

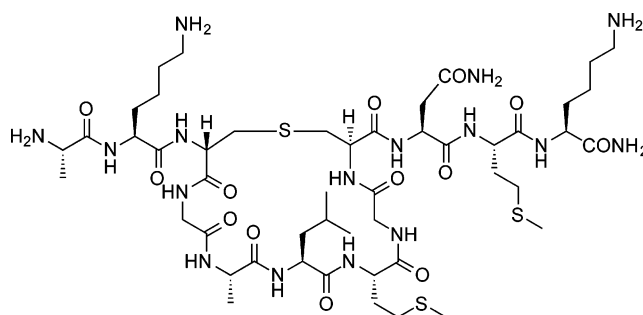
## Orthogonally Protected Lanthionines: Synthesis and Use for the Solid-Phase Synthesis of an Analogue of Nisin Ring C

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Lanthionine, a thioether analogue of cystine, is a key component of the lantibiotics, a family of modified peptides bearing multiple thioether bridges resulting from posttranslational modifications between side chains. It is also used as a conformational constraint in medicinally active peptides. We have explored two synthetic routes to give lanthionine, orthogonally protected with Alloc/allyl and Fmoc groups. One route utilized a carbamate-protected iodoalanine that yielded a mixture of diastereoisomers, and one utilized a trityl-protected iodoalanine, formed via a Mitsunobu reaction, that gave the single desired lanthionine, in complete regio- and diastereoselectivity. We then used this orthogonally protected lanthionine in the solid-phase synthesis of an analogue of a fragment of nisin containing its ring C. The chemoselective deprotection of the allyl and Alloc groups of the incorporated lanthionine unit was followed by regio- and stereoselective cyclization on resin to give the desired lanthionine-bridged peptide.

### Introduction

The unusual bisamino acid lanthionine, a thioether analogue of cystine, is a key component of the lantibiotics,<sup>1</sup> a group of multiply bridged peptides with a range of biological properties, and it is also found as a cross-linking unit in bacterial cell walls.<sup>2</sup> Incorporation of both amino acid moieties of lanthionine into a linear peptide sequence necessarily results in a cyclic peptide with a thioether linkage between two side chains. Lan-

tibiotics typically include between two and five lanthionine, or  $\beta$ -methylanthionine, residues, and therefore have two to five thioether bridges. These bridges can be mostly sequential, as in Type A lantibiotics such as nisin and subtilisin, or highly overlapping, as in Type B lantibiotics such as cinnamycin. The thioether linkage acts as a conformational constraint, which mimics the disulfide bridge of cystine but is stable to reducing agents. For this reason, lanthionine and analogues have also been incorporated into medicinally relevant peptides to afford biologically active, thioether-bridged peptidomimetics.<sup>3</sup>

The development of methodology for the synthesis of peptides containing lanthionine and indeed for the synthesis of lanthionine and methylanthionine themselves are therefore of great interest. To date, however, the formidable challenge presented by the lantibiotics has

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meant that only one total synthesis has been disclosed. Shiba et al. have reported the total synthesis of nisin, by solution-phase synthesis of individual cystine-bridged cyclic peptides, followed by desulfurization to give lanthionine-bridged fragments, and finally by segment coupling of these fragments.<sup>4</sup> This desulfurization approach for the in situ generation of lanthionine bridges (and related structures) from disulfide-bridged cyclic peptides has been explored in detail by Spatola et al., who have demonstrated that the mechanism of this transformation involves generation of a dehydroalanine residue, followed by Michael addition to form the thioether. Under these (base-catalyzed) conditions, however, the reaction does not appear to be diastereoselective.<sup>5</sup> The methodology has also recently been adapted for solid-phase methods.<sup>6</sup> Several other groups have approached the synthesis of lanthionine-bridged cyclic peptides by explicitly generating dehydroalanine residues within a preassembled peptide and subsequently carrying out a Michael addition of cysteine sulfhydryl to form the thioether bridge.<sup>7–11</sup> This biomimetic approach has been most extensively explored by Bradley et al.<sup>11</sup> using mildly basic conditions (buffer, pH 8) for the cyclization step. Under these conditions, the majority of the precursor peptides appear to be preorganized such that the Michael addition proceeds in a diastereoselective fashion to give a single diastereoisomer. This approach has also recently been adapted for solid-phase methods.<sup>11b</sup> Finally, the PCOR (peptide cyclization on an oxime resin) method has also been employed.<sup>3</sup> In this method, a linear peptide containing an orthogonally protected lanthionine is first synthesized, and head-to-tail cyclization, with simultaneous cleavage of the cyclic peptide from the resin, then gives the desired cyclic peptide. All of these approaches have shown potential for the synthesis of *singly* bridged lanthionine-containing peptides. However, the expedient and regioselective formation of *multiple* and *overlapping* thioether bridges, as seen in the families of lantibiotics, still presents a considerable challenge.

The synthesis of lanthionine itself also presents several challenges. Michael addition of cysteine to separate dehydroalanine residues has been shown to give a 1:1 mixture of lanthionine diastereoisomers,<sup>12</sup> in the absence of any conformational preference that arises when these

residues are within a linear peptide.<sup>7–11</sup> The ring opening of serine  $\beta$ -lactones<sup>13</sup> with protected cysteines gives mixtures of lanthionines and thioesters, although sterically hindered analogues may be successfully prepared.<sup>13,14</sup> The ring opening of aziridines with cysteine has also been studied;<sup>15</sup> however, mixtures of regioisomers arising from competing attack at the  $\alpha$ - and  $\beta$ -positions have been observed. Recent reports have suggested that lanthionine can be expediently synthesized from the attack of suitably protected cysteine on  $\beta$ -iodo-<sup>16,17</sup> or  $\beta$ -bromoalanine.<sup>18</sup>

We have developed a flexible, general method for the solid-phase synthesis of other side chain bridged peptides using orthogonally protected bisamino acids as building blocks.<sup>19,20</sup> In this general approach, the required bisamino acid is first synthesized with the side chain linkage (Z: e.g., Z = aliphatic) in place. One amino acid moiety is protected for conventional Fmoc-based synthesis, and the other amino acid moiety is protected with allyl and Alloc groups, orthogonal to both the transient (Fmoc) and permanent (<sup>t</sup>Bu/Boc) protecting groups used in solid-phase peptide synthesis. This bisamino acid is then incorporated into a linear peptide using standard solid-phase peptide synthesis conditions (Scheme 1). The ring closure is formed by sequential selective deprotection of the allyl/Alloc, and Fmoc groups followed by on-resin cyclization, affording the desired side chain bridged cyclic peptide, which can then be further chain-extended.

In this paper, we report two new approaches to the synthesis of orthogonally protected lanthionine (Z = S), and in an extension of our solid-phase approach, we demonstrate the use of this residue in the solid-phase synthesis of an analogue of ring C of the lantibiotic nisin.

## Results and Discussion

**Synthesis of Lanthionines via Carbamate-Protected Iodoalanine.** We have previously shown<sup>20</sup> that the synthesis<sup>16</sup> of protected lanthionines from the reaction of *N*-trityl- $\beta$ -iodoalanine esters with Fmoc-Cys-O<sup>t</sup>-Bu and Cs<sub>2</sub>CO<sub>3</sub> is prone to rearrangement during the synthesis of the *N*-trityl- $\beta$ -iodoalanine esters. This gives  $\alpha$ -iodo- $\beta$ -alanine derivatives and hence nor-lanthionines as the major product and not a mixture of lanthionine rotamers, as previously reported. This rearrangement is assumed to take place via an aziridine intermediate, which is generated both during the synthesis of *N*-trityl- $\beta$ -iodoalanine from *N*-trityl-*O*-methanesulfonyl serine esters and during its reaction with thiol nucleophiles.

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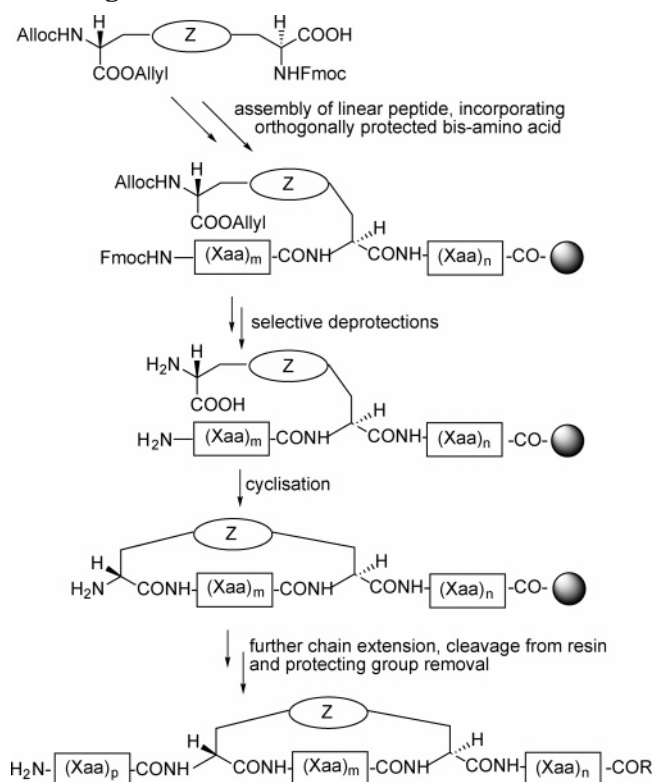
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**SCHEME 1. General Scheme for the Solid-Phase Synthesis of Side Chain Bridged Peptides Using Orthogonally Protected Bis-amino Acids as Building Blocks**

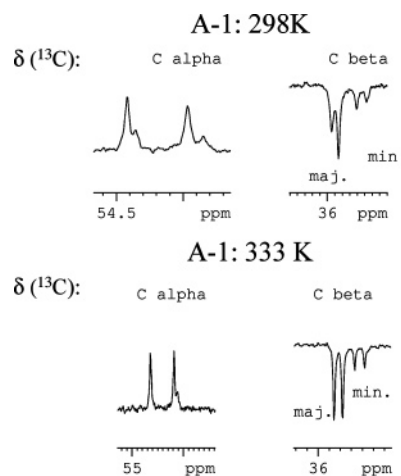
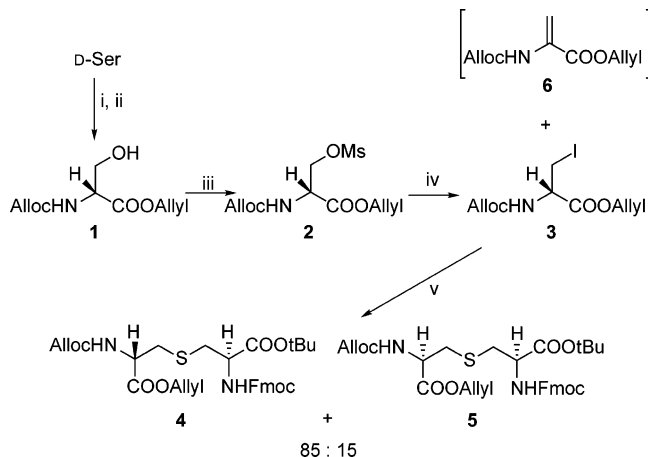


To circumvent this problem, as the electron-donating trityl group is believed to promote the formation of aziridines, we decided to explore routes using carbamate-protected  $\beta$ -iodoalanine. Since our approach to the solid-phase synthesis of side chain bridged peptides required Alloc/allyl protection on one amino acid moiety, we envisaged that this combination of protecting groups on the precursor  $\beta$ -iodoalanine should enable a very concise synthesis of the desired protected lanthionine.

D-Serine was sequentially protected to give alcohol **1**. This was used to access mesylate **2**, which was subsequently converted to  $\beta$ -iodoalanine **3** using NaI (Scheme 2). Under these conditions, only the  $\beta$ -iodo derivative was obtained, as expected, and neither  $\beta$ -alanine nor the corresponding aziridine was formed. The only side product formed during the latter reaction corresponded to the dehydroalanine **6** resulting from a  $\beta$ -elimination process on **3**. This side product was easily removed by flash chromatography, and the unstable iodo derivative **3** was isolated. The freshly obtained iodo derivative **3** was reacted with Fmoc-Cys-O<sup>t</sup>Bu in the presence of cesium carbonate as a base to afford the thioether in 82% yield. However, careful inspection of the <sup>13</sup>C NMR revealed the presence of two isomeric lanthionines, **4** and **5**, in an approximately 85:15 ratio, as determined by integration of the <sup>13</sup>C signals (Figure 1).

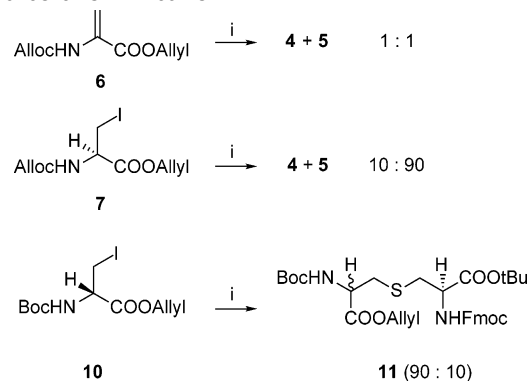
Variable temperature NMR experiments (Figure 1) showed no coalescence of the <sup>13</sup>C signals at temperatures up to 333 K, indicating that the additional peaks arise from the presence of two diastereomers rather than conformers. To establish that the two inseparable compounds obtained were diastereoisomeric at the C $\alpha$ -

**SCHEME 2. Synthesis of Lanthionines via Carbamate-Protected Iodoalanine<sup>a</sup>**



**FIGURE 1.** Excerpt of the <sup>13</sup>C DEPT (100.61 MHz) VT experiments on the mixture A-1: **4** + **5** (85:15).

**SCHEME 3. Different Routes to Mixtures of **4** and **5** and to the Mixture **11**<sup>a</sup>**



position of the Alloc/allyl-protected amino acid moiety, dehydroalanine **6** was reacted with Fmoc-Cys-O<sup>t</sup>Bu (Scheme 3). The <sup>13</sup>C peaks corresponding to the C $\beta$  of the mixture obtained indicated that **4** and **5** were produced in a 1:1 ratio, indicating that the formation of **5** under

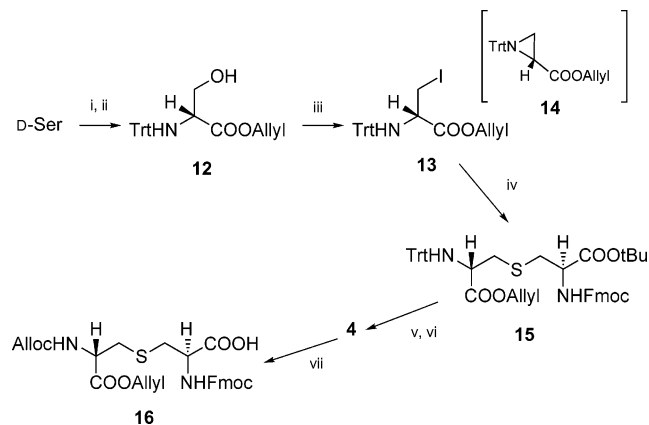
our original conditions must arise from Michael addition to the dehydroalanine **6** formed under the coupling reaction conditions. As a further test, the enantiomeric iodoalanine **7** was reacted with Fmoc-Cys-O<sup>t</sup>Bu and Cs<sub>2</sub>CO<sub>3</sub> under the same conditions, affording **4** and **5** in a 10:90 ratio. Boc-protected iodoalanine **10** was also synthesized from D-serine (via Boc-Ser-Oallyl **8** and Boc-Ser-(Ms)-Oallyl **9**) and subjected to the same coupling reaction. This gave **11**, also as two diastereoisomers, which in this case could be differentiated in both the <sup>13</sup>C and <sup>1</sup>H spectra.

Clearly, the abstraction of the α-hydrogen from **3** to give dehydroalanine **6** cannot be completely avoided under these coupling reaction conditions and, as has been previously observed, Michael additions between separate dehydroalanines and cysteine nucleophiles show no diastereoselectivity.<sup>12</sup> Although a range of conditions were investigated (solvent, base, temperature, excess of cysteine nucleophile), we could not avoid the competing elimination reaction leading to formation of an appreciable amount of the undesired diastereomer **5**. In contrast, Schmidt has reported<sup>18</sup> that similar reactions between less reactive electrophilic bromoalanine derivatives and cysteine nucleophiles are highly diastereoselective, implying that in this case elimination to the dehydroalanine does not occur. However, we concluded that partial elimination from the more reactive carbamate-protected iodoalanines could not be avoided, making these unsuitable for direct nucleophilic reaction with cysteine nucleophiles. We therefore revisited the use of trityl-protected iodoalanines, for which this problem does not arise.

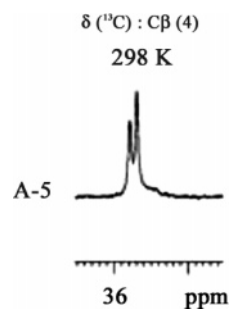
**Synthesis of Lanthionines via the Mitsunobu Reaction.** We have previously reported<sup>21</sup> that trityl serine allyl ester can be reacted with Fmoc-Cys-O<sup>t</sup>Bu, using modified Mitsunobu conditions with zinc tartrate as a cocatalyst, to give the desired lanthionine regioisomer directly. This reaction proceeded with complete selectivity to give a unique coupling compound. Unfortunately, although this route would undoubtedly be the most direct method to give protected lanthionines, we could not further optimize the moderate (50%) yield of this key step as the reaction was too slow; competing Fmoc deprotection and cysteine dimerization led instead to the consumption of the starting materials.

We therefore returned to the synthesis of the key intermediate, *N*-trityl-β-iodo serine **13**, and found that it can be directly synthesized via a Mitsunobu reaction from the alcohol **12** (Scheme 4). (This avoids the synthesis of an intermediate mesylate; we previously showed<sup>21</sup> that displacement of the mesylate by I<sup>-</sup> is sufficiently slow that the resulting iodo alanine can rearrange.) Provided the reaction temperature was carefully controlled and did not exceed -2 °C during the entire reaction, only the β-iodo-alanine **13** was produced and it was possible to avoid the production of the undesired regioisomeric α-iodo-β-alanine. Only trace amounts of aziridine **14** were observed as a byproduct. Formation of the aziridine **14** was observed during purification and was also occasion-

#### SCHEME 4. Synthesis of Lanthionine via the Mitsunobu Reaction<sup>a</sup>



<sup>a</sup> (i) Me<sub>3</sub>SiCl, Et<sub>3</sub>N, then MeOH, then Trt-Cl; (ii) Cs<sub>2</sub>CO<sub>3</sub>, allyl bromide (72% over 2 steps); (iii) MeI, Ph<sub>3</sub>P, DEAD, CH<sub>2</sub>Cl<sub>2</sub> (72%); (iv) FmocCysO<sup>t</sup>Bu, Cs<sub>2</sub>CO<sub>3</sub>, DMF (90%); (v) TFA/CH<sub>2</sub>Cl<sub>2</sub> (1:10 v/v); (vi) Alloc-Cl, NaHCO<sub>3</sub>, H<sub>2</sub>O/dioxane (85% over 2 steps); (vii) TFA/CH<sub>2</sub>Cl<sub>2</sub> (1:1 v/v) (41% after HPLC).



**FIGURE 2.** Excerpt of the <sup>13</sup>C (100.61 MHz) spectrum (*C*<sub>β</sub> domain) of **4**, prepared via the route shown in Scheme 4.

ally observed during the reaction if the temperature or the duration of the reaction was not carefully controlled.

Once **13** had been successfully produced as a single regioisomer, reaction with Fmoc-Cys-O<sup>t</sup>Bu/Cs<sub>2</sub>CO<sub>3</sub> then gave the desired lanthionine **15** as a single regio- and diastereoisomer.<sup>22</sup> Subsequent manipulation of the protecting groups allowed us to access **4** as a single isomer, confirmed by its <sup>13</sup>C NMR spectrum showing only one set of peaks (A-5, Figure 2). Finally, removal of the <sup>t</sup>Bu ester allowed lanthionine **16** to be obtained via a direct, regio- and stereocontrolled route.

**Synthesis of an Analogue of Ring C of Nisin.** To illustrate the utility of such orthogonally protected lanthionines for the solid-phase synthesis of lantibiotics, we undertook the synthesis of a cyclic fragment of nisin

(H-Ala-Lys-Lan-Gly-Ala-Leu-Met-Gly-Lan-Asn-Met-Leu-NH<sub>2</sub>). Linear peptide **C** was first assembled manually on Sieber resin with a loading of 0.16 mmol g<sup>-1</sup>. Standard coupling techniques (preactivation of the Fmoc-protected amino acid with HBTU using DIEA, followed by coupling for 25 min), followed by capping with acetylimidazole and subsequent deprotection with 20% piperidine in DMF, were used. Lanthionine **16** was incorporated at the fourth

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(22) We have previously shown<sup>21</sup> that the undesired aziridine **14** cannot subsequently react with Fmoc-Cys-O<sup>t</sup>Bu/Cs<sub>2</sub>CO<sub>3</sub>. It is also clear that under these conditions the β-iodo-α-alanine **13** cannot rearrange (via the aziridine) to give the undesired nor-lanthionine regioisomer.

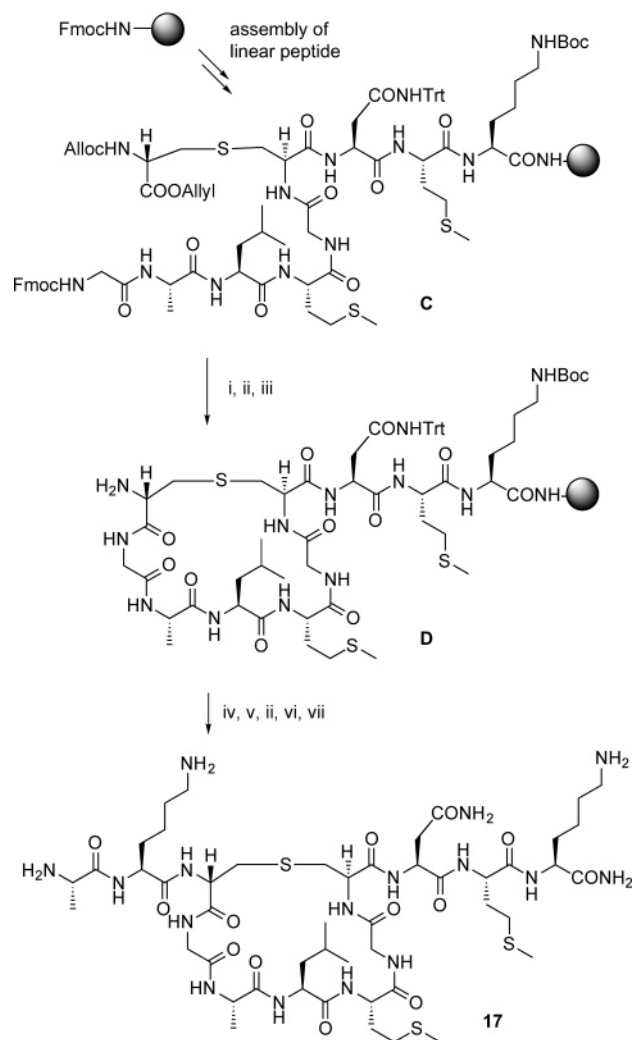


TABLE 1.  $^1\text{H}$  NMR Parameters of Peptide **17** Deduced from Experiments in  $\text{D}_2\text{O}$  (277 K) and  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1) (275 K)

amino acid residue	$^1\text{H}$ NMR chemical shifts, $\delta$ (ppm)							$J_{\text{H,H}}$ (Hz)	
	NH <sup>a</sup>	H $\alpha$	H $\beta$	H $\gamma$	H $\delta$	H $\epsilon$	other H	$^3J_{\alpha,\beta}$	$^3J_{\alpha,\text{NH}}^a$
Lys1	8.49	4.26	1.84; 1.78	1.46; 1.41	1.68	2.98		8.8; 5.4	6.7
Met2	8.54	4.48	2.12; 1.99	2.60; 2.51			2.09 SCH <sub>3</sub>	9.6; 4.7	6.8
Asn3	8.75	4.67	2.85; 2.76					7; 6.9	6.6
Lan4	8.30	4.55	3.07; 2.95					9.0; 4.6	7.5
Gly5	8.58	4.05, 3.85						17.5 ( $^2J$ )	6.1
Met6	8.15	4.54	2.08; 2.02	2.61; 2.49			2.09 SCH <sub>3</sub>	9.3; 5.3	7
Leu7	8.39	4.22	1.77; 1.74	1.63	0.88; 0.93 CH <sub>3</sub>			10; 5.4	5.5
Ala8	8.45	4.41	1.43 CH <sub>3</sub>					7.1	6
Gly9	8.90	4.09, 3.82						16.8 ( $^2J$ )	6.1
Lan10	8.81	4.60	2.99; 2.94					7.6; 6.3	6.3
Lys11	8.80	4.31	1.81; 1.77	1.45; 1.41	1.68	2.97		8.0; 6.4	5.6
Ala12	8.19	4.07	1.51 CH <sub>3</sub>					7.2	nd

<sup>a</sup> From  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1) experiments

### SCHEME 5. Synthesis of an Analogue of Nisin Ring C<sup>a</sup>



<sup>a</sup> (i)  $\text{Pd}(\text{PPh}_3)_4$ ,  $\text{CHCl}_3/\text{AcOH}/\text{NMM}$ ; (ii) 20% piperidine in DMF; (iii) PyAOP, HOAt, DIEA; (iv) Fmoc-Lys(Boc)OH, HBTU/DIEA; (v) acetylimidazole, DMF; (vi) Boc-Ala-OH, HBTU/DIEA; (vii) 5% TFA/5%TES/ $\text{CH}_2\text{Cl}_2$ , then TFA (90%) (19% overall).

residue (Scheme 5) using the same coupling protocol. After linear extension of the peptide sequence to give resin-bound intermediate C, the allyl and Alloc protecting groups were then selectively removed with  $\text{Pd}(0)$ .<sup>23</sup>

Removal of the Fmoc group was then followed by intramolecular cyclization using PyAOP/HOAt<sup>24</sup> to give the cyclic intermediate D. The remaining amino group was further extended first with Fmoc-Lys(Boc)OH and then with Boc-Ala-OH, using the same standard coupling techniques as previously described.

The peptide was then cleaved from the resin, purified by reverse-phase HPLC (C18), and freeze-dried. This afforded the desired cyclic peptide **17** as a single isomer, as a light powder with a yield of 19% (relative to the mmol of resin functionality used). The peptide was characterized by mass spectrometry using electrospray positive mode (ESI+), and a single mass, corresponding to the desired peptide, was observed. It is theoretically possible for interstrand cross-linking to take place between two separate strands of the partially deprotected, resin-bound peptide (with two free  $\text{NH}_2$  and one free COOH group) during the cyclization with PyAOP to give peptide D. We therefore also analyzed both the crude peptide (prior to reverse-phase HPLC) and a sample of peptide cleaved from the resin after the cyclization step by ESI+ mass spectrometry. In all cases, no evidence of peptides with molecular weights higher than that expected was seen, indicating that only intrastrand bond formation occurs during the cyclization step. This selective bond formation may be favored by the low substitution of the resin.

The structure and connectivity of the peptide **17** was established by NMR. NMR experiments were performed in a  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1) mixture and in  $\text{D}_2\text{O}$  on a static sample at 275 and 277 K, respectively. The complete assignment of all proton resonances of **17**, summarized in Table 1, was based on the combined use of TOCSY and NOESY experiments performed in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1). Coupling constants between NH and H $\alpha$  ( $^3J_{\alpha,\text{NH}}$ ) were estimated from the 1D  $^1\text{H}$  spectrum recorded in the  $\text{H}_2\text{O}/\text{D}_2\text{O}$  mixture, whereas coupling constants between H $\alpha$  and H $\beta$  were found in the 1D  $^1\text{H}$  spectrum recorded in  $\text{D}_2\text{O}$  (Table 1).

NOESY experiments allowed us to prove the cyclic character of the target peptide by clearly showing a cross-peak (marked with a square in Figure 3) between the NH of the Gly9 and the H $\alpha$  of the Lan10 units.

The assignment of the  $\alpha$  and side chain carbon resonances (Table 2) was deduced using gradient hetero-

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TABLE 2.  $^{13}\text{C}$  NMR Parameters of Peptide 17 in  $\text{D}_2\text{O}$  at 277 K

amino acid residue	$^{13}\text{C}$ NMR chemical shifts, $\delta$ (ppm)						
	CO	C $\alpha$	C $\beta$	C $\chi$	C $\delta$	C $\epsilon$	other C
Lys1	179.29	56.20	32.95	24.95	28.98	41.87	
Met2	176.47	55.46	32.60	32.12			16.90 or 16.79 SCH <sub>3</sub>
Asn3	175.24/177.31 <sup>a</sup>	53.31	38.38				
Lan4	174.35	56.38	35.85				
Gly5	174.90	45.75					
Met6	176.79	55.28	32.46	31.96			16.90 or 16.79 SCH <sub>3</sub>
Leu7	177.74	56.20	41.87	27.17	23.30, 24.86		
Ala8	178.16	52.34	19.8 CH <sub>3</sub>				
Gly9	173.63	45.60					
Lan10	175.16	55.99	35.57				
Lys11	176.30	56.59	33.29	24.95	29.16	41.87	
Ala12	173.57	51.50	19.3CH <sub>3</sub>				

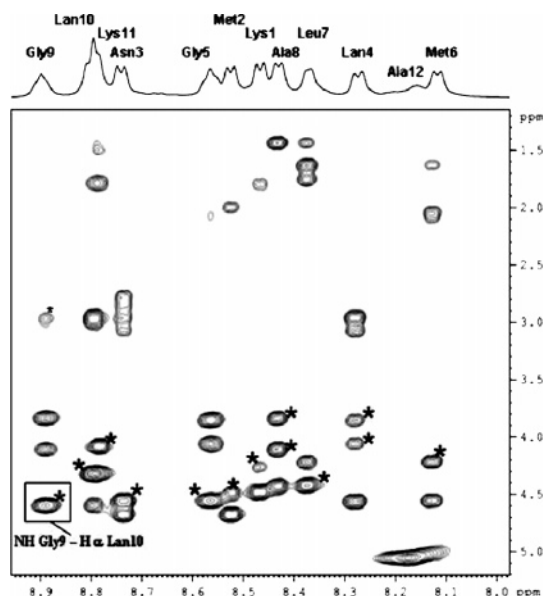
<sup>a</sup> Side chain.

FIGURE 3. NH-H $\alpha$  domain of NOESY spectrum of **17** ( $\text{H}_2\text{O}/\text{D}_2\text{O}$ , 9:1, 275 K) Inter-residue NOE cross-peaks ( $\text{NH}_i\text{-H}\alpha_{i+1}$ ) are labeled with a  $\star$ .

nuclear single quantum coherence experiments ( $^1\text{H}\text{-}^{13}\text{C}$  gHSQC) performed in  $\text{D}_2\text{O}$ . The assignment of each carbonyl resonance was established by performing gradient heteronuclear multiple bond coherence experiments ( $^1\text{H}\text{-}^{13}\text{C}$  gHMBC). The latter confirmed the desired connectivity of the core backbone.

Finally, we have also utilized the mixture of inseparable diastereomeric lanthionines obtained via the carbamate-protected iodoalanine (Scheme 2) in a further synthesis of an analogue of ring C of nisin. The mixture of **4** and **5** was deprotected with TFA, as described above, to give the protected lanthionine monomer as an inseparable mixture of (2*S*,6*R*) **16** and its (2*R*,6*R*) diastereoisomer (85:15 ratio). This mixture was then used to synthesize the sequence Ac-Lys-Lan8-Gly-Ala-Leu-Met-Gly-Lan2-Asn-H, starting from Novasyn TGT resin, preloaded with Fmoc-Asn(Trt), in a manner analogous to that described above for the synthesis of **17**. Cleavage from the resin and purification by reverse-phase HPLC (C18) afforded two peptide diastereoisomers in an approximately 80:20 ratio. These were separated and

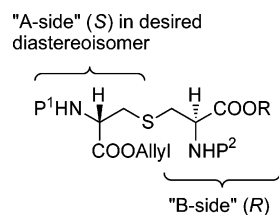
characterized by mass spectroscopy (ESI+). The major isomer has been characterized by 1D and 2D  $^1\text{H}$  NMR (NOESY, TOCSY). As the synthesis was carried out using the inseparable mixture of lanthionine diastereoisomers, the diastereomeric character of the two resulting peptides can be assumed to come from the two isomers at the C $\alpha$  position of the original Alloc/allyl-protected amino acid moiety.

### Conclusion

In summary, a very concise route to protected lanthionines as a C-2 diastereomeric mixture has been reported, using a carbamate-protected iodo-alanine. An alternative route, using a trityl-protected iodo-alanine formed by a Mitsunobu reaction, gave the single desired lanthionine, in complete regio- and diastereoselectivity. We have used this orthogonally protected amino acid in an efficient strategy for the synthesis of side chain bridged peptides. In this approach, a linear peptide, incorporating the orthogonally protected lanthionine, is first synthesized, and at an appropriate point, chemoselective deprotection of the allyl and Alloc groups is followed by regio- and stereoselective cyclization on resin to give the desired lanthionine-bridged peptide. In this way, the position of on-resin cyclization may be completely controlled, and furthermore, competing interchain coupling is not observed. This approach was successfully used to synthesize an analogue of nisin ring C.

### Experimental Section

For ease of assignment, the two amino acid moieties of all lanthionine derivatives have been indicated thus:



**N-Allyloxycarbonyl- $\beta$ -iodo-(*S*)-alanine Allyl Ester (3).** *N*-Allyloxycarbonyl-(*R*)-serine(*O*-methanesulfonyl) allyl ester **2** (381 mg, 1.24 mmol) and NaI (931 mg, 6.2 mmol, 5 equiv) were dissolved in dry acetone (10 mL). The reaction mixture was stirred for 29 h at room temperature.  $\text{Et}_2\text{O}$  (40 mL) was added, and the resulting organic layer was washed with 10% thiosulfate aqueous solution (1  $\times$  20 mL) and with brine (2  $\times$

15 mL). It was then dried over MgSO<sub>4</sub>, filtered through Celite, and concentrated to afford a crude mixture as pale yellow oil. Purification by flash chromatography on silica gel (hexane/EtOAc 10:1 to 8:1) afforded the desired compound **3** as a white sticky oil (329 mg, 0.97 mmol, 78% isolated) which turned to a white solid at low temperature.  $R_f = 0.4$  (hexane/EtOAc 8:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.88 (m, 1H, CH=CH<sub>2</sub>), 5.59 (bd, 1H, <sup>3</sup>J = 6.2 Hz, NHAlloc), 5.33 and 5.25 (2dq, 2H, <sup>3</sup>J<sub>trans</sub> = 17.2 Hz, <sup>2</sup>J = 1.4 Hz, <sup>3</sup>J<sub>cis</sub> = 10.4 Hz, <sup>4</sup>J = 1.2 Hz, CH=CH<sub>2</sub>), 5.29 and 5.19 (2dq, 2H, <sup>3</sup>J<sub>trans</sub> = 17.2 Hz, <sup>3</sup>J<sub>cis</sub> = 10.5 Hz, <sup>4</sup>J = 1.3 Hz, CH=CH<sub>2</sub>), 5.22 (4dd, 4H, CH=CH<sub>2</sub>), 4.65 (m, 2H, CH<sub>2</sub>-CH=CH<sub>2</sub>), 4.55 (m, 3H, <sup>3</sup>J = 5.2 Hz, CH<sub>2</sub>-CH=CH<sub>2</sub> + H<sub>α</sub>), 3.56 (m, 2H, CH<sub>2</sub> β); <sup>13</sup>C NMR (100.61 MHz, CDCl<sub>3</sub>) δ 168.8 (CO allyl), 154.2 (CO Boc), 132.3 and 131.0 (CH<sub>2</sub>-CH=CH<sub>2</sub>), 119.3 and 117.9 (CH<sub>2</sub>-CH=CH<sub>2</sub>), 66.7 and 65.9 (CH<sub>2</sub>-CH=CH<sub>2</sub>), 53.9 (C<sub>α</sub>), 7.21 (C<sub>β</sub>); IR ν<sub>max</sub> 1750, 1720, 1500 +1200 cm<sup>-1</sup>. C<sub>10</sub>H<sub>14</sub>NO<sub>4</sub>I (ES+)  $m/z$ : [M - HI + H]<sup>+</sup> = 212, [M - HI + Na]<sup>+</sup> = 234. HRMS (FAB) C<sub>10</sub>H<sub>14</sub>NO<sub>4</sub>I calcd for [MH]<sup>+</sup> 340.00458, found 340.00507.

The only impurity formed during this reaction and its purification corresponds to the corresponding *N*-allyloxycarbonyl dehydroalanine allyl ester **6**:  $R_f = 0.7$  (hexane/EtOAc 10:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.22 (s, 1H, NHAlloc), 6.18 (bs, 1H, C<sub>β</sub>-H), 5.87 (m, 1H, CH=CH<sub>2</sub>), 5.75 (d, 1H, <sup>2</sup>J = 1.3 Hz, C<sub>β</sub>-H), 5.29 and 5.21 (2dq, 2H, <sup>3</sup>J<sub>trans</sub> = 17.3 Hz, <sup>2</sup>J = 1.6 Hz, <sup>3</sup>J<sub>cis</sub> = 10.4 Hz, <sup>4</sup>J = 1.3 Hz, CH=CH<sub>2</sub>), 5.26 and 5.17 (2dq, 2H, <sup>3</sup>J<sub>trans</sub> = 17.3 Hz, <sup>2</sup>J = 1.6 Hz, <sup>3</sup>J<sub>cis</sub> = 10.4 Hz, <sup>4</sup>J = 1.3 Hz, CH=CH<sub>2</sub>), 4.65 (dt, 2H, <sup>3</sup>J = 5.7 Hz, <sup>4</sup>J = 1.3, CH<sub>2</sub>-CH=CH<sub>2</sub>), 4.55 (dt, 2H, <sup>3</sup>J = 5.7 Hz, <sup>4</sup>J = 1.3, CH<sub>2</sub>-CH=CH<sub>2</sub>); <sup>13</sup>C NMR (100.61 MHz, CDCl<sub>3</sub>) δ 163.2 (CO allyl), 152.8 (CO Alloc), 132.2 (CH=CH<sub>2</sub> Alloc), 131.11 (C<sub>α</sub>), 130.9 (CH=CH<sub>2</sub> allyl), 118.7 (CH=CH<sub>2</sub> allyl), 118.0 (CH=CH<sub>2</sub> Alloc), 105.8 (C<sub>β</sub>), 66.3 (CH<sub>2</sub>-CH=CH<sub>2</sub> allyl), 65.6 (CH<sub>2</sub>-CH=CH<sub>2</sub> Alloc); IR ν<sub>max</sub> 1735, 1716, 1527 + 1310 cm<sup>-1</sup>. C<sub>10</sub>H<sub>13</sub>NO<sub>5</sub> (ES+)  $m/z$ : [MH]<sup>+</sup> = 212, [MNa]<sup>+</sup> = 234. HRMS (CI-methane) C<sub>10</sub>H<sub>13</sub>NO<sub>5</sub> calcd for [MH]<sup>+</sup> 212.09228, found 212.09265.

**3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*S*)-(allyloxycarbonylamino)-propionic Acid Allyl Ester (**4**) and 3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]-(*R*)-(allyloxycarbonylamino)propionic Acid Allyl Ester (**5**).** (4:5, 85:15) A mixture of *N*-9-fluorenylmethoxycarbonyl-*R*-cysteine *tert*-butyl ester (45 mg, 1.13 × 10<sup>-4</sup> mol, 0.96 equiv) and *N*-allyloxycarbonyl-β-iodo-*S*-alanine allyl ester **3** (40 mg, 1.18 × 10<sup>-4</sup> mmol, 1 equiv) were dissolved in DMF (0.5 mL). Cesium carbonate (36 mg, 1.11 × 10<sup>-4</sup> mmol, 0.94 equiv) was added portionwise over 2 h. The reaction was monitored by TLC and was complete after 2.5 h. EtOAc (5 mL) was then added to the reaction mixture, and the organic layer was washed with 5% aqueous citric acid (4 × 10 mL) and brine (1 × 5 mL), dried over MgSO<sub>4</sub>, and concentrated in vacuo. Purification by flash chromatography on silica gel (hexane/EtOAc 4:1 to 2:1) afforded a mixture of two inseparable diastereomers (*C*-2 epimers) **4** (**M**) and **5** (**m**) in a ratio [M]:[m] = 85:15 (62 mg, 0.99 × 10<sup>-4</sup> mol, 88% overall):  $R_f = 0.3$  (hexane/EtOAc 2:1); <sup>1</sup>H NMR (500.13 MHz, CDCl<sub>3</sub>) δ 7.78–7.25 (m, 8H, H arom. Fmoc), 5.86 (m, 2H, CH=CH<sub>2</sub> allyl and Alloc), 5.77 (d, 1H, <sup>3</sup>J = 7.65 Hz, NHFmoc), 5.69 (d, 1H, <sup>3</sup>J = 7.10 Hz, NHAlloc), 5.30 and 5.22, (2m, 2H, <sup>3</sup>J<sub>trans</sub> = 17.1 Hz, <sup>3</sup>J<sub>cis</sub> = 10.4 Hz, CH<sub>2</sub>-CH=CH<sub>2</sub> allyl), 5.28 and 5.18 (2m, 2H, <sup>3</sup>J<sub>trans</sub> = 17.1 Hz, <sup>3</sup>J<sub>cis</sub> = 10.4 Hz, CH<sub>2</sub>-CH=CH<sub>2</sub> Alloc), 4.61 (m, 3H, CH<sub>2</sub>-CH=CH<sub>2</sub> Alloc + H<sub>α</sub> A side), 4.56 (m, 2H, CH<sub>2</sub>-CH=CH<sub>2</sub> allyl), 4.47 (m, 1H, H<sub>α</sub> B side), 4.37 (m, 2H, CH<sub>2</sub> Fmoc), 4.21 (t, 1H, <sup>3</sup>J = 7 Hz, CH Fmoc), 3.09–2.91 (m, 4H, CH<sub>2</sub> β A and B sides), 1.47 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>) δ 170.12 (CO allyl m.), 170.09 (CO allyl M), 169.33 (CO <sup>t</sup>Bu m), 169.27 (CO <sup>t</sup>Bu M), 155.7 (CO Fmoc M + m), 155.6 (CO Alloc M + m), 143.8, 143.8, 141.21, 141.20 (Cq. Fmoc M + m), 132.4 (CH=CH<sub>2</sub> Alloc M + m), 131.2 (CH=CH<sub>2</sub> allyl M + m), 128.8, 127.6, 127.0, 125.1, 119.9 (C. arom. Fmoc M + m), 119.2 (CH=CH<sub>2</sub> allyl M + m), 117.9 (CH=CH<sub>2</sub> Alloc M + m), 83.0 (Cq. <sup>t</sup>Bu M + m), 67.1 (CH<sub>2</sub> Fmoc M), 67.1 (CH<sub>2</sub>

Fmoc m), 66.4 (CH<sub>2</sub> allyl m), 66.3 (CH<sub>2</sub> allyl M), 65.9 (CH<sub>2</sub> Alloc M + m), 54.3 (C<sub>α</sub> M, B side), 54.2 (C<sub>α</sub> m, B side) 53.8 (C<sub>α</sub> M, A side), 53.7 (C<sub>α</sub> m, A side), 47.0 (CH Fmoc M + m), 35.8 (C<sub>β</sub> M, B side), 35.7 (C<sub>β</sub> M, A side), 35.4 (C<sub>β</sub> m, B side), 35.3 (C<sub>β</sub> m, A side), 27.9 (CH<sub>3</sub> <sup>t</sup>Bu M + m). C<sub>32</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub>S (ES+)  $m/z$ : [MH]<sup>+</sup> = 611, [MNa]<sup>+</sup> = 633, [MK]<sup>+</sup> = 649. HPLC (reverse phase, flow rate of 1.5 mL min<sup>-1</sup>, λ = 214 nm,  $t_R$  (mixture) = 42.8 min using a linear gradient buffer B (CH<sub>3</sub>CN) in A (H<sub>2</sub>O) [90:10 to 10:90 within 60 min],  $t_R$  (mixture) = 14.6 min using a isocratic buffer B (CH<sub>3</sub>CN) in A (H<sub>2</sub>O) [40:60].

***N*-Triphenylmethyl-*S*-alanine Allyl Ester (**12**).**<sup>17</sup> To a suspension of *D*-serine (3.85 g, 36.4 mmol, 1 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (150 mL) was added trimethylsilyl chloride (14.25 mL, 112.7 mmol, 3.1 equiv) at room temperature. The mixture was heated under reflux for 40 min and allowed to cool to room temperature. Et<sub>3</sub>N (15.7 mL, 112.7 mmol, 3.1 equiv) diluted in dry CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was then added, and the reaction mixture was heated under reflux for a further 1 h. It was then cooled to 0 °C using an ice–water bath before being subjected to the dropwise addition of dry MeOH (1.5 mL, 36.4 mmol, 1 equiv). The mixture was allowed to warm to room temperature. Et<sub>3</sub>N (5.07 mL, 36.4 mmol, 1 equiv) was then added followed by a portionwise addition of triphenylmethyl chloride (10.13 g, 36.4 mmol, 1 equiv). After stirring at room temperature for 27 h, Et<sub>3</sub>N (20 mL) and MeOH (200 mL) were added to the reaction mixture. Removal of the solvents in vacuo led to a residue that was partitioned between EtOAc (500 mL) and 5% aqueous citric acid, precooled to 4–5 °C using a water–ice bath (300 mL). The organic layer was washed with further 5% precooled aqueous citric acid (2 × 300 mL), dried over MgSO<sub>4</sub> and concentrated to afford a crude solid that was used in the next step without further purification.

This solid was dissolved in dry MeOH (100 mL) in the presence of cesium carbonate (5.92 g, 18.2 mmol, 0.5 equiv). The reaction mixture was stirred at room temperature for 1 h before being concentrated under vacuum. The resulting cesium salt was dissolved in DMF (50 mL), and allylbromide (3.5 mL, 39.99 mmol, 1.1 equiv) was then added dropwise at room temperature. The reaction mixture was stirred for 14 h, and then EtOAc (250 mL) was added directly to the mixture. The layer was washed with 5% aqueous citric acid, precooled to 4–5 °C using a water–ice bath (5 × 200 mL), dried over MgSO<sub>4</sub>, and concentrated in vacuo. Purification by flash chromatography on silica gel afforded the compound **12** as a white sticky foam (10.85 g, 26.92 mmol, 74% over two steps):  $R_f = 0.2$  (hexane/EtOAc 4:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.61 (m, 6H, Trt), 7.33 (m, 6H, Trt), 7.26 (m, 3H, Trt), 5.78 (m, 1H, CH=CH<sub>2</sub>), 5.27 and 5.18 (2dq, 2H, <sup>3</sup>J<sub>trans</sub> = 17.2 Hz, <sup>2</sup>J = 1.5 Hz, <sup>3</sup>J<sub>cis</sub> = 10.5 Hz, <sup>4</sup>J = 1.5 Hz, CH=CH<sub>2</sub>), 4.28 and 4.17 (2 dd, 2H, <sup>2</sup>J = 13.2 Hz, <sup>3</sup>J = 5.9, 5.7 Hz, CH<sub>2</sub>-CH=CH<sub>2</sub>), 3.72 (m, 1H, H<sub>α</sub>), 3.87 and 3.68 (2 dd, 2H, <sup>2</sup>J = 10.4 Hz, <sup>3</sup>J = 4.3, 5.6 Hz, CH<sub>2</sub> β), 3.16 (s, 1H, OH); <sup>13</sup>C NMR (100.61 MHz, CDCl<sub>3</sub>) δ 173.1 (CO), 145.9 (Cq. arom. Trt), 131.9 (CH=CH<sub>2</sub>), 129.9, 128.1, 126.7 (C arom. Trt), 118.5 (CH=CH<sub>2</sub>), 71.5 (Cq. aliph. Trt), 65.8 (CH<sub>2</sub>-CH=CH<sub>2</sub>), 65.2 (C<sub>β</sub>), 58.1 (C<sub>α</sub>). C<sub>33</sub>H<sub>42</sub>N<sub>2</sub>O<sub>8</sub>S (ES+)  $m/z$ : [Trt]<sup>+</sup> = 243, [MH]<sup>+</sup> = 388, [MNa]<sup>+</sup> = 410.

***N*-Triphenylmethyl-β-iodo-*S*-alanine Allyl Ester (**13**).** *N*-Triphenylmethyl-*S*-alanine allyl ester **12** (1.57 g, 4.06 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at room temperature in the presence of triphenylphosphine (1.60 g, 6.09 mmol, 1.5 equiv). After 10 min, the reaction mixture was cooled to –10 °C using an ethanol bath refrigerated with a cryostat probe. DEAD (937 μL, 6.04 mmol, 1.5 equiv) was added dropwise to the reaction mixture over 1 min. After a further 5 min this was followed by a careful addition of iodomethane (375 μL, 6.04 mmol, 1.5 equiv). The temperature of the reaction mixture was then maintained at –2 °C over 3 h. Formation of the undesired aziridine **14** can occur during the reaction if the temperature of the reaction is not properly controlled; this must be avoided, as if the aziridine forms



during the reaction itself, this can lead to rearrangement to give the undesired and inseparable  $\alpha$ -iodo- $\beta$ -alanine.<sup>21</sup>

The whole reaction mixture was directly filtered by chromatography on silica gel using a nonresolving eluant (hexane/EtOAc 4:1) to remove the phosphorus derivatives. The fractions collected containing the target compound were concentrated, and the residue obtained was purified by flash chromatography on silica gel (hexane/EtOAc 20:1) to afford the desired iodo derivative **13** as a white foam (1.45 g, 2.92 mmol, 72%). Iodo derivative **13** has to be kept and stored at a low temperature ( $-4\text{ }^{\circ}\text{C}$ ). At this temperature, the derivative is a solid.

The main side product of this reaction was the aziridine derivative **14**. As well as forming during the Mitsunobu reaction itself if the temperature was not carefully controlled, **14** was observed if the iodo derivative is allowed to stand for prolonged periods in solvents. Collected fractions from the column chromatography steps must therefore be concentrated quite quickly. It is preferable to use moderate heating to perform the concentration and avoid the addition of dichloromethane, as aziridine formation is favored by high temperature and (surprisingly) by acidic conditions, even slight.

**N-Triphenylmethyl- $\beta$ -iodo-(S)-alanine Allyl Ester (13).**  $R_f = 0.6$  (hexane/EtOAc 15:1);  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.59 (m, 6H, Trt), 7.31 (m, 6H, Trt), 7.23 (m, 3H, Trt), 5.74 (m, 1H,  $\text{CH}=\text{CH}_2$ ), 5.18 and 5.11 (2dq, 2H,  $^3J_{\text{trans}} = 17.2\text{ Hz}$ ,  $^3J_{\text{cis}} = 10.3\text{ Hz}$ ,  $^2J = 1.5\text{ Hz}$ ,  $\text{CH}=\text{CH}_2$ ), 4.31 and 4.17 (2 dd, 2H,  $^2J = 13.0\text{ Hz}$ ,  $^3J = 5.9, 4.5\text{ Hz}$ ,  $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 3.51 (dd, 1H,  $^3J = 6.9, 3.4, \text{H}\alpha$ ), 3.33 and 3.22 (2 dd, 2H,  $^2J = 9.8\text{ Hz}$ ,  $^3J = 6.7, 3.4\text{ Hz}$ ,  $\text{CH}_2\text{I}$ );  $^{13}\text{C NMR}$  (100.61 MHz,  $\text{CDCl}_3$ )  $\delta$  171.8 (CO), 145.5 (Cq, Trt), 131.6 ( $\text{CH}=\text{CH}_2$ ), 129.0, 127.9, 126.6 (C arom. Trt), 118.6 ( $\text{CH}=\text{CH}_2$ ), 71.0 (Cq. aliph. Trt), 65.9 ( $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 56.1 (C $\alpha$ ), 9.8 (C $\beta$ ).  $\text{C}_{25}\text{H}_{24}\text{NO}_2\text{I}$  (ES+)  $m/z$ :  $[\text{Trt}]^+ = 243$ ,  $[\text{MNa}]^+ = 520$ . IR  $\nu_{\text{max}}$  1730, 1510  $\text{cm}^{-1}$ . HRMS (FAB)  $\text{C}_{25}\text{H}_{24}\text{NO}_2\text{I}$  calcd for  $[\text{MNa}]^+ 520.0732$ , found 520.0750.

**(R)-1-Triphenylmethylaziridine-2-carboxylic Acid Allyl Ester (14).**<sup>21</sup>  $R_f = 0.5$  (hexane/EtOAc 15:1);  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.35 (m, 6H, Trt), 7.29 (m, 6H, Trt), 7.23 (m, 3H, Trt), 5.95 (m, 1H,  $\text{CH}=\text{CH}_2$ ), 5.36 and 5.27 (2dq, 2H,  $^3J_{\text{trans}} = 17.2\text{ Hz}$ ,  $^2J = 1.5\text{ Hz}$ ,  $^3J_{\text{cis}} = 10.5\text{ Hz}$ ,  $^4J = 1.2\text{ Hz}$ ,  $\text{CH}=\text{CH}_2$ ), 4.69 (m, 2H,  $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 2.29 (dd, 1H,  $^3J = 2.7\text{ Hz}$ ,  $^2J = 1.6\text{ Hz}$ , H $\beta$ u), 1.94 (dd, 1H,  $^3J = 2.7, 6.2\text{ Hz}$ , H $\alpha$ ), 1.44 (dd, 1H,  $^3J = 6.2\text{ Hz}$ ,  $^2J = 1.6\text{ Hz}$ , H $\beta$ d);  $^{13}\text{C NMR}$  (125.75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.1 (CO), 143.6 (Cq arom.), 131.9 ( $\text{CH}=\text{CH}_2$ ), 129.0, 129.8, 126.9 (C arom.), 118.5 ( $\text{CH}=\text{CH}_2$ ), 74.3 (Cq aliph.), 65.5 ( $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 31.7 (C $\alpha$ ), 28.7 (C $\beta$ ).  $\text{C}_{25}\text{H}_{23}\text{NO}_2$  (ES+)  $m/z$ :  $[\text{Trt}]^+ = 243$ ,  $[\text{MH}]^+ = 370$ ,  $[\text{MNa}]^+ = 392$ .

**3-[(R)-2-tert-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]--(S)-(triphenylmethylamino)propionic Acid Allyl Ester (15).** *N*-Triphenylmethyl- $\beta$ -iodo-(S)-alanine allyl ester **13** (450 mg,  $9.1 \times 10^{-4}$  mol, 1.1 equiv) and *N*-9-fluorenylmethoxycarbonyl-(*R*)-cysteine *tert*-butyl ester (328 mg,  $8.23 \times 10^{-4}$  mol, 1 equiv) were dissolved in DMF (15 mL). Cesium carbonate (267 mg,  $8.23 \times 10^{-4}$  mol, 1 equiv) was added portionwise over 30 min at room temperature, and the reaction was complete within 4 h. EtOAc (80 mL) was then added to the reaction mixture, and the resulting organic layer was washed with distilled water (7  $\times$  30 mL) and brine (1  $\times$  30 mL) before being dried over  $\text{MgSO}_4$  and concentrated under vacuum. The residue was purified by chromatography on silica gel (hexane/EtOAc 10:1, then 4:1 to 2:1) to afford the desired compound **15** as a light white solid (569 mg,  $7.41 \times 10^{-4}$  mol, 90%):  $R_f = 0.15$  (hexane/EtOAc 10:1);  $R_f = 0.8$  (hexane/EtOAc 2:1);  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.95–7.17 (m, 15H, H arom.), 5.68 (m, 1H,  $\text{CH}=\text{CH}_2$ ), 5.62 (d, 1H,  $^3J = 7.8\text{ Hz}$ , NHFmoc), 5.18 and 5.14 (2dq, 2H,  $^3J_{\text{trans}} = 17.2\text{ Hz}$ ,  $^2J = 1.5\text{ Hz}$ ,  $^3J_{\text{cis}} = 10.5\text{ Hz}$ ,  $^4J = 1.5\text{ Hz}$ ,  $\text{CH}=\text{CH}_2$ ), 4.48 (m, 1H, H $\alpha$  B side), 4.38 (m, 2H,  $\text{CH}_2$  Fmoc), 4.23 (t, 1H,  $^3J = 7.2\text{ Hz}$ , CH Fmoc), 4.15 and 3.98 (2dd, 2H,  $^2J = 13.1\text{ Hz}$ ,  $^3J = 6, 5.9\text{ Hz}$ ,  $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 3.54 (dd, 1H,  $^3J = 7.4, 4.8, \text{H}\alpha$  A side), 2.97 and 2.84 (m, 4H,  $^2J = 13.1, 14\text{ Hz}$ ,  $^3J = 7.4, 6.2, 4.8, 4.4\text{ Hz}$ , 4H $\beta$ ), 1.6 (bs, 1H, NHTrt), 1.30 (s, 9H,  $\text{C}(\text{CH}_3)_3$ );  $^{13}\text{C NMR}$  (125.75 MHz,  $\text{CDCl}_3$ )  $\delta$  173.0, 169.5 (CO

allyl and  $^t\text{Bu}$ ), 155.7 (CO Fmoc), 145.6, 145.5, 143.7, 141.2 (Cq arom.), 131.6 ( $\text{CH}=\text{CH}_2$ ), 129.2, 129.0, 128.4, 128.2, 127.6, 127.0, 126.9, 125.7, 125.7, 120.4 (C arom. Fmoc and Trt.), 119.0 ( $\text{CH}=\text{CH}_2$ ), 83.2 (Cq. aliph.  $^t\text{Bu}$ ), 71.0 (Cq aliph. Trt), 67.1 ( $\text{CH}_2$  Fmoc), 65.6 ( $\text{CH}_2-\text{CH}=\text{CH}_2$ ), 56.1 (C $\alpha$  A side), 54.0 (C $\alpha$  B side), 47.0 (CH Fmoc), 37.8 (C $\beta$  A side), 35.2 (C $\beta$  B side), 28.9 ( $\text{CH}_3$   $^t\text{Bu}$ ).  $\text{C}_{47}\text{H}_{48}\text{N}_2\text{O}_6\text{S}$  (ES+)  $m/z$ :  $[\text{Trt}]^+ = 243$ ,  $[\text{MH}]^+ = 769$ ,  $[\text{MNa}]^+ = 791$ .

**3-[(R)-2-tert-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]--(S)-(allyloxycarbonylamino)propionic Acid Allyl Ester (4).**<sup>17</sup> An excess of TFA (0.5 mL) was added at room temperature to a solution of **15** (300 mg,  $3.9 \times 10^{-4}$  mol) dissolved in 5 mL of  $\text{CH}_2\text{Cl}_2$  (5 mL). (The rate of the reaction could be increased by the addition of TES (triethylsilane) in the mixture 5% v/v.) After 3 h, the reaction mixture was concentrated, helped by several additions of MeOH. The residue obtained was then suspended in water (4 mL) in the presence of sodium hydrogen carbonate (100 mg,  $15.6 \times 10^{-4}$  mol, 4 equiv). Dioxane (4 mL) was then added to the reaction mixture, which was cooled to  $1\text{ }^{\circ}\text{C}$  using an ethanol bath refrigerated with a cryostat probe. At this temperature, allylchloroformate (84  $\mu\text{L}$ ,  $7.8 \times 10^{-4}$  mol, 2 equiv) was added, and the reaction was stirred overnight at  $+1\text{ }^{\circ}\text{C}$ . The dioxane was then carefully removed under vacuum, and the aqueous layer obtained was diluted in further water (25 mL). The layer was extracted with EtOAc (2  $\times$  50 mL). The collected organic layers were then washed with water (5  $\times$  35 mL), dried over  $\text{MgSO}_4$  and concentrated. Purification of the resulting residue by flash chromatography on silica gel (hexane/EtOAc 4:1 to 2:1) afforded the desired compound **4** as a single isomer (183 mg,  $3.3 \times 10^{-4}$  mol, 85%):  $R_f = 0.3$  (hexane/EtOAc 2:1);  $^1\text{H NMR}$  (500.13 MHz,  $\text{CDCl}_3$ )  $\delta$  7.78–7.25 (m, 8H, H arom. Fmoc), 5.86 (m, 2H,  $\text{CH}=\text{CH}_2$  allyl and Alloc), 5.77 (d, 1H,  $^3J = 7.65\text{ Hz}$ , NHFmoc), 5.69 (d, 1H,  $^3J = 7.1\text{ Hz}$ , NHAlloc), 5.30 and 5.22, (2m, 2H,  $^3J_{\text{trans}} = 17.1\text{ Hz}$ ,  $^3J_{\text{cis}} = 10.4\text{ Hz}$ ,  $\text{CH}_2-\text{CH}=\text{CH}_2$  allyl), 5.28 and 5.18 (2m, 2H,  $^3J_{\text{trans}} = 17.1\text{ Hz}$ ,  $^3J_{\text{cis}} = 10.4\text{ Hz}$ ,  $\text{CH}_2-\text{CH}=\text{CH}_2$  Alloc), 4.61 (m, 3H,  $\text{CH}_2-\text{CH}=\text{CH}_2$  allyl + H $\alpha$  A side), 4.56 (m, 2H,  $\text{CH}_2-\text{CH}=\text{CH}_2$  Alloc), 4.47 (m, 1H, H $\alpha$  B side), 4.37 (m, 2H,  $\text{CH}_2$  Fmoc), 4.21 (t, 1H,  $^3J = 7\text{ Hz}$ ), 3.09–2.91 (m, 4H,  $\text{CH}_2$   $\beta$  A and B sides), 1.47 (s, 9H,  $\text{C}(\text{CH}_3)_3$ );  $^{13}\text{C NMR}$  (125.75 MHz,  $\text{CDCl}_3$ )  $\delta$  170.1 (CO allyl), 169.3 (CO  $^t\text{Bu}$ ), 155.7 (CO Fmoc), 155.6 (CO Alloc), 143.78, 143.78, 141.21, 141.20 (Cq. Fmoc), 132.4 ( $\text{CH}=\text{CH}_2$  Alloc), 131.2 ( $\text{CH}=\text{CH}_2$  allyl), 128.8, 127.6, 127.0, 125.1, 119.9 (C. arom. Fmoc), 119.2 ( $\text{CH}=\text{CH}_2$  allyl), 117.9 ( $\text{CH}=\text{CH}_2$  Alloc), 83.0 (Cq.  $^t\text{Bu}$ ), 67.1 ( $\text{CH}_2$  Fmoc), 66.3 ( $\text{CH}_2$  allyl), 65.9 ( $\text{CH}_2$  Alloc), 54.3 (C $\alpha$ , B side), 53.8 (C $\alpha$ , A side), 47.0 (CH Fmoc), 35.8 (C $\beta$ , B side), 35.7 (C $\beta$ , A side), 27.9 ( $\text{CH}_3$   $^t\text{Bu}$ ). HRMS (FAB)  $\text{C}_{32}\text{H}_{38}\text{N}_2\text{O}_8\text{S}$  calcd for  $[\text{MNa}]^+ 633.2265$ , found 633.2247.

**3-[(R)-2-tert-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]--(S)-(allyloxycarbonylamino)propionic Acid (16).** 3-[(*R*)-2-tert-Butoxycarbonyl-2-(fluoren-9-ylmethoxycarbonylamino)ethylsulfanyl]--(*S*)-(allyloxycarbonylamino)propionic acid allyl ester **4** (200 mg,  $3.28 \times 10^{-4}$  mol) was treated with a mixture of TFA/ $\text{CH}_2\text{Cl}_2$  (1:1, 10 mL), and the resulting mixture was stirred at room temperature for 5 h. The solvents were then removed under high vacuum. Removal of TFA was helped by several additions of toluene followed by evaporation; MeOH has to be excluded for coevaporation to help to avoid the formation of the methyl ester derivative. This gave **16** in quantitative yield. The final traces of TFA were removed by purifying the crude mixture either by reverse phase chromatography (isocratic eluant  $\text{CH}_3\text{-CN}/\text{H}_2\text{O}$  60:40) or by HPLC using a C-18 column, monitoring at 214, 254, or 301 nm. Purification was performed at a flow rate of  $15\text{ mL min}^{-1}$  using a linear gradient buffer B in A (A:B from 50:50 to 30:70 within 20 min; A = 0.05% aqueous TFA; B = 0.05% TFA in  $\text{CH}_3\text{CN}$ ). The  $\text{CH}_3\text{CN}$  was then removed under vacuum, and the aqueous layer either evaporated with moderate heating or freeze-dried. This procedure yielded **16** (72 mg, 41%) suitable for solid-phase peptide synthesis:  $R_f =$



0.2 (CH<sub>2</sub>Cl<sub>2</sub>/MeOH/ε AcOH 10:1); <sup>1</sup>H NMR (500.13 MHz, MeOD) (7.78–7.25 (m, 8H, H arom. Fmoc), 5.90 (m, 2H, CH=CH<sub>2</sub> allyl and Alloc), 5.29 and 5.17, (2m, 2H, <sup>3</sup>J<sub>trans</sub> = 17.2 Hz, <sup>3</sup>J<sub>cis</sub> = 10.5 Hz, CH<sub>2</sub>–CH=CH<sub>2</sub> allyl), 5.28 and 5.18 (2m, 2H, <sup>3</sup>J<sub>trans</sub> = 17.4 Hz, <sup>3</sup>J<sub>cis</sub> = 10.7 Hz, CH<sub>2</sub>–CH=CH<sub>2</sub> Alloc), 4.58 (m, 2H, CH<sub>2</sub>–CH=CH<sub>2</sub> allyl), 4.52 (m, 2H, CH<sub>2</sub>–CH=CH<sub>2</sub> Alloc), 4.44 (m, 1H, Hα A side), 4.33 (m, 1H, Hα B side), 4.37 and 4.28 (2dd, 2H, <sup>2</sup>J = 10.4 Hz, <sup>3</sup>J = 7.1 Hz, CH<sub>2</sub> Fmoc), 4.22 (t, 1H, <sup>3</sup>J = 7 Hz, CH Fmoc), 3.09 and 2.93 (2dd, 2H, <sup>2</sup>J = 13.6 Hz, <sup>3</sup>J = 3.6 Hz, CH<sub>2</sub> β A side), 3.03 and 2.94 (2dd, 2H, <sup>2</sup>J = 14.3 Hz, <sup>3</sup>J = 4.9 Hz, CH<sub>2</sub> β B side); <sup>13</sup>C NMR (125.75 MHz, MeOD) δ 175.4 (COOH), 172.0 (CO allyl), 158.4 (CO Fmoc), 158.3 (CO Alloc), 145.3, 142.6 (Cq. Fmoc), 134.2 (CH=CH<sub>2</sub> Alloc), 133.1 (CH=CH<sub>2</sub> allyl), 128.8, 128.2, 126.4, 120.9 (C. arom. Fmoc), 118.8 (CH=CH<sub>2</sub> allyl), 117.7 (CH=CH<sub>2</sub> Alloc), 68.2 (CH<sub>2</sub> Fmoc), 67.0 (CH<sub>2</sub> allyl), 66.7 (CH<sub>2</sub> Alloc), 56.0 (Cα, B side), 55.7 (Cα, A side), 49.0 (CH Fmoc estimated from HMQC), 36.0 (Cβ, B side); IR ν<sub>max</sub> 3446, 2590, 1668, 1446, 1110+973 cm<sup>-1</sup>; HPLC (reverse phase, flow rate of 1.5 mL min<sup>-1</sup>, λ = 214 or 254 nm, t<sub>R</sub> = 19.2 min using a linear gradient buffer B (CH<sub>3</sub>CN) in A (H<sub>2</sub>O) [90:10 to 0:100 within 20 min], t<sub>R</sub> = 5.91 min using a linear gradient buffer B (CH<sub>3</sub>CN) in A (H<sub>2</sub>O) [50:50 to 30:70 within 10 min]: HRMS (ES+) C<sub>28</sub>H<sub>30</sub>-N<sub>2</sub>O<sub>2</sub>S calcd for [MNa]<sup>+</sup> 577.16151, found 577.16182.

**Peptide Synthesis and Characterization.** Unless mentioned otherwise, all numbers of equivalents of reagents are given relative to the resin loading (mmol g<sup>-1</sup>). Peptide **17** was synthesized manually using solid-phase Fmoc strategy starting from Fmoc-Sieber resin (0.3 g, 0.048 mmol, loading 0.16 mmol g<sup>-1</sup>). Fmoc was removed by a treatment with 20% piperidine in dimethylformamide. Apart from the lanthionine unit (used in a 1.5-fold excess), a 4-fold excess of the respective commercially available Fmoc amino acids was preactivated for 3 min using HBTU (0.95 equiv relative to the amount of Fmoc amino acid) and diisopropylethylamine (DIEA, 2 equiv relative to the amount of Fmoc amino acid) in DMF. The whole mixture was then added to the growing *N*-terminus of the unprotected peptide, and each coupling was allowed to proceed for 25 min. Capping of unreacted amino groups was performed by acetylation using *N*-acetyl imidazole in DMF (0.3 M) during 20 min. Prior to the cyclization step, the fully protected, resin-bound peptide **C** (Scheme 5) was treated with tetrakis(triphenylphosphine)palladium<sup>0</sup> (2 equiv) previously dissolved in a mixture of DMF/CHCl<sub>3</sub>/NMM/AcOH (1.85/1.85/1/2, [Pd<sup>0</sup>] = 0.050 M). After 2 h protected from light, the resin was then washed several times successively with a solution of DIEA in DMF (5% v/v) followed by DMF, diethyldithiocarbamate trihydrate in DMF (0.5% w/v), and further DMF. Subsequent Fmoc removal lead to the cyclization substrate. Intramolecular cyclization was allowed to occur in the presence of DIEA (10 equiv) using a PyAOP/HOAt mixture (5 equiv) previously dissolved in DMF and added to the resin. The resin was subjected twice to this treatment to give the cyclized resin-bound peptide **D**. The resin was then washed several times with DMF, and the following Fmoc lysine activated unit was added. For the final alanine residue incorporated, Boc-Ala-OH was used, activated as described above. Cleavage of the peptide from the solid support was carried out by treating the resin several times with a solution of TFA (5%) in CH<sub>2</sub>Cl<sub>2</sub> in the presence of 2.5% of water and 2.5% of triethylsilane. The collected layers were then concentrated in vacuo to a small volume mainly consisting of an aqueous layer. (Coevaporation of TFA was facilitated by addition of MeOH.) The resulting solution was then treated with pure TFA for 30 min at room

temperature in order to cleave the side chain protecting groups. The presence of the remaining water allowed the trityl carbocation, generated by the asparagine side chain deprotection, to be trapped. TFA was removed under high vacuum, and the resulting aqueous layer was extracted twice with ether, allowing an expedient removal of the trityl subproduct.

The resulting mixture was then purified via reverse-phase HPLC using a C-18 column, monitoring at 214 nm. Purification was performed at a flow rate of 15 mL min<sup>-1</sup> using a linear gradient buffer B in A (A:B from 90:10 to 0:100 within 40 min; A = 0.05% aqueous TFA in CH<sub>3</sub>CN; B = 0.05% TFA in CH<sub>3</sub>CN). The CH<sub>3</sub>CN was then removed under vacuum, and the peptide solution was freeze-dried. The lyophilization yielded the peptide **17** as white light solid (11 mg purified, 19% isolated). HPLC (reverse phase, flow rate of 1.5 mL min<sup>-1</sup>, λ = 214 or 254 nm, t<sub>R</sub> = 6.1 min using a linear gradient buffer B (CH<sub>3</sub>CN) in A (H<sub>2</sub>O) [90:10 to 0:100 within 20 min]. C<sub>48</sub>H<sub>86</sub>N<sub>16</sub>O<sub>13</sub>S<sub>3</sub> (ES+) *m/z*: [MH]<sup>+</sup> = 1191, [MNa]<sup>+</sup> = 1213, [MK]<sup>+</sup> = 1229.

All NMR experiments were performed on a NMR spectrometer equipped with a pulse field gradient, operating at 500.13 and 125.75 MHz, respectively, for <sup>1</sup>H and <sup>13</sup>C observations. Data acquisition and processing were realized using Xwin NMR software. All experiments were performed in D<sub>2</sub>O or in a mixture of H<sub>2</sub>O/D<sub>2</sub>O on a static sample at 277 and 275 K, respectively. Temperature was kept constant using air flow. External reference signals were used for calibration of both 1D and 2D spectra. <sup>1</sup>H and <sup>13</sup>C chemical shifts were measured relative to the methyl resonance present in <sup>1</sup>H and <sup>13</sup>C spectra of 3-(trimethylsilyl)-1-propane sulfonic acid (TMS-PSA, Aldrich) recorded in D<sub>2</sub>O at 277 and 275 K respectively (<sup>1</sup>H: sr = -162.89 (277 K), sr = -177.31 (275 K). <sup>13</sup>C: sr = -338.13 (277 K)). Mixing times were set to 80 ms for total correlation spectroscopy (TOCSY) and 600 ms for nuclear Overhauser enhancement spectroscopy (NOESY). For 1D and 2D experiments in H<sub>2</sub>O/D<sub>2</sub>O, the water resonance was suppressed with a presaturation pulse. Complete assignments of <sup>1</sup>H and <sup>13</sup>C are summarized in Tables 1 and 2 of Results and Discussion.

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**Supporting Information Available:** Descriptions of general procedures and experimental for the preparation of **1**, **2**, and **8–11**; experimental procedures for the formation of **4** and **5** (1:1 from dehydroalanine **6**, and 1:9 from **7**); <sup>13</sup>C NMR spectra of all compounds described in the Experimental Section and Supporting Information; <sup>1</sup>H NMR of the mixture of **4** and **5** (85:15 and 50:50 ratios); excerpts from <sup>13</sup>C DEPT and <sup>13</sup>C NMR spectra (Cβ region, around 36 ppm) of mixtures of **4** and **5** (85:15, 50:50, 10:90 ratios); sample <sup>1</sup>H NMR spectra at various stages during the synthesis and purification of **13**; <sup>1</sup>H NMR spectra of **13**, **15**, and **16**. For peptide **17**: <sup>1</sup>H spectra in D<sub>2</sub>O (500.13 MHz, 277 K), showing Hα assignments, <sup>1</sup>H spectra in H<sub>2</sub>O:D<sub>2</sub>O 9:1 (500.13 MHz, 275 K), showing NH assignments, TOCSY spectrum (H<sub>2</sub>O:D<sub>2</sub>O 9:1, 275K), <sup>13</sup>C spectrum (D<sub>2</sub>O, 125.75 MHz, 277 K). <sup>1</sup>H NMR of diastereomeric mixture of peptides (80:20 ratio). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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