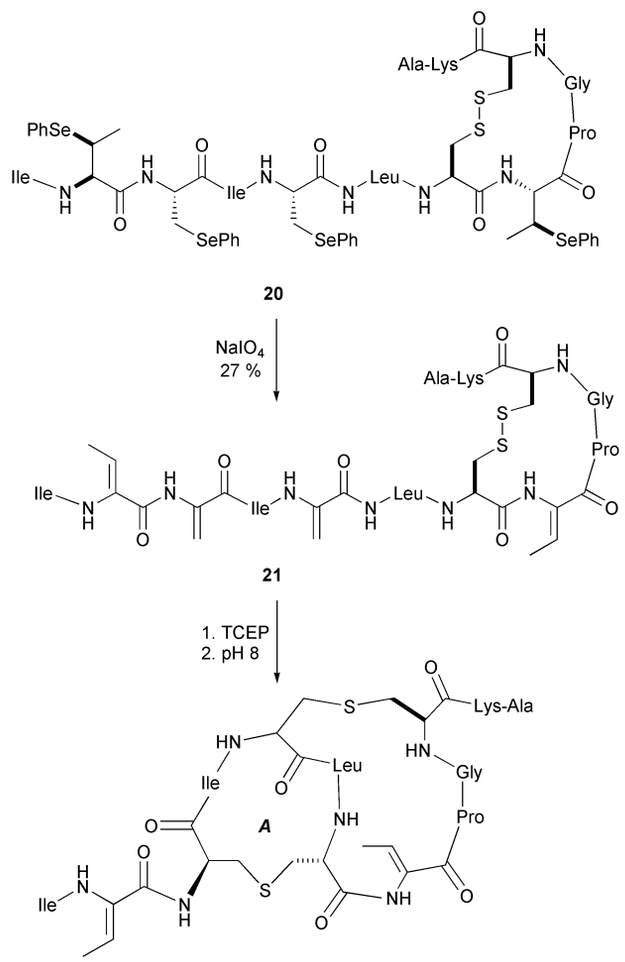
Scheme 6



Scheme 7

lanthionine formed from *E*-**1**. On the basis of this result, the cyclization precursor was assigned structure **18** since its products would be different from those that can be accessed from *E*-**1**. Indeed, the NMR spectra of the cyclization products from the final diastereomeric peptide **19** showed that one of the two methyllanthionine isomers displayed an identical ^{1}H NMR spectrum as that formed from *E*-**1** and the two compounds also coeluted in HPLC experiments. Since only **4** can be formed in the cyclizations of both **19** and *E*-**1**, we conclude that enolates **6** and **7** are both protonated from the *Re*-face irrespective of the configuration at C3 of the methyllanthionine. Because the face selectivity of the initial attack by the thiolate and the protonation of the resulting enolate are the same for both isomers of **1**, the observed stereochemistry in these reactions is dominated by the conformation of the peptide backbone.

Given the stereoselective non-enzymatic formation of the natural diastereomers of individual lanthionines and methyllanthionines shown in this and previous work,[12–16] we decided to investigate to what extent the regio- and stereoselectivity is governed by the dehydropeptide itself. To this end, using our synthetic methodology we prepared dehydropeptide **21** (Scheme 8), a potential precursor to the A and B rings of nisin



Scheme 8

(Scheme 1). The prospect of preparing an *N*-terminal fragment of nisin by biomimetic cyclization is intriguing given the reported importance of this region for binding to its lipid II target.[31] In whole cell assays, nisin 1–12 acts as an antagonist for binding of wild-type nisin.[32] The biosynthetic machinery does not allow non-conservative mutations in this region of the antibiotic,[33,34] and hence a biomimetic synthetic route would be extremely useful for structure–activity relationship studies.

Peptide **20** was prepared by SPPS and the two Dha and two Dhb residues were oxidatively unmasked in 27% yield after HPLC purification. Although the overall yield is low, four dehydro amino acids were installed in one step. The intramolecular disulfide was reduced and upon addition of base,

cyclization took place as evidenced by HPLC and MS-MS. ^{1}H NMR analysis of the crude reaction mixture showed 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[13] prevents the biomimetic cyclization in which one Ln (A-ring) and one MeLn (B-ring) would have been formed (Scheme 1). At present, we tentatively assign the structure drawn in Scheme 8 to the product. Bradley and coworkers have shown that Cys^7 reacts preferentially with Dha^3 over Dha^5 to give a five-residue lanthionine ring as a 3 : 1 mixture of diastereomers.[15] We postulate that after this initial cyclization, Cys^{11} reacts with Dha^5 to form a six-residue lanthionine ring. LC-MS analysis showed that at least two product isomers are formed, which could be either diastereomers or constitutional isomers. Since the cyclization did not lead to the formation of the rings found in nisin, no further attempts were made to elucidate the exact structures of the products. The important conclusion with respect to the biosynthesis of lantibiotics drawn from these studies is that lantibiotics biosynthesis requires enzymatic control over the regioselectivity and/or processivity of the cyclization reactions.

Summary

We demonstrated the use of *syn*- and *anti*-**8** for the stereospecific introduction of *E*- and *Z*-dehydrobutyrine residues into peptides. The method installs the dehydro amino acids after global deprotection in the ultimate step of the synthesis of dehydropeptides, minimizing possible side reactions, and is fully compatible with all physiological amino acids including appropriately protected cysteines. The oxidation step can be carried out on a solid support and can be conveniently monitored by solid state magic angle spinning NMR spectroscopy. The utility of the methodology is demonstrated by the solution phase biomimetic syntheses of lanthionines and methyllanthionines. These studies show that while the precursor peptides to the lantibiotics have an inherent propensity to cyclize to the naturally occurring stereoisomers, enzymatic control must be exerted to ensure proper regioselectivity and to overcome the much lower reactivity of dehydrobutyrines compared to dehydroalanines. Our mechanistic studies indicate that the observed highly selective stereochemical outcome in the intramolecular Michael additions has a kinetic rather than a thermodynamic origin.

Experimental

General details

Protected amino acids were purchased from either Advanced ChemTech or Chem-Impex. Wang resins pre-loaded with the *C*-terminal Fmoc-protected amino acid were obtained from Advanced ChemTech. MeOH and EtOH were purified *via* treatment with Mg and I_2 followed by distillation, THF was distilled from sodium/benzophenone, CH_2Cl_2 was distilled from CaH_2, and pyridine was distilled from CaH_2 onto 4 Å molecular sieves and stored under Ar. Automated SPPS were performed on a Rainin model PS3 peptide synthesizer. RP-HPLC purification was performed on either a Rainin Dynamax system or a Beckman System Gold with Vydac C18 analytical or preparative columns. ^{1}H and ^{13}C NMR data were obtained on either a Varian U400 or U500 spectrophotometer in $CDCl_3$ or D_2O. Optical rotations were recorded on a JASCO DIP-360 Digital Polarimeter. Melting points were measured on a Fisher melting apparatus without correction. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX. Mass spectrometry of peptides was performed by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. For larger peptides, matrix assisted laser desorption ionization (MALDI) MS was carried out on an Applied Biosystems Voyager DE STR or electrospray ionization (ESI) MS was used employing a Finnigan LCQ Deca XP or a Micromass Quattro. Fast atom bombardment (FAB) ionization was used for smaller peptides and organic molecules employing a Micromass 70-VSE instrument for low resolution and a Micromass 70-SE-4F for high resolution measurements. CHN elemental analysis was performed by the Microanalysis Laboratory, University of Illinois with a Leeman Labs Inc. Model DE 440 elemental analyzer. Described in the electronic supplementary information † are the procedures used to determine the stereochemical purity of *anti*-**8** and a COSY spectrum of compound **4**.

Fmoc-*allo*-(±)-Thr-ODpm

Fmoc-(±)-*allo*-threonine (1.65 g, 4.85 mmol) was placed in a round bottom flask with diphenylmethyl hydrazone (1.86 g, 9.70 mmol), and the mixture was suspended in CH_2Cl_2 (16 mL). A 1% (w/v) solution of I_2 in CH_2Cl_2 (1.0 mL) was added to the mixture at 0 °C. Iodobenzenediacetate (3.12 g, 9.70 mmol) was added slowly over 1.5 h as the mixture was stirred under Ar. The reaction was stirred at 0 °C for 3 h. The solvent was evaporated and the resulting yellow oil was dissolved in EtOAc (25 mL). The organic layer was washed with H_2O (20 mL), saturated aqueous $NaHCO_3$ (3 × 20 mL), and H_2O again (20 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated to give a yellow oil. The oil was dissolved in EtOAc and loaded onto a silica plug. Impurities were washed through with 8 : 1 hexanes : EtOAc, then the plug was washed with 30 : 1 CH_2Cl_2 : CH_3OH to elute the product (2.20 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.09 (d, J = 6.57 Hz, 3H), 1.19–1.23 (m, 2H), 4.23 (t, J = 7.05 Hz, 1H), 5.61 (br s, 1H), 5.69 (d, J = 7.22 Hz, 1H), 6.93 (s, 1H), 7.27–7.43 (m, 13H), 7.46–7.48 (m, 1H), 7.58 (d, J = 5.54 Hz, 2H), 7.76 (d, J = 7.71 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 20.1, 47.4, 59.5, 67.5, 68.3, 78.6, 120.2, 125.4, 127.3, 128.0, 128.4, 128.9, 139.6, 141.5, 143.9, 144.1, 157.0, 170.5; FAB-HRMS *m*/*z* calcd for $C_{32}H_{29}O_5NNa$ $(M + Na)^+$ 530.1943, found 530.1945.

Fmoc-*allo*-(±)-Thr(OTs)-ODpm

Fmoc-*allo*-(±)-threonine-ODpm (2.8 g, 5.52 mmol) was dissolved in pyridine (20 mL), cooled to 0 °C, and TsCl (2.6 g, 13.8 mmol) was added. The mixture was stirred at 0 °C for 2 d. The mixture was diluted with EtOAc (100 mL) and washed with 5% $KHSO_4$ (50 mL × 5). The organic layer was dried with $MgSO_4$ and concentrated. Crystallization from ether afforded 2.9 g (79%) of a white solid. ^{1}H NMR (500.0 MHz, $CDCl_3$): δ ppm 1.35 (d, J = 6.44 Hz, 3H), 2.36 (s, 3H), 4.17 (t, J = 7.09 Hz, 1H), 4.26–4.35 (m, 2H), 4.56 (d, J = 7.39 Hz, 1H), 4.98 (m, 1H), 5.60 (d, J = 8.52 Hz, 1H), 6.90 (s, 1H), 7.27–7.43 (m, 16H), 7.57 (d, J = 7.40 Hz, 2H), 7.62 (d, J = 8.07 Hz, 2H), 7.67 (d, J = 7.46 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 18.19, 22.02, 47.46, 58.26, 68.01, 78.70, 79.36, 120.24, 125.43, 127.24, 127.39, 127.54, 128.01, 128.15, 128.49, 128.56, 128.85, 130.08, 139.20, 141.50, 143.94, 145.23, 155.80, 167.44.

Fmoc-(±)-*syn*-MeSec-ODpm

KOH (0.426 g, 7.60 mmol) was dissolved in MeOH (10 mL), and PhSeH (1.37 g, 8.76 mmol) was added under Ar. The mixture was stirred at room temperature for 10 min. The solvent was removed to afford an off-white solid. Fmoc-*allo*-(±)-Thr(OTs)-ODpm (2.52 g, 3.80 mmol) in DMF (12 mL) was cooled to 0 °C and transferred to the above solid. The orange mixture was stirred at room temperature for 4 h. Ice-cold 5% aqueous $KHSO_4$ (100 mL) was added, and the mixture was extracted with EtOAc (250 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL), brine (50 mL), dried, and

concentrated. Purification by flash chromatography[35] (hexanes : EtOAc = 5 : 1) afforded a white solid (1.71 g, 70%; R_f = 0.36, 5 : 1 hexanes : EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.50 (d, J = 7.09 Hz, 3H), 3.82 (qd, J = 7.12 Hz, 3.81 Hz, 1H), 4.23 (t, J = 7.07 Hz, 1H), 4.39 (m, 2H), 4.79 (dd, J = 8.86 Hz, 3.65 Hz, 1H), 5.60 (d, J = 9.00 Hz, 1H), 6.77 (s, 1H), 7.20 (t, J = 7.49 Hz, 2H), 7.27–7.42 (m, 10H), 7.48 (d, J = 7.15 Hz, 2H), 7.59 (d, J = 7.48 Hz, 2H), 7.79 (d, J = 7.41 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 20.46, 42.11, 47.36, 59.82, 67.69, 79.17, 120.25, 125.41, 127.30, 127.33, 127.59, 127.96, 128.35, 128.42, 128.72, 128.81, 129.33, 135.79, 139.56, 139.67, 141.57, 156.58, 170.02.

Fmoc-(±)-*syn*-MeSec-OH (*syn*-8)

Fmoc-(±)-syn-MeSec-ODpm (1.20 g, 1.86 mmol) was dissolved in 10 mL of CH_2Cl_2/TFA (1/1), and triisopropylsilane was dropped in until the mixture turned colorless. The solution was stirred at room temperature for 1 h, and the solvent was removed by rotary evaporation. Purification by flash chromatography (CH_2Cl_2 : MeOH = 20 : 1) afforded a solid (0.737 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.55 (d, J = 7.19 Hz, 3H), 3.88 (qd, J = 7.14 Hz, 3.44 Hz, 1H), 4.28 (t, J = 7.30 Hz, 1H), 4.46 (d, J = 7.33 Hz, 2H), 4.69 (dd, J = 9.17 Hz, 3.37 Hz, 1H), 5.63 (d, J = 9.52 Hz, 1H), 7.23–7.29 (m, 5H), 7.36 (t, J = 7.33 Hz, 3H), 7.44 (td, J = 7.27 Hz, 2.04 Hz, 2H), 7.62 (dd, J = 12.33 Hz, 7.40 Hz, 4H), 7.81 (d, J = 7.42 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 20.18, 41.27, 47.28, 59.33, 67.61, 120.21, 125.40, 127.34, 127.97, 128.56, 129.35, 135.90, 141.52, 143.86, 144.07, 156.75, 175.39.

Fmoc-(±)-*syn*-MeSecOMe (±-*syn*-9)

An Aldrich Mini Diazald Apparatus was used. A solution of diazald (0.044 g, 0.21 mmol) in 3 mL of Et_2O was used to generate diazomethane, which was condensed and dropped into a solution of Fmoc-(±)-*syn*-MeSec-OH (0.020 g, 0.042 mmol) dissolved in 5 mL of ethyl ether in the receiving flask cooled in an ice/salt bath at −15 °C. After the solvent was evaporated, the product was obtained (0.020 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.52 (d, J = 7.13 Hz, 3H), 3.33 (s, 3H), 3.86 (qd, J = 7.20 Hz, 3.60 Hz, 1H), 4.25 (t, J = 7.21 Hz, 1H), 4.39 (d, J = 7.09 Hz, 2H), 4.64 (dd, J = 9.28 Hz, 3.44 Hz, 1H), 5.64 (d, J = 9.24 Hz, 1H), 7.29–7.42 (m, 5H), 7.41 (m, 2H), 7.61 (m, 4H), 7.78 (d, J = 7.19 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 20.63, 42.58, 47.46, 52.68, 58.96, 67.67, 120.30, 125.49, 127.43, 128.05, 128.49, 129.37, 135.79, 141.60, 144.06, 144.24, 156.64, 170.90; HRMS-ESI for $C_{26}H_{26}NO_4Se$ (M + H) calcd. 496.1027, found 496.1046.

BocCys(Trt)OMe

The procedure for the synthesis of compound ±-*syn*-**9** was employed. Starting with 1.50 g of Boc-Cys(Trt)-OH, the reaction produced 1.34 g (86%) of Boc-Cys(Trt)-OMe. M.p.: 41-42 °C. $[a]_D^{20}$ = +18.8 (c = 0.55, $CHCl_3$). IR ($CHCl_3$): 3419, 2977, 1748, 1717, 1492, 1166 cm^{-1}. ^{1}H NMR (400 MHz) δ ppm 1.43 (s, 9 H, tBu), 2.57 (d, 2 H, J = 5.52, CH_2), 3.70 (s, 3 H, CH_3), 4.28 (m, 1 H, CH), 5.01 (d, 1 H, J = 6.5, NH), 7.20–7.40 (m, 15 H, trityl). ^{13}C NMR (100 MHz) δ ppm 28.5 (CH_3/CH, C(CH_3)$_3$), 34.3 (C/CH_2, CH_2), 52.6 (CH_3/CH, OCH_3), 52.7 (CH_3/CH, CH), 67.0 (C/CH_2, CPh_3), 80.2 (C/CH_2, CMe_3), 127.1 (CH_3/CH, Ph), 128.2 (CH_3/CH, Ph), 129.7 (CH_3/CH, Ph), 144.5 (C/CH_2, Ph), 155.2 (C/CH_2, CO amide), 171.5 (C/CH_2, CO ester). FAB-HRMS m/z calcd for $C_{28}H_{32}NO_4S$ (M + 1)$^+$ 478.2052, found 478.2050. Anal. Calcd for $C_{28}H_{31}NO_4S$ C 70.41, H 6.54, N 2.93, found C 70.67, H 6.24, N 2.71%.

BocSec(Ph)Cys(Trt)OMe

Boc-Cys(Trt)-OMe (0.44 g, 0.92 mmol) was treated with CH_2Cl_2/TFA (4 mL, 50/50) for 30 min at rt. The solvent was evaporated under reduced pressure, and DIEA (4 mL) and CH_3CN (10 mL) were added to the flask. BocSec(Ph)-OH[12] (0.31 g, 0.92 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.40 g, 0.92 mmol) were added. The mixture was stirred at 25 °C for 2 h, and the solvent was evaporated. The crude product was then dissolved in EtOAc and washed with 3 M HCl, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$. The residue obtained after filtration and concentration of the filtrate was purified by flash column chromatography eluting with CH_2Cl_2/MeOH (40/1) to afford the desired compound (0.65 g, 85%) as a white solid. M.p.: 52-54 °C. $[a]_D^{20}$ = −20.2 (c = 0.52, $CHCl_3$). IR ($CHCl_3$): 3325, 2977, 1744, 1676, 1491, 1167 cm^{-1}. ^{1}H NMR (500 MHz) δ ppm 1.41 (s, 9 H), 2.62 (d, 2 H, J = 4.03), 3.15–3.30 (m, 2 H), 3.70 (s, 3 H), 4.33 (br, 1 H), 4.39–4.44 (m, 1 H), 5.20 (br, 1 H), 6.63 (d, 1 H, J = 8.21), 7.19–7.53 (m, 20 H). ^{13}C NMR (125 MHz) δ ppm 28.5, 30.2, 33.8, 51.5, 52.8, 53.2, 54.5, 67.1, 127.2, 127.7, 128.3, 129.5, 129.7, 133.3, 144.5, 155.4, 170.3, 170.6. FAB-HRMS m/z calcd for $C_{37}H_{41}N_2O_5S^{80}Se$ (M + 1)$^+$ 705.1901, found 705.1904. Anal. Calcd for $C_{37}H_{40}N_2O_5SSe$ C 63.15, H 5.73, N 3.98, found C 62.84, H 5.57, N 3.59%.

BocDhaCys(Trt)OMe

BocSec(Ph)Cys(Trt)OMe (30 mg, 0.04 mmol) placed in a 10 mL round bottom flask was dissolved in 0.6 mL of THF. $NaIO_4$ (34 mg, 0.16 mmol) dissolved in a few drops of water was added. The mixture was stirred at 0 °C for 6 h, then CH_2Cl_2 was added. The organic solution was washed with water and aqueous $NaHCO_3$, then dried over $MgSO_4$. The title compound (19 mg, 82%) was obtained after removal of solvent and purified by flash column chromatography eluting with hexane/EtOAc (85/15). M.p.: 43–45 °C. $[a]_D^{20}$ = −1.6 (c = 0.72, $CHCl_3$). IR ($CHCl_3$) 3401, 1731, 1667, 1632, 1494, 1444, 1392, 1217, 1159 cm^{-1}. ^{1}H NMR (400 MHz) δ ppm 1.48 (s, 9 H), 2.64 (dd, 1 H, J = 18, 4.5 Hz), 2.72 (dd, 1 H, J = 18, 5.68 Hz), 3.73 (s, 3 H), 4.56–4.60 (m, 1 H), 5.09 (t, 1 H, J = 1.7 Hz), 6.05 (s, 1 H), 6.60 (d, 1 H, J = 7.46 Hz), 7.17 (br, 1 H), 7.20–7.39 (m, 15 H). ^{13}C NMR (125 MHz) δ ppm 28.5, 33.8, 51.8, 53.1, 67.3, 80.8, 98.9, 127.2, 128.3, 129.7, 134.5, 144.4, 152.9, 163.8, 170.7. The product fragments during mass spectrometry give CPh_3^+ (detected by positive FAB-LRMS) and (M − CPh_3)$^-$ (detected by negative FAB-LRMS).

[BocSec(Ph)CysOMe]$_2$

BocSec(Ph)Cys(Trt)OMe (0.128 g, 0.178 mmol) in 4 mL of MeOH was dropped into a solution of I_2 in MeOH (0.0452 g, 0.178 mmol, 2 mL MeOH). The mixture was stirred at rt for 2 h. An aqueous solution of $Na_2S_2O_3$ was dropped into the reaction to remove the orange color. Water (30 mL) was added to the reaction, and EtOAc (60 mL) was used to extract the organic compounds. The organic layer was dried over $MgSO_4$ and filtered. The solution was concentrated to afford the crude product. Purification by flash chromatography with 20 : 1 CH_2Cl_2 : MeOH provided the product (R_f = 0.32, 20 : 1 CH_2Cl_2 : MeOH; yield 81%). M.p.: 145–146 °C. $[a]_D^{20}$ = +2.9 (c = 0.46, $CHCl_3$). IR ($CHCl_3$): 3308, 2977, 1744, 1664, 1508, 1165 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 1.45 (9H, s), 3.06–3.16 (m, 2H), 3.27 (m, 2H), 3.74 (s, 3H), 4.48 (br, 1H), 4.73–4.78 (dt, 1H, J = 7.4, 5.4), 5.60 (d, 1H, J = 7.81), 7.22–7.27 (m, 3H), 7.30–7.35 (br s, 1H), 7.53–7.55 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 28.5, 30.0, 40.8, 52.2, 53.0, 54.5, 80.6, 127.63, 129.4, 129.7, 133.4, 155.8, 170.5, 171.1; FAB-HRMS m/z calcd for $C_{36}H_{51}N_4O_{10}S_2{}^{80}Se_2$ (M + 1)$^+$ 923.1377, found 923.1377. Anal. Calcd. for $C_{36}H_{50}N_4O_{10}S_2Se_2$ C 46.95, H 5.47, N 6.08, found C 46.55, H 5.45, N 5.98%.

[BocDhaCys(OMe)]$_2$

[BocSec(Ph)CysOMe]$_2$ (29.3 mg, 0.055 mmol) in 2 mL of MeOH was mixed with $NaIO_4$ (95.0 mg, 0.444 mmol) in a few drops of water at 0 °C. The solution was warmed to rt and stirred overnight. EtOAc was added into the reaction, and the resulting solution was washed with water, aqueous $NaHCO_3$ and dried over $MgSO_4$. Filtration and concentration of the filtrate gave the crude product. Purification by flash column chromatography eluting with CH_2Cl_2/MeOH (20/1) afforded 25.5 mg (75%) of the product. M.p.: 62–65 °C. $[a]_D^{20}$ = +3.6 (c = 1.00, $CHCl_3$). IR ($CHCl_3$): 3392, 1731, 1663, 1630, 1499, 1368, 1247, 1160. ^{1}H NMR (400 MHz) δ ppm 1.47 (s, 9 H), 3.23 (d, 2 H, J = 5.16 Hz), 3.78 (s, 3 H), 4.89 (dt, 1 H, J = 7, 5 Hz), 5.21 (t, 1 H, J = 1.7 Hz), 6.07 (s, 1 H), 6.99 (d, 1 H, J = 7.29 Hz), 7.21 (s, 1 H). ^{13}C NMR (125 MHz) δ ppm 28.4, 40.6, 52.2, 53.2, 80.9, 99.2, 134.5, 153.0, 164.1, 170.6. FAB-HRMS m/z calcd for $C_{24}H_{39}N_4O_{10}S_2$ (M + 1)$^+$ 607.2107, found 607.2115.

Boc-(2*R*,3*S*)-MeSec(Ph)-OBn

The procedure described by Wakamiya *et al.*[36] was followed starting with (2*S*,3*R*)-2-*tert*-butoxycarbonylamino-3-(phenylselanyl)butyric acid benzyl ester[13] giving 67% yield. $[a]_D^{20}$ = 19.2° (c = 1.79 w/v%, $CHCl_3$);^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.40 (d, J = 6.89 Hz, 3H), 1.43 (s, 9H), 3.61 (qd, J = 7.05 Hz, 4.60 Hz, 1H), 4.56 (dd, J = 8.66 Hz, 4.29 Hz, 1H), 5.15 (AB, J_{ab} = 12.2 Hz, 2H), 5.25 (d, J = 8.46 Hz, 1H), 7.25–7.38 (m, 8H), 7.58 (dd, J = 7.58 Hz, 1.37 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 18.6, 28.5, 41.5, 58.4, 67.5, 128.3, 128.5, 128.8, 129.4, 135.4, 135.6, 156.4, 170.8; IR ($CHCl_3$): 3432, 3057, 2982, 2931, 1732, 1498, 1478, 1456, 1373, 1266, 1161, 1047, 732, 704 cm^{-1}; HRMS (FAB$^+$): calcd for [M + H] $C_{22}H_{27}N_1O_4Se_1$ 449.1105, found 449.1106.

Fmoc-(±)-*syn*-MeSec-OBn

The title compound was prepared using a similar procedure as for Boc-(2*R*,3*S*)-MeSec(Ph)-OBn. Yield: 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.54 (d, J = 7.19 Hz, 3H), 3.88 (qd, J = 7.22 Hz, 3.10 Hz, 1H), 4.26 (t, J = 7.32 Hz, 1H), 4.41 (d, J = 7.52 Hz, 2H), 4.62 (d, J = 12.19 Hz, 1H), 4.72 (dd, J = 9.23, 3.40 Hz, 1H), 4.99 (d, J = 12.20 Hz, 1H), 5.69 (d, J = 9.32 Hz, 1H), 7.24–7.45 (m, 12H), 7.62 (m, 4H), 7.80 (d, J = 7.14 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 20.52, 42.49, 47.34, 59.05, 67.53, 67.56, 120.25, 125.39, 125.43, 127.35, 127.97, 128.11, 128.48, 128.64, 128.73, 128.79, 129.38, 129.72, 135.12, 135.85, 135.89, 141.55, 143.92, 144.15, 156.64, 170.32; FAB-HRMS calcd for $C_{32}H_{30}N_1O_4Se_1$ [M + H] 572.1340, found 572.1340.

Ac-Gly-(2*R*,3*S*)-MeSec(Ph)-OBn

Boc-(2*R*,3*S*)-MeSec(Ph)-OBn (0.056 g, 0.15 mmol) was dissolved in 9 mL of CH_2Cl_2 : TFA (2 : 1) and stirred at room temperature for 1 h. The solvent was removed by rotary evaporation. The residue obtained was dissolved in 3 mL of CH_3CN. Ac-Gly-OH (0.018 g, 0.15 mmol) and benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (0.067 g, 0.15 mmol), were added followed by diisopropylethylamine (0.039 g, 0.30 mmol). The mixture was stirred for 1 h and diluted with CH_2Cl_2 (80 mL), washed with 1 M HCl (30 mL), sat. $NaHCO_3$ (30 mL), water and brine. The organic layer was dried and concentrated. Purification over silica gel (CH_2Cl_2 : MeOH = 40 : 1) provided the product (0.052 g, 92%; R_f = 0.26, CH_2Cl_2 : MeOH = 40 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.40 (d, J = 7.26 Hz, 3H), 1.99 (s, 3H), 3.68 (qd, J = 7.13 Hz, 4.25 Hz, 1H), 3.88 (B of ABX, J_{ab} = 18.41 Hz, J_{bx} = 8.66 Hz, 1H), 3.91 (A of ABX, J_{ab} = 18.41 Hz, J_{ax} = 8.44 Hz, 1H), 4.85 (dd, J = 8.40 Hz, 4.32 Hz, 1H), 5.14 (AB, J_{ab} = 12.22 Hz, 2H), 6.48 (X of ABX, 1H), 7.13 (d, J = 8.39 Hz, 1H), 7.25–7.37 (m, 8H), 7.55–7.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 18.2, 23.1, 40.5, 43.4, 57.2, 67.7, 128.4, 128.5, 128.7, 128.8, 128.9, 129.5, 135.3, 135.5, 169.2, 170.0, 171.0; IR ($CHCl_3$): 3300, 3056, 2927, 1738, 1658, 1520, 1375, 1266, 743, 703 cm^{-1}; HRMS (FAB$^+$): calcd [M + H] $C_{21}H_{25}N_2O_4Se_1$ 449.0980, found 449.0980.

Ac-Gly-*Z*-Dhb-OBn

Ac-Gly-(2*R*,3*S*)-MeSec(Ph)-OBn (0.052 g, 0.140 mmol) was dissolved in 6.0 mL of CH_2Cl_2 and 6.0 mL of MeOH. $NaIO_4$ (0.120 g, 0.560 mmol) was dissolved in 3.0 mL of water. The $NaIO_4$ solution was added to the dipeptide solution at room temperature and the mixture was stirred for 0.5 h. Water (10 mL) was added and the mixture was extracted with CH_2Cl_2 (80 mL). The organic layer was washed with water (30 mL), brine (30 mL), and dried with $MgSO_4$. The solvent was removed under reduced pressure. Purification over silica gel (CH_2Cl_2 : MeOH = 20 : 1) afforded the desired product (Yield: 83%; R_f = 0.29, CH_2Cl_2 : MeOH = 20 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.76 (d, J = 7.24 Hz, 3H), 2.00 (s, 3H), 4.06 (d, J = 5.28 Hz, 2H), 5.17 (s, 2H), 6.63 (t, J = 5.20 Hz, 1H), 6.85 (q, J = 7.21 Hz, 1H), 7.31–7.37 (m, 5H), 7.87 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 14.7, 23.1, 43.8, 67.4, 126.2, 128.4, 128.6, 128.8, 135.3, 135.8, 164.3, 170.0, 171.3; IR ($CHCl_3$): 3286, 3034, 1722, 1662, 1520, 1386, 1269, 1145, 1070, 1029, 968, 737, 699 cm^{-1}; HRMS (FAB$^+$): calcd. for [M + H] $C_{15}H_{19}N_2O_4$ 291.1345, found 291.1345.

[Ac-Gly-(±)-*syn*-MeSec(Ph)-OBn]

Fmoc-(±)-*syn*-MeSec-OBn (0.049 g, 0.086 mmol) was dissolved in 1 mL of $CHCl_3$ and treated with 1 mL of 4-aminomethylpiperidine (4-AMP) at rt for 30 min. The mixture was washed with 10% NaH_2PO_4 (5 × 20 mL) and concentrated. The residue was dissolved in 2 mL of CH_2Cl_2. Ac-Gly-OH (0.0096 g, 0.082 mmol) and BOP (0.036 g, 0.082 mmol) were added followed by diisopropylethylamine (0.032 g, 0.25 mmol). The mixture was stirred overnight and diluted with CH_2Cl_2 (80 mL), washed with 1 M HCl (30 mL), sat. $NaHCO_3$ (30 mL), water and brine. The organic layer was dried and concentrated. Purification over silica gel (CH_2Cl_2 : MeOH = 20 : 1) provided the product (0.037 g, 96%; R_f = 0.25, CH_2Cl_2 : MeOH = 20 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.48 (d, J = 7.29 Hz, 3H), 2.07 (s, 3H), 3.87 (qd, J = 7.24 Hz, 3.71 Hz, 1H), 4.00 (qd, J = 16.77 Hz, 5.18 Hz, 2H), 4.58 (B of AB, J_{ab} = 12.19 Hz, 1H), 4.93 (dd, J = 8.83 Hz, 3.61 Hz, 1H), 4.95 (A of AB, J_{ab} = 12.19 Hz, 1H), 6.36 (br, 1H), 6.81 (d, J = 8.94 Hz, 1H), 7.21–7.36 (m, 8H), 7.57–7.59 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.33, 23.15, 42.33, 56.99, 57.76, 67.59, 127.95, 128.56, 128.65, 128.76, 128.80, 129.39, 135.03, 135.88, 169.46, 169.86, 170.81; FAB-HRMS calcd for $C_{21}H_{25}N_2O_4{}^{80}Se$ [M + H] 449.0982, found 449.0980.

Ac-Gly-*E*-Dhb-OBn

Ac-Gly-(±)-*syn*-MeSec(Ph)-OBn (0.018 g, 0.038 mmol) was dissolved in 2.0 mL of CH_2Cl_2 and 2.0 mL of MeOH. $NaIO_4$ (0.041 g, 0.154 mmol) was dissolved in 1.0 mL of water. The $NaIO_4$ solution was added to the dipeptide solution at room temperature and the mixture was stirred for 0.5 h. Water (10 mL) was added and the mixture was extracted with EtOAc (140 mL). The organic layer was washed with water (40 mL), brine (30 mL), and dried with $MgSO_4$. The solvent was removed under reduced pressure. Purification over silica gel (CH_2Cl_2 : MeOH = 20 : 1) afforded the desired product (0.0092 g, 79%; R_f = 0.29, CH_2Cl_2 : MeOH = 20 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.02 (s, 3H), 2.10 (d, J = 7.73 Hz, 3H), 3.99 (d, J = 6.26 Hz, 2H), 5.27 (s, 2H), 6.31 (br, 1H), 7.16 (q, J = 7.39 Hz, 1H), 7.37–7.42 (m, 5H), 7.92 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.70, 23.06, 44.22, 67.70, 125.41, 128.67,

128.81, 128.93, 129.89, 135.35, 164.21, 167.62, 171.01; FAB-HRMS calcd for $C_{15}H_{19}N_2O_4$ [M + H] 291.1345, found 291.1345.

Fmoc-(±)-*syn*-*S*-benzyl-3-methylcysteine (14)

Boc-*syn*-(±)-*S*-benzyl-3-methylcysteine methyl ester (0.60 g, 1.8 mmol) was treated with 10 mL of TFA/CH_2Cl_2 at rt for 1 h. The mixture was then concentrated under reduced pressure. The residue was treated with 12 M HCl at 65 °C for 5 h, then concentrated. $NaHCO_3$ (0.16 g, 1.9 mmol) in 18 mL of H_2O and 12 mL of acetone was added to the residue. FmocOSu was added to the mixture. The reaction was kept at pH ~ 9 and stirred at rt overnight. The reaction was diluted with EtOAc, and washed with 1 M HCl. The aqueous layer was extracted with EtOAc (2 × 50 mL). The organic layer was dried over $MgSO_4$, concentrated and purified by silica gel chromatography to afford 0.64 g (81%, R_f = 0.32, 15 : 1 CH_2Cl_2 : MeOH) of the product. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 1.33 (d, J = 7.12 Hz, 3 H), 3.40 (qd, J = 7.88 Hz, 3.92 Hz, 1 H), 3.77 (B of AB, J = 13.38 Hz, 1 H), 3.81 (A of AB, J = 13.38 Hz, 1 H), 4.27 (t, J = 6.99 Hz, 1 H), 4.44 (d, J = 7.16 Hz, 2 H), 4.60 (dd, J = 8.85 Hz, 3.08 Hz, 1 H), 5.61 (d, J = 8.92 Hz, 1 H), 7.25–7.33 (m, 7 H), 7.42 (m, 2 H), 7.63 (t, J = 6.85 Hz, 2 H), 7.79 (d, J = 7.13 Hz, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 19.94, 36.14, 42.20, 47.35, 58.64, 67.70, 120.31, 125.40, 127.41, 127.49, 127.60, 128.05, 128.88, 129.15, 137.77, 141.59, 143.87, 144.06, 156.90, 175.91; FAB-HRMS calcd for $C_{26}H_{25}N_1O_4S_1Na_1$ [M + Na] 470.1402, found 470.1403. Fmoc-(±)-*anti*-*S*-benzyl-3-methylcysteines were synthesized similarly.

Fmoc-*syn*-3-methylcystines (15)

$SiCl_4$ (2.86 g, 16.82 mmol) and Ph_2SO (0.85 g, 4.20 mmol) in 12 mL of TFA were added to Fmoc-*syn*-(±)-*S*-benzyl-3-methylcysteine (0.37 g, 0.84 mmol) at 0 °C and the resulting mixture was stirred for 1 h. The reaction mixture was diluted with 40 mL of Et_2O. The solution was treated with saturated aq. $NaHCO_3$. The aqueous layer was then acidified to pH ~ 1, extracted with EtOAc, dried over $MgSO_4$ and concentrated to afford 0.29 g (98%) of the title compound. FAB-LRMS calcd for $C_{38}H_{36}N_2O_8S_2$ [M + H] 713.8, found 714.2. The material was used without further purification. Fmoc-*anti*-*S*-benzyl-3-methylcystines were synthesized similarly.

Solid phase peptide synthesis

Peptides were synthesized in an automated peptide synthesizer using standard Fmoc protocols.[37] Resins (generally 0.6 mmol g^{-1} loading capacity) were swollen in DMF (3 × 6 mL, 10 min). Fmoc groups were removed with 20% v/v piperidine in DMF (2 × 6 mL, 7 min). After deprotection, the resins were rinsed with DMF (6 × 6 mL, 30 s). Amino acids were activated by reaction with *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and 0.4 M *N*-methylmorpholine (3 mL, 30 s). The activated solutions were transferred into the reaction vessel. The amino acid cartridges were rinsed with DMF (3 mL, 30 s) and this solution was also transferred to the reaction vessel. Coupling of the amino acids in general proceeded for 30 min or until complete as determined by a Kaiser test.[38] For every coupling, four equivalents of each amino acid and HBTU with respect to the resin loading capacity were used. Following each coupling, the resins were rinsed with DMF (3 × 6 mL, 30 s).

Ac-Ala-Dha-Ala-Wang resin

The precursor peptide was synthesized on a 0.4 mmol scale using standard Fmoc protocols. MAS ^{1}H NMR spectra were obtained using a Varian nanoprobe on a Varian UI500WB instrument. Resin loaded with the peptide (3–5 mg) was placed in the nanoprobe cell and swollen in 40 μL of DMF-d_7. Spin echo enhancement was applied with a delay of 4 ms between 180° pulses (26 μs). A recycle delay of 9.5 s was used between acquisitions. MAS- ^{1}H NMR (DMF-d_7, 500 MHz) δ ppm 1.36 (6H, s, βCH_3), 1.96 (3H, s, CH_3-Ac), 3.28 (2H, s, βCH_2), 4.39 (2H, s, αCH-Ala), 4.72 (1H, s, αCH-PhSec), 8.25 (3H, s, NH). The resin bound peptide (0.72 g, 0.43 mmol) was swollen in DMF (11 mL) for 30 min, and then 30% H_2O_2 (0.25 mL, 2.16 mmol) was added. The resin mixture was agitated every 10 s by purging the reaction with N_2. Small aliquots of resin were removed, washed with DMF, EtOH, and CH_2Cl_2 every 15 min for 3 h. ^{1}H NMR spectroscopy showed the reaction to be complete in 45 min. A small amount of resin was subjected to cleavage conditions (5% TIS in TFA, 90 min), and FAB MS was obtained on the crude cleavage product. MAS-^{1}H NMR (DMF-d_7, 500 MHz) δ ppm 1.36 (3H, s, βCH_3-Ala), 1.44 (3H, s, βCH_3-Ala), 1.97 (3H, CH_3-Ac), 4.54 (2H, s, αCH), 6.39 (1H, s, CH_2-vinyl), 8.42 (1H, s, NH), 8.84 (1H, s, NH), 9.21 (1H, s, NH); FAB-MS 274 $(M + 1)^+$.

Michael addition to Ac-Ala-Dha-Ala-Wang resin

The Ac-Ala-Dha-Ala-Wang resin (0.42 g, 0.25 mmol) was swollen in DMF (7 mL) for 30 min. 2-Mercaptoethylamine hydrochloride salt (0.29 g, 2.53 mmol) and *N*-methyl morpholine (0.69 mL, 6.32 mmol) were dissolved in DMF (3 mL) and added to the mixture. The resin was agitated every 10 s by purging with N_2 for 7.5 h. Small aliquots of resin were withdrawn every 10 min, washed with DMF, CH_2Cl_2, and EtOH and dried in a vacuum dessicator. ^{1}H NMR spectroscopy and quantitative ninhydrin tests showed the reaction to be complete after 6 h. The product resin was subjected to cleavage conditions (5% TIS/TFA, 90 min) and the product was purified by preparative RP-HPLC (Vydac C-18 column) to give the peptide as an oil (66 mg, 75%). ^{1}H NMR (DMF-d_7, 500 MHz) δ ppm 1.38 (6H, s, βCH_3-Ala), 1.96 (3H, s, CH_3-Ac), 3.05 (2H, s, CH_2), 3.13 (1H, br m, CH_2), 3.26 (2H, s, βCH_2), 4.44 (2H, s, αCH-Ala), 4.74 (2H, br m, αCH), 8.41 (1H, br m, NH), 8.58 (1H, s, NH-diastereomer 1), 8.67 (1H, s, NH-diastereomer 2); FAB-MS 349 (100%) $(M + 1)^+$, 371 (42%) $(M + Na)^+$, 387 (14%) $(M + K)^+$. The stock solutions and procedures used for the quantitative ninhydrin tests were those described by Merrifield and coworkers.[28] The dried resin (3–4 mg) was subjected to the reaction conditions at 100 °C for 10 min. The resin was then washed and the mother liquor diluted as described. The color yield of the reaction was monitored at 570 nm by UV/VIS spectrophotometry.

Preparation of Ac-Gly-Ala-Cys(β-glucosyl)-Ala-Ser-Thr-Ser-OH (12) on solid phase

Ac-Gly-Ala-Sec-Ala-Ser(OtBu)-Thr(OtBu)-Ser(OtBu)-Wang resin (**11**, 0.576 g, 0.29 mmol) was obtained by SPPS. A small quantity of beads (10 mg) was cleaved in 3 mL of TFA/CH_2Cl_2 (95/5) with 3 drops of TIS for 1 h. Removal of TFA by rotary evaporation afforded an oily residue, which turned to a white solid upon addition of ether. MALDI-MS *m*/*z* calcd for $C_{29}H_{43}N_7O_{12}SeNa$ $(M + Na)^+$ 784.2, found 783.9. Ac-Gly-Ala-Sec-Ala-Ser(OtBu)-Thr(OtBu)-Ser(OtBu)-Wang resin (0.251 g, 0.127 mmol) was swelled in DMF (7 mL) for 0.5 h. H_2O_2 (30% aq., 71 μL) was added to the resin. The mixture was purged periodically with N_2 for 2 h. Ac-Gly-Ala-Dha-Ala-Ser(OtBu)-Thr(OtBu)-Ser(OtBu)-Wang resin (0.226 g) was washed with DMF, MeOH and CH_2Cl_2, and dried under reduced pressure. A small quantity of beads (3 mg) was cleaved in 3 mL of TFA/CH_2Cl_2 (95/5) with 3 drops of TIS for 1 h. Removal of TFA by rotary evaporation afforded an oily residue, which turned to a white solid upon addition of ether. MALDI-MS *m*/*z* calcd for $C_{23}H_{37}N_7O_{12}Na$ $(M + Na)^+$ 626.2, found 626.1. The sodium salt of 1-thio-β-glucosylpyranose (50 mg, 0.23 mmol) was passed through an HCl-regenerated cation exchange column and collected into 0.1 M aqueous Et_3N. The solution was

lyophilized to give a white foam, which was dissolved in 0.1 mL of DMF and transferred to 40 mg (0.022 mmol) of Ac-Gly-Ala-Dha-Ala-Ser(OtBu)-Thr(OtBu)-Ser(OtBu)-Wang resin. The mixture was stirred for 2 d. The resin was filtered and washed with DMF, MeOH and CH_2Cl_2. The peptide was cleaved from the resin (60 mg) with 10 mL of 95/3/2 TFA/CH_2Cl_2/TIS. Removal of TFA by rotary evaporation afforded an oily residue, which turned to a white solid upon addition of ether. The peptide was purified by HPLC to provide 8.0 mg (overall 45%) of the desired peptide. MALDI-MS *m*/*z* calcd for $C_{29}H_{49}N_7O_{17}SNa$ $(M + Na)^+$ 822.2, found 821.9.

[A-*syn*-MeSec(Ph)-PGCVA]$_2$

The HPLC-purified peptide A-*syn*-MeSec(Ph)-PGC(Acm)VA (0.049 g, 0.048 mmol) was dissolved in 4 mL of 50% aq. MeOH. Acetic acid (0.2 mL) was added followed by a 0.05 M solution of I_2 in 1.5 mL of MeOH. The reaction mixture was stirred at room temperature for 45 min and quenched with 10% aq. sodium thiosulfate until the mixture was colorless. The mixture was concentrated by evaporation under reduced pressure and purified by preparative RP-HPLC (0.027 g). Yield: 74%; FAB-MS calcd for $C_{62}H_{93}N_{14}O_{16}S_2Se_2$ [M + H] 1513.46, found 1513.64.

(A-*E*-DhbPGCVA)$_2$ (*E*-1)

Peptide [A-*syn*-MeSec(Ph)-PGCVA]$_2$ (5.0 mg, 3.3 μmol) was dissolved in 6 mL of H_2O and $NaIO_4$ (0.008 g, 0.037 mmol) in 0.6 mL of H_2O was added. The reaction mixture was stirred at room temperature for 5.5 h and purified by semi-preparative RP-HPLC. Yield: 2.7 mg, 68%. ^{1}H NMR (D_2O, 500 MHz): δ ppm 0.78 (d, 3H, J = 6.81 Hz, Val-CH_3), 0.79 (d, 3H, J = 7.53 Hz, Val-CH_3), 1.23 (d, 3H, J = 7.24 Hz, Ala-CH_3), 1.41 (d, 3H, J = 7.03 Hz, Ala-CH_3), 1.59 (d, 3H, J = 7.36 Hz, Dhb-CH_3), 1.77–1.97 (m, 5H, Pro-2H$_\gamma$ + Pro-H$_\beta$ + Val-H$_\beta$), 2.23 (m, 1H, Pro-H$_\beta$), 2.81 (B of ABX, 1H, J = 13.92 Hz, 8.74 Hz, Cys-H$_\beta$), 3.03 (A of ABX, 1H, J = 13.92 Hz, 4.74 Hz, Cys-H$_\beta$), 3.40–3.57 (m, 2H, Pro-2H$_\delta$), 3.84 (s, 2H, Gly-H), 3.97 (q, 1H, J = 4.74 Hz, Ala-H$_\alpha$), 4.00 (d, J = 6.74 Hz, 1H, Val-H$_\alpha$), 4.12 (q, 1H, J = 6.78 Hz, Ala-H$_\alpha$), 4.36 (t, 1H, J = 6.48 Hz, Pro-H$_\alpha$), 4.56 (m, 1H, Cys-H$_\alpha$), 5.58 (q, 1H, J = 7.20 Hz, Dhb-H$_\beta$); FAB-MS calcd. for $C_{50}H_{81}N_{14}O_{16}S_2$ [M + H] 1197.53, found 1197.58.

Methyllanthionine 4

Peptide *E*-**1** (1.0 mg, 0.8 μmol) was dissolved in 0.52 mL of degassed CH_3CN/20 mM aq. NH_4OAc (1 : 1). A solution of TCEP (1.7 mg, 6.0 μmol) in 20 mM NH_4OAc (0.09 mL) was added and the reaction mixture was stirred at room temperature until complete as determined by RP-HPLC. Aqueous NH_4OAc (20 mM, 3.0 mL) was added to the mixture and the pH of the mixture was adjusted to 8–9 by the addition of Et_3N. The cyclization was complete in about 10 h as determined by RP-HPLC. The mixture was purified by RP-HPLC to give the product **4** (0.7 mg). Yield: 70%. ^{1}H NMR (D_2O, 500 MHz): δ ppm 0.78 (d, J = 6.62 Hz, 3H, Val-CH_3), 0.79 (d, J = 6.46 Hz, 3H, Val-CH_3), 1.13 (d, J = 9.57 Hz, 3H, Cys(Me)-CH_3), 1.16 (d, J = 8.07 Hz, 3H, Ala-CH_3), 1.39 (d, J = 6.46 Hz, 3H, Ala-CH_3), 1.74 (m, 1H, Pro-H$_\beta$), 1.83 (m, 1H, Pro-H$_\beta$), 1.91 (m, 1H, Val-H$_\beta$), 2.02 (m, 1H, Pro-2H$_\gamma$), 2.26 (m, 1H, Pro-H$_\alpha$), 2.65 (AB of ABX, 1H, J = 4.78 Hz, Cys-H$_\beta$), 2.89 (qd, 1H, J = 6.89, 3.22, MeLan-H$_\beta$), 3.12 (AB of ABX, J = 3.09 Hz, 1H, MeLan-H$_\beta$), 3.40 (m, 2H, Pro-H$_\delta$), 3.82 (q, 2H, J = 6.69 Hz, Gly-H$_\alpha$), 3.92 (q, 1H, J = 6.12 Hz, Ala-H$_\alpha$), 3.95 (d, 1H, J = 8.03 Hz, Val-H$_\alpha$), 3.99 (q, 1H, J = 7.08 Hz, Ala-H$_\alpha$), 4.80 (d, 1H, J = 9.75 Hz, Pro-H$_\alpha$), 4.84 (t, J = 3.78 Hz, 1H, Cys-H$_\alpha$), 4.90 (d, J = 3.22 Hz, 1H, MeLan-H$_\alpha$); FAB-HRMS calcd for $C_{25}H_{42}N_7O_8S$ [M + H] 600.2816, found 600.2818.

Ile-(2*R*,3*S*)-Me-Sec(Ph)-Sec(Ph)-Ile-Sec(Ph)-Leu-Cys(Acm)-(2*R*,3*S*)-methyl-Sec(Ph)-Pro-Gly-Cys(Acm)-Lys-Ala (23)

The title compound was prepared on solid phase. Fmoc-Ala-Wang resin (0.167 g, 0.1 mmol) was swollen in DMF. Fmoc-(2*R*,3*S*)-methyl-Sec(Ph)-OH, Fmoc-Sec(Ph)-OH and Fmoc-Cys(Acm)-OH were coupled manually with HOBT and DIC. Couplings were allowed to proceed for 2 h each. The completion of coupling was monitored by bromophenyl blue. The peptide was cleaved from the resin with water (0.5 mL), anisole (0.5 mL), thioanisole (0.5 mL), triisopropylsilane (0.3 mL) and TFA (10 mL). TFA was removed by rotary evaporation and ice-cold Et_2O was added to precipitate the peptide, which was purified by RP-HPLC (41.1 mg, 21%). LRMS (MALDI): calculated $C_{84}H_{122}N_{16}O_{16}S_2Se_4$ 1994.53, found 1995.50 [M + H].

Ile-(2*R*,3*S*)-Me-Sec(Ph)-Sec(Ph)-Ile-Sec(Ph)-Leu-Cys-(2*R*,3*S*)-methyl-Sec(Ph)-Pro-Gly-Cys-Lys-Ala (20)

Compound **23** (8 mg, 4.0 μmol) was placed in a 50 mL, round bottomed flask and dissolved in 80% aq. HOAc (3 mL). I_2 (30.6 mg, 120 μmol) was dissolved in 24 mL of 80% aq. HOAc and added to the peptide solution. The reaction mixture was stirred at rt for 25 min. Water (50 mL) was added and the aqueous layer was washed with CCl_4 (4 × 30 mL) to remove excess I_2. The aqueous layer was lyophilized and then purified by RP-HPLC. Yield: 54%. LRMS (MALDI): calculated $C_{78}H_{110}N_{14}O_{14}S_2Se_4$ 1850.44, found 1851.76 [M + H].

Ile-Dha-Dha-Ile-Dha-Leu-Cys-Dhb-Pro-Gly-Cys-Lys-Ala (21) and cyclization product 22

A similar procedure was conducted as for compound *E*-**1**. LRMS (MALDI): calculated $C_{54}H_{86}N_{14}O_{14}S_2$ 1218.59, found 1219.28 [M + H]. The same procedure was conducted for the cyclization of **21** as described for methyllanthionine **4**. LRMS (MALDI): calculated $C_{54}H_{88}N_{14}O_{14}S_2$ 1220.60, found 1221.13 [M + H]. ^{1}H NMR analysis showed the presence of the quartets corresponding to the vinyl protons of the two Dhb residues.

A-MeCys-PGU(Ph)VA (13a–d)

The mixture of peptides was synthesized on an automated peptide synthesizer by standard Fmoc chemistry using either *syn*-**15** or a mixture of *syn*/*anti*-**15**. Fmoc-Ala-Wang resin (0.16 g, 0.10 mmol) was swollen in DMF for 30 min. Couplings were allowed to proceed for 40 min for Fmoc-Val-OH and Fmoc-Gly-OH, 60 min for Fmoc-Ala-OH and Fmoc-Pro-OH, and 120 min for Fmoc-MeCystine-OH and FmocSec(Ph)-OH. Coupling of *syn*-Fmoc-MeCystine-OH (**15**, 0.071 g, 0.1 mmol) was carried out in the presence of HOBt (0.027 g, 0.2 mmol) and DIC (0.032 mL, 0.2 mmol). The resin was isolated by filtration, washed with DMF, ethanol and CH_2Cl_2, and dried *in vacuo* in a desiccator. The peptide was cleaved from the resin using 10 mL of 95% TFA and 5% CH_2Cl_2 for 1.5 h, and the solvent was removed (10 mg, 13%). MALDI-MS for [M + H] calcd. 1513.6, found 1513.5. The mixture of diastereomeric disulfides (4.2 mg, 0.0028 mmol) was dissolved in 4 mL of H_2O. Zn powder (41 mg, 0.63 mmol) and 0.1 mL of TFA were added to the mixture. The reaction was stirred at rt for 5 h and purified on RP-HPLC (**13a**, 1.0 mg, 24%, **13b**, 1.2 mg, 29%). FAB-HRMS for $C_{31}H_{48}N_7O_8S^{80}Se$ [M + H] calcd. 758.2450, found 758.2450. Compound **13a** ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.71 (d, J = 6.83 Hz, 3 H, Val-CH_3), 0.74 (d, J = 6.76 Hz, 3 H, Val-CH_3), 1.20 (d, J = 7.07 Hz, 3 H, Ala-CH_3), 1.21 (d, J = 6.23 Hz, 3 H, MeCys-CH_3), 1.42 (d, J = 7.17 Hz, 3 H, Ala-CH_3), 1.83–1.92 (m, 4 H, Pro-H$_\beta$, Pro-2H$_\gamma$ + Val-H$_\beta$), 2.14–2.19 (m, 1 H, Pro-H$_\beta$), 3.09 (B of ABX, J_{ab} = 13.67 Hz, J_{bx} = 9.45 Hz, 1 H, Sec-CH_2), 3.20 (A of ABX, J_{ab} = 13.67 Hz, J_{ax} = 3.91 Hz,

1 H, Sec-CH_2), 3.22 (m, 1 H, MeCys-H_β), 3.65 (AB, J_{ab} = 17.10 Hz, 2 H, Gly-CH_2), 3.61–3.71 (m, 2 H, Pro-H_δ), 3.87 (d, J = 7.10 Hz, 1 H, Val-H_α), 3.99-4.01 (m, 2 H, Ala-H_α), 4.28 (dd, J = 8.35, 4.75 Hz, 1 H, Pro-H_α), 4.36-4.39 (m, 1 H, Sec-H_α), 4.62 (m, 1 H, MeCys-H_α), 7.17–7.22 (m, 3 H, Ph), 7.40–7.43 (m, 2 H, Ph). Compound **13b**: ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.71 (d, J = 6.73 Hz, 3 H, Val-CH_3), 0.74 (d, J = 6.76 Hz, 3 H, Val-CH_3), 1.20 (d, J = 7.11 Hz, 3 H, Ala-CH_3), 1.23 (d, J = 6.96 Hz, 3 H, MeCys-CH_3), 1.35 (d, J = 7.08 Hz, 3 H, Ala-CH_3), 1.74–1.83 (m, 1 H, Pro-H_β), 1.83–1.91 (m, 2 H, Pro-H_γ + Val-H_β), 1.91–1.99 (m, 1 H, Pro-H_γ), 2.14–2.22 (m, 1 H, Pro-H_β), 3.10 (B of ABX, J_{ab} = 14.30 Hz, J_{bx} = 7.34 Hz, 1 H, Sec-CH_2), 3.21 (A of ABX, J_{ab} = 14.30 Hz, J_{ax} = 6.56 Hz, 1 H, Sec-CH_2), 3.30 (m, 1 H, MeCys-H_β), 3.55–3.60 (m, 1 H, Pro-H_δ), 3.67–3.71 (m, 1 H, Pro-H_δ), 3.68 (AB, J_{ab} = 17.10 Hz, 2 H, Gly-CH_2), 3.87 (d, J = 7.40 Hz, 1 H, Val-H_α), 3.98–4.02 (m, 2 H, Ala-H_α), 4.25 (t, J = 7.40 Hz, 1 H, Pro-H_α), 4.39–4.42 (m, 1 H, Sec-H_α), 4.62 (m, 1 H, MeCys-H_α), 7.19–7.20 (m, 3 H, Ph), 7.41–7.43 (m, 2 H, Ph). A-(2*R*,3*S*)-MeCys-PGU(Ph)VA (**13c**) and A-(2*S*,3*R*)-MeCys-PGU(Ph)VA (**13d**) were prepared similarly using the four diastereomers of Fmoc-(±)-MeCystine-OH (**15**). The corresponding disulfides were obtained in 20% yield (138 mg, 0.45 mmol synthesis) and after reduction with TCEP the four diastereomers were separated by RP-HPLC (C18 column, gradient 2–100% MeCN in H_2O, 0.1% TFA, elution order **13b**, **13a**, **13c**, **13d**). A-(2*R*,3*S*)-MeCys-PGU(Ph)VA was obtained in 19% yield, FAB-HRMS for $C_{31}H_{48}N_7O_8S^{80}Se$ [M + H] calcd. 758.2450, found 758.2447 and A-(2*S*,3*R*)-MeCys-PGU(Ph)VA was obtained in 32% yield, FAB-HRMS for $C_{31}H_{48}N_7O_8S^{80}Se$ [M + H] calcd. 758.2450, found 758.2447. **13c** ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.73 (d, J = 6.64 Hz, 3 H, Val-CH_3), 0.75 (d, J = 6.81 Hz, 3 H, Val-CH_3), 1.19 (d, J = 6.84 Hz, 3 H, Ala-CH_3), 1.26 (d, J = 7.48 Hz, 3 H, MeCys-CH_3), 1.34 (d, J = 7.03 Hz, 3 H, Ala-CH_3), 1.83–1.95 (m, 4 H, Pro-H_β, Pro-2H_γ + Val-H_β), 2.16–2.18 (m, 1 H, Pro-H_β), 3.09 (B of ABX, J_{ab} = 14.74 Hz, J_{bx} = 8.77 Hz, 1 H, Sec-CH_2), 3.21 (A of ABX, J_{ab} = 14.74 Hz, J_{ax} = 6.64 Hz, 1 H, Sec-CH_2), 3.15 (m, 1 H, MeCys-H_β), 3.69 (AB, J_{ab} = 16.97 Hz, 2 H, Gly-CH_2), 3.61–3.71 (m, 2 H, Pro-H_δ), 3.87 (d, J = 7.66 Hz, 1 H, Val-H_α), 3.97 (q, J = 7.32 Hz, 1 H, Ala-H_α), 4.11 (q, J = 7.56 Hz, 1 H, Ala-H_α), 4.28 (m, 1 H, Pro-H_α), 4.40 (m, 1 H, Sec-H_α), 4.62 (m, 1 H, MeCys-H_α), 7.17–7.26 (m, 3 H, Ph), 7.40–7.46 (m, 2 H, Ph). **13d** ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.73 (d, J = 6.70 Hz, 3 H, Val-CH_3), 0.75 (d, J = 6.99 Hz, 3 H, Val-CH_3), 1.20 (d, J = 6.91 Hz, 3 H, Ala-CH_3), 1.25 (d, J = 7.40 Hz, 3 H, MeCys-CH_3), 1.40 (d, J = 7.07 Hz, 3 H, Ala-CH_3), 1.83–1.95 (m, 4 H, Pro-H_β, Pro-2H_γ + Val-H_β), 2.16–2.18 (m, 1 H, Pro-H_β), 3.08–3.12 (m, 2 H, Sec-CH_2 and MeCys-H_β), 3.19–3.22 (m, 1 H, Sec-CH_2), 3.67 (AB, J_{ab} = 16.89 Hz, 2 H, Gly-CH_2), 3.68–3.79 (m, 2 H, Pro-H_δ), 3.87 (d, J = 7.61 Hz, 1 H, Val-H_α), 3.95 (q, J = 7.13 Hz, 1 H, Ala-H_α), 4.10 (q, J = 7.15 Hz, 1 H, Ala-H_α), 4.33 (m, 1 H, Pro-H_α), 4.39 (m, 1 H, Sec-H_α), 4.62 (m, 1 H, MeCys-H_α), 7.19–7.23 (m, 3 H, Ph), 7.40-7.46 (m, 2 H, Ph).

[A-(2*R*,3*R*)-MeCys-PGU(Ph)VA]$_2$

A-(2*R*,3*R*)-MeCys-PGU(Ph)VA **13b** (1.2 mg, 0.0016 mmol) was dissolved in 1 mL of H_2O. A solution of I_2 in MeOH (0.0028 M) was dropped into the reaction until the yellow color was sustained (after the 3rd drop). A drop of saturated $Na_2S_2O_3$ was added to quench excess I_2. The mixture was concentrated and purified by RP-HPLC (0.6 mg, 50%). FAB-HRMS for [M + H] calcd. 1513.4666, found 1513.4666. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.70 (d, J = 6.87 Hz, 3 H, Val-CH_3), 0.74 (d, J = 6.85 Hz, 3 H, Val-CH_3), 1.20 (d, J = 6.15 Hz, 3 H, Ala-CH_3), 1.26 (d, J = 7.08 Hz, 3 H, MeCys-CH_3), 1.34 (d, J = 7.27 Hz, 3 H, Ala-CH_3), 1.76–1.92 (m, 3 H, Pro-H_β + Pro-H_γ + Val-H_β), 1.92–1.98 (m, 1 H, Pro-H_γ), 2.12–2.17 (m, 1 H, Pro-H_β), 3.05–3.13 (m, 2 H, Sec-CH_2 + MeCys-H_β), 3.18 (m, 1 H, Sec-CH_2), 3.65 (AB, J_{ab} = 17.10 Hz, 2 H, Gly-CH_2), 3.67–3.81 (m, 2 H, Pro-2H_δ), 3.88 (d, J = 7.90 Hz, 1 H, Val-H_α), 3.98–4.02 (m, 2 H, Ala-H_α), 4.22 (t, J = 7.40 Hz, 1 H, Pro-H_α), 4.39–4.42 (m, 1 H, Sec-H_α), 7.16–7.20 (m, 3 H, Ph), 7.34–7.45 (m, 2 H, Ph). [A-(2*S*,3*S*)-MeCys-PGU(Ph)VA]$_2$ was prepared similarly from **13a** (1.0 mg, 0.0013 mmol). The product was obtained in 40% yield (0.4 mg). FAB-HRMS for [M + H] calcd. 1513.4666, found 1513.4666. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.71 (d, J = 6.70 Hz, 3 H, Val-CH_3), 0.74 (d, J = 6.69 Hz, 3 H, Val-CH_3), 1.18 (d, J = 6.97 Hz, 3 H, Ala-CH_3), 1.22 (d, J = 7.22 Hz, 3 H, MeCys-CH_3), 1.39 (d, J = 6.95 Hz, 3 H, Ala-CH_3), 1.81–1.95 (m, 4 H, Pro-H_β, Pro-2H_γ + Val-H_β), 2.10-2.18 (m, 1 H, Pro-H_β), 3.08–3.14 (m, 1 H, Sec-CH_2), 3.17–3.24 (m, 2 H, Sec-CH_2 + MeCys-H_β), 3.65 (s, 2 H, Gly-CH_2), 3.61–3.70 (m, 2 H, Pro-2H_δ), 3.86 (d, J = 7.20 Hz, 1 H, Val-H_α), 3.96 (q, J = 7.40, 1 H, Ala-H_α), 4.04 (q, J = 7.59, 1 H, Ala-H_α), 4.25 (dd, J = 8.45, 4.85 Hz, 1 H, Pro-H_α), 4.38 (m, 1 H, Sec-H_α), 4.74 (d, J = 7.70, 1 H, MeCys-H_α), 7.16–7.23 (m, 3 H, Ph), 7.37–7.43 (m, 2 H, Ph). [A-(2*R*,3*S*)-MeCys-PGU(Ph)VA]$_2$ was prepared from **13c** (7.4 mg, 0.010 mmol) in 84% yield (6.2 mg). MS-HRESI for $[M + H]^{2+}$ calcd. 757.2375, found 757.2357. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.73 (d, J = 6.85 Hz, 3 H, Val-CH_3), 0.76 (d, J = 6.73 Hz, 3 H, Val-CH_3), 1.11 (d, J = 7.07 Hz, 3 H, MeCys-CH_3), 1.25 (d, J = 7.33 Hz, 3 H, Ala-CH_3), 1.37 (d, J = 7.12 Hz, 3 H, Ala-CH_3), 1.80 (m, 1 H, Pro-H_β), 1.81–1.95 (m, 2 H, Pro-2H_γ), 1.95–1.98 (m, 1 H, Val-H_β) 2.16–2.18 (m, 1 H, Pro-H_β), 3.07 (B of ABX, J_{ab} = 13.31 Hz, J_{bx} = 7.48 Hz, 1 H, Sec-CH_2), 3.19 (A of ABX, J_{ab} = 13.31 Hz, J_{ax} = 6.58 Hz, 1 H, Sec-CH_2), 3.45 (m, 1 H, MeCys-H_β), 3.59 (m, 1 H, Pro-H_δ), 3.69 (s, 2 H, Gly-CH_2), 3.74 (m, 1 H, Pro-H_δ), 3.89 (d, J = 7.28 Hz, 1 H, Val-H_α), 4.02 (q, J = 7.16 Hz, 1 H, Ala-H_α), 4.10 (q, J = 6.95 Hz, 1 H, Ala-H_α), 4.28 (t, J = 7.55 Hz, 1 H, Pro-H_α), 4.40 (m, 1H, Sec-H_α), 5.11 (d, J = 3.60 Hz, 1H, MeCys-H_α), 7.14–7.22 (m, 3H, Ph), 7.35–7.44 (m, 2 H, Ph). [A-(2*S*,3*R*)-MeCys-PGU-(Ph)VA]$_2$ was prepared from **13d** (7.6 mg, 0.010 mmol) in 90% yield (6.8 mg). MS-HRESI for $[M + H]^{2+}$ calcd. 757.2375, found 757.2353. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.73 (d, J = 6.76 Hz, 3 H, Val-CH_3), 0.76 (d, J = 6.83 Hz, 3 H, Val-CH_3), 1.14 (d, J = 6.95 Hz, 3 H, MeCys-CH_3), 1.25 (d, J = 7.32 Hz, 3 H, Ala-CH_3), 1.41 (d, J = 7.09 Hz, 3 H, Ala-CH_3), 1.83–1.95 (m, 4 H, Pro-H_β, Pro-2H_γ + Val-H_β), 2.11–2.16 (m, 1 H, Pro-H_β), 3.12 (B of ABX, J_{ab} = 13.22 Hz, J_{bx} = 7.34 Hz, 1 H, Sec-CH_2), 3.22 (A of ABX, J_{ab} = 13.22 Hz, J_{ax} = 6.26 Hz, 1 H, Sec-CH_2), 3.29 (m, 1 H, MeCys-H_β), 3.69 (AB, J_{ab} = 17.05 Hz, 2 H, Gly-CH_2), 3.64–3.76 (m, 2 H, Pro-H_δ), 3.88 (d, J = 7.05 Hz, 1 H, Val-H_α), 4.01 (q, J = 7.17 Hz, 1 H, Ala-H_α), 4.10 (q, J = 7.30 Hz, 1 H, Ala-H_α), 4.33 (dd, J = 8.30, 5.10 Hz, 1 H, Pro-H_α), 4.39 (m, 1 H, Sec-H_α), 5.05 (d, J = 5.60 Hz, 1 H, MeCys-H_α), 7.18–7.23 (m, 3 H, Ph), 7.39-7.43 (m, 2 H, Ph).

[A-(2*R*,3*R*)-MeCys-PGDhaVA]$_2$ (17) and isomers 16, 18 and 19

[A-(2*R*,3*R*)-MeCys-PGU(Ph)VA]$_2$ (0.6 mg, 0.0004 mmol) was dissolved in 1 mL of H_2O. H_2O_2 (30%, 10 µL) was added and the reaction was stirred for 30 min. Another 10 µL of H_2O_2 was added and the mixture was stirred for another 30 min. The mixture was then purified by RP-HPLC (0.3 mg, 64%). MALDI-MS for [M + H] calcd. 1197.5, found 1197.7. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.79 (d, J = 7.90 Hz, 3 H, Val-CH_3), 0.82 (d, J = 6.85 Hz, 3 H, Val-CH_3), 1.18 (d, J = 7.31 Hz, 3 H, Ala-CH_3), 1.25 (d, J = 7.01 Hz, 3 H, MeCys-CH_3), 1.34 (d, J = 6.99 Hz, 3 H, Ala-CH), 1.79–2.02 (m, 4 H, Pro-H_β + Pro-2H_γ + Val-H_β), 2.13–2.20 (m, 1 H, Pro-H_β), 3.06 (m, 1 H, MeCys-H_β), 3.63 (m, 1 H, Pro-H_δ), 3.73 (m, 1 H, Pro-H_δ), 3.86 (s, 2 H, Gly-CH_2), 3.97–4.04 (m, 3 H, Val-H_α + 2 Ala-H_α), 4.30 (m, 1 H, Pro-H_α), 5.51 (d, J = 3.80 Hz, 2 H, vinyl-H). [A-(2*S*,3*S*)-MeCys-PGDhaVA]$_2$ (**16**) was prepared similarly from [A-(2*S*,3*S*)-MeCys-PGU(Ph)VA]$_2$ (0.4 mg, 0.00026 mmol). MALDI-MS for [M + H] calcd. 1197, found 1197.7. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.79 (d, J = 6.75 Hz, 3 H, Val-CH_3), 0.82 (d, J = 6.76 Hz, 3 H, Val-CH_3), 1.19 (d, J = 6.72 Hz, 3 H,

Ala-CH_3), 1.23 (d, J = 7.37 Hz, 3 H, MeCys-CH_3), 1.41 (d, J = 7.06 Hz, 3 H, Ala-CH_3), 1.87–1.94 (m, 3 H, Pro-H_β + Pro-2H_γ), 1.95–2.02 (m, 1 H, Val-H_β), 2.13–2.20 (m, 1 H, Pro-H_β), 3.20 (m, 1 H, MeCys-H_β), 3.65 (m, 1 H, Pro-H_δ), 3.73 (m, 1 H, Pro-H_δ), 3.85 (s, 2 H, Gly-CH_2), 3.96 (q, J = 7.13 Hz, 1 H, Ala-H_α), 4.03 (d, J = 7.90 Hz, 1 H, Val-H_α), 4.07 (q, J = 7.33 Hz, 1 H, Ala-H_α), 4.30 (m, 1 H, Pro-H_α), 5.51 (d, J = 7.82 Hz, 2 H, vinyl-H). [A-(2*R*,3*S*)-MeCys-PGDhaVA]$_2$ (**18**) was prepared from [A-(2*R*,3*S*)-MeCys-PGU(Ph)VA]$_2$ (6.2 mg, 0.0041 mmol) in 86% yield (4.2 mg). MS-ESI for $[M + H]^{2+}$ calcd. 599.3, found 599.7. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.78 (d, J = 6.76 Hz, 3 H, Val-CH_3), 0.80 (d, J = 6.81 Hz, 3 H, Ala-CH_3), 1.06 (d, J = 7.03 Hz, 3 H, Val-CH_3), 1.23 (d, J = 7.24 Hz, 3 H, MeCys-CH_3), 1.34 (d, J = 7.09 Hz, 3 H, Ala-CH_3), 1.72–2.00 (m, 4 H, Pro-H_β + Pro-2H_γ + Val-H_β), 2.13–2.21 (m, 1 H, Pro-H_β), 3.42 (m, 1 H, MeCys-H_β), 3.55 (m, 1 H, Pro-H_δ), 3.74 (m, 1 H, Pro-H_δ), 3.84 (s, 2 H, Gly-CH_2), 3.99 (m, 1 H, Ala-H_α), 4.02 (d, J = 7.20 Hz, 1 H, Val-H_α), 4.10 (m, 1 H, Ala-H_α), 4.28 (m, 1 H, Pro-H_α), 5.09 (d, J = 3.05 Hz, 1 H, MeCys-H_α), 5.46 (d, J = 11.8 Hz, 2 H, vinyl-H). [A-(2*S*,3*R*)-MeCys-PGDhaVA]$_2$ (**19**) was prepared from [A-(2*S*,3*R*)-MeCys-PGU(Ph)VA]$_2$ (6.8 mg, 0.0045 mmol) in 82% yield (4.5 mg). MS-ESI for $[M + H]^+$ calcd. 1197.5, found 1197.7. ^{1}H NMR: (D_2O, 500 MHz): δ ppm 0.78 (d, J = 6.79 Hz, 3 H, Val-CH_3), 0.80 (d, J = 6.86 Hz, 3 H, Ala-CH_3), 1.10 (d, J = 7.03 Hz, 3 H, Val-CH_3), 1.19 (d, J = 7.22 Hz, 3 H, MeCys-CH_3), 1.38 (d, J = 7.11 Hz, 3 H, Ala-CH_3), 1.82–1.91 (m, 3 H, Pro-H_β + Pro-2H_γ), 1.92–2.00 (m, 1 H, Val-H_β), 2.13–2.21 (m, 1 H, Pro-H_β), 3.25 (m, 1 H, MeCys-H_β), 3.63 (m, 1 H, Pro-H_δ), 3.72 (m, 1 H, Pro-H_δ), 3.83 (s, 2 H, Gly-CH_2), 3.95 (q, J = 7.17 Hz, 1 H, Ala-H_α), 4.01 (d, J = 6.70 Hz, 1 H, Val-H_α), 4.02 (q, J = 7.13 Hz, 1 H, Ala-H_α), 4.28 (m, 1 H, Pro-H_α), 5.01 (d, J = 5.80 Hz, 1 H, MeCys-H_α), 5.49 (d, J = 16.10 Hz, 2 H, vinyl-H).

Preparation of methyllanthionines from 17, 18 and 19

[A-(2*R*,3*R*)-MeCys-PGDhaVA]$_2$ (**17**) (0.3 mg, 0.00025 mmol) was dissolved in 0.5 mL of degassed CH_3CN/20 mM aq. NH_4OAc (1 : 1). A solution of TCEP (1.7 mg, 6.0 μmol) in 20 mM NH_4OAc (0.09 mL) was added and the reaction mixture was stirred at room temperature until complete as determined by RP-HPLC. Aqueous NH_4OAc (20 mM, 3.0 mL) was added to the mixture and the pH of the mixture was adjusted to 8–9 by the addition of Et_3N. The cyclization furnished two products as determined by RP-HPLC, none of which coeluted with the product obtained from *E*-**1**. The cyclization of [A-(2*R*,3*S*)-MeCys-PGU(Ph)VA]$_2$ (**18**) also afforded two peaks, neither of which coeluted with the product from *E*-**1**. The cyclization of [A-(2*S*,3*R*)-MeCys-PGDhaVA]$_2$ (**19**) also led to the formation of two compounds. The first peak coeluted with the cyclization product of *E*-**1** and showed identical ^{1}H and COSY NMR spectra.

Acknowledgements

This work was supported by grants from the Beckman Foundation, the National Institutes of Health (GM58822), and the Research Corporation (R10187). YZ acknowledges support from a Zeneca Graduate Fellowship. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence, funded in part by the W.M. Keck Foundation, NIH (PHS 1 S10 RR10444), and NSF (CHE 96-10502). The purchase of the mass spectrometers was funded in part by the National Institutes of Health (Voyager, RR 11966; Quattro, RR 07141; Micromass 70-SE-4F, GM 27029; Micromass 70 VSE, RR 04648). Any opinions, findings, and conclusions expressed in this publication are those of the authors and do not necessarily reflect the views of the Beckman Foundation, NIH, NSF, or the Research Corporation.

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