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The stereospecific synthesis of 'orthogonally' protected lanthionines☆

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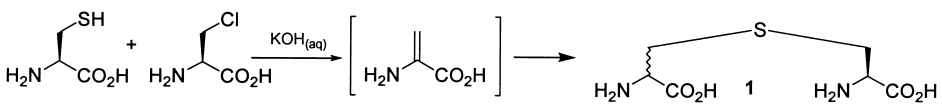
Abstract—Lanthionine is an attractive monomer for the design and synthesis of novel conformationally constrained peptidomimetics, since unlike cystine, the monosulfur bridge of lanthionine is chemically far more robust and also displays a greater degree of conformational rigidity. The synthesis of lanthionine residues for use in peptide synthesis is non-trivial due to the protectional requirements necessary for this tetra-functional amino acid. In this paper an efficient stereo-specific route to orthogonally protected lanthionine is described.

1. Introduction

Lanthionine is a non-proteinogenic amino acid comprised of two alanyl residues bridged by a thioether linkage. This unusual amino acid was first detected in 1941 in alkaline hydrolysates of wool,[1] and was subsequently found in human hair,[2] feathers,[3] chick embryos[4] and epidermal keratin.[5] Lanthionine has also been found in cataractous human lenses, and it is believed that the photo-oxidative degradation of cystine residues results in the formation of lanthionine; the subsequent increase in the extent of protein cross-bridging leading to increased tissue rigidity and hardening of the eye lens.[6] Other bridged amino acids such as lysinoalanine have also been implicated in the ageing process.[7] Biosynthetically, lanthionine is derived from the Michael addition of cysteine onto dehydroalanine.[8] The processing of many foods involves treatment with heat or alkali at some stage, treatments which are known to give rise to novel amino acids such as histidinoalanine, lysinoalanine and lanthionine,[9] which in turn have consequences for nutrition, food safety and health.[7] Lanthionine is also of interest in the silk[10] and leather[11] industries. In contrast to the labile disulfide bond of cystine, the monosulfur bridge of lanthionine is chemically far more robust, while cyclic structures based on lanthionines also display a greater degree of conformational rigidity. For these reasons, lanthionine is an attractive monomer for the design of novel conformationally constrained peptidomimetics. However, the synthesis of lanthionine residues for use in peptide synthesis is non-trivial for two main reasons. Often, the reactive synthons employed in the construction of the lanthionine skeleton can undergo eliminative side reactions, thus the maintainance of stereochemical integrity throughout the reaction course is frequently a problem. A second issue is the discrimination of the two amine and two acid functions of the lanthionine residue. The selection of orthogonal protecting groups can be pivotal to the success of lanthionine forming reactions.

The first synthesis of lanthionine was performed by du Vigneaud and Brown[12,13] in 1940 (Scheme 1). Their strategy involved the *S*-alkylation of L-cysteine with L-β-chloroalanine. However, the strongly basic conditions required resulted in the formation of dehydroalanine from



Scheme 1. The first synthesis of lanthionine.

☆ Swali, V. PhD Thesis, University of Southampton, 2000.

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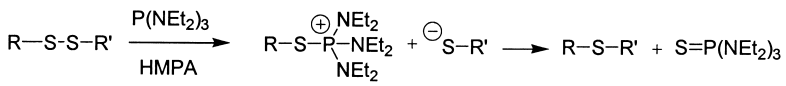
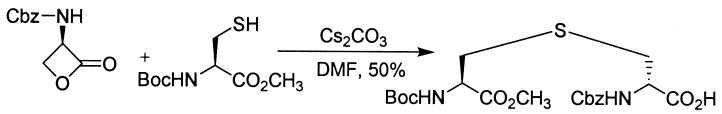


Scheme 2. Mono-desulfurisation of disulfides.

the chloroalanine and the subsequent 1,4-addition of the thiolate anion afforded a diastereomeric mixture of lanthionine **1** in poor yield.

An attractive approach to the synthesis of lanthionines involves the extrusion of sulfur from cystine residues, a methodology which was reported by Harpp and Gleason for a range of alkyl thioethers[14] (Scheme 2).

However the application of this method to lanthionine synthesis has proven to be problematic for several groups, including our own, with large amounts of the dehydroamino acid being formed.[15] Despite these problems, Shiba et al. accomplished the total synthesis of the lantibiotic nisin using the sulfur extrusion method to generate lanthionine ring fragments which were duly condensed.[16] Another route to lanthionines involves the opening of a serine β-lactone[17] with a protected cysteine residue. This method was utilised by Goodman to prepare a series of tri-protected lanthionine residues (Scheme 3).[18]



Scheme 3. Synthesis of lanthionine by ring opening of serine β-lactones.

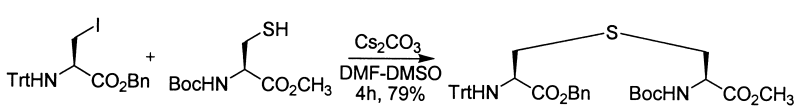
This methodology was used to generate lanthionine derivatives for the solid phase synthesis of peptidomimetics, although only moderate yields were obtained due to competing *O*-acyl fission.[19] However, the use of the Cbz protecting groups limits application. Bradley utilised a biomimetic synthesis to allow the preparation of a fully protected lanthionine derivative suitable for solid phase synthesis. Thus the Michael reaction between Boc-Dha-OMe and Fmoc-Cys-OAll afforded the desired lanthionine in 70% yield.[15] The two diastereoisomers were separable by HPLC, however drawbacks to this approach included the low overall yield of the desired *meso* isomer. Recently, Dugave and Menez detailed a solution phase synthesis of lanthionines[20] involving the alkylation of cysteinyl derivatives with *N*-triphenylmethyl (trityl) protected iodoalanines (Scheme 4). It was found that steric buttressing by the trityl group protected the iodoalanine residue from β-elimination, though small amounts of the aziridine were isolated. Analysis of the lanthionines showed the stereochemistry of the iodoalanine chiral centre to be retained during substitution. However, the scope of this thioalkylation methodology was limited due to the protecting groups utilised.

Despite the interest surrounding the lantibiotic family of peptides, relatively little has been reported concerning the solid phase incorporation of lanthionine into synthetic peptides. The reasons for this as discussed earlier being the formation of an optically homogeneous lanthionine skeleton and the chemo-differentiation of the two acid and two amino functions. Goodman has reported methodology which allows for the solid phase synthesis of cyclolanthionines: the technique utilised lanthionyl monomers derived from serine β-lactones which were subsequently cyclised and cleaved using an oxime linker.[21] Mayer and co-workers have detailed a procedure for the in situ synthesis of lanthionines on the solid phase.[22] Their approach involved the conversion of a serine unit within the peptide sequence into bromoalanine. This residue was then used to alkylate a cysteinyl monomer, also contained within the peptide. However, the stereochemical integrity of these conversions is in doubt, it being probable that the reactions proceed via a dehydroalanine residue. It is thus evident that despite much activity in this area, there is still a requirement for a high yielding synthesis of a lanthionine derivative, which is appropriately protected for solid phase initiatives. Furthermore, it is essential that the reaction proceeds without epimerisation of either chiral centre and that the desired lanthionine product can be isolated without difficulty.

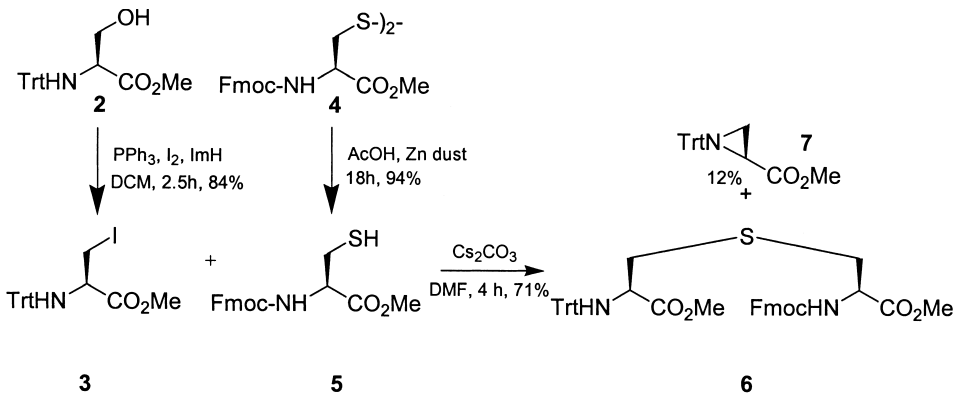
2. Results and discussion

2.1. Lanthionine synthesis

Our first attempts to prepare lanthionine derivatives used Mitsunobu chemistry. A major consideration in Mitsunobu reactions performed on serine is the fate of the activated alcohol. In the case of N-protected serines the activated intermediate decomposes intramolecularly, yielding lactones. Bis-protected derivatives however are highly prone to elimination, forming dehydroalanines. In order to circumvent this general problem the amino protecting group, triphenylmethyl (Trt) has been used. The steric bulk of this functionality shields the α-carbon centre of amino acids, thus protecting them from base promoted racemisation.[23] *N*-Trityl amino acid esters have also been shown to withstand saponification, again due to the spatial properties of the trityl group. In light of these results, we attempted the



Scheme 4. The Dugave–Menez approach to lanthionines utilising β-iodoalanines.



Scheme 5. Successful synthesis of lanthionine by thioalkylation.

synthesis of lanthionine derivatives via the Mitsunobu reaction of *N*-trityl-serine methyl ester[24] using Fmoc-Cys-OMe as the nucleophile, with DIAD and PPh_3. However, this reaction failed to generate the desired lanthionine. The reaction was repeated with a variety of redox carriers (DEAD, DIAD, TMAD, PPh_3, PBu_3) without success. It was believed that following the initial activation of the triphenylphosphine by DIAD, the nucleophilic thiol component reacted at the phosphorus centre. At this stage, a report on the synthesis of thioethers using modified Mitsunobu chemistry caused us to change our strategy. Falck demonstrated that a variety of aliphatic thiols could undergo Mitsunobu reactions with primary alcohols using ADDP/PMe_3 in the presence of imidazole.[25] However the only material isolated when these conditons were applied was, surprisingly, the trityl protected dehydroamino acid. Other *N*-trityl protected alanine β-cation equivalents were therefore utilised in subsequent attempts to synthesise lanthionines. Sulfonate esters of serine in thioalkylations were fruitless and resulted in formation of the aziridine. Dugave and Menez[20] reported similar findings during this work, however, they showed that the iodide, generated from the serine mesylate was much more pliable. It was envisaged therefore that iodoalanine could be a potentially useful intermediate in the synthesis of lanthionines. The literature route to the iodide via the mesylate was found to be capricious, often giving significant amounts of aziridine or poor conversion. A simple one-step phosphine mediated iodination[26] of *N*-Trt-Ser-OMe (**2**) was therefore developed to afford the desired iodide (Scheme 5). The iodide (**3**) was found to be highly photosensitive and thermally unstable, but could be stored for up to a month at $-20^{\circ}C$ without appreciable degradation. NMR analysis indicated the presence of two rotamers arising from restricted rotation about the $C_\alpha-C_\beta$ bond, but variable temperature NMR experiments showed no coalescence of rotameric signals below 323 K, while above this temperature the sample decomposed to the aziridine.

The synthesis of lanthionines utilising the iodide (**3**) via solution phase thioalkylation was attempted. It was hoped that dehydroalanine formation would be suppressed by the steric protection of the trityl group, while aziridine formation would be minimised by the selection of reaction conditions. Thus cystine was suitably protected to give (**4**) prior to conversion into cysteine (**5**) by reduction with zinc in acetic acid. The coupling of the iodide (**3**) with cysteine (**5**) was performed in DMF with 1 equiv. of Cs_2CO_3 (Scheme 5), which after purification by column chromatography afforded the desired lanthionine (**6**) in 71% yield. A small quantity (12%) of aziridine (**7**) was also isolated.

2.2. Stereochemical course of lanthionine formation

The other lanthionine diastereomer was prepared in similar fashion. Optical rotations correlated well with those of the corresponding compounds from the L-series and with literature data. Analysis was also carried out by NMR and, to this end, the two diastereomeric lanthionines were converted into their Mosher amides by detritylation and amidation with (2*R*)-2-methoxy-2-phenyl-3,3,3-trifluoroacetyl chloride.[27] Analysis confirmed that lanthionine synthesis had transpired stereoselectively, in particular, the resonance of the methoxy group was found to vary significantly according to the lanthionine stereochemistry. By recording the ^1H NMR of a mixed sample it was possible to demonstrate the optical purity of the individual samples was >95% and hence the stereo-integrity of the thioalkylation step.

2.3. An orthogonally protected lanthione

Following successful stereospecific synthesis of lanthionines it was necessary to extend the methodology. In particular, we envisaged a Trt/Allyl/Fmoc strategy would facilitate entry into the cyclo-lanthionyl systems via immobilisation onto a solid support via esterification or

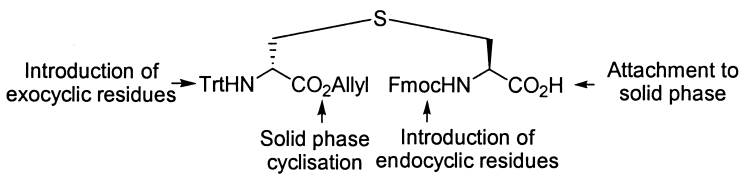
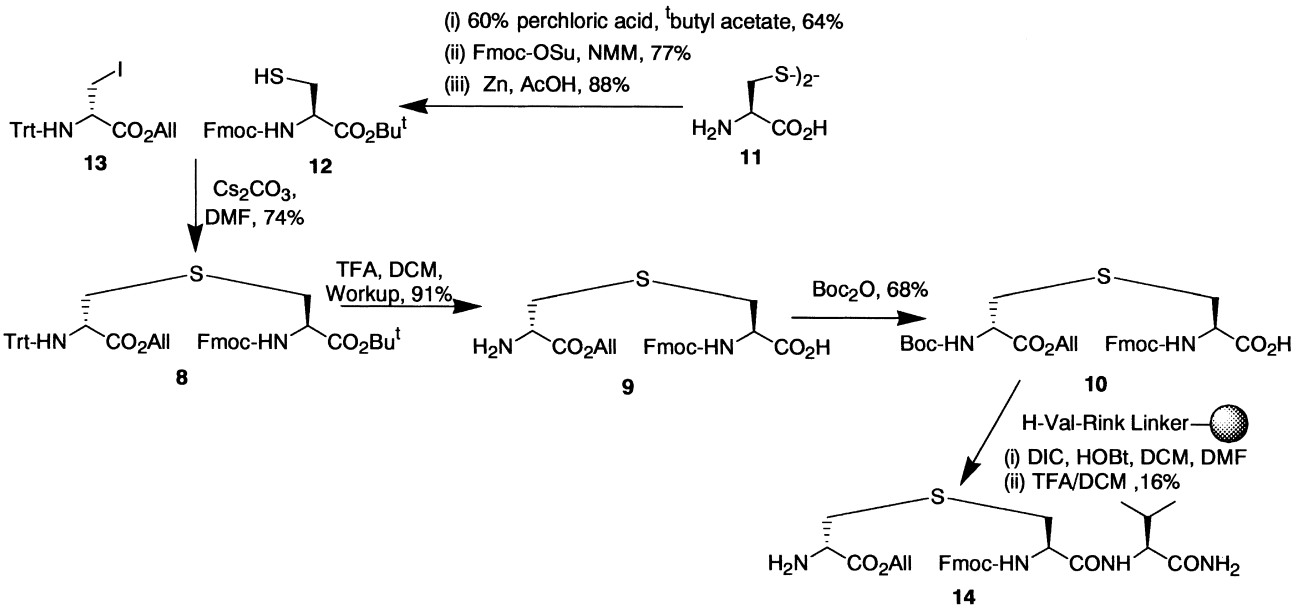


Figure 1. Protecting group strategy for synthesis of cyclic lanthionine fragments.



Scheme 6. Orthogonally protected lanthionine synthesis.

amidation of the unprotected C-terminus (Fig. 1), followed by Fmoc peptide chemistry, Pd(0) removal of the Allyl ester, cyclisation and chain extension following Trityl removal.

Initial attempts at generating tri-protected lanthionines utilising Fmoc-Cys-OH as the nucleophilic synthon partnered with an iodoalanine furnished poor yields of the required lanthionine with the mass balance being accounted for by aziridine formation. Another strategy was therefore adopted subjecting the fully protected lanthionine residue (**6**) to regioselective saponification in an attempt to provide a suitable monomer for solid phase attachment and synthesis. Although lanthionine (**6**) contains three potentially base labile functionalities, two esters and the Fmoc group, Zervas[8] demonstrated that the steric properties of the *N*-Trt group will block α-methyl ester hydrolysis. Two groups have reported the selective removal of a methyl ester in the presence of an Fmoc group. Burke for example saponified a tyrosine derivative without significant loss of the Fmoc group using ice-cold 0.2 M LiOH in dioxane,[28] while Pascal and Sola[29] observed that the addition of high concentrations of calcium chloride to the reaction media dramatically increased the lifetime of the Fmoc group in basic solution. The regioselective saponification of (**6**) in alkaline solution containing calcium chloride was therefore attempted. Unfortunately, work-up of the reaction furnished only starting material, due to the limited solubility of the substrate under the reaction conditions (^{i}PrOH/H_2O, 7:3), while other mixed solvent systems were also unsuccessful. Attempts at performing selective hydrolysis with lithium hydroxide in dioxane were equally fruitless, a single equivalent of base failed to afford the tri-protected monomer while an excess led to deprotection of the Fmoc group. A chemoenzymatic approach to the lanthionyl acids was also considered. The use of hydrolytic enzymes, especially esterases and lipases, in organic synthesis has been well documented,[30] in particular, porcine liver esterase (PLE) has found considerable use in a variety of stereo and regioselective tranformations. To this end the enzymatic monohydrolysis of lanthionine (**6a**) with PLE and PPL was attempted. Despite using a variety of reaction conditions, it was not possible to isolate the requisite acid, the lack of hydrolysis being ascribed to either the poor solubility in acetone/water mixtures or the bulky nature of the substrate. The problems associated with the regioselective monohydrolysis of lanthionyl diesters led to a reassessment of the route to the lanthionine acid. Differential protection of the acid moieties in (**6**) would afford a tetra-functionalised lanthionine unit (**8**) (Scheme 6). Acidolysis would furnish (**9**), which could be re-protected to give (**10**).

Synthesis of iodoalanine (**13**) began with esterification of D-serine under Dean–Stark conditions with allyl alcohol,[31] followed by *N*-tritylation of the ester affording di-protected serine, which was transformed into the desired, photosensitive, iodide (**13**) as previously described. As expected, the iodide exhibited rotational isomerism by NMR, and degraded to the corresponding aziridine at elevated temperatures. The thiol component was realised from cystine (**11**) by protection of both acid functions with *tert*-butyl acetate in perchloric acid,[32] double *N*-protection under standard conditions (Fmoc-OSu) to furnish the symmetrical cystine derivative, which was reduced with zinc/acetic acid[33] to give the desired thiol (**12**). The cesium salt of the thiol (**12**) was alkylated with the β-iodoalanine (**13**) in DMF, to afford the desired tetra-functional lanthionine (**8**) as a white foam (74%). The aziridine, formed by the intra-molecular degradation, was also obtained (16%). Acidolytic deprotection of the trityl group was effected with TFA in DCM (our fears that the TIS/TFA system could reduce the allyl olefin[34] were assuaged by ES–MS and NMR analysis) and *N*-protection with Boc_2O in dioxane proved facile, affording the orthogonally protected monomer in 68% yield. Following the successful synthesis of the lanthionine monomer its utility in solid phase peptide synthesis was tested. Thus the lanthionine monomer (**10**) was coupled onto a solid support

(Fmoc-Val-Rink linker-PS). TFA cleavage gave the expected lanthionine derivative (2*S*,6*R*)-N^6-(9*H*-fluorenylmethoxycarbonyl)-lanthionyl-(O^1-allyl)-valinamide (**14**).

3. Conclusion

Despite the simplicity of its structure, the synthesis of lanthionine derivatives, which have utility as peptidomimetics, is by no means trivial. We have demonstrated the utility of iodoalanines in the generation of synthetically valuable lanthionines, useful for solid phase synthesis. Such iodide synthons may be rapidly accessed from the chiral pool, though their instability should not be forgotten. It has been shown by chemical correlation and the method of Mosher that the formation of lanthionines by thioalkylation of iodides is a stereospecific process. These lanthionines are now available for the synthesis of a range of constrained peptido-mimetics and other synthetic targets, including the lantibiotics.

4. Experimental

4.1. General

NMR spectra were obtained at 298 K using a Bruker AC-300 spectrometer (300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR and 282 MHz for ^{19}F NMR) and a Bruker DPX-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR). Chemical shifts (δ) were referenced to the residual proton signals of deuterated solvents. ES–MS was performed on a VG Platform quadrupole electrospray ionisation mass spectrometer. FAB mass spectra were obtained on a VG analytical 70-250-SE normal geometry double focusing mass spectrometer, using argon as a bombarding gas. High resolution mass measurements were recorded at 10,000 resolution using mixtures of polyethylene glycol and/or polyethylene glycol methyl ethers as mass calibrants for FAB. Infra-red spectra were recorded on a Bio-Rad Golden Gate FTS 135 spectrophotometer with neat solids or solutions. Optical rotations were measured using an AA-100 polarimeter from Optical Activity Ltd using a path length of 5 cm. Melting points were determined using open capillaries on a Gallenkamp apparatus and remain uncorrected. TLC was performed using Alugram® silica gel 60 F_{254} (0.25 mm) plates and visualised using UV, phosphomolybdic acid, ninhydrin or bromocresol green.

4.1.1. (2*R*)-*N*-Triphenylmethyl-3-iodoalanine methyl ester (3). The title compound was prepared by a modification to the Garegg procedure.[26] Thus (2*S*)-*N*-triphenylmethyl serine methyl ester (2.00 g, 5.54 mmol), triphenylphosphine (1.45 g, 5.54 mmol, 1 equiv.) and imidazole (0.38 g, 5.54 mmol, 1 equiv.) were dissolved in dry DCM (50 mL) with stirring. The solution was cooled on ice to 0°C and stirring continued for 5 min. Iodine (1.41 g, 5.54 mmol, 1 equiv.) was added portionwise over 2 min and stirring continued for 2.5 h in the dark. Solvent was removed in vacuo and the residue was purified by column chromatography to afford the title compound as a photosensitive amorphous off white solid solid (2.19 g, 84%). Data agreed with the literature.[20] R_f (ether/hexane, 1:9 v/v): 0.41; LRMS (ESI+) *m/z*: 243 $(Trt)^+$; 472 $(M+H)^+$; δ_H (300 MHz; $CDCl_3$; * denotes minor rotamer): 2.20 (bs, 1H, N*H*); 2.51* (dd, *J*=6, 13 Hz)+3.14 (dd, *J*=7, 10 Hz) (1H, CH_β); 2.68* (dd, *J*=9, 13 Hz)+3.27 (dd, *J*=4, 10 Hz) (1H, C$H_{\beta'}$); 3.23 (s)+3.69* (s) (3H, CO_2CH_3); 3.39 (m)+4.39* (m) (1H, CH_α); 7.09–7.28 (m, 9H, Trt Ar*H*); 7.41–7.56 (m, 6H, Trt Ar*H*). δ_C (75 MHz; $CDCl_3$; * denotes minor rotamer): 9.8*+48.5 (CH_β); 20.3+56.4* (CH_α); 52.1*+53.0 (CO_2CH_3); 71.2*+77.4 (Ph_3CNH); 126.7–129.4 (complex, Ar *C*H); 145.6+145.8* (*ipso* Ar-*C*); 171.3+172.9* (CO_2CH_3); IR (neat) ν (cm^{-1}): 3100–2800 (br, w); 1733 (m; C=O); 1595 (w); 1489 (w); 1447 (w); 1208 (m); 1158 (m); 1028 (w); 900 (w); 743 (s); 701 (s); $[\alpha]_D^{20}$=+23 (*c*=1, $CHCl_3$), lit. $[\alpha]_D^{25}$=+21 (*c*=1, $CHCl_3$).

4.1.2. (2*S*)-*N*-Triphenylmethyl-3-iodoalanine methyl ester (3b). Repetition of the above methodology afforded the title compound in 85% yield. Data as above except: $[\alpha]_D^{20}$=−21.0 (*c*=1, $CHCl_3$).

4.1.3. (2*R*)-*N*-(9*H*-Fluorenylmethoxycarbonyl) cysteine methyl ester (5). The title compound was prepared using a modification of the Kihlberg procedure.[33] To a solution of (2*R*,7*R*)-*N*,*N*-bis-(9*H*-fluorenylmethoxycarbonyl) cystine dimethyl ester (5.00 g, 7.0 mmol) in acetic acid (50 mL) and DCM (25 mL) was added acid washed zinc dust (4.56 g, 70.2 mmol, 10 equiv.) portionwise. The mixture was stirred under nitrogen overnight and the solvent removed in vacuo. 2 M HCl (250 mL) was added to the residue and the mixture extracted with DCM (2×250 mL). The combined organics were washed with water, dried over $MgSO_4$ and concentrated in vacuo to afford the title compound as a white solid (4.71 g, 94%) which was used immediately.

4.1.4. (2*R*,6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-lanthionine dimethyl ester (6a). The title compound was prepared using a modification of the Dugave and Menez procedure.[20] Thus, (2*R*)-*N*-triphenylmethyl-3-iodoalanine methyl ester (1.70 g, 3.61 mmol) and (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine methyl ester (1.29 g, 3.61 mmol, 1 equiv.) were dissolved in dry DMF (20 mL). Cesium carbonate (1.18 g, 3.61 mmol, 1 equiv.) was added and the reaction mixture stirred under nitrogen in the dark for 4 h. 10% citric acid solution (100 mL) was added and the solution extracted with ether (2×50 mL). The combined organics were washed with brine, dried over $MgSO_4$ and the solvent removed in vacuo. The residue was purified by column chromatography to afford the title compound as a white foam (1.80 g, 71%); R_f (ethyl acetate/hexane, 3:17 v/v): 0.12; LRMS (ESI+) *m/z*: 243 $(Trt)^+$; 701 $(M+H)^+$; 723 $(M+Na)^+$; 739 $(M+K)^+$; HRMS (FT-MS) calcd for $C_{42}H_{41}O_6N_2S$ 701.2680. Found 701.2682; δ_H (400 MHz; $CDCl_3$): 2.27 (br s, 1H, Trt N*H*); 2.40–3.01 (m, 4H, CH_2SCH_2); 3.14 (s, 3H, TrtNHCH(R)CO_2CH_3); 3.39–3.51 (m, 1H, CH_α); 3.81 (s, 3H, Fmoc NHCH(R)CO_2CH_3); 4.15 (t, *J*=7 Hz, 1H, Fmoc *H*-9); 4.21–4.43 (m, 2H, Fmoc CH_2O); 4.52 (m, 1H, C$H_{\alpha'}$); 5.70 (d, *J*=7 Hz, 1H, Fmoc N*H*); 7.24 (t, *J*=7 Hz, 3H, Trt Ar *p*-*H*); 6.95–7.51 (m, 16H, Trt Ar*H*+Fmoc *H*); 7.68 (br d, *J*=7 Hz, 2H, Fmoc *H*-1+*H*-8); 7.83 (d, *J*=7 Hz, 2H, Fmoc *H*-4+*H*-5); δ_C (100 MHz; $CDCl_3$): 34.5 (CH_2SCH_2); 35.8 (CH_2SCH_2); 47.5 (Fmoc *C*H); 52.3 (CO_2CH_3); 53.2

(CO_2CH_3); 54.2 (CH_α); 56.9 ($CH_{\alpha'}$); 67.6 (Fmoc CH_2); 71.6 (Ph_3C); 120.4, 125.6, 127.0, 127.5, 128.2, 128.4, 129.2 (Fmoc Ar *C*H+Trt Ar *C*H); 141.7, 144.2 (Fmoc Ar-*C*); 146.0 (Trt *ipso* Ar-*C*); 156.2 (Fmoc O*C*ONH); 171.4 (CO_2CH_3); 174.3 (CO_2CH_3); IR (neat) ν (cm^{-1}): 1722 (br; s); 1493 (m); 1446 (m); 1205 (br; s); 1028 (br; m); 739 (s); 704 (s); $[\alpha]_D^{20}$=+48.8 (*c*=0.5, ethyl acetate).

4.1.5. (2*S*,6*R*)-*N*^{2}-Triphenylmethyl-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine dimethyl ester (6b). White foam, LRMS (ESI+) *m*/*z*: 243 (Trt)$^+$; 701 (M+H)$^+$; 723 (M+Na)$^+$; 739 (M+K)$^+$; δ_H (400 MHz; $CDCl_3$): 1.95 (br s, 1H, Trt N*H*); 2.80 (dd, *J*=8, 13 Hz, 1H, $CH_\beta H_{\beta'}S$); 2.94–3.03 (m, 2H, $CH_\beta H_{\beta'}S$+$SCH_\beta H_{\beta'}$); 3.09 (dd, *J*=5, 14 Hz, 1H, $SCH_\beta H_{\beta'}$); 3.30 (s, 3H, TrtNHCH(R)CO_2CH_3); 3.59 (dd, *J*=5, 7 Hz, 1H, CH_α); 3.81 (s, 3H, Fmoc NHCH(R)CO_2CH_3); 4.30 (t, *J*=7 Hz, 1H, Fmoc *H*-9); 4.48 (d, *J*=7 Hz, 2H, Fmoc CH_2O); 4.69 (m, 1H, $CH_{\alpha'}$); 5.74 (d, *J*=8 Hz, 1H, Fmoc N*H*); 7.24 (t, *J*=7 Hz, 3H, Trt Ar *p*-*H*); 7.32 (dd, *J*=7, 7 Hz, 6H, Trt Ar *m*-*H*); 7.35–7.40 (m, 2H, Fmoc *H*-2+*H*-7); 7.46 (dd, *J*=7, 7 Hz, 2H, Fmoc *H*-3+*H*-6); 7.56 (d, *J*=7 Hz, 6H, Trt Ar *o*-*H*); 7.68 (br d, *J*=7 Hz, 2H, Fmoc *H*-1+*H*-8); 7.83 (d, *J*=7 Hz, 2H, Fmoc *H*-4+*H*-5); δ_C (100 MHz; $CDCl_3$): 34.2 (CH_2SCH_2); 35.0 (CH_2SCH_2); 47.2 (Fmoc *C*H); 52.0 (CO_2CH_3); 52.6 (CO_2CH_3); 53.7 (CH_α); 56.3 ($CH_{\alpha'}$); 67.4 (Fmoc CH_2); 71.3 (Ph_3C); 120.1, 125.3, 126.7, 127.2, 127.9, 128.1, 128.9 (Fmoc Ar *C*H+Trt Ar *C*H); 141.4, 143.9 (Fmoc Ar-*C*); 145.7 (Trt *ipso* Ar-*C*); 155.9 (Fmoc O*C*ONH); 171.2 (CO_2CH_3); 174.1 (CO_2CH_3); IR (DCM) ν (cm^{-1}): 1721 (br; s); 1495 (m); 1444 (m); 1205 (br; s); 1027 (br; m); 738 (s); 699 (s); $[\alpha]_D^{20}$=−51.2 (*c*=0.5, ethyl acetate).

4.1.6. (2*R*,6*R*)-*N*^{2}-[(2*R*)-(2-Methoxy-2-phenyl-3,3,3-trifluoropropionoyl]-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine dimethyl ester. The title compound was prepared by using a modification of the Mosher procedure.[27] Thus, (2*R*,6*R*)-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-*O*^{1},*O*^{7}-dimethyl lanthionine (**6a**), (50 mg, 109 μmol) in DMF (5 mL) was treated with (2*R*)-(2-methoxy-2-phenyl-3,3,3-trifluoroacetyl chloride 36 mg, 141 μmol) and DIPEA (57 μL, 328 μmol). The reaction mixture was stirred overnight and ethyl acetate (15 mL) added. The solution was extracted with 10% citric acid (3×15mL), 10% $NaHCO_3$ (3×15 mL), brine (2×15 mL) dried over $MgSO_4$ and the solvent removed in vacuo to afford a white foam (41 mg, 57%); LRMS (ESI+) *m*/*z*: 675 (M+H)$^+$; 697 (M+Na)$^+$; 713 (M+K)$^+$; 1371 (2M+H)$^+$; HRMS (FT-MS) calcd for $C_{33}H_{34}F_3O_8N_2S$ 675.1988. Found 675.1998; δ_H (300 MHz; $CDCl_3$): 2.79–3.21 (m, 4H, CH_2S+SCH_2); 3.52 (br s, 3H, OCH_3); 3.75 (s, 3H, Fmoc NHCH(R)CO_2CH_3); 3.77 (s, 3H, CONHCH(R)CO_2-CH_3); 4.24 (t, *J*=7 Hz, 1H, Fmoc *H*-9); 4.46 (d, *J*=7 Hz, 2H, Fmoc CH_2O); 4.51 (m, 1H, CH_α); 4.89 (m, 1H, $CH_{\alpha'}$); 5.60 (d, *J*=8 Hz, 1H, Fmoc N*H*); 7.28–7.70 (m, 11H, C_6H_5+Fmoc Ar*H*); 7.78 (d, *J*=7 Hz, 2H, Fmoc *H*-4+*H*-5); δ_C (75 MHz; $CDCl_3$): 34.9 (CH_2SCH_2); 35.0 (CH_2SCH_2); 47.2 (Fmoc *C*H); 52.0 (CH_α); 53.0 (CO_2CH_3); 53.1 (CO_2CH_3); 53.8 ($CH_{\alpha'}$); 55.4 (OCH_3); 67.3 (Fmoc CH_2); 77.4 (CF_3); 120.2, 125.2, 127.3, 127.8, 127.9, 128.7, 129.8, 132.6 (Ph *C*H+Fmoc Ar *C*H); 155.8 (Fmoc O*C*ONH); 166.7 (*C*ONH); 170.5 (CO_2CH_3); 171.0 (CO_2CH_3); δ_F ($CDCl_3$): 8.65 (s, 3F, CF_3); IR (neat) ν (cm^{-1}): 3346 (br, w); 1742 (s); 1721 (s); 1692 (s); 1511 (s); 1448 (m); 1440 (m); 1342 (m); 1317 (w); 1264 (m); 1213 (s); 1164 (s); 1105 (m); 911 (m); 734 (s); 697 (m).

4.1.7. (2*S*,6*R*)-*N*^{2}-[(2*R*)-(2-Methoxy-2-phenyl-3,3,3-trifluoropropionoyl]-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine dimethyl ester. The title compound was prepared as above from (**6b**) (selected data): (33 mg, 45%); white foam; HRMS (FAB) calcd for $C_{33}H_{34}F_3O_8N_2S$ 675.1988. Found 675.1988; δ_F ($CDCl_3$): 8.38 (s, 3F, CF_3).

4.1.8. (2*R*)-*N*-Triphenylmethyl serine allyl ester. The title compound was prepared by using a modification of the Baldwin procedure.[24] (2*R*)-Serine allyl ester toluenesulfonate salt (5.60 g, 16.7 mmol) was dissolved in dry DCM (100 mL) with stirring and cooled to 0°C. Triethylamine (3.38 g, 4.66 mL, 2 equiv.) was added dropwise, followed by triphenylmethyl chloride (5.12 g, 18.4 mmol, 1.1 equiv.) in DCM (50 mL). The solution was stirred at 0°C overnight and filtered. The filtrate was washed with 1 M citric acid (2×100 mL), water (2×100 mL), dried over $MgSO_4$ and solvent removed in vacuo. The residue was purified by column chromatography to afford the title compound as a clear oil (4.39 g, 68%); LRMS (ESI+) *m*/*z*: 243 (Trt$^+$); 388 (M+H)$^+$; 410 (M+Na)$^+$; HRMS (FAB) calcd for $C_{25}H_{26}O_3N$ 388.1913. Found 388.1907; $[\alpha]_D^{20}$=+28 (*c*=1, MeOH); δ_H (400 MHz; $CDCl_3$): 2.42 (br s, 1H, O*H* or N*H*); 3.07 (br s, 1H, O*H* or N*H*); 3.62 (m, 2H, CH_2OH); 3.77 (dd, *J*=7, 13 Hz, 1H, CH_α); 4.14 (dd, *J*=6, 13 Hz, 1H, allyl $CHH'CH{=}CH_2$); 4.26 (dd, *J*=6, 13 Hz, 1H, allyl $CHH'CH{=}CH_2$); 5.23 (dd, *J*=1, 11 Hz, 1H, allyl CH_2-$CH{=}CHH'$); 5.24 (dd, *J*=2, 17 Hz, 1H, allyl CH_2-$CH{=}CHH'$); 5.76 (ddt, *J*=6, 12, 17 Hz, 1H, allyl $CH_2CH{=}CHH'$); 7.21–7.43 (m, 9H, Trt Ar*H*); 7.45–7.62 (m, 6H, Trt Ar*H*); δ_C (100 MHz; $CDCl_3$): 58.2 (CH_α); 65.4 (CH_2OH or allyl $CH_2CH{=}CH_2$); 66.1 (CH_2OH or allyl $CH_2CH{=}CH_2$); 71.4 (Ph_3C); 119.0 (allyl $CO_2CH_2CH{=}CH_2$); 127.1, 128.4, 129.1 (Ar*C*H); 132.0 (allyl $CO_2CH_2CH{=}CH_2$); 146.0 (*ipso* Ar-*C*); 173.6 (CO_2CH_2); IR (neat) ν (cm^{-1}): 3056 (br, w); 1731 (s); 1489 (w); 1447 (w); 1169 (s); 1154 (s); 984 (m); 933 (m); 745 (s); 697 (s).

4.1.9. (2*S*)-*N*-Triphenylmethyl-3-iodoalanine allyl ester (13). The title compound was prepared by using a modification of the Garegg procedure.[26] (2*S*)-*N*-Triphenylmethyl serine allyl ester (1.19 g, 3.07 mmol), triphenylphosphine (0.80 g, 3.07 mmol, 1 equiv.) and imidazole (0.29 g, 3.07 mmol, 1 equiv.) were dissolved in dry DCM (50 mL) with stirring. The solution was cooled to 0°C and stirring continued for 5 min. Iodine (0.78 g, 3.07 mmol, 1 equiv.) was added portionwise over 2 min and stirring continued for 2.5 h in the dark. The solvent was removed in vacuo and the residue purified by column chromatography (SiO_2, ether/hexane, 1:15 v/v) to afford the title compound as a clear photosensitive gum (1.28 g, 84%); LRMS (ESI+) *m*/*z*: 243 (Trt)$^+$; 498 (M+H)$^+$; HRMS (FAB) calcd for $C_{25}H_{25}O_2NI$ 498.0930. Found 498.0925; δ_H (300 MHz; $CDCl_3$; rotamers 2:1; * denotes minor rotamer): 2.25–2.49* (br s)+2.93 (d, *J*=10 Hz) (1H, TrtN*H*); 2.54–2.65* (m)+3.25 (dd, *J*=7, 10 Hz) (1H, H_β); 2.70–2.82* (m)+3.36 (dd, *J*=4, 10 Hz) (1H, $H_{\beta'}$); 3.50–3.58 (m)+4.45* (dd, *J*=7, 9 Hz) (1H, H_α); 4.12 (dddd, *J*=1, 1, 6, 13 Hz)+4.27 (dddd, *J*=1, 1, 6, 13 Hz)+4.68* (ddd, *J*=1,

1, 6 Hz) (2H, allyl $CH_2CH{=}CH_2$); 5.20 (dd, J=1, 10 Hz)+5.32* (dd, J=1, 10 Hz) (1H, allyl $CH_2CH{=}CHH'$); 5.24 (dd, J=1, 18 Hz)+5.42* (dd, J=1, 18 Hz) (1H, allyl $CH_2CH{=}CHH'$); 5.77 (dddd, J=6, 6, 10, 18 Hz)+5.97* (dddd, J=6, 6, 10, 18 Hz) (1H, allyl $CH_2CH{=}CH_2$); 7.18–7.25 (m, 3H, Trt Ar*H*); 7.26–7.36 (m, 6H, Trt Ar*H*); 7.45–7.57 (m, 6H, Trt Ar*H*); δ_C (75 MHz; $CDCl_3$; * denotes minor rotamer): 10.1+48.6* (CH_β); 20.6*+56.3 (CH_α); 66.2+66.5* (allyl $CO_2CH_2CH{=}CH_2$); 71.0*+71.3 (Ph_3CNH); 118.9+119.1* (allyl $CO_2CH_2CH{=}CH_2$); 126.7, 126.8, 128.2, 128.6, 128.8 (Ar *C*H); 131.5*+131.8 (allyl $CO_2CH_2CH{=}CH_2$); 145.7*+145.8 (*ipso* Ar-*C*); 170.7*+172.1 (CO_2CH_2); IR (neat) ν (cm^{-1}): 3039 (br, w); 1730 (s); 1596 (w); 1490 (m); 1448 (m); 1415 (w); 1364 (w); 1323 (w); 1272 (w); 1206 (m); 1171 (s); 1115 (w); 1031 (w); 985 (w); 935 (w); 901 (w); 774 (w); 747 (m); 705 (s).

4.1.10. (2*R*)-*N*-(9*H*-Fluorenylmethoxycarbonyl) cysteine *tert*-butyl ester (12). Cystine (**11**) was protected according to the method of Amaral.[32] Thus (2*R*,7*R*)-cystine (1.92 g, 8.0 mmol) was dissolved in 60% perchloric acid (5.88 g, 35.2 mmol, 4.4 equiv.) with stirring. To the solution was added *tert*-butyl acetate (50 mL) and the mixture stirred for 2 h to give a homogeneous solution. The solution was cooled to 0°C for 24 h and filtered. The white solid was dissolved in ether (50 mL) and 1 M $NaHCO_3$ (25 mL) added, the organic layer was washed with 1 M $NaHCO_3$ (2×25 mL) and saturated sodium chloride solution (3×25 mL). The organic phase was dried over $MgSO_4$ and reduced in vacuo to furnish the desired compound as a clear oil (1.81 g, 64%); LRMS (ESI+) *m*/*z*: 353 $(M+H)^+$; 375 $(M+Na)^+$; 705 $(2M+H)^+$; δ_H (300 MHz; $CDCl_3$): 1.50 (s, 18H, $C(CH_3)_3$); 1.71 (s, 4H, NH_2); 2.85 (dd, J=7, 14 Hz, 2H, H_β); 3.12 (dd, J=4, 14 Hz, 2H, $H_{\beta'}$); 3.67 (dd, J=4, 7 Hz, 2H, H_α); δ_C (75 MHz; $CDCl_3$): 28.2 ($C(CH_3)_3$); 44.2 ($CH_\beta H_{\beta'}$); 54.4 (CH_α); 81.2 ($C(CH_3)_3$); 173.0 (CO_2^tBu); IR (neat) ν (cm^{-1}): 2977 (w); 1725 (s); 1478 (w); 1459 (w); 1393 (w); 1368 (m); 1251 (m); 1152 (s); 991 (w); 845 (w); 752 (w); $[\alpha]_D^{20}=-7.8$ (c=2, CH_3OH). (2*R*,7*R*)-cystine di-*tert*-butyl ester was protected according to the method of Jung.[32] *N*-(9*H*-Fluorenylmethoxycarbonyloxy) succinimide (5.40 g, 16.0 mmol, 0.8 equiv.) and (2*R*,7*R*)-cystine di-*tert*-butyl ester (3.52 g, 10.0 mmol) were dissolved in THF (10 mL). A solution of *N*-methylmorpholine (2.02 g, 2.20 mL, 20.0 mmol, 1.0 equiv.) in THF (5 mL) was added dropwise and the solution stirred for 3 h. Solvent was removed in vacuo and the residue taken up in ethyl acetate (100 mL). The solution was washed with 2 M $KHSO_4$ (3×100 mL) and water (2×100 mL), dried over $MgSO_4$ and reduced in vacuo. Further purification by flash column chromatography (SiO_2, chloroform) afforded the requisite compound as a white foam (6.11 g, 77%); mp: 150–152°C (lit. 151.5–152°C); LRMS (ESI+) *m*/*z*: 797 $(M+H)^+$; 814 $(M+NH_4)^+$; 819 $(M+Na)^+$; 835 $(M+K)^+$; δ_H (300 MHz; $CDCl_3$): 1.50 (s, 18H, $C(CH_3)_3$); 3.18 (dd, J=6, 14 Hz, 2H, H_β); 3.26 (dd, J=5, 14 Hz, 2H, $H_{\beta'}$); 4.22 (t, J=7 Hz, 2H, Fmoc *H*-9); 4.46 (d, J=7 Hz, 4H, Fmoc CH_2O); 4.60 (m, 2H); 5.78 (d, J=7 Hz, 2H, Fmoc N*H*); 7.30 (dd, J=7, 7 Hz, 4H, Fmoc *H*-2+*H*-7); 7.40 (dd, J=7, 7 Hz, 4H, Fmoc *H*-3+*H*-6); 7.60 (d, J=7 Hz, 4H, Fmoc *H*-1+*H*-8); 7.76 (d, J=7 Hz, 4H, Fmoc *H*-4+*H*-5); δ_C (75 MHz; $CDCl_3$): 28.2 ($C(CH_3)_3$); 42.0 ($CH_\beta H_{\beta'}$); 47.2 (Fmoc *C*H); 54.3 (CH_α); 67.3 (Fmoc CH_2); 83.3 ($C(CH_3)_3$); 120.1, 125.3, 127.2, 127.9 (Fmoc Ar *C*H); 141.4, 143.9 (Fmoc Ar-*C*); 155.9 (Fmoc O*C*ONH); 169.5 (CO_2^tBu); IR (neat) ν (cm^{-1}): 3341 (br, w); 2979 (br, w); 1708 (s); 1503 (m); 1449 (m); 1394 (w); 1368 (m); 1341 (m); 1221 (s); 1149 (s); 1045 (m); 842 (m); 756 (m); 736 (s); $[\alpha]_D^{20}=-5.1$ (c=2, $CHCl_3$), lit. $[\alpha]_D^{23}=-6.4$ (c=0.56, $CHCl_3$).

The title compound was prepared using a modification of the Kihlberg procedure.[33] Thus to a solution of (2*R*,7*R*)-*N*,*N*-bis-(9*H*-fluorenylmethoxycarbonyl) cystine di-*tert*-butyl ester (3.00 g, 3.9 mmol) in acetic acid (30 mL) was added zinc dust (2.53 g, 39.0 mmol, 10 equiv.) portionwise. The mixture was stirred under nitrogen overnight and the solvent removed in vacuo. 2 M $KHSO_4$ (100 mL) was added to the residue and the mixture extracted with DCM (2×50 mL). The combined organics were washed with water (2×50 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (SiO_2, ether/hexane, 1:4 v/v) afforded the title compound as a white foam (2.64 g, 88%); LRMS (ESI+) *m*/*z*: 417 $(M+NH_4)^+$; 438 $(M+K)^+$; 799 $(2M+H)^+$; 816 $(2M+NH_4)^+$; 821 $(2M+Na)^+$; 837 $(2M+K)^+$; HRMS (FAB) calcd for $C_{22}H_{25}O_4NS$ 400.1583. Found 400.1584; δ_H (400 MHz; $CDCl_3$): 1.26 (dd, J=9, 9 Hz, 1H, CH_2SH); 1.42 (s, 9H, $C(CH_3)_3$); 2.86–2.98 (m, 2H, $CH_\beta H_{\beta'}$); 4.15 (t, J=7 Hz, 1H, Fmoc *H*-9); 4.28–4.40 (m, 2H, Fmoc CH_2O); 4.43–4.49 (m, 1H, CH_α); 5.61 (d, J=7 Hz, 1H, Fmoc N*H*); 7.24 (ddd, J=8, 8, 2 Hz, 2H, Fmoc *H*-2+*H*-7); 7.33 (dd, J=8, 8 Hz, 2H, Fmoc *H*-3+*H*-6); 7.53 (d, J=8 Hz, 2H, Fmoc *H*-1+*H*-8); 7.68 (d, J=8 Hz, 2H, Fmoc *H*-4+*H*-5); δ_C (100 MHz; $CDCl_3$): 27.2 ($CH_\beta H_{\beta'}$); 28.4 ($C(CH_3)_3$); 47.6 (Fmoc *C*H); 55.9 (CH_α); 67.5 (Fmoc CH_2); 83.5 ($C(CH_3)_3$); 120.4, 125.5, 127.5, 128.2 (Fmoc Ar *C*H); 141.8, 144.1, 144.3 (Fmoc Ar-*C*); 156.1 (Fmoc O*C*ONH); 169.4 (CO_2^tBu); IR (neat) ν (cm^{-1}): 2978 (br, w); 2360 (br, w); 1721 (br, s); 1511 (m); 1345 (m); 1249 (w); 1220 (w); 1155 (s); 1060 (w); 913 (w); 844 (w); 740 (m).

4.1.11. (2*S*,6*R*)-N^2-Triphenylmethyl-N^6-(9*H*-fluorenylmethoxycarbonyl)-lanthionine O^1-allyl-O^7-*tert*-butyl esters (8). The title compound was prepared using a modification of the Dugave and Menez procedure.[20] Thus, (2*S*)-*N*-triphenylmethyl-3-iodoalanine allyl ester (497 mg, 1 mmol, 1eq) and (2*R*)-*N*-(9*H*-fluorenylmethoxycarbonyl) cysteine *tert*-butyl ester (399 mg, 1 mmol, 1 equiv.) were dissolved in dry DMF (10 mL). Cesium carbonate (326 mg, 1 mmol, 1 equiv.) was added and the reaction mixture stirred under nitrogen in the dark for 4 h. 10% Citric acid solution (100 mL) was added and the solution extracted with ether (2×50 mL). The combined organics were washed with brine, dried over $MgSO_4$ and solvent removed in vacuo. The residue was purified by column chromatography (SiO_2, ethyl acetate/hexane, 3:17 v:v) to afford the title compound as a white foam (568 mg, 74%) and the aziridine (59 mg, 16%); LRMS (ESI+) *m*/*z*: 769 $(M+H)^+$; 791 $(M+Na)^+$; 807 $(M+K)^+$; 1537 $(2M+H)^+$; HRMS (FAB) calcd for $C_{47}H_{48}O_6N_2S$ 769.3311. Found 769.3293; δ_H (300 MHz; $CDCl_3$): 1.49 (s)+1.50 (s) (9H, $C(CH_3)_3$); 2.25 (br s, 1H, N*H*); 2.42–2.73 (m, 2H, CH_2SCH_2); 2.83–3.19 (m, 2H, CH_2SCH_2); 3.53 and 3.61 (2t, J=9, 9 Hz, 1H, CH_α); 4.24 (dd, J=7, 15 Hz, 1H, Fmoc *H*-9); 4.32–4.44 (m, 2H, Fmoc CH_2O); 4.48–4.60 (m, 1H, $CH_{\alpha'}$); 4.68 (dd, J=4, 6 Hz, 2H, allyl $CO_2CH_2CH{=}CH_2$); 5.27 (dd, J=1, 10 Hz,

1H, allyl $CO_2CH_2CH{=}CHH'$); 5.36 (ddd, J=1, 1, 18 Hz, 1H, allyl $CO_2CH_2CH{=}CHH'$); 5.63 (d, J=8 Hz)+5.74 (d, J=8 Hz) (1H, conformers, N*H*); 5.95 (m, allyl $CO_2CH_2CH{=}CHH'$); 7.14–7.83 (m, 23H, Fmoc ArC*H*+Trt C*H*); δ_C (75 MHz; $CDCl_3$): 28.2 ($C(CH_3)_3$); 34.1+34.6 (CH_2SCH_2); 44.4+44.7 (CH_2SCH_2); 47.3 (Fmoc *C*H-9); 47.8+48.6 (CH_α); 54.1+54.4 ($CH_{\alpha'}$); 66.1+66.2 (allyl $CO_2CH_2CH{=}CH_2$); 67.4 (Fmoc CH_2O); 80.0 ($C(CH_3)_3$); 83.1 (Ph_3C); 119.0+119.1 (allyl $CO_2CH_2CH{=}CH_2$); 120.1, 125.3, 126.6, 127.3, 128.12, 128.7 (Fmoc ArC*H*+Trt C*H*); 131.8+131.9 (allyl $CO_2CH_2CH{=}CH_2$); 141.4, 144.0, 145.8 (Fmoc ArC*H*+Trt C*H*); 155.9 (Fmoc O*C*ONH);169.6, 171.6 (CO_2R); IR (neat) ν (cm^{-1}): 3308 (w); 2974 (w); 1720 (s); 1492 (w); 1448 (w); 1216 (m); 1148 (s); 1032 (m); 739 (s); 704 (s).

4.1.12. (2*S*,6*R*)-*N*^{6}-(9*H*-Fluorenylmethoxycarbonyl)-lanthionine *O*^{1}-allyl ester (9). (2*S*,6*R*)-*N*^{2}-Triphenylmethyl-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine *O*^{1}-allyl-*O*^{7}-*tert*-butyl esters (2.00 g, 2.71 mmol) was stirred with TFA (4.9 mL), DCM (4.9 mL) and TIS (0.2 mL) for 1 h. Solvent was removed in vacuo and the residue purified by column chromatography (SiO_2, MeOH/$CHCl_3$, 1:9 v:v) to afford the title compound as a white solid (1.16 g, 91%); LRMS (ESI+) *m*/*z*: 471 $(M+H)^+$; 493 $(M+Na)^+$; HRMS (FAB) calcd for $C_{24}H_{27}O_6N_2S$ 471.1590. Found 471.1571; δ_H (400 MHz; $CDCl_3$): 2.71–3.40 (br m, 4H, CH_2SCH_2); 4.01–4.47 (br m, 5H, Fmoc C*H*-9+Fmoc OCH_2+CH_α+C$H_{\alpha'}$); 4.50–4.71 (br m, 2H, allyl $CO_2CH_2CH{=}CH_2$); 5.00–5.47 (br m, 2H, allyl $CO_2CH_2CH{=}CHH'$+allyl $CO_2CH_2CH{=}CHH'$); 5.61–5.91 (br m, 1H, allyl $CO_2CH_2CH{=}CH_2$); 6.13–6.35 (br s, 1H, N*H*); 7.10–7.84 (m, 8H, Fmoc Ar*H*); δ_C (100 MHz; $CDCl_3$): 33.1 (CH_2SCH_2); 36.2 (CH_2SCH_2); 47.3 (Fmoc *C*H-9); 53.4 (CH_α); 54.7 ($CH_{\alpha'}$); 67.1+67.9 (allyl $CO_2CH_2CH{=}CH_2$+Fmoc CH_2O); 120.3 (allyl $CO_2CH_2CH{=}CH_2$); 120.6 (allyl $CO_2CH_2CH{=}CH_2$); 125.5, 127.5, 128.2, 130.6 (Fmoc Ar*C*H); 141.6, 143.9 (Fmoc Ar*C*); 157.1 (Fmoc O*C*ONH); 167.9 (allyl $CO_2CH_2CH{=}CH_2$); 174.2 (CO_2H); IR (neat) ν (cm^{-1}): 1673 (br; s); 1512 (br; m); 1181 (br; s); 1133 (br; s); 721 (br; m); 739 (br; m).

4.1.13. (2*S*,6*R*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine *O*^{1}-allyl ester (10). The title compound was prepared using a modification of the procedure of Yamamoto et al.[35] To a solution of (2*S*,6*R*)-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-lanthionine *O*^{1}-allyl ester (1.35 g, 2.87 mmol, 1 equiv.) in dioxane at 0°C was added di-*tert*-butyl dicarbonate (626 mg, 1 equiv.) and DIPEA (0.50 mL, 372 mg, 1 equiv.). The solution was stirred for 3 h and the solvent removed in vacuo. The residue was purified by column chromatography (SiO_2, methanol/chloroform, 1:19 v:v) to yield the title compound as a white solid (1.12 g, 68%); LRMS (ESI+) *m*/*z*: 571 $(M+H)^+$; 593 $(M+Na)^+$; 609 $(M+Na)^+$; 1163 $(2M+Na)^+$; HRMS (FAB) calcd for $C_{29}H_{35}O_8N_2S$ 571.2114. Found 571.2090; δ_H (300 MHz; $CDCl_3$): 1.38 (br s, 9H, $C(CH_3)_3$); 2.61–3.20 (br m, 4H, CH_2SCH_2); 3.81–4.63 (br m, 7H, Fmoc C*H*-9+Fmoc OCH_2+CH_α+C$H_{\alpha'}$+allyl $CO_2CH_2CH{=}CH_2$); 4.89–5.31 (br m, 2H, allyl $CO_2CH_2CH{=}CHH'$+allyl $CO_2CH_2CH{=}CHH'$); 5.40–5.91 (br m, 1H, allyl $CO_2CH_2CH{=}CH_2$); 7.00–7.71 (m, 8H, Fmoc Ar*H*); δ_C (75 MHz; $CDCl_3$): 28.3 ($C(CH_3)_3$); 35.1 (CH_2SCH_2); 41.2 (CH_2SCH_2); 47.1 (Fmoc *C*H-9); 53.7 (CH_α); 56.3 ($CH_{\alpha'}$); 66.3+67.3 (allyl $CO_2CH_2CH{=}CH_2$+Fmoc CH_2O); 80.4 ($C(CH_3)_3$); 119.1 (allyl $CO_2CH_2CH{=}CH_2$); 119.9 (allyl $CO_2CH_2CH{=}CH_2$); 125.3, 127.2, 127.7, 131.5 (Fmoc Ar*C*H); 141.2, 144.1 (Fmoc Ar *C*); 156.1 (Fmoc O*C*ONH); 157.5 (Boc O*C*ONH); 171.3 (allyl $CO_2CH_2CH{=}CH_2$); 177.2 (CO_2H).

4.1.14. (2*S*,6*R*)-*N*^{6}-(9*H*-Fluorenylmethoxycarbonyl)-lanthionyl-(*O*^{1}-allyl)-valinamide (14). Fmoc-Val-Rink Linker-Polystyrene Resin (1.20 g, 0.99 mmol) was shaken with 20% piperidine in DMF (20 mL) for 5 min. The resin was filtered and again shaken with 20% piperidine in DMF for 15 min. The resin was filtered and washed with DMF (2×10 mL×1 min), DCM (2×10 mL×1 min) and ether (2×10 mL×1 min). (2*S*,6*R*)-*N*^{2}-*tert*-Butoxycarbonyl-*N*^{6}-(9*H*-fluorenylmethoxycarbonyl)-*O*^{1}-allyl lanthionine (1.13 g, 1.98 mmol) and HOBt (0.30 g, 1.98 mmol) were dissolved in DCM (9 mL) and DMF (1 mL) with stirring for 5 min. DIC (0.31 mL, 1.98 mmol) was added and stirring continued for a further 5 min. The coupling mixture was added to the resin and the reaction mixture shaken for 2 h. The resin was filtered and washed as above. A small aliquot of resin gave a negative ninhydrin test. A sample of the resin (200 mg) was shaken with TFA (1.9 mL) and TIS (0.1 mL) for 3 h the mixture filtered and the resin was washed with TFA (0.5 mL). The organic fractions were pooled and reduced in vacuo. The residue was dissolved in TFA (0.2 mL) and cold ether (5 mL) added dropwise. The supernatant was decanted and the procedure repeated. The resultant white solid was purified by column chromatography (SiO_2, methanol/chloroform, 1:19 v:v) to give the title compound as a white foam (15 mg, 16%); LRMS (ESI+) *m*/*z*: 569 $(M+H)^+$; 591 $(M+Na)^+$; HRMS (FAB) calcd for $C_{29}H_{37}O_6N_4S$ 569.2434. Found 569.2447; δ_H (400 MHz; CD_3OD): 0.87 (d, J=6 Hz, 3H, Val CH_3); 0.89 (d, J=6 Hz, 3H, Val CH_3); 1.91–2.10 (m, 1H, Val CH_β); 2.71–2.91 (m, 4H, CH_2SCH_2); 3.60–3.71 (m, 1H, Lan CH_α); 4.09–4.17 (m, 2H, Val CH_α+Fmoc C*H*-9); 4.21–4.38 (m, 3H, Lan $CH_{\alpha'}$+Fmoc CH_2O); 4.59 (d, J=5 Hz, 2H, allyl $CO_2CH_2CH{=}CH_2$); 5.10–5.32 (m, 2H, allyl $CO_2CH_2CH{=}CH_2$); 5.78–5.92 (m, 1H, allyl CO_2CH_2-$CH{=}CH_2$); 7.14–7.73 (m, 8H, Fmoc Ar*H*); δ_C (100 MHz; CD_3OD): 18.7+20.2 (2×Val CH_3); 32.3 (Val CH_β); 36.0+37.5 (CH_2SCH_2); 48.7 (Fmoc *C*H-9); 55.2+56.5+60.1 (2×Lan CH_α+Val CH_α); 67.6 (allyl $CO_2CH_2CH{=}CH_2$); 68.6 (Fmoc CH_2O); 119.6 (allyl $CO_2CH_2CH{=}CH_2$); 121.4 allyl $CO_2CH_2CH{=}CH_2$); 126.6, 128.6, 129.2, 133.5 (Fmoc Ar *C*H); 142.9, 145.5 (Fmoc Ar *C*); 158.9 (Fmoc O*C*ONH); 163.9, 173.3, 176.3 (2×*C*ONH+CO_2R); IR (neat) ν (cm^{-1}): 3352 (br, w); 1732 (br s); 1727 (s); 1687 (s); 1521 (s); 1432 (m); 1324 (m); 1326 (w); 1254 (m); 1161 (s); 1105 (w); 914 (m); 704 (m); 697 (m).

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References

1. Horn, M. J.; Jones, D. B.; Ringel, S. J. *J. Biol. Chem.* **1941**, *138*, 141.

2. Horn, M. J.; Jones, D. B. *J. Biol. Chem.* **1941**, *139*, 473.
3. Horn, M. J.; Jones, D. B.; Ringel, S. J. *J. Biol. Chem.* **1942**, *144*, 87.
4. Sloane, N. H.; Untch, K. G. *Biochemistry* **1966**, *5*, 2658.
5. Steinert, P. M.; Idler, W. W. *Biochemistry* **1979**, *18*, 5664.
6. Bessems, G. J. H.; Rennen, H. J. J. M.; Hoenders, H. J. *Exp. Eye Res.* **1987**, *44*, 691.
7. Friedman, M. *J. Agric. Food Chem.* **1999**, *47*, 1295.
8. Zervas, L.; Ferderigos, N. *Isr. J. Chem.* **1974**, *12*, 139.
9. Hurrell, R. F.; Carpenter, K. J.; Sinclair, W. J.; Otterburn, M. S.; Asquith, R. S. *Br. J. Nutr.* **1976**, *35*, 383.
10. Shinbo, H. *Comp. Biochem. Physiol.* **1998**, *91*, 301.
11. Kamal, A. M. S.; Nomura, Y.; Ishii, Y.; Shirai, K. *J. Am. Leather Chem. As.* **1998**, *93*, 272.
12. Brown, G.; du Vigneaud, V. *J. Biol. Chem.* **1940**, *134*, 413.
13. Brown, G.; du Vigneaud, V. *J. Biol. Chem.* **1941**, *140*, 767.
14. Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1970**, *35*, 3259. Harpp, D. N.; Gleason, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 2437. Harpp, D. N.; Gleason, J. G. *J. Org. Chem.* **1971**, *36*, 73.
15. Probert, J. M.; Rennex, D.; Bradley, M. *Tetrahedron Lett.* **1996**, *37*, 1101. Cavelier-Frontin, F.; Daunis, J.; Jacquier, R. *Tetrahedron: Asymmetry* **1992**, *3*, 85.
16. Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Wakamiya, T.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shiba, T. *Bull. Chem. Soc. Jpn* **1992**, *65*, 2227.
17. Arnold, L. D.; Kalantar, T. H.; Vederas, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 7105.
18. Shao, H.; Wang, S. H. H.; Lee, C. W.; Osapay, G.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 2956.
19. Osapay, 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.
20. Dugave, C.; Menez, A. *J. Org. Chem.* **1996**, *61*, 6067. Dugave, C.; Menez, A. *Tetrahedron: Asymmetry* **1997**, *8*, 1453.
21. Melacini, G.; Zhu, Q.; Osapay, G.; Goodman, M. *J. Med. Chem.* **1997**, *40*, 2252.
22. Mayer, J. P.; Zhang, J.; Groeger, S.; Liu, C. F.; Jarosinski, M. A. *J. Pept. Res.* **1998**, *51*, 432.
23. Guthrie, R. D.; Nicolas, E. C. *J. Am. Chem. Soc.* **1981**, *103*, 4638.
24. Baldwin, J. E.; Spivey, A. C.; Schofield, C. J.; Sweeney, J. B. *Tetrahedron* **1993**, *49*, 6309.
25. Falck, J. R.; Lai, J. Y.; Cho, S. D.; Yu, J. *Tetrahedron Lett.* **1999**, *40*, 2903.
26. Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2866.
27. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
28. Ye, B.; Burke, T. R. *Tetrahedron Lett.* **1995**, *36*, 4733.
29. Pascal, R.; Sola, R. *Tetrahedron Lett.* **1998**, *39*, 5031.
30. Whitesides, G. M.; Wong, C. H. *Enzymes in Synthetic Organic Chemistry*. Pergamon: Oxford, 1984. Danieli, B.; Lesma, G.; Passarella, D.; Silvani, A. *J. Org. Chem.* **1998**, *63*, 3492.
31. Waldmann, H.; Kunz, H. *Liebigs Ann. Chem.* **1983**, 1712.
32. Amaral, M. J. S. A.; Macedo, M. A.; Oliveira, M. I. A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 205. Metzger, J. W.; Wiesmuller, K-H.; Jung, G. *Int. J. Pept. Protein Res.* **1991**, *38*, 545.
33. Salvador, L. A.; Elofsson, M.; Kihlberg, J. *Tetrahedron* **1995**, *51*, 5643.
34. Kursanov, D. N.; Parnes, Z. N.; Loin, N. M. *Synthesis* **1974**, 633.
35. Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Nat. Acad. Sci. USA* **1972**, *69*, 730.