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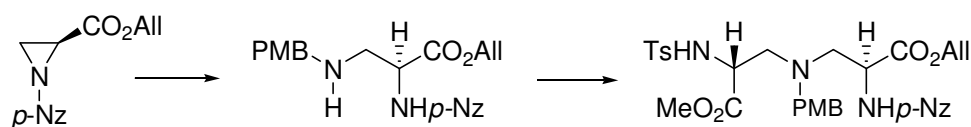
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Graphical abstract :

Synthesis of orthogonally protected azalanthionines (lanazanines) by sequential ring-opening of *N*-substituted aziridine 2-carboxylates

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Abstract:

Orthogonally protected azalanthionines (lanazanines, 4-azadiaminopimelic acids or β-aminoalaninoalanines) have been synthesised in good yields by the ring-opening of *N*-protected aziridine 2-carboxylates with suitably protected diaminopropanoic acids (DAPs). The required DAPs were also synthesised by ring-opening of *N*-protected aziridine 2-carboxylates with *para*-methoxybenzylamine.

Keywords:

Azalanthionine; Lanthionine; Aziridine; Ring-opening

Many peptides contain a disulfide bridge, formed by the oxidative linking of two cysteine residues, but in lanthionine-containing peptides (lanthipeptides)[1] this is a bridge with a single sulfur atom (thioether). An azalanthionine (lanazanine) is thus the *N*-linked analogue of this more common lanthionine moiety. There have been very few reports of the synthesis, or use, of azalanthionines (Figure 1). Pleixats described the isolation of an azalanthionine, as a by-product, from the $FeCl_3$-catalysed addition of an amine to a didehydroalanine (Dha),[2] while Paulus has reported the observation of azalanthionines, as intermediates in the formation of imidazolines, from the non-selective addition of ammonia to a Dha moiety.[3] A stereoselective synthesis of another protected azalanthionine analogue, though not orthogonally protected, was reported by Kim via the ring-opening of cyclic sulfamidates with benzylamines, en route to the synthesis of chiral peraza-macrocycles.[4] The lantibiotics (*lan*thionine-containing *antibiotics*) are a subset of antimicrobial peptides which are active against a wide range of bacterial species, and contain a number of unusual amino acid residues in their structures.[5] These residues are formed by post-translational modifications and include lanthionine, β-methyllanthionine, Dha and

didehydrobutyrine (Dhb), amongst others. Vederas has recently shown that it is possible to replace the thioether linker in lanthionines with either a hydrocarbon or ether linker (oxalanthionine, Figure 1), while maintaining biological activity, when they are incorporated into peptides.[6] The goal of our studies was to extend the range of possible thioether replacements in lanthionines by preparing analogues containing an amine linker. The efficient incorporation of an amine-linked analogue into peptides would first require the preparation of a suitably orthogonally protected azalanthionine (lanazanine, 4-azadiaminopimelic acid or β-aminoalaninoalanine) for subsequent use in solid-phase peptide synthesis. Tabor has reported a multi-step synthetic route for orthogonally protected lysinoalanines, which are chain-extended analogues of the azalanthionines.[7]

It was envisaged that the preparation of the required orthogonally protected azalanthionines would be possible from the ring-opening of suitably protected aziridine 2-carboxylates with protected DAPs (Scheme 1). The required protected aziridine 2-carboxylates were prepared from serine derivatives by literature methods. The *N*-trityl precursor **1** was prepared in two steps from L-serine methyl ester, following the method of Zwanenburg.[8] Following removal of the trityl group, the aziridine nitrogen was protected separately with the tosyl or *p*-nosyl group (*para*-nitrobenzenesulfonyl) to give the aziridines **2** and **3**. Synthesis of the desired azalanthionines would therefore require the ring-opening of these aziridine 2-carboxylates with a suitably protected DAP. This involved thermal ring-opening of the previously prepared aziridine 2-carboxylates with *p*-methoxybenzylamine in acetonitrile at 80 °C for 24 h, following the method of Kim.[9] To avoid double addition two molar equivalents of the benzylamine was used. Using aziridine **2**, attack at the less hindered β-position of the aziridine gave an isolated yield of only 32% of the desired DAP **4**, along with 41% of the regioisomeric DAP **5**, formed by attack of the benzylamine at the α-position of the aziridine **2**. When the same reaction was undertaken at room temperature, for 24 h, there was a reversal in the selectivity with the desired DAP **4** being obtained in a 70% isolated yield, with only a 23% yield of the regioisomer **5**. The regioselectivity at elevated temperatures was not unexpected, but the fact that there was still a significant amount of reaction at the more hindered α-position, even at room temperature, was somewhat of a surprise. Literature reports

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of the reaction of activated aziridines at room temperature usually show that nucleophilic attack occurs at the less hindered β-position, unless the α-position is allylic or benzylic.[10]

With the required DAP in hand the first synthesis of an azalanthionine was achieved by reaction of **4** with the *N*-nosyl aziridine **3**. When the reaction was performed at room temperature, no product formed due to the extra steric hindrance of the secondary *p*-methoxybenzyl substituted amine of the DAP **4**. However, heating to 80 °C gave the azalanthionine **6** in a 51% yield, as the sole product, with no evidence of attack at the α-position being observed.

Previously, we showed that protected DAPs could also be prepared from serine derivatives by the Mitsunobu reaction with sulfonamides, but these syntheses were somewhat difficult to reproduce with large variations in the isolated yields being obtained.[11] However, the fully protected DAP **8** was prepared from serine derivative **7** using this method, in a 63% yield (Scheme 2). Removal of the *p*-Ns group of **8** was achieved in an 80% yield, using thiophenol, giving the secondary PMB-protected DAP **9**. It was noted that attempts to prepare **9** directly by ring-opening of *N*-alloc aziridine 2-allyl ester with *p*-methoxybenzylamine were unsuccessful. Surprisingly, no thermal reaction of **9** with aziridine **2** was observed, but when BF$_3$.OEt$_2$ was used as a Lewis acid catalyst, in CH$_2$Cl$_2$ at reflux temperature, the azalanthionine **10** was obtained in a poor yield of 30%, even after 24 hours.

Neither of the prepared azalanthionines (**6** and **10**) were orthogonally protected which would be advantageous for their use in peptide synthesis. Previously, Vederas successfully used the *para*-nitrobenzyloxycarbonyl (*p*-Nz) protecting group in the synthesis of orthogonally protected oxalanthionine analogues.[6] As the route to the required DAPs, using ring-opening of aziridine 2-carboxylates with PMB-NH$_2$, was more efficient than the Mitsunobu reaction route, the orthogonally protected DAP **13** (Scheme 3), containing the *p*-Nz group, was prepared from the *p*-Nz protected aziridine **12**.[12] In this case when the reaction was performed at room temperature the product from attack at the β-position was the sole product obtained, in a 66% yield. There was no evidence for the product from attack at the α-position, which, in this

case, is in agreement with the literature reports of similar types of reactions.[10] However, the outcome was quite different to that found for the synthesis of the Dap **4** (using *N*-tosyl aziridine methyl ester **2**, Scheme 1), where attack at both the α- and β-position was observed at room temperature. Currently, we are expanding the study of these reactions with different combinations of protecting groups on the aziridine 2-carboxylates, to see how the choice of the activating protecting groups influences both the regioselectivity and the overall success, or otherwise, of the ring-opening reactions. The results of these studies will be reported in due course.

With the DAP **13** in hand, subsequent reaction with aziridine **2**, in acetonitrile at reflux temperature for 24 h, gave the fully orthogonally protected azalanthionine **14** in a 38% yield. The azalanthionine **15**, which contained the more peptide-synthesis-friendly *p*-Ns protecting group in place of the *N*-tosyl group, was prepared in a similar manner using aziridine **3**, but in a diminished yield of 32%. Since both partners used for the azalanthionine synthesis were derived from L-serine, the stereochemistry of the two stereocentres of the product was not analogous to that found in natural lanthionines. Therefore, the synthesis was repeated with the enantiomer of aziridine **3**, which was prepared from D-serine. This gave the fully orthogonally protected diastereoisomeric azalanthionine **16** with the correct stereochemistry, in a yield of 38%. Finally, it was found that the azalanthionines prepared in this study were prone to decomposition during their purification and characterisation, when stored at ambient temperature, both as pure samples and in solutions of organic solvents, particularly solvents used for column chromatography and NMR analysis. For example, azalanthionine **6** was the most stable azalanthionine synthesised but was still fully decomposed after two weeks when stored at ambient temperature, under a nitrogen atmosphere, with multiple products being observed by chromatographic and NMR analyses. It seems that the same very strongly electron-withdrawing aziridine *N*-substituents required in order to activate the aziridines sufficiently for nucleophilic attack are also the substituents responsible for the observed decomposition. However, when compound **6** was stored at -20 °C it showed an increase in stability with the shelf-life increasing to greater than one month, instead of less than two weeks, as seen by HPLC and NMR analyses. It is likely that when the azalanthionines are

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incorporated into peptide structures, in the future, they will be more stable due to the absence of these highly electron-withdrawing substituents.

In conclusion, we have demonstrated the first synthesis of orthogonally protected azalanthionines, amine-linked analogues of the biologically important lanthionines. Currently, this chemistry is being extended to the synthesis of the corresponding orthogonally protected β-methylazalanthionines as analogues of the β-methyllanthionines found in the ring structures of many important lantibiotics.[5] The results of these studies will be reported in due course.

*Typical procedure for azalanthionine synthesis, exemplified by the synthesis of **6***. *N*-p-Nosyl aziridine methyl ester **3** (0.07 g, 0.25 mmol) was dissolved in MeCN (5 ml) before adding DAP **4** (0.2 g, 0.5 mmol, 2 eq.) after which the solution was stirred at reflux temperature for 24 h, before removing the solvent *in vacuo*. The resulting oil was dissolved in EtOAc (20 ml), washed with brine (2 x 20 ml) and dried over anhydrous MgSO$_4$, before removing the solvent *in vacuo*. The crude residue was purified by preparative TLC on silica gel (petroleum ether:EtOAc 4:1) to yield **6** as a pale yellow oil (0.09 g, 51%). R_f: 0.37 petroleum ether: EtOAc (1:1). $[\alpha]_D^{20}$ = +32.96 (*c* = 1 in CHCl$_3$). **^{1}H NMR** (CDCl$_3$) δ ppm; 8.34 (d, 2H, *J* = 8.7 Hz), 8.06 (d, 2H, *J* = 8.7 Hz), 7.73 (d, 2H, *J* = 8.4 Hz), 7.28 (d, 2H, *J* = 8.3 Hz), 7.13 (d, 2H, *J* = 8.5 Hz), 6.82 (d, 2H, *J* = 8.5 Hz), 4.14 (s, 1H), 4.06 (s, 1H), 3.80 (s, 3H), 3.80 (d, 1H, *J* = 12.9), 3.50 (d, 1H, *J* = 12.9 Hz), 3.46 (s, 3H), 3.41 (s, 3H), 3.02 (m, 3H), 2.76 (m, 1H), 2.42 (s, 3H). **^{13}C NMR** (CDCl$_3$) δ ppm; 171.1, 170.8, 159.1, 149.9, 146.1, 143.8, 136.6, 130.8, 130.6, 129.6, 128.4, 127.2, 124.1, 113.8, 58.4, 56.7, 56.2, 55.2, 55.1, 52.68, 52.63, 21.6. **IR** (thin film, NaCl) cm^{-1}, 3356, 3089, 2982, 1744, 1732, 1532, 1379, 1173, 1133. **HRMS:** ES+ for C$_{29}$H$_{34}$N$_4$O$_{11}$S$_2$, expected [M+H] 679.1738, observed [M+H] 679.1748.

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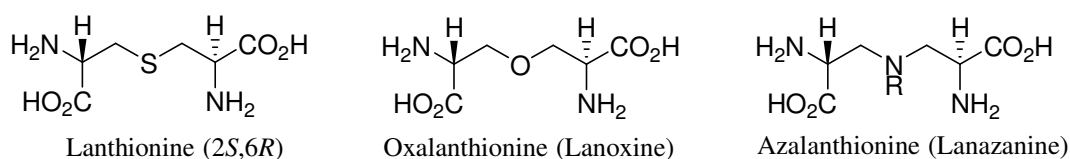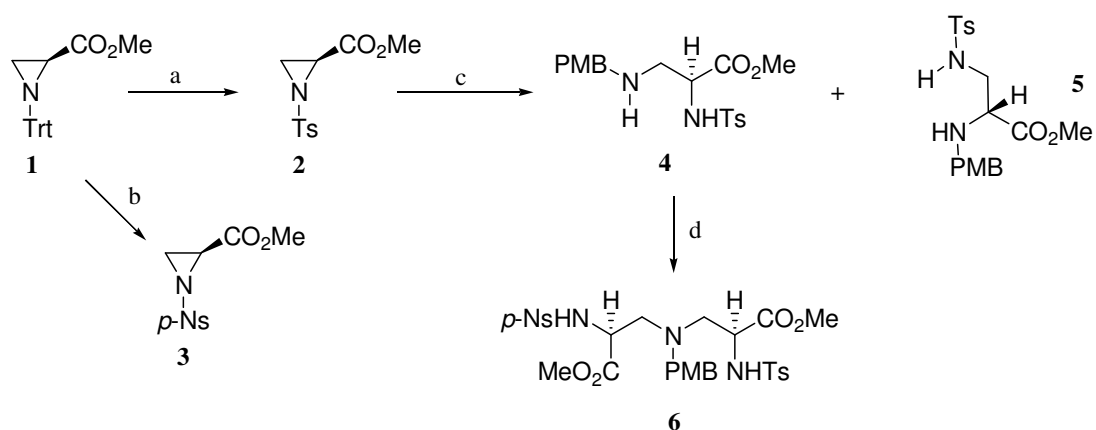
**Figure 1**. Structures of Lanthionine, Oxalanthionine and Azalanthionine

Scheme 1: Reagents and conditions; (a) i) 50% TFA in CH_2Cl_2/MeOH (1:1), rt, 30 min., ii) $NaHCO_3$, H_2O, rt, iii) *p*-toluenesulfonyl chloride, EtOAc, rt, 24 h, 92% from **1**; (b) i) 50% TFA in CH_2Cl_2/MeOH (1:1), rt, 30 min., ii) $NaHCO_3$, H_2O, rt, iii) *p*-nitrobenzenesulfonyl chloride, EtOAc, rt, 24 h, 85% from **1**; (c) *p*-methoxybenzylamine, MeCN, rt, 24 h (70% **4** and 23% **5**); (d) **3**, MeCN, 80 °C, 24 h, 51%.

Scheme 1
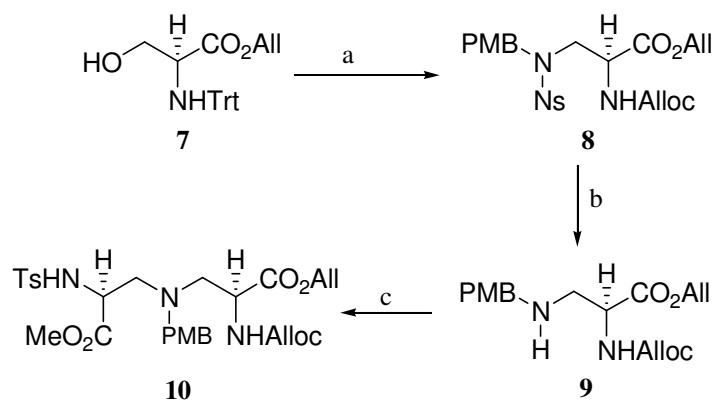
Scheme 2: Reagents and conditions; (a) Ref 11; (b) thiophenol, K_2CO_3, DMF, rt, 16 h, 80%; (c) **2**, $BF_3 \cdot OEt_2$, CH_2Cl_2, reflux temperature, 24 h, 30%.

Scheme 2
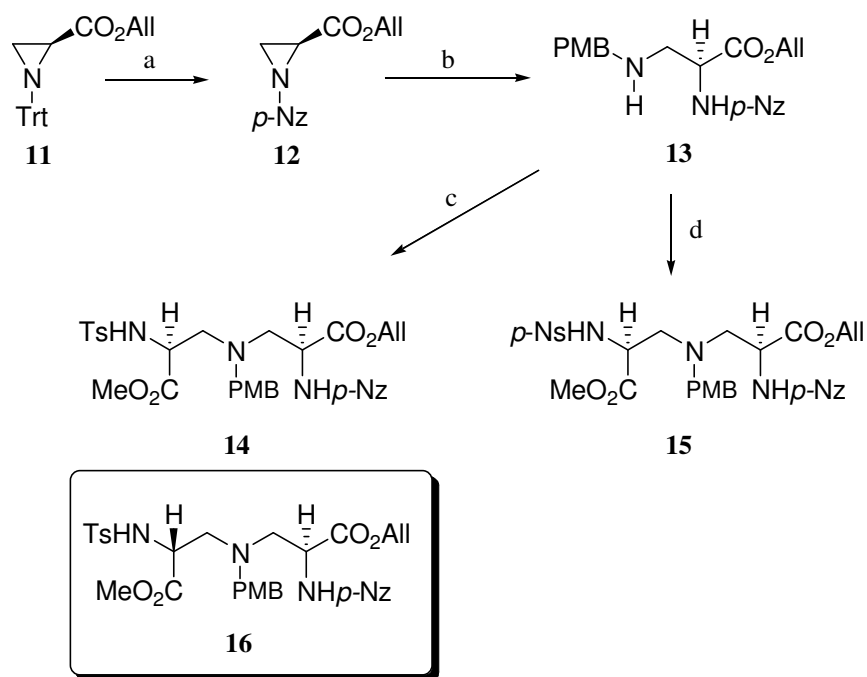
Scheme 3: Reagents and conditions; (a) i) 50% TFA in CH_2Cl_2/MeOH (1:1), rt, 30 min., ii) $NaHCO_3$, H_2O, rt, iii) *p*-nitrobenzylchloroformate, EtOAc, rt, 24 h, 82% from **11**; (b) *p*-methoxybenzylamine, MeCN, rt, 24 h, 66%; (c) **2**, MeCN, 80 °C, 24 h, 38%; (d) **3**, MeCN, 80 °C, 24 h, 32%.

Scheme 3