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# Synthesis of Orthogonally Protected Lanthionines

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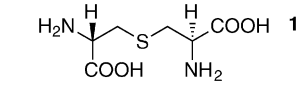
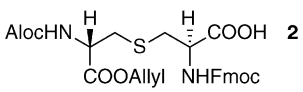
Synthetic approaches to the lantibiotics, a family of thioether-bridged antimicrobial peptides, require flexible synthetic routes to a variety of orthogonally protected derivatives of lanthionine **1**. The most direct approaches to lanthionine involve the reaction of cysteine with an alanyl $\beta$-cation equivalent. Several possibilities exist for the alanyl $\beta$-cation equivalent, including direct activation of serine under Mitsunobu conditions: however, the low reactivity of sulfur nucleophiles in the Mitsunobu reaction has previously precluded its use in the synthesis of the lantibiotics. We report here a new approach to the synthesis of protected lanthionine, using a novel variant of the Mitsunobu reaction in which catalytic zinc tartrate is used to enhance the nucleophilicity of the thiol. In the course of these studies, we have also demonstrated that the synthesis of lanthionine from trityl-protected $\beta$-iodoalanines is prone to rearrangement, via an aziridine, to give predominantly trityl-protected α-iodo-$\beta$-alanines, and hence norlanthionines, as the major products.

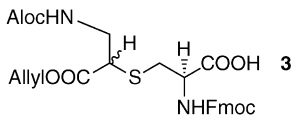
## Introduction

The unusual amino acid lanthionine (**1**) is the key component of the lantibiotics, a family of antimicrobial peptides such as nisin, duramycin, and subtilin produced by Gram-positive bacteria.[1] Many of these peptides show potent antibiotic properties; others are believed to act as enzyme inhibitors. Peptides-containing lanthionine residues are polycyclic structures, with multiple thioether bridges between side-chains resulting from the lanthionine residues. As part of an ongoing program to develop a new approach for the solid-phase synthesis of cyclic peptides with unnatural side-chain linkages,[2] we are currently investigating the synthesis of mono-and polycyclic lanthionine-bridged peptides.

For our approach to the solid-phase synthesis of lantibiotics, we required the orthogonally protected lanthionine **2**. Various approaches to the synthesis of lanthionine have previously been published. The use of




sulfur extrusion, from cystine and from suitably protected cyclic analogues,[3] afforded protected lanthionines, in low to moderate yields. Ring opening of serine $\beta$-lactone with protected cysteine derivatives afforded lanthionines in good yields;[4] however, competing formation of the undesired thioester analogue was also observed, and the procedure was not compatible with the Fmoc-protecting group. Approaches based on Michael additions of cysteine to dehydroalanine have also been reported[5] but these of necessity afford a mixture of diastereoisomers. Recently, a strategy based on the use of iodoalanine as an alanyl $\beta$-cation equivalent was reported by Dugave and Ménez.[6] This route had many apparent advantages; it was direct, high-yielding, and amenable to large-scale synthesis, and it was compatible with a very wide range of protecting

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[#] Ludwig Institute for Cancer Research.

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(6) Dugave, C.; Ménez, A. *Tetrahedron: Asymmetry* **1997**, *8*, 1453.

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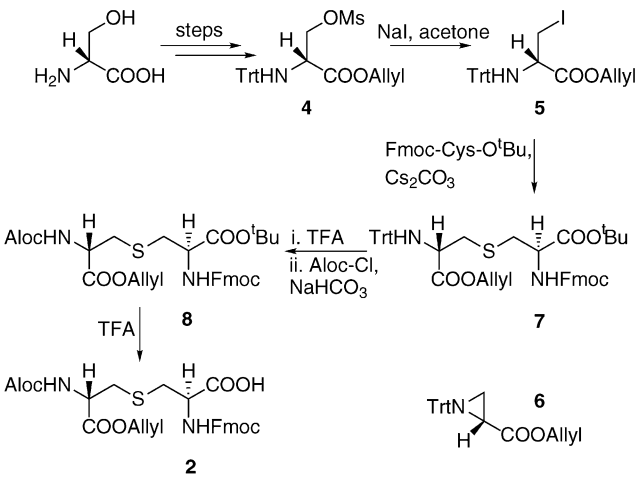
**SCHEME 1**




groups. We therefore elected to use this route to synthesize the required orthogonally protected lanthionine **2**. However, during the course of our synthetic studies, problems became evident, and we therefore carried out a full investigation of the regio- and stereochemical outcome of the key reaction between the iodoalanine and cysteine derivatives. This led us to reassess this reaction, and to the conclusion that, in fact, the regioisomeric norlanthionine **3** is predominantly produced via this route. In this paper, we report full details of this investigation[7] and propose an alternative approach to lanthionine synthesis which circumvents these problems.

## Results and Discussion

**Attempted Synthesis of Lanthionine 2 from Iodoalanine.** We undertook the synthesis of lanthionine **2** (Scheme 1) following the route reported by Dugave and Ménez[6] and adjusting the protecting groups to suit the requirements of our strategy for solid-phase synthesis of side-chain linked peptides.[2] Thus, (*R*)-serine was converted by standard methods to the mesylate **4**. Reaction with sodium iodide afforded iodoalanine **5**, with up to 10% of aziridine **6** as a byproduct that could be removed by recrystallization. As previously described,[6] two distinct sets of peaks were observed in the $^1$H NMR spectrum of the purified product **5**. The peaks were in an uneven ratio, with a major component and a minor component initially in a 2:1 ratio; after recrystallization, the ratio of major isomer to minor isomer was 5:1. In the previous work,[6] the same doubling of the peaks in the $^1$H NMR spectra of similar protected iodoalanines, formed via this route, was observed, and this was attributed to the presence of two rotameric forms of these iodoalanines. Compound **5** was then reacted with Fmoc-Cys-O$^t$Bu in the presence of $Cs_2CO_3$ to give lanthionine **7**; again, aziridine **6** was observed as a byproduct. Removal of the trityl group and replacement with Aloc afforded **8**. Finally, removal of the *tert*-butyl ester gave the desired lanthionine **2**, with the requisite orthogonal protecting groups for SPPS. Dugave and Ménez had also reported the presence of two rotameric forms for other protected lanthionine derivatives formed from iodoalanines similar to **5**, and indeed, we again also observed two clearly distinct sets of signals, a major component and a minor component, in the $^1$H NMR spectrum of **7**. However, even when the bulky trityl group had been replaced by Aloc in **8**, several sets of peaks were still observed in the $^1$H NMR spectrum, although the differences in chemical shifts between the isomers were less pronounced. This caused us to question whether the isomeric forms observed in the $^1$H NMR spectra of **5**, **7**, and **8** were, in fact, due to the presence of two rotameric forms of each compound and raised doubts concerning the identity of **5**, **7**, and **8**. We therefore undertook a careful investigation of the iodoalanine and lanthionine derivatives.

**Rotamers, Diastereoisomers, or Regioisomers?** To eliminate the possibility of the additional $^1$H NMR signals arising from impurities, the purity and homogeneity of the samples were confirmed. Although **7** and **8** were purified by flash column chromatography, and **5** by recrystallization, and appeared homogeneous by TLC, HPLC analysis revealed the presence of two distinct peaks. Although these could not be detected or resolved by flash column chromatography, a partial separation of the two components observed in the NMR spectrum of **5** was achieved by normal-phase preparative HPLC, enabling the NMR spectrum to be analyzed. In the case of **8**, the two isomers could be separated and isolated by normal-phase preparative HPLC, which allowed us to access sufficient material for the characterization of each of these compounds.

We also sought to establish whether the major and minor isomers were rotamers by VT NMR experiments. Predictably, the thermal instability of **5** precluded a successful outcome. Extensive decomposition of the sample, giving aziridine **6** and other unidentified byproducts, was observed at temperatures in excess of 50 °C. VT experiments with lanthionine derivative **7** in DMSO-$d_6$ were also performed, and even at temperatures up to 100 °C, no coalescence of the signals was observed.[8]

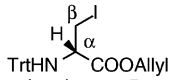
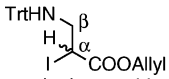
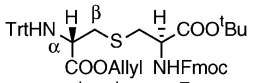
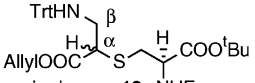
These results suggested that the two isomers formed in the conversion of **4** to **7** via **5** were unlikely to be rotamers. It was therefore necessary to consider the possibility that the two isomers observed in the $^1$H NMR spectrum of **7** were either diastereoisomers or regioisomers. The most likely route for diastereoisomer formation would be as a result of competing elimination of HI from **5** to form the dehydroalanine; subsequent Michael addition[5] would result in a mixture of isomers α to the −NHTrt group. However, Dugave and Ménez had unambiguously previously demonstrated, via desulfurization, derivatization and chiral HPLC measurements of the resulting alanines, that racemization of this chiral center does not occur during the coupling of iodoalanines to cysteine.[6]

We confirmed these observations by the independent synthesis of the diastereoisomer **10**. L-Serine was again protected and converted via the mesylate to **9**, and two sets of peaks were again observed in the $^1$H NMR spectrum. Using the same reaction sequence (Scheme 2),

(7) For preliminary results reassessing this synthesis, see: Mohd Mustapa, M. F.; Harris, R.; Mould, J.; Chubb, N. A. L.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *Tetrahedron Lett.* **2002**, *43*, 8359.

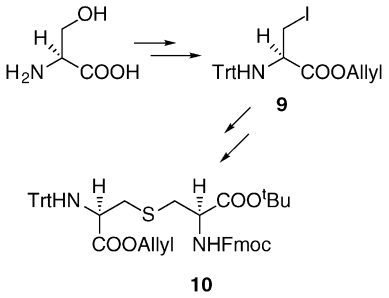
(8) We have also prepared a range of other lanthionine derivatives following this method: in all cases two isomers were observed in the $^1$H NMR, and VT experiments failed to show coalescence of the peaks. Details are given in the Supporting Information.

**TABLE 1. Key Chemical Shifts for α- and *β*-H and -C for the Major and Minor Iodoalanine and Lanthionine Isomers**

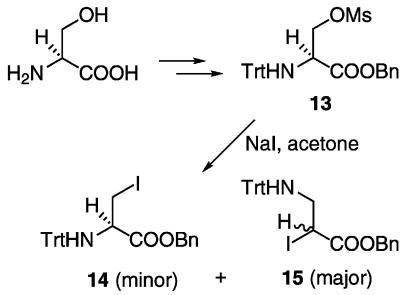
| |  minor isomer **5** |  major isomer **11** |  minor isomer **7** |  major isomer **12** |
|---|---|---|---|---|
| $\delta(H_\alpha)$/ppm | 3.50 | 4.42 | 3.56 | 3.49 and 3.56[b] |
| $\delta(C_\alpha)$/ppm | 55.8 | 20.1 | 56.2 | 48.0 and 48.8[b] |
| $\delta(H_\beta)$/ppm | 3.23, 3.30 | 2.57, 2.73 | 2.70-2.89 | 2.45 and 2.61[b] |
| $\delta(C_\beta)$/ppm | 9.55 | 48.2 | [a] | 44.6 and 44.9[b] |

[a] The signal was too weak to be unambiguously determined. [b] Two signals were observed, due to the presence of two diastereoisomers.

**SCHEME 2**



**SCHEME 3**



**9** was then converted to **10**.[9] Two sets of peaks were also observed in the NMR spectra of **10**. However, the chemical shifts of the signals corresponding to the minor isomer were different from those observed for **7**,[10] indicating that the two sets of signals in **7** do not arise from the presence of the undesired diastereoisomer **10**.

The only possibility remaining was that the two sets of signals arose from two structural isomers of the iodoalanine **5**, and likewise from structural isomers of the lanthionines **7**, **8** and **2**. As it was not possible to obtain crystals suitable for X-ray diffraction, the structures of **5** and **7** were examined in detail by means of $^1H-^{13}C$ NMR correlation experiments. A $^1H-^{13}C$ HSQC spectrum was acquired on iodoalanine **5**, which revealed that the chemical shift of the α-carbon in the major component was ~30 ppm upfield of that in the minor component and that of the *β*-carbon was ~40 ppm downfield (Table 1). These chemical shifts are only compatible with attachment of iodine at the α-carbon in the major component, implying that the major component is the regioisomer α-iodo-*β*-alanine **11**. Conversely, the iodine atom must be attached at the *β*-carbon in the minor component, implying that this is the desired iodoalanine **5**. To confirm these observations, we carried out an HMBC experiment on the mixture of lanthionine isomers **7**. $^3J$ correlations between the *β*-C$H_2$ on the Fmoc-protected side of the lanthionine and the α-*C* on the trityl-protected side, and also between the α-C*H* on the Fmoc-protected side of the lanthionine and the *β*-*C* on the trityl-protected side, were observed for the major isomer. This is only compatible with the formation of the norlanthionine regioisomer **12** from attack of Fmoc-Cys-O$^t$Bu on the major regioisomer α-iodo-*β*-alanine **11**. Similar HSQC and HMBC experiments were carried out on the mixtures of isomers for **8** and **2**, with the same pattern of chemical shifts and connectivities observed for the major components in each case. To verify that the unexpected synthesis of **11** and **12** was not an effect arising from the different protecting groups used in our work, L-serine was protected and converted to the mesylate **13**.[6] Treatment of this material with sodium iodide again led to a mixture of iodoalanine isomers, identified by $^1H$ NMR as *N*-trityl-*β*-iodo-α-alanine benzyl ester **14** (minor isomer) and *N*-trityl-α-iodo-*β*-alanine benzyl ester **15** (major isomer: Scheme 3).

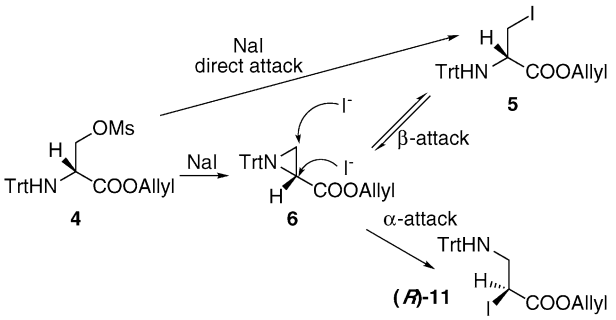
Inspection of the $^{13}C$ NMR spectrum of the mixture of **7** and **12** revealed a total of three peaks for many of the carbon signals. A sample of pure **12** (synthesized from the partially purified α-iodo-*β*-alanine **11**) showed doubling of the carbon signals, indicating that a diastereomeric mixture of norlanthionines had been formed.

**Formation of the Major Regioisomer, α-Iodo-*β*-alanine 12, via Aziridine Ring Opening.** It is likely that the aziridine **6**, rather than being a minor byproduct of the reaction, plays a key role as an intermediate in the formation of the two iodoalanine regioisomers **5** and **11**. We hypothesized that during the course of the reaction mesylate **4** is in fact converted to aziridine **6**. α-Attack would then lead to the formation of the major regioisomer α-iodo-*β*-alanine **11**, whereas the minor

(9) Satisfactory microanalytical data were produced for both products.

(10) Previous work in which two lanthionine diastereoisomers (with different protecting groups) were unambiguously prepared have shown the chemical shifts to be similar in value, but distinguishable: Ösapay, G.; Zhu, Q.; Shao, H.; Chadha, R. K.; Goodman, M. *Int. J. Pept. Prot. Res.* **1995**, *46*, 290.

**SCHEME 4**

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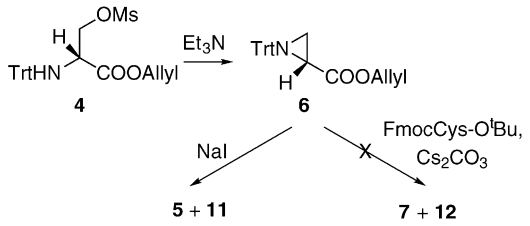
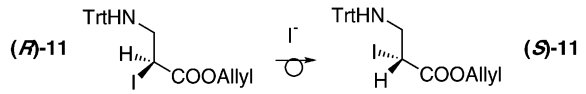



regioisomer, *β*-iodo-α-alanine **5**, could be formed either by *β*-attack or by direct reaction on mesylate **4** (Scheme 4).

Literature precedent supported this rationale. Righi et al. demonstrated that $C_3$-substituted (2*R*,3*R*)-1-ethoxycarbonylaziridine-2-carboxylic acid methyl and ethyl esters undergo nucleophilic ring opening with sodium iodide and Amberlyst 15 in acetone at −40 °C to give a mixture of α- and *β*-amino acids, with the *β*-amino acid isomers predominating.[11] We confirmed this by synthesizing the aziridine **6**, treating mesylate **4** with $Et_3N$ at reflux for 18 h (Scheme 5).[12] The pure aziridine was then treated with 10 equiv of sodium iodide, under the conditions used for the iodination of **4**, affording a mixture of the major regioisomer α-iodo-*β*-alanine **11** and the minor regioisomer, *β*-iodo-α-alanine **5** in a 3:1 ratio, together with 75% of unreacted aziridine. This confirms that both **5** and **11** may arise from an aziridine intermediate. The ratio of the minor isomer **5** to the major isomer **11** is, however, greater with the original synthetic route (Scheme 1, reaction of mesylate **4** with sodium iodide), indicating that some of the minor isomer **5** must be formed by direct $S_N2$ attack on the mesylate (direct reaction pathway, Scheme 4). Clearly the slow reaction of the aziridine, with incomplete conversion even with 10 equiv of NaI over 72 h (attributable to the electron-withdrawing nature of the aziridine protecting group) also indicates that a direct reaction pathway must operate and that re-conversion of the iodoalanines to aziridine must occur.

The aziridine **6** is also formed during the reaction with Fmoc-Cys-O$^t$Bu, and it was possible that it might also be an intermediate in this reaction. Several groups have reported the ring opening of aziridines with thiols, either

**SCHEME 6**




giving exclusively *β*-attack[13−15] or exclusively α-attack,[16] depending on the substitution of the aziridines and the presence of Lewis acids. Hata and Watanabe[17] utilized the ring opening of N-unprotected aziridines with unprotected cysteine to afford mixtures of lanthionine (via *β*-attack) and norlanthionine (via α-attack). We therefore treated the pure aziridine **6** with Fmoc-Cys-O$^t$Bu in the presence of $Cs_2CO_3$, under the conditions of the original synthesis (Scheme 5); however, no reaction was observed. This must indicate that norlanthionine **12** must derive from the major regioisomer α-iodo-*β*-alanine **11**, and lanthionine **7** from the minor regioisomer *β*-iodo-α-alanine **5**.

Both our work and that of Dugave and Ménez show that the minor desired lanthionine isomer **7** is a single diastereoisomer. In light of the preceding discussion, this must indicate that the minor regioisomer *β*-iodo-α-alanine **5** is formed without racemization. However, the major norlanthionine regioisomer **12** is formed as a mixture of two diastereoisomers, indicating that the major regioisomer α-iodo-*β*-alanine **11** is in turn a mixture of enantiomers. The cyclization of **5** to **6**, ring-opening of **6** to afford **11**, and the ring-closure of **11** to give **6** (Scheme 4) should all occur via $S_N2$ mechanisms, preserving the chirality of the intermediate aziridine 6 and initially affording a single enantiomer, (*R*)-**11**. However, once (*R*)-**11** has been formed, it is in turn susceptible to nucleophilic attack, and we believe that under the conditions used for the reaction (10 equiv of NaI) it undergoes Walden inversion, via further attack of the excess $I^-$, leading to partial racemization at this center. Both the nucleophilicity of $I^-$ relative to other halides[18] and the facile racemization of optically active alkyl iodides by excess iodide[19] are precedented (Scheme 6).

Righi et al. report that enantiopure α-iodo-*β*-amino acid derivatives were formed during the reaction of enantiopure derivatives of aziridine-2-carboxylates with a single equivalent of NaI in the presence of Amberlyst 15.[11] We treated **6** under the same conditions and obtained **5** and **11** in good yield as an inseparable mixture (1:9 ratio) of regioisomers.

Having shown that attempted preparation of iodoalanine **5** from mesylate **4** leads to an inseparable mixture of iodoalanine regioisomers **5** and **11**, and thence to a mixture of lanthionine **7** and norlanthionine **12** (Scheme 7), we then turned our attention to developing methodology that would unambiguously produce only the desired lanthionine.

**Lanthionine Synthesis via the Mitsunobu Reaction.** In light of the above observations, we sought an

(11) (a) Righi, G.; D'Achille, R.; Bonini, C. *Tetrahedron Lett.* **1996**, *37*, 6893. (b) Righi, G.; Chionne, A.; D'Achille, R.; Bonini, C. *Tetrahedron*: *Asymmetry* **1997**, *8*, 903.

(12) (a) Olofsson, B.; Wijtmans, R.; Somfai, P. *Tetrahedron* **2002**, *58*, 5979. (b) Willems, J. G. H.; Hersmis, M. C.; de Gelder, R.; Smits, J. M. M.; Hammink, J. B.; Dommerholt, F. J.; Thjis, L.; Zwanenburg, B. *J. Chem. Soc., Perkin Trans. 1* **1997**, 963.

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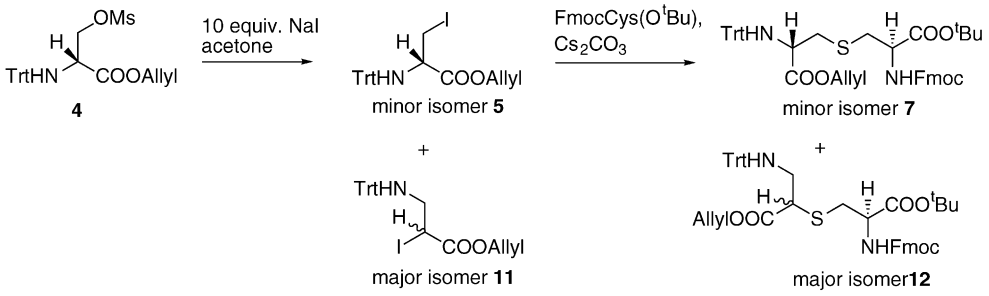
(17) Hata, Y.; Watanabe, M. *Tetrahedron* **1987**, *43*, 3881.

(18) Edwards, J. O.; Pearson, R. G. *J. Am. Chem. Soc.* **1962**, *84*, 16.

(19) Hughes, E. D.; Juliusburger, F.; Masterman, S.; Topley, B.; Weiss, J. *J. Chem. Soc.* **1935**, 1525.

**SCHEME 7**

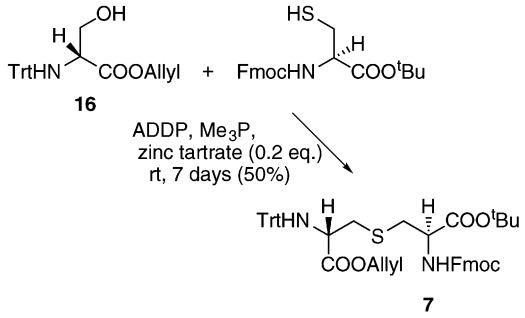



alternative pathway for the synthesis of the desired protected lanthionines that would be both stereo- and regioselective. In addition to providing a route to the necessary amino acid building blocks, it was also envisaged that this would provide further proof of the structures of the lanthionines and norlanthionines synthesized above.

The Mitsunobu reaction[20] is well-known as a mild method for conversion of alcohols into a range of different functionalities. Cherney and Wang[21] have demonstrated that Mitsunobu reactions under standard conditions with *N*-trityl-protected serine are extremely efficient for producing amino acids functionalized at the $\beta$-position. For the formation of carbon−sulfur bonds, however, masked reagents such as thio acids or thioamides, or aryl thiols, are generally used,[20b] as aliphatic thiols generally lack sufficient acidity to react under the standard Mitsunobu conditions.[22,23] However, recently aliphatic sulfides and thioglycosides have been successfully prepared using 1,1′-(azodicarbonyl)dipiperidine (ADDP) and $Me_3P$ in the presence[24] or absence[25,26] of imidazole. We therefore studied the Mitsunobu reaction as a potential methodology for the synthesis of protected lanthionines.

Reaction of *N*-trityl-(*R*)-serine allyl ester (**16**) with ADDP, $Me_3P$, and Fmoc-Cys-O$^t$Bu, in the presence or absence of imidazole, using the range of conditions previously described,[24−26] failed to produce the desired lanthionine **7**. Recovery of unreacted starting materials, with some dimerization of the cysteine, resulted. Zinc chloride is known[27] to enhance sluggish Mitsunobu reactions, but under the conditions described (between 0.2 and 4 equiv of $ZnCl_2$), extensive detritylation of **16** was observed, presumably as a result of the generation of HCl. Catalytic zinc tartrate has been used to increase the nucleophilicity of thiols in epoxide ring-opening reactions,[28] and as a less acidic byproduct would thereby be generated, this seemed a viable alternative. Accordingly, **16** was added to the preformed ADDP/$Me_3P$ adduct, followed by addition of a preformed mixture of zinc tartrate (0.2 equiv) and Fmoc-Cys-O$^t$Bu (Scheme 8). After the mixture was stirred at room temperature for 7 days, the desired lanthionine **7** was produced, as a single isomer, in 50% yield. The reaction was then repeated using the corresponding (*S*)-serine derivative; this also yielded only one isomer, the desired lanthionine **10**. HSQC experiments carried out as before on both diastereoisomers, **7** and **10**, synthesized via this Mitsunobu route, confirmed the correct thioether bridge connectivity in each case, with similar $^{13}C$ chemical shift values observed between the $\alpha$ and $\beta$-Cs on both sides of the thioether bridge. HMBC experiments confirmed this with the presence of $^3J$ couplings between $\beta$-Hs and $\beta$-Cs across the thioether bridge. Comparison of the $^1H$ NMR spectrum of **7** with the $^1H$ NMR spectrum of the mixture of isomers produced via the iodoalanine route (Scheme 1) confirmed that the minor isomer produced from this latter route is, in fact, the desired lanthionine. Similarly, comparison of the $^1H$ NMR spectrum of **10** synthesized via the Mitsunobu route with the $^1H$ NMR spectrum of the mixture of isomers produced via the iodoalanine route (Scheme 2) also confirmed that the minor isomer produced from this latter route is the diastereomeric lanthionine. As expected,[10] small differences were observed in the $^1H$ NMR chemical shift values between the two diastereoisomers **7** and **10**.

**SCHEME 8**




## Conclusions

In this paper, we have shown that the reaction of *N*-trityl-*O*-methanesulfonyl-(*R*)-serine allyl ester **4** with sodium iodide leads to a mixture of two iodoalanine regioisomers. The major product from this reaction is $\alpha$-iodo-$\beta$-alanine **11** and not, as previously reported,[6] $\beta$-iodo-$\alpha$-alanine **5**, which is present as a minor component. We have demonstrated that the complexity of the $^1H$ NMR spectra observed with these reactions arises from the inseparable mixture of regioisomers produced,

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(21) Cherney, R. J.; Wang, L. *J. Org. Chem.* **1996**, *61*, 2544.
(22) Tanigawa, Y.; Kanamaru, H.; Murahashi, S.-I. *Tetrahedron Lett.* **1975**, 4655.
(23) For an exception, see: Alpegiani, M.; Perrone, E.; Franceschi, G. *Heterocycles* **1988**, *27*, 49.
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(28) Yamashita, H.; Mukaiyama, T. *Chem. Lett.* **1985**, 1643.

and not from the presence of two rotameric forms of **5**. We have presented evidence that the reaction proceeds via an intermediate aziridine, such as **6**, with concomitant partial racemization of the chiral center of the predominant α-iodo-*β*-alanine regioisomer **11**. The mixture of regioisomers, **5** and **11**, in turn leads to a mixture of lanthionine **7** and norlanthionine **12**, on treatment with Fmoc-Cys-O$^t$Bu in the presence of $Cs_2CO_3$. These mixtures of regioisomers are difficult to separate; however, in our hands purification at each subsequent synthetic stage resulted in the isolation of norlanthionine **3**, suitable for a three-dimensional orthogonal protecting group strategy for the synthesis of cyclic peptides. In the following paper, we have demonstrated the use of this approach to synthesize norlanthionine bridged cyclic peptides.[29] Finally, we have developed a new approach to the synthesis of protected lanthionine, using a variant of the Mitsunobu reaction, which gives exclusively the correct lanthionine regioisomer. Further studies to optimize this reaction and to synthesize lanthionine-bridged peptides are in hand.

## Experimental Section

***N*-Triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) Allyl Ester (4).** *N*-Triphenylmethyl-(*R*)-serine allyl ester **16** (19 g, 49 mmol) was dissolved in dry THF (200 mL) under Ar, and the solution was cooled to 0 °C. Triethylamine (7.0 mL, 49 mmol) and methanesulfonyl chloride (8.3 mL, 98 mmol, 2 equiv) were added, the ice bath removed, and the mixture stirred for 4 h. The reaction mixture was diluted with ether (400 mL) and washed with ice-cold water (6 × 100 mL) and then brine (4 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, and removal of the solvent yielded a pale yellow liquid. Recrystallization of the crude product was carried out in DCM and MeOH (1:4) yielding white crystals ($R_f$ 0.3 (hexane/ethyl acetate, 2:1)) (21 g, 44 mmol, 91%): mp 116−118 °C dec; $[\alpha]^{20}_D$ −32.7 (*c* 0.46, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) *δ* 7.55 (6H, m, Trt), 7.31 (6H, m, Trt), 7.23 (3H, m, Trt), 5.74 (1H, m, C*H*=$CH_2$), 5.24 (1H, dd, *J* = 15.3, 1.4 Hz, CH=C$H_2$), 5.20 (1H, dd, *J* = 8.3, 1.1 Hz, CH=C$H_2$), 4.47 (1H, dd, *J* = 4.3 Hz, 10.1 Hz, C$H_2$CH=$CH_2$), 4.27 (1H, dd, *J* = 6.1, 10.1 Hz, C$H_2$−CH=$CH_2$), 4.24 (1H, dd, *J* = 4.4, 12 Hz, C$H_2SO_2$), 4.01 (1H, dd, *J* = 5.8, 12 Hz, C$H_2SO_2$), 3.74 (1H, dd, *J* = 5.8, 4.4 Hz, HNC*H*$CO_2$), 2.95 (3H, s, $CH_3SO_2$); $^{13}$C NMR ($CDCl_3$, 100.61 MHz) *δ* 171.1, 145.2, 131.4, 129.5, 128.0, 126.7, 118.7, 71.0, 70.6, 66.0, 55.3, 37.4; IR $\nu_{max}$ ($CHCl_3$) 1736, 1489, 1346 + 1177 cm$^{-1}$; mass spectrum *m*/*z* (FAB) 488 ([M + Na]$^+$, 1), 243 (Trt$^+$, 100); HRMS (FAB) calcd for $C_{26}H_{27}NO_5SNa$ ([M + Na]$^+$) 488.1520, found 488.1508.

**Mixture of *N*-Triphenylmethyl-*β*-iodo-(*S*)-alanine Allyl Ester (5) and (*R*/*S*)-2-Iodo-3-(triphenylmethylamino)-propionic Acid Allyl Ester (11).** A solution of *N*-triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester **4** (22 g, 46 mmol) in acetone (80 mL) was added to a solution of sodium iodide (70 g, 0.46 mol, 10 equiv) in dry acetone (100 mL) under argon. The mixture was stirred for 72 h at 25 °C, and the resulting yellow-brown slurry was then concentrated in vacuo. Ether (400 mL) was added, and sodium thiosulfate (10% aq w/v, approximately 25 mL) was then slowly added, dissolving the solids and substantially decolorizing the organic layer to afford a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to give a yellow oil. This was purified by flash column chromatography (hexane/ethyl acetate, 2:1, $R_f$ 0.60) followed by precipitation from $CH_2Cl_2$/MeOH (1:5) to give **5** and **11** in a ratio of 1:5 (19.9 g, 40 mmol, 87% overall yield for two regioisomers). A partial separation of the two regioisomers was possible using preparative normal-phase HPLC, with a gradient of 2−3% ethyl acetate in hexane over 10 min, 15 mL/min: the retention time of **5** was12.1 min and that of **11** 12.84 min: $^1$H NMR ($CDCl_3$, 600 MHz) for **5** *δ* 7.51 (6H, m, Trt), 7.29 (6H, m, Trt), 7.20 (3H, m, Trt), 5.73 (1H, m, C*H*=$CH_2$), 5.22 (1H, dd, *J* = 17.3, 1.4 Hz, CH=C$H_2$), 5.19 (1H, dd, *J* = 10.2, 1.1 Hz, CH=C$H_2$), 4.22 (1H, dd, *J* = 13.1, 6.1 Hz, OC$H_2$CH), 4.09 (1H, dd, *J* = 13.1, 6.1 Hz, OC$H_2$CH), 3.50 (1H, m, HNC*H*$CO_2$), 3.30 (1H, dd, *J* = 9.8, 3.4 Hz, CHC$H_2$I), 3.23 (1H, dd, *J* = 9.8, 6.9 Hz, CHC$H_2$I), 2.88 (1H, d, *J* = 9.8 Hz, TrtN*H*CH); for **11** *δ* 7.52 (6H, m, Trt), 7.30 (6H, m, Trt), 7.21 (3H, m, Trt), 5.94 (1H, m, C*H*=$CH_2$), 5.40 (1H, dd, *J* = 17.2, 1.4 Hz, CH=C$H_2$), 5.30 (1H, dd, *J* = 10.5, 1.1 Hz, CH=C$H_2$), 4.67 (2H, m, OC$H_2$CH), 4.42 (1H, m, C*H*I$CO_2$), 2.73 (1H, dd, *J* = 12.9, 8.5 Hz, HNC$H_2$CHI), 2.57 (1H, dd, *J* = 12.9, 5.9 Hz, HNC$H_2$CHI), 2.27 (TrtN*H*$CH_2$); $^{13}$C NMR ($CDCl_3$, 150 MHz) for **5** *δ* 171.9, 145.6, 131.3, 128.4, 127.9, 126.4, 118.6, 70.9, 65.8, 55.8, 9.55; for **11** *δ* 170.4, 145.4, 131.1, 128.3, 128.0, 126.5, 118.8, 70.8, 66.1, 48.2, 20.1; IR $\nu_{max}$ 1730, 1489 cm$^{-1}$; mass spectrum *m*/*z* (APCI$^+$) 498 ([M + H]$^+$, 2), 243 (Trt$^+$, 100), 256 ([M − Trt + 2]$^+$, 2); HRMS (FAB) calcd for $C_{25}H_{24}NO_2INa$ ([M + Na]$^+$) 520.0732, found 520.0750.

**Mixture of 3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-2-(triphenylmethylamino)propionic Acid Allyl Ester (7) and (*R*/*S*)-2-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-3-(triphenylmethylamino)propionic Acid Allyl Ester (12).** *N*-9-Fluorenylmethoxycarbonyl-(*R*)-cysteine *tert*-butyl ester (5.6 g, 14 mmol) was dissolved in dry DMF (75 mL) under inert conditions. A solution of the regioisomeric mixture of *N*-triphenylmethyl-*β*-iodo-(*S*)-alanine allyl ester (**5**) and (*R*/*S*)-2-iodo-3-(triphenylmethylamino)propionic acid allyl ester (**11**) (7.7 g, 16 mmol, 1.1 equiv) (1:5 ratio) in DMF (75 mL) was added. Cesium carbonate (4.6 g, 14 mmol) was then added, and the mixture was stirred for 4 h at 25 °C. The solvent was removed in vacuo, and the residue was dissolved in a mixture of ethyl acetate (250 mL) and citric acid (5% aq w/v, 100 mL). The organic layer was separated, washed with water (8 × 100 mL), and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo yielded a pale yellow oil. This was purified via flash column chromatography (hexane/ethyl acetate, 4:1, $R_f$ 0.28) to give a 1:5 mixture of the regioisomers **7** and **12** as a very pale yellow foam (9.7 g, 12.6 mmol, 90%): $^1$H NMR ($CDCl_3$, 500 MHz) for **7** *δ* 7.74 (2H, m, Fmoc (Ar)), 7.60 (2H, m, Fmoc (Ar)), 7.49 (6H, m, Trt), 7.38 (2H, m, Fmoc (Ar)), 7.20−7.31 (2H, m, Fmoc (Ar) + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.69 (1H, m, C*H*=$CH_2$), 5.63 (1H, bd, FmocN*H*), 5.18 (1H, m, CH=C$H_2$), 5.13 (1H, m, CH=C$H_2$), 4.49 (1H, m, FmocNHC*H*), 4.34 (2H, m, C$H_2$OCONH), 4.18 (1H, m, C*H*C$H_2$OCONH), 4.04 (2H, m, OC$H_2$CH=$CH_2$), 3.56 (1H, m, TrtNHC*H*), 2.89−3.03 (2H, m, CHC$H_2$S), 2.70−2.89 (2H, m, TrtNHCHC$H_2$), 2.20 (1H, bs, TrtN*H*), 1.50 (9H, s, C(C$H_3$)$_3$); for **12** (diastereoisomeric mixture) *δ* 7.73 (2H, m, Fmoc (Ar)), 7.56 (2H, m, Fmoc (Ar)), 7.43 (6H, m, Trt), 7.36 (2H, m, Fmoc (Ar)), 7.20−7.31 (2H, m, Fmoc (Ar) + 6H, m, Trt), 7.16 (3H, m, Fmoc (Ar)), 5.91 (1H, m, C*H*=$CH_2$), 5.58 + 5.69 (1H, bd, FmocN*H*), 5.33 (1H, m, CH=C$H_2$), 5.23 (1H, m, CH=C$H_2$), 4.66 (2H, m, OC$H_2$-CH=$CH_2$), 4.50 (1H, m, FmocNHC*H*), 4.29−4.41 (2H, m, C$H_2$-OCONH), 4.18−4.25 (1H, m, C*H*C$H_2$OCONH), 3.49 + 3.56 (1H, t, *J* = 6.4 + 6.7, TrtNHC$H_2$C*H*), 2.81−3.12 (2H, m, CHC$H_2$S), 2.61 (1H, m, TrtNHC$H_2$), 2.45 (1H, m, TrtNHC$H_2$), 1.48−1.49 (9H, s, C(C$H_3$)$_3$); $^{13}$C NMR ($CDCl_3$, 126 MHz) for **7** *δ* 171.9, 169.7, 156.1, 146.0, 144.2, 141.7, 132.1, 129.2, 128.4, 128.1, 127.0, 127.5, 125.6, 120.4, 119.2, 83.4, 71.2, 67.6, 66.3, 54.3, 48.9, 47.5, 44.9, 34.3, 28.4; for **12** *δ* 171.8, 169, 156.1, 146, 144.1, 141.6, 132, 129, 128, 128.1, 127.5, 127, 125.5, 120.3, 119, 83, 71, 67.6, 66, 54, (48.8 + 48.0), 47.5, (44.9 + 44.6), (34.8 + 34.3), 28.3; IR $\nu_{max}$1724, 1504, 1450, 1215, 1153 cm$^{-1}$; mass spectrum *m*/*z* (FAB) 527 ([M − Trt + 1]$^+$, 0.4), 471 ([M − Trt

(29) Mohd Mustapa, M. F.; Harris, R.; Esposito, D.; Mould, J.; Chubb, N. A. L.; Schultz, D.; Driscoll, P. C.; Tabor, A. B. *J. Org. Chem.* **2003**, *68*, 8193.

$-$ $^t$Bu + 2]$^+$, 0.3), 243 (Trt$^+$, 100); HRMS (FAB) calcd for $C_{47}H_{48}N_2O_6SCs$ ([M + Cs]$^+$) 901.2264, found 901.2287.

**Mixture of 3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-2-(allyloxycarbonylamino)propionic Acid Allyl Ester (8) and the Regioisomer (*R*/*S*)-2-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-3-(allyloxycarbonylamino)propionic Acid Allyl Ester.** The mixture (1:5) of 3-[(*R*)-2-*tert*-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-2-(triphenylmethylamino)propionic acid allyl ester **7** and (*R*/*S*)-2-[(*R*)-2-*tert*-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-3-(triphenylmethylamino)propionic acid allyl ester **12** (3.5 g, 4.6 mmol) was treated with a 5% solution of trifluoroacetic acid (1.4 mL, 18 mmol, 4 equiv) in $CHCl_3$ (27 mL) under inert conditions for 4 h. The resulting solution was diluted with $CHCl_3$ (200 mL) and washed with sodium hydrogen carbonate (5% aq w/v, 2 × 75 mL) and water (2 × 50 mL). The solvent was removed in vacuo. The material was then redissolved in $CHCl_3$ (20 mL) and MeOH (20 mL), and the solvents were again removed in vacuo to yield a pale yellow liquid. This was then treated with sodium hydrogen carbonate (1.5 g, 18 mmol, 4 equiv) in water (30 mL). The mixture was then cooled to 0 °C, and a solution of allyl chloroformate (0.80 mL, 9.2 mmol, 2 equiv) in dioxane (30 mL) was added. The resulting mixture was stirred at below 5 °C for 18 h. The solvents were then removed in vacuo, and the residue was redissolved in ethyl acetate (250 mL). The organic layer was washed with water (8 × 50 mL), dried over anhydrous sodium sulfate, and concentrated yielding a yellow liquid. This was purified via flash column chromatography (hexane/ethyl acetate, 4:1 then 2:1, $R_f$ 0.29) yielding a mixture of **8** and the regioisomer in a 1:6 ratio as a viscous yellow oil (2.4 g, 4.0 mmol, 87%). Separation of the regioisomers was carried out via preparative normal-phase HPLC (20−23% ethyl acetate in hexane over 45 min, 15 mL/min) yielding both products as viscous, pale yellow liquids. The retention time of **8** was 16.6 min and that of the regioisomer (*R*/*S*)-2-[(*R*)-2-*tert*-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-3-(allyloxycarbonylamino)propionic acid allyl ester 20.1 min: $^1$H NMR ($CDCl_3$, 400 MHz) for **8** $\delta$ 7.78 (2H, m), 7.63 (2H, m), 7.42 (2H, m), 7.33 (2H, m), 5.80−5.96 (2H, m, C*H*=CH$_2$), 5.75 (1H, m, FmocN*H*), 5.66 (1H, m, AlocN*H*), 5.17−5.35 (4H, m, CH=C$H_2$), 4.56−4.65 (4H, m, OC$H_2$CH=CH$_2$), 4.61 (1H, m, AlocNHC*H*), 4.50 (1H, m, FmocNHC*H*), 4.39 (2H, m, C$H_2$OCONH), 4.25 (1H, m), 2.94−3.10 (4H, m, C$H_2$SC$H_2$), 1.50 (9H, s, C(C$H_3$)$_3$); for the regioisomer $\delta$ 7.76 (2H, m), 7.62 (2H, m), 7.40 (2H, m), 7.32 (2H, m), 5.90 (2H, m, C*H*=CH$_2$), 5.76 (1H, br m, FmocNH), 5.14−5.36 (4H, m, CH=C$H_2$), 5.31 (1H, m, AlocNH), 4.50−4.70 (4H, m, OC$H_2$CH=CH$_2$), 4.54 (1H, m, FmocNHCH), 4.40 (2H, m, C$H_2$OCONH), 4.25 (1H, m, C*H*CH$_2$OCONH), 3.45−3.66 (2H, m, SCHC$H_2$), 3.52 (1H, m, AlocNHCH), 2.97−3.25 (2H, m, SC$H_2$), 1.50 (9H, s, C(C$H_3$)$_3$); $^{13}$C NMR ($CDCl_3$, 126 MHz) for **8** $\delta$ 170.2, 169.2, 156.1, 155.7, 143.8, 141.3, 132.5, 131.3, 127.7, 127.1, 125.1, 120.0, 119.1, 117.7, 83.1, 67.2, 66.4, 66.1, 54.0, 47.1, 35.5, 28.0; for the regioisomer $\delta$ 170.7, (169.2 + 169.1), 156, 157, 143.7, 141.2, 132.6, (131.4 + 131.3), 127.7, 127.0, 125.1, 119.9, (119.0 + 118.9), (117.72 + 117.65), 83.1, (67.24 + 67.16), (66.1 + 66.0), 65.7, (54.3 + 53.9), 47.0, (46.7 + 46.0), (41.5 + 41.4), (34.34 + 34.26), 27.9; IR $\nu_{max}$ 3429, 1724, 1512, 1450, 1226 + 1153 cm$^{-1}$; mass spectrum *m*/*z* 633 ([M + Na]$^+$, 31), 611 ([M + H]$^+$, 38), 555 ([M $-$ $^t$Bu + 2]$^+$, 56), 333 ([M $-$ $^t$Bu $-$ Fmoc + 3]$^+$, 100); HRMS (FAB) calcd for $C_{32}H_{38}N_2O_8SNa$ ([M + Na]$^+$) 633.2265, found 633.2247.

**3-[(*R*/*S*)-1-Allyloxycarbonyl-2-(allyloxycarbonylamino)-ethylsulfanyl]-(*R*)-2-(fluoren-9-ylmethoxycarbonylamino)-propionic Acid (3).** A 1:6 mixture of 3-[(*R*)-2-*tert*-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl-(*S*)-2-(allyloxycarbonylamino)propionic acid allyl ester **8** and the regioisomer (*R*/*S*)-2-[(*R*)-2-*tert*-butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-3-(allyloxycarbonylamino)propionic acid allyl ester (2.1 g, 3.4 mmol) was treated with a solution of TFA (5 mL) in $CHCl_3$ (5 mL), and the mixture was stirred for 4 h. The mixture was concentrated in vacuo and redissolved in $CHCl_3$ (100 mL) and MeOH (100 mL). The solvents were again removed in vacuo. The crude material was purified via reversed-phase flash column chromatography (0−35% MeCN, 20% saturated $NaHCO_3$, $H_2O$, $R_f$ 0.31) yielding only **3** as a colorless foam (1.4 g, 2.6 mmol, 76%): $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 7.76 (2H, m), 7.62 (2H, m), 7.35 (2H, m), 7.27 (2H, m), 5.80−5.96 (2H, m, 2 × C*H*=CH$_2$), 5.10−5.35 (4H, m, 2 × CH=C$H_2$), 4.60 (2H, m, OC$H_2$CH=CH$_2$), 4.48 (2H, m, OC$H_2$CH=CH$_2$), 4.38 (1H, m, FmocNHC*H*), 4.28−4.41 (2H, m, C$H_2$OCONH), 4.22 (1H, m), 3.64 (1H, m, AlocNHCH$_2$C*H*), 3.44 (2H, m, AlocNHC$H_2$), 2.88−3.24 (2H, m, CHC$H_2$S); $^{13}$C NMR ($CD_3OD$, 125.8 MHz) $\delta$ (172.5 + 172.4), 172.0, 158, 145.2, 142.5, (134.2 + 134.1), (133.2 + 133.0), 129.2, (128.7 + 128.6), 126.3, 120.6, 118.8, (117.7 + 117.6), (68.23 + 68.18), (66.99 + 66.96), (66.6 + 66.5), 55.6, 48.3, 47.2, (43.1 + 42.8), (34.7 + 34.2); IR $\nu_{max}$ 3433, 3337, 1720, 1512, 1450, 1223 + 1150 cm$^{-1}$; mass spectrum *m*/*z* (ES+) 577 ([M + Na]$^+$, 100), 555 ([M + H]$^+$, 4), 333 ([M $-$ Fmoc + 2]$^+$, 4). Anal. Calcd for $C_{44}H_{48}N_2O_8S_2$: C, 60.64; H, 5.45; N, 5.05; S, 5.78. Found: C, 61.06; H, 6.02; N, 4.68; S, 5.22.

**(*R*)-1-Triphenylmethylaziridine-2-carboxylic Acid Allyl Ester (6).** Triethylamine (1.8 mL, 12.9 mmol, 3 equiv) was added at room temperature to a solution of *N*-triphenylmethyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester **4** (2 g, 4.3 mmol) in THF (20 mL). The solution was heated at reflux for 12 h. The mixture was then diluted with EtOAc (20 mL), washed with citric acid (5% aq, 2 × 10 mL) and brine (2 × 10 mL), dried over $MgSO_4$, and concentrated to afford a viscous oil. Silica chromatography purification (hexane/EtOAc, 12:1) afforded the pure aziridine **6** (1.4 g, 88%) as a colorless oil. Starting material was recovered in 8% yield: $R_f$ 0.60 (hexane/ethyl acetate, 2:1); $^1$H NMR ($CDCl_3$ 500 MHz) $\delta$ 7.60 (6H, m, Trt), 7.34 (6H, m, Trt), 7.26 (3H, m, Trt), 5.95 (1H, m, C*H*=CH$_2$), 5.36 (1H, dd, $J$ = 17.2, 1.5 Hz, CH=C$H_2$), 5.27 (1H, dd, $J$ = 10.4 Hz, 1.3 Hz, CH=C$H_2$), 4.69 (m, 2H, C$H_2$CH=CH$_2$), 2.29 (1H, dd, $J$ = 2.7, 1.6 Hz, NC$H_2$), 1.94 (1H, dd, $J$ = 2.7, 6.15 Hz, NC*H*CO$_2$), 1.44 (1H, dd, $J$ = 6.15, 1.6 Hz, NC$H_2$);$^{13}$C NMR ($CDCl_3$, 126 MHz) $\delta$ 171.1, 143.6, 131.9, 129.0, 129.8, 126.9, 118.5, 74.3, 65.5, 31.7, 28.7; mass spectrum *m*/*z* (ES+) 370 ([M + H]$^+$, 9), 243 (Trt$^+$, 100); (FAB) 393 ([M + Na + H]$^+$, 13), 243 (Trt$^+$, 100); HRMS (FAB) calcd for $C_{25}H_{23}NO_2Na$ ([M + Na]$^+$) 392.1645, found 392.1626.

**Reaction of Aziridine 6 with 10 equiv of NaI To Give 5 and 11.** (*R*)-*N*-Triphenylmethylaziridine-2-carboxylic acid allyl ester **6** (0.012 g, 33 $\mu$mol) was treated with sodium iodide (0.049 g, 0.33 mmol, 10 equiv) in acetone (0.5 mL) under Ar, and the mixture was stirred for 72 h at 25 °C. The solvent was then removed in vacuo. Ether (10 mL) was added, and sodium thiosulfate (10% aq w/v, 1 mL approximately) was then slowly added, decolorizing the organic layer to a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The crude product was purified by preparative normal-phase HPLC (2−3% ethyl acetate in hexane over 60 min, 15 mL/min). A mixture of **5** (46.0 min) and **12** (47.1 min) was isolated and partially resolved (combined mass 0.0044 g, 8.8 $\mu$mol, 27%) in a 1:3 ratio, identical by NMR to the mixture analyzed previously. The starting material was also recovered at 53.6 min as the predominant species.

**Reaction of Aziridine 6 with 1 equiv of NaI To Give 5 and 11.** A solution of **6** (235 mg, 6.38 mmol), NaI (96 mg, 6.38 mmol, 1 equiv), and Amberlyst 15 (80 mg, 6.38 mmol, 1 equiv) in distilled acetone (7 mL) was stirred for 12 h at −40 °C. After filtration of the beads, the solution was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The purification by silica chromatography of the resulting residue (hexane/EtOAc: 12/1) afforded **5** (10%) and **11** (90%) as an inseparable mixture (2.79 g, 88%).

**Synthesis of 3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-2(Triphenylmethylamino)propionic Acid Allyl Ester (7) via the Mitsunobu Reaction.** To a precooled solution of ADDP (0.12 g, 0.5 mmol, 2 equiv) in anhydrous $CHCl_3$ (3.0 mL) under $N_2$ was added $PMe_3$ (1 M solution in toluene, 0.5 mL, 0.5 mmol, 2 equiv). The reaction was stirred at 0 °C for 1 h, during which time the solution changed its color from yellow to almost transparent. At the same time, a solution of *N*-9-fluorenylmethoxycarbonyl-(*R*)-cysteine *tert*-butyl ester (0.10 g, 0.25 mmol, 1 equiv) and zinc tartrate (0.01 g, 0.05 mmol, 0.2 equiv) in dry $CHCl_3$ (2 mL) under $N_2$ were stirred at room temperature for 1 h. *N*-Triphenylmethyl-(*R*)-serine allyl ester **16** (0.09 g, 0.25 mmol, 1 equiv) was then added to the $PMe_3$−ADDP mixture. After being stirred for a further 5 min, this mixture was transferred to the zinc tartrate−cysteine mixture. The reaction was then stirred for 7 days at room temperature under inert conditions. The solvents were removed in vacuo, and the crude material was redissolved in ethyl acetate (10 mL). Hexane (40 mL) was added, causing precipitation of the hydrazide, which was removed by filtration. The solvents were then removed in vacuo to afford the crude product, which was purified by flash chromatography (hexane/ethyl acetate, 4:1) to give pure **7** as a yellow oil (0.100 g, 0.13 mmol, 52%), spectroscopically identical to the minor isomer described above.

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**Supporting Information Available:** Full experimental details and characterization for the preparation of **16** from (*R*)-serine, the preparation of Fmoc-Cys-O$^t$Bu, and the preparation of veratryl-protected lanthionines, together with the complete experimental details and characterization for the pathways, based on (*S*)-serine, described in Schemes 2 and 3. $^1$H NMR spectra of all compounds described in the Experimental Section and in the Supporting Information (both mixtures of regioisomers, pure **8** and the regioisomer after HPLC separation, and pure lanthionines **7** and **10** prepared via the Mitsunobu route) and the benzyl ester **14/15**; VT NMR of the regioisomer mixtures **5**/**11**, **7**/**12**, and veratryl-protected lanthionine; $^1$H−$^{13}$C HSQC spectra of the regioisomer mixtures **5**/**11**, **7**/**12**, and pure lanthionines **7** and **10** prepared via the Mitsunobu route; HMBC spectra of the regioisomer mixtures **7**/**12** and pure lanthionines **7** and **10** prepared via the Mitsunobu route. This material is available free of charge via the Internet at http://pubs.acs.org.

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