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Cystine mimetics—solid phase lanthionine synthesis

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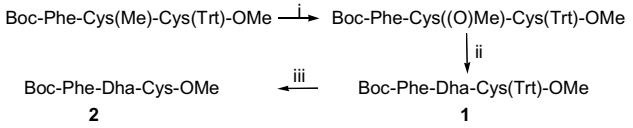
Abstract—The total solid phase synthesis of an analogue of the B ring of nisin was achieved, in a biomimetic fashion, via the solid phase diastereoselective cyclisation of a dehydrothiol-containing peptide.

Lanthionines are a group of unnatural amino acids found in a range of natural products, most notably in the lantibiotics,[1] a class of polycyclic peptide antibacterials possessing a broad range of biological activities. Lanthionine is also a motif, which has been widely adopted as a peptidomimetic of cystine.[2]

Several approaches have been explored to access lanthionines in solution phase methods[3–7] and have included a total synthesis of nisin,[8] while several biomimetic solution phase syntheses via the Michael addition of a cysteine residue onto a dehydroalanine-containing peptide have been reported.[6]

On the solid phase, there are few examples of cyclic lanthionine synthesis.[2d,9] One of the most recent advances in this field focused on the preparation of an analogue of the B ring of nisin but, for synthetic reasons, the precursor dehydrothiol was assembled via a segment condensation approach.[6e]

Recently, a mild and highly selective oxidation procedure was described by Matteucci[10] that enabled the synthesis of *S*-methylcysteine sulfoxides in the presence of a variety of acid labile protecting groups, including *S*-trityl protected cysteine residues. This method was exploited here to prepare the dehydrothiol tripeptide **2**[11] (Scheme 1) by selective oxidation, base mediated elimination and acidic removal of the cysteine protecting group, all in reasonable yield. The ability to use excess oxidant and a solvent system rich in dichloromethane, rendered this approach highly suitable for solid phase applications.



Scheme 1. Reagents and conditions: (i) H_2O_2 (60% solution in H_2O, 5 equiv)/$Sc(OTf)_3$ (0.2 equiv) in CH_2Cl_2/10% EtOH, 15 min, 96%; (ii) DBU (2 equiv) in CH_3OH, 1 h, 44%; (iii) 1% TFA, TIS (3 equiv) in CH_2Cl_2, 55%. Trt = triphenylmethyl; $Sc(OTf)_3$ = scandium trifluoromethane sulfonate; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; Dha = dehydroalanine; TFA = trifluoroacetic acid; TIS = triisopropylsilane.

The high chemoselectivity of this method enabled the generation of a resin bound dehydrothiol peptide using methyl cysteine as a 'masked' dehydroalanine moiety. This peptide, following further synthetic elaborations, was cyclised to give the desired lanthionyl derivative both in solution and on the solid phase.

To achieve these goals, peptide **3** was assembled on PS resin equipped with a Rink linker. This was exposed to the oxidizing conditions (H_2O_2/cat. $Sc(OTf)_3$) and treated with 5% DBU for 5 h (Scheme 2) to give, following acidic cleavage, peptide **5** in good purity (76% by RP-HPLC, UV detecton) proving the efficiency of the oxidation/elimination sequence and achieving, for the first time, the solid phase synthesis of a dehydrothiol without the need to prepare fragments in solution.[12]

Keywords: Solid phase synthesis; Lanthionine; Dehydro peptides; Lantibiotics.

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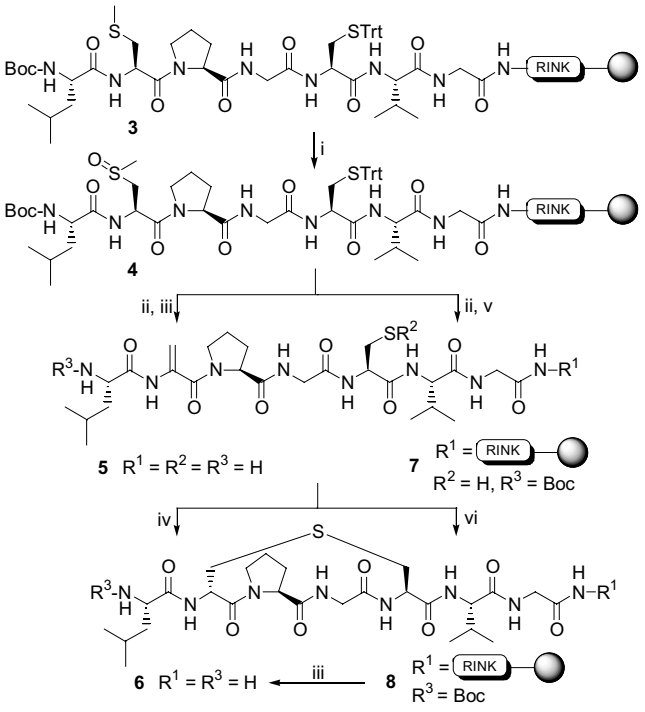

Scheme 2. Reagents and conditions: (i) H_2O_2 (60% solution in H_2O, 5 equiv)/$Sc(OTf)_3$ (0.2 equiv) in CH_2Cl_2/10% EtOH, 20 min; (ii) 5% DBU in CH_2Cl_2/CH_3OH (1/1), 5 h; (iii) TFA/TIS/CH_2Cl_2 (95/2/3), 2 h; (iv) H_2O (pH = 8.3), 20 min, 65%; (v) TFA/TIS/CH_2Cl_2 (2/2/96), 2 h; (vi) 10% DBU in DMF, 15 h.

Before attempting the synthesis of cyclic lanthionine **6** on the solid phase, cyclisation was first carried out in solution. Dehydrothiol peptide **5** was dissolved in water (pH = 8.3) and cyclisation was observed to occur rapidly and cleanly (monitored by RP-HPLC, C_8 column) to give the expected *meso*-lanthionine **6** in 65% yield (after RP-HPLC purification)[12] as a single diastereoisomer, as observed previously.[2a,6b,e–g,9b]

Cyclisation was then attempted on the solid phase (Scheme 2). Thiol **7** was generated by treating the *S*-trityl protected precursor resin with a mildly acidic cocktail (TFA/ TIS/CH_2Cl_2, 2/2/96). Several bases (NMM, DIPEA, TEA) were used in order to promote cyclisation. Surprisingly, very limited conversion to the expected lanthionine were observed under these conditions. This was believed to be due to residual acid remaining in the resin and therefore the resins were thoroughly washed with various solvents and solutions of the bases in DMF prior to the basic treatment. However, even this did not improve conversions. When DBU was used as the base, the reaction was complete within 15 h, showing for the first time that elimination and cyclisation could be achieved on the solid phase.

Following acidic cleavage, the lanthionyl derivative was purified by semi-preparative RP-HPLC and obtained as a single diastereoisomer in 33% isolated overall yield (from the starting aminomethyl resin). Data obtained for the solution and solid phase cyclisation products were identical (Fig. 1).[13]

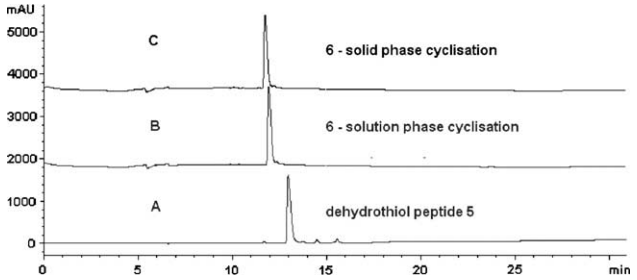

Figure 1. RP-HPLC traces (C_8 column, UV detector, $\lambda = 220$ nm) of (A) crude dehydrothiol peptide **5**; (B) solution phase cyclisation; (C) solid phase cyclisation.

The methods described above enable the efficient synthesis of peptides containing free thiols and dehydroamino acids using the two orthogonally protected, commercially available cysteine residues (*S*-Me and *S*-Trt), which were easily incorporated using Fmoc chemistry. The dehydrothiol peptide **5** could be cleaved into solution for analysis and cyclisation or cyclised directly on the solid phase to yield, stereoselectively, the cyclic lanthionyl derivative **6**, an analogue of the B ring of nisin. This approach proves a general method for lanthionyl synthesis (a stable mimetic of cystine) and is amenable to peptides containing multiple *S*-Me and *S*-Trt cysteine residues.

Acknowledgements

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11. Data for **2**: R_f: 0.23 (Et_2O/hexanes, 7/3); IR (neat): ν_{max}: 2570 (SH), 1736, 1686, 1656, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.39 (br s, 1H, Dha-N*H*), 7.33–7.10 (m, 5H, Phe-Ar*H*), 7.00 (m, 1H, Cys-N*H*), 6.51 (s, 1H, Dha-H_β), 5.41 (s, 1H, Dha-H_β), 5.06–4.89 (br s, 1H, Phe-N*H*), 4.85 (m, 1H, Cys-H_α), 4.56–4.33 (br s, 1H, Phe-H_α), 3.81 (s, 3H, CO_2CH_3), 3.22–2.89 (m, 4H, Phe-H_β + Cys-H_β), 1.38 (9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.8 (CO_2Me), 170.6 (Phe-*C*ONH), 163.7 (Dha-*C*ONH), 155.5 (O*C*ONH), 136.36 + 136.33 (Phe-*ipso*-Ar*C*), 133.5 (Dha-C_α), 129.3 + 128.9 (Phe-*o* + *m*-Ar*C*H), 103.4 (Dha-C_β), 80.6 (*C* $(CH_3)_3$), 56.7 (Phe-C_α), 54.2 (Cys-C_α), 53.2 (OCH_3), 38.4 (Phe-C_β), 28.3 ($C(CH_3)_3$), 27.01 + 27.00 (Cys-C_β); m/z (ES^+): 474.2 $(M+Na)^+$, 925.5 $(2M+Na)^+$; HRMS (ES^+): calcd for $C_{21}H_{29}N_3O_6SNa$ $(M+Na)^+$, 474.1669, found, 474.1671; RP-HPLC: 14.7 min (ELSD).
12. Data for **5** after cleavage from resin and semi-preparative RP-HPLC purification (16%); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.96 (d, 1H, $J = 9$ Hz, Val-NH), 7.83 (d, 1H, $J = 8$ Hz, Cys-NH), 5.43 (s, 1H, Dha-H_β), 5.11 (s, 1H, Dha-H_β), 4.47 (m, 1H, Cys-H_α), 4.30 (m, 1H, Leu-H_α), 4.10 (m, 1H, Val-H_α), 3.95–3.40 (m, 7H, Pro-H_α + 2Pro-H_δ + 4Gly-H_α), 2.83–2.63 (m, 2H, Cys-H_β), 2.27 (t, 1H, $J = 9$ Hz, Cys-SH), 2.20–2.08 (m, 1H, Leu-H_β), 2.07–1.73 (m, 4H, Leu-H_β + Val-H_β + Pro-H_γ), 1.64–1.45 (m, 2H, Pro-H_β), 0.94–0.80 (m, 13H, Leu-CH_3 + Val-CH_3 + Leu-H_γ); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.8, 170.9, 170.6, 169.9, 168.9, 168.2, 165.0 (6CONH + $CONH_2$), 137.2 (Dha-C_α), 104.5 (Dha-C_β), 59.9 (Leu-C_α), 58.2 (Val-C_α), 54.9 (Cys-C_α), 51.0 (Pro-C_α), 49.5 (Pro-C_δ), 42.1 (Gly-C_α), 41.7 (Gly-C_α), 40.1 (Pro-C_β), 30.0 (Val-C_β), 29.4 (Leu-C_β), 26.1 (Cys-C_β), 24.6 (Pro-C_γ), 23.5 + 22.6 + 21.8 + 19.2 + 18.1 (Leu-CH_3 + Val-CH_3 + Leu-C_γ); m/z (ES^+): 613.7 $(M+H)^+$; HRMS (ES^+): calcd for $C_{26}H_{44}N_8O_7S_1Na$ $(M+Na)^+$, 635.2946, found, 635.2944; RP-HPLC (1220): 13.1 mins.
13. Data for **6** after solid-phase cyclisation, cleavage and purification by semi-preparative RP-HPLC purification (33%); ^1H NMR (DMSO-d_6, 400 MHz): δ 8.99 (d, 1H, $J = 8$ Hz), 8.75 (t, 1H, $J = 6$ Hz), 8.18–8.02 (m, 3H), 7.94 (d, 1H, $J = 8$ Hz), 7.59 (d, 1H, $J = 9$ Hz), 7.19 + 6.97 (2s, 2H), 4.92 (m, 1H), 4.69–4.53 (m, 2H), 4.10 (m, 1H), 3.85–3.55 (m, 5H), 3.54–3.43 (m, 1H), 3.34 (m, 1H), 2.97 (dd, 1H, $J = 15$, 7 Hz), 2.86 (dd, 1H, $J = 14$, 4 Hz), 2.69–2.54 (m, 2H), 2.27–2.12 (m, 1H), 2.00 (m, 2H), 1.85–1.70 (m, 2H), 1.71–1.43 (m, 3H), 0.94–0.77 (m, 12H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.9, 170.8, 170.7, 170.0, 169.3, 169.1, 168.8, 59.5, 58.0, 53.7, 50.7, 50.7, 46.9, 43.9, 41.7, 40.5, 34.0, 33.5, 31.5, 30.2, 23.5, 22.6, 22.0, 21.8, 19.2, 18.0; m/z (ES^+): 613.3 $(M+H)^+$, 635.3 $(M+Na)^+$; HRMS (ES^+): calcd for $C_{26}H_{45}N_8O_7S$ $(M+H)^+$, 613.3127, found, 613.3123; RP-HPLC (λ_{220}): 11.7 min.