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Concise Preparation of Tetra-orthogonally Protected (2*S*,6*R*)-Lanthionines

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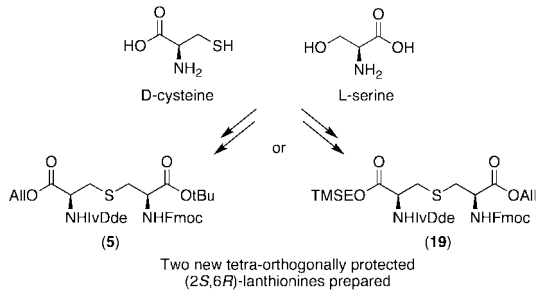

Lantibiotics are antimicrobial peptides containing the unique bis-amino acids lanthionine and β-methyllanthionine. While previous syntheses of lanthionine have often involved the coupling of precursors derived from D-serine and L-cysteine, we here report an inverted strategy whereby D-cysteine and L-serine are employed as building blocks. This approach provides for a concise preparation of tetra-orthogonally protected (2R,6S)-lanthionines while allowing convenient introduction of orthogonal protecting groups not previously incorporated into lanthionines.

The rapid emergence of drug-resistant bacteria poses a serious threat to human health and underscores the importance of developing new antibiotics. In this regard, the lantibiotic family of antimicrobial peptides is rapidly gaining recognition.[1−3] Lantibiotics are ribosomally synthesized and highly modified bacterial defense peptides with potent antibacterial activity against a wide range of infectious (and drug-resistant) bacteria.[4,5] The most thoroughly studied lantibiotic nisin (Figure 1) contains a number of unique structural features including thioether linkages and dehydrated amino acid side chains.[6−8]

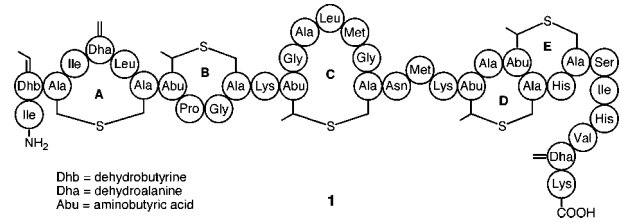

FIGURE 1. Structure of the lantibiotic peptide nisin (**1**).

The biosynthetic origin of these structural features involves the enzymatic dehydration of serine and threonine residues to dehydroalanine (Dha) and dehydrobutyrine (Dhb), respectively.[9−11] Subsequent enzyme-mediated Michael addition of cysteine thiolates to Dha and Dhb residues generates the thioether cross-links lanthionine and β-methyllanthionine respectively (these moieties provide lantibiotic peptides their family name).[12] Given the interest in lantibiotic peptides, various methods for their synthetic preparation have been pursued. Critical to such investigations is the ability to incorporate lanthionine residues containing the (2*S*,6*R*) stereochemistry found in nature (Figure 2).

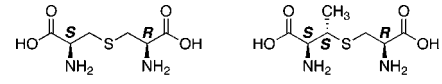

FIGURE 2. Naturally occurring (2*S*,6*R*)-lanthionine and (2*S*,3*S*,6*R*)-β-methyllanthionine.

In the only total synthesis of a lantibiotic to date, the Shiba group utilized a desulfurizing ring-contraction approach to convert cystines into lanthionines for the preparation of nisin.[13] This methodology provides lanthionines in moderate yields and is not amenable to solid-phase peptide synthesis (SPPS). As an alterative, dehydro-residues have been incorporated into peptides, after which Michael addition by a neighboring cysteine thiolate can provide lanthionine-containing peptides.[14−17] The diastereoselectivity of lanthionine bridge formation in such cases, however, has been shown to be highly dependent upon preorganization of the peptide, and the desired stereochemical outcome cannot be guaranteed in all cases. An alternate strategy toward the synthesis of lantibiotic peptides involves the

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incorporation of orthogonally protected lanthionine building blocks. Methods reported for the preparation of such building blocks include the ring openings of serine β-lactones, [18−20] aziridines,[21,22] and cyclic sulfamidates[23,24] by protected cysteines. Apart from these ring-opening approaches, β-bromo-[20,25,26] and β-iodoalanines[27−29] derived from D-serine have also been described as useful precursors in lanthionine preparation. In this regard, the orthogonally protected lanthionine **4** was recently employed by Tabor's group in the solid-phase synthesis of the nisin C-ring (Figure 3).[29]

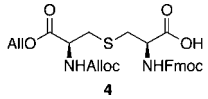
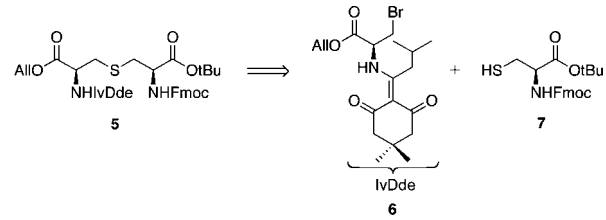


FIGURE 3. Tabor's protected lanthionine building block for use in SPPS.[29]

The development of additional methods for the preparation of orthogonally protected lanthionines continues to be of importance, and our recent progress in this area is here reported. To date, compound **4** is the only protected lanthionine building block described as suitable for use in SPPS. While the reported preparation of **4** stereoselectively provides the requisite (2*S*,6*R*) lanthionine stereochemistry, the route employed is a multistep process providing **4** in a moderate overall yield.[29] As well, given that compound **4** contains both allyl ester and allyloxy carbamate protection, its use in SPPS requires a relatively low resin loading so as to avoid the interstrand cross-linking possible after simultaneous allyl/alloc deprotection.[29] Our objective was therefore the development of a concise approach toward lanthionine building blocks compatible with SPPS while employing an improved orthogonal protection strategy in place of the allyl/alloc system. To this end the IvDde group developed by Bycroft and co-workers[30−32] was investigated as protection for one of the two α-amines present in lanthionine **5** (Scheme 1). IvDde protection is stable to conditions used for removal of *tert*-butyl and allyl esters as well as Fmoc deprotection, and no racemization is observed when it is used to protect the α-amine of amino acid building blocks used in SPPS.[30] As removal of the IvDde group is facilitated by treatment with dilute hydrazine, compound **5** was designed such that Fmoc removal would precede IvDde deprotection, thus avoiding protecting group incompatibility issues.

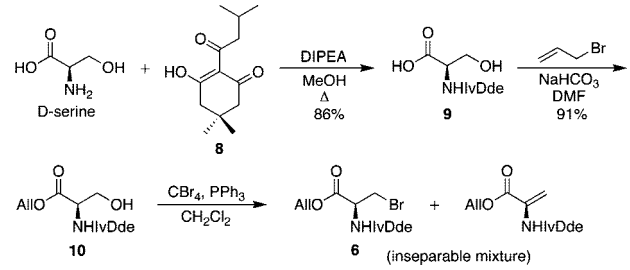
Protected lanthionine **5** was envisioned to be assembled from the previously reported cysteine **7**[29] and the D-serine derived

SCHEME 1. Proposed Route to Tetra-orthogonally Protected Lanthionines Employing IvDde Protection Strategy



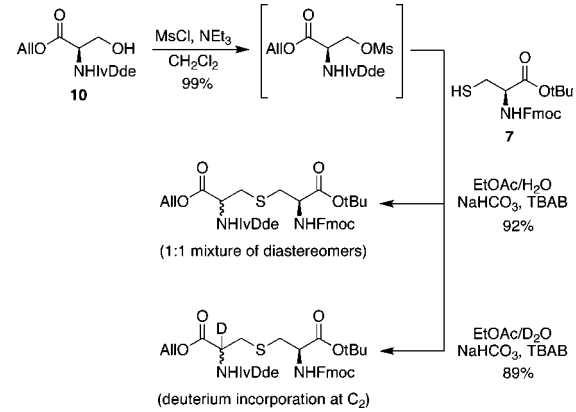
bromide **6** (Scheme 1). To this end, D-serine was *N*-IvDde protected by treatment with isovaleryl substituted dimedone **8** followed by conversion to allyl ester **10** (Scheme 2). Subsequent conversion of **10** to the bromide **6**, however, was problematic. Under all reaction conditions explored, the undesired dehydro elimination product was formed in significant quantities. Complicating matters was the observation that bromide **6** was found to further decompose to the dehydro side product upon attempted chromatographic purification.

SCHEME 2. Attempted Preparation of Bromide 6



Given that protected cysteine **7** would be expected to react nonspecifically with the dehydro compound (yielding a diastereomeric mixture of products), bromide **6** was not pursued further. Somewhat surprisingly, treatment of **10** with MsCl/NEt_3 led to near quantitative formation of the mesylate with no detectable formation of the dehydro elimination product (Scheme 3). Subsequent treatment of the mesylate with protected L-cysteine **7** under the mildly basic phase transfer conditions previously described by Zhu and Schmidt[25] yielded the lanthionine product in high yield. Close inspection of the ^{13}C NMR spectrum, however, revealed a number of duplicate peaks suggesting that the product was either formed as an inseparable mixture of diastereomers or exists in a rotameric equilibrium. To distinguish between these two possibilities the coupling of

SCHEME 3. Preparation of Lanthionine 5 (Diastereomeric Mixture) via Mesylate Approach



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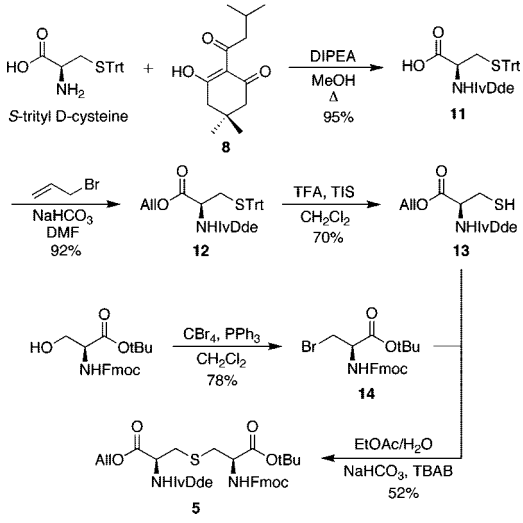
the mesylate with **7** was repeated using D_2O in place of H_2O. This investigation revealed complete loss of the C_2 α-proton indicating that formation of the lanthionine proceeds via an elimination/addition mechanism to give the product as the undesired diastereomeric mixture.

Reasoning that the *N*-IvDde protecting group present in compound **10** was responsible for the instability of the corresponding bromide and mesylate, an alternate approach for the incorporation of IvDde protection into lanthionine target **5** was considered. This approach involved an inverted strategy whereby IvDde and allyl protection could be introduced via a D-cysteine derived building block. *S*-Trityl D-cysteine was thus *N*-IvDde protected followed by conversion to allyl ester **12** (Scheme 4). Treatment with TFA/TIS provided thiol **13**, which despite difficulty in handling (decomposing to give unidentified species other than the disulfide) could be used directly in the subsequent step. Employing the same phase transfer conditions described above, thiol **13** was then reacted with known bromide **14**[33] to yield lanthionine **5**.

SCHEME 4. Stereoselective Preparation of Tetra-orthogonally Protected Lanthionine 5



Despite the modest yield of **5** obtained, we were encouraged by the optical purity of the product. Examination of the ^{13}C NMR spectrum obtained for **5** indicated formation of a single diastereomer (Figure 4).

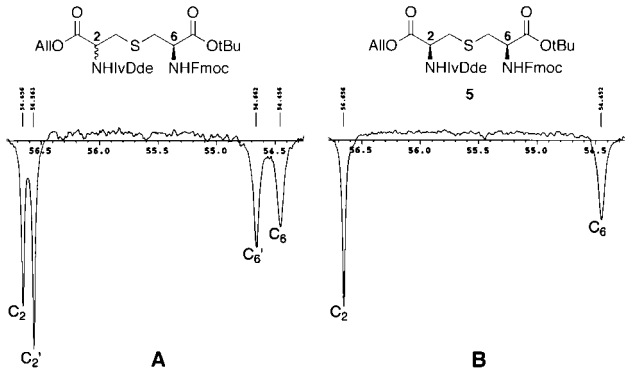
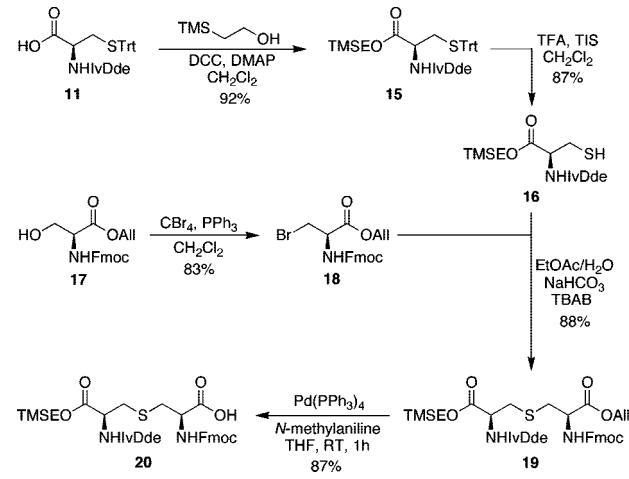


FIGURE 4. Expansions of the C_α region in the ^{13}C APT spectra acquired for (A) the diastereomeric mixture of lanthionine **5** obtained via mesylate (Scheme 3) and (B) diastereomerically pure lanthionine **5** obtained via inverted strategy (Scheme 4).

Furthermore, during the course of the reaction in forming lanthionine **5**, periodic TLC analysis showed complete disappearance of thiol **13** while a significant quantity of bromide **14** remained unreacted. This observation suggested that the low yield of **5** was due to a competing degradation of thiol **13** and prompted us to explore an alternate protecting group strategy for the D-cysteine derived precursor. In place of allyl protection, *N*-IvDde protected *S*-trityl D-cysteine **11** was converted to trimethylsilylethyl ester **15** (Scheme 5). Trityl group removal proceeded cleanly (20 equiv of TFA, 2 equiv of TIS) to yield thiol **16**, which was found to be much more stable than **13**. The use of thiol **16** for the synthesis of an orthogonally protected lanthionine in turn required the preparation of a new, alternately protected L-serine derived β-bromoalanine. Fmoc-L-Serine allyl ester **17** was thus transformed into bromide **18** in good yield. Coupling of thiol **16** and bromide **18** was performed as before providing lanthionine **19** in a yield much improved over that obtained for lanthionine **5**. The optical purity of **19** was also verified by ^{13}C NMR showing selective formation of a single diastereomer. Repeated synthesis of **19** following this approach proved to be readily scalable, allowing for the preparation of multigram quantities of lanthionine **19** in similar overall yield. Compound **19** was readily deprotected using $Pd(PPh_3)_4$ and *N*-methylaniline in THF[34] to provide lanthionine building block **20** in a form suitable for SPPS.

SCHEME 5. Stereoselective Preparation of Tetra-orthogonally Protected lanthionine 19



In conclusion, we have developed a concise route for the preparation of new tetra-orthogonally protected (2*S*,6*R*)-lanthionines. Different from most previously reported lanthionine syntheses, we here describe an "inverse strategy" utilizing D-cysteine and L-serine derived building blocks. This approach provides for a more concise route toward protected lanthionines while allowing for the incorporation of orthogonal and SPPS-compatible protecting groups (TSME, IvDde) not previously utilized in the preparation of such compounds. Access to these new orthogonally protected lanthionines serves to expand the repertoire of available tools for future explorations into the lantibiotic family of antimicrobial peptides. In this regard, we are currently investigating the use of compounds **5** and **19** in the construction of lanthionine containing peptides. Our progress toward this end will be reported in due course.

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Experimental Section

Details pertaining to the preparation of the diastereomerically pure, orthogonally protected lanthionine **19** are provided below as a representative procedure.

(*R*)-Allyl 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-((*S*)-2-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butylamino)-3-oxo-3-(2-(trimethylsilyl)-ethoxy)-propylthio)propanoate (19). Protected D-cysteine **15** (5.02 g, 7.5 mmol) was dissolved in CH_2Cl_2 (100 mL), cooled on ice, and treated with triisopropylsilane (3.07 mL, 15.0 mmol, 2 equiv) followed by addition of TFA (11.14 mL, 150 mmol, 20 equiv) and warming to room temperature. After 1 h, TLC analysis (4:1 hexanes/EtOAc) indicated complete consumption of starting material and formation of a more polar product (R_f 0.50). The mixture was diluted with water (100 mL) followed by slow addition of solid $NaHCO_3$ (12.6 g, 150 mmol, 20.0 equiv) to neutralize the excess TFA. The CH_2Cl_2 layer was separated, dried over Na_2SO_4, filtered, and evaporated. The residue was applied to a silica column eluting with a gradient of 8:1 → 4:1 hexane/EtOAc. Product-containing fractions were pooled and evaporated to provide thiol **16** as a colorless oil (2.80 g, 87%), which was used immediately in the next step so as to avoid possible disulfide formation. The intermediate thiol **16** (2.80 g, 6.5 mmol) and bromide **18** (3.10 g, 7.2 mmol, 1.1 equiv) were dissolved in EtOAc (75 mL) and treated with 75 mL of a saturated $NaHCO_3$ solution containing tetrabutylammonium bromide (8.40 g, 26.0 mmol, 4.0 equiv). The mixture was stirred vigorously under N_2 (g) for 14 h after which TLC analysis (4:1 hexane/EtOAc) indicated complete consumption of thiol **16** and formation of a more polar product (R_f 0.30). The mixture was diluted with EtOAc (75 mL) and water (75 mL), and the layers were separated. The EtOAc layer was dried over Na_2SO_4, filtered, and evaporated. The residue was applied to a silica column and eluted with a gradient of 3:1 → 2:1 hexane/EtOAc. Product containing fractions were pooled and evaporated to provide compound **19** as a colorless oil that solidified upon storage at 4 °C (4.42 g, 88%): mp 73−75 °C; R_f 0.30 (4:1 hexanes/EtOAc); ^{1}H (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 7.4 Hz), 7.59 (d, 2H, J = 7.2 Hz), 7.39 (t, 2H, J = 7.2 Hz), 7.30 (t, 2H, J = 7.4 Hz), 6.0−5.82 (m, 1H), 5.78 (br d, 1H, J = 8.0 Hz); 5.40−5.20 (m, 2H), 4.71−4.54 (m, 4H), 4.45−4.33 (m, 2H), 4.33−4.19 (m, 3H), 3.18−2.83 (m, 6H), 2.43 (s, 2H), 2.33 (s, 2H), 1.93 (septet, 1H, J = 6.6), 1.05−0.93 (m, 14H), 0.02 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.5, 196.5, 176.2, 170.2, 169.1, 156.0, 144.0, 143.9, 141.5, 131.5, 128.0, 127.3, 125.4, 120.2, 119.6, 108.0, 67.6, 66.8, 65.4, 56.8, 54.2, 54.1, 52.6, 47.3, 37.7, 36.0, 35.7, 30.1, 29.5, 28.5, 28.4, 22.7, 17.6, −1.3; $[\alpha]_D$ = +14.4° (*c* 1.2, $CHCl_3$); HRMS (MALDI) calcd for $C_{42}H_{56}N_2O_8SSi$ $[M + H]^+$ 777.3605, found 777.3610.

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Supporting Information Available: Experimental procedures for preparation of new compounds including spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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