Pergamon

Tetrahedron Letters 39 (1998) 2831–2834

TETRAHEDRON LETTERS

A Highly Efficient Route To Dehydroalanine Containing Peptides

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Received 9 January 1998; accepted 30 January 1998

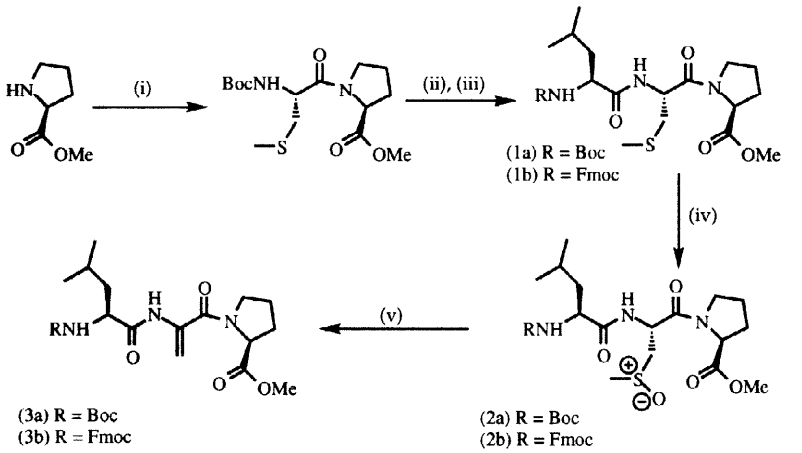
Abstract: A routine method for the synthesis of dehydroalanine containing peptides has been developed. These residues are introduced as S-methyl cysteines which are subsequently oxidised and eliminated. The approach is compatible with both Boc and Fmoc protection strategies and can allow the introduction of a number of αβ unsaturated amino acids into peptides during synthesis.

Dehydroamino acids such as dehydroalanine and dehydroaminobutyrate are found in many naturally occurring biologically active peptides[1] and are predominately formed *via* dehydration of serine and threonine residues. They have been demonstrated to be intermediates in the formation of many unusual amino acids,[2,3] instilling conformational rigidity as well as introducing a potential site for nucleophilic attack. They have therefore found application in the synthesis of peptidomimetics.[4,5]

Since dehydroamino acids are relatively unstable and polymerise and/or decompose to pyruvate and amides[6], their incorporation at an early stage in peptide synthesis may limit later reactions. A number of biomimetic routes to dehydroalanine have been published which require conversion from a protected serine/threonine to the αβ unsaturated amino acid.[3,7] Dehydroalanine has also been accessed by oxidation and elimination of β-phenylselenoalanine[7] as well as by Wittig chemistry[1] and most recently by the oxidation of a resin linked cysteine residue followed by elimination.[8] Although these first two methods are synthetically useful we have never found them to be particularly efficient nor amenable to solid phase chemistry or the synthesis of multiple dehydroamino acid containing peptides. Here we describe the incorporation of dehydroalanine residues from commercially available S-methyl cysteine. The methodology we report here allows for the inherent instability of dehydroalanine containing peptides by allowing conversion to the αβ unsaturated amino acid on completion of peptide synthesis. S-methyl cysteine was incorporated during peptide synthesis and subsequently oxidised and eliminated to yield the desired αβ unsaturated amino acid. The approach developed can utilise either Boc or Fmoc protection strategies. We have applied this technique to the synthesis of a tripeptide containing one dehydroamino acid and a pentapeptide containing two dehydroalanine residues for biomimetic studies associated with lantibiotic biosynthesis.

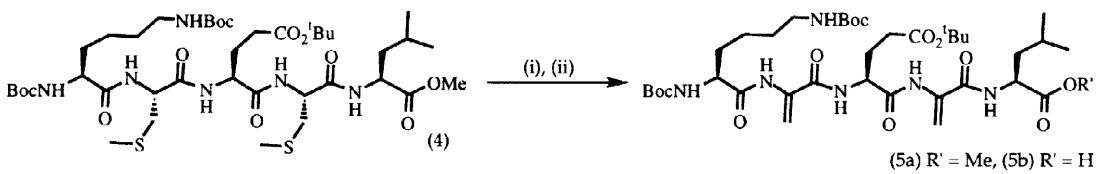
0040-4039/98/$19.00
PII: S0040-4039(98)00258-5

Tripeptide **1** was synthesised from the Boc protected amino acids by solution couplings. Treatment of tripeptide **1** with sodium metaperiodate (1.1 equivalents) for 1 hour produced the sulfoxide **2** in quantitative yield. Elimination of the sulfoxide **2** was effected using two different approaches.[10] Pyrolysis of the sulfoxides (**2a** and **b**) in refluxing dioxane for 15 hours yielded the desired peptides **3a** and **3b** respectively in good yield (88% and 84%). This method was found to be applicable with either Fmoc or Boc strategies. The yield and purity of **3a** was improved by treating the sulfoxide **2a** with diazabicycloundec-7-ene (DBU) (2 equivalents) in methanol. Elimination in this case was complete within one hour and gave the desired product **3a** in excellent yield and purity (96%).



Scheme 1. (i) Boc-S-Methyl Cysteine, HOBt, DCC, DCM, 65%; (ii) TFA, DCM, (99%); (iii) Boc-Leu-OH or Fmoc-Leu-OH, HOBt, EDC, DCM, (76% or 65%); (iv) $NaIO_4$, H_2O, Dioxane, (quant).; (v) DBU, MeOH (R = Boc), (96%) or Dioxane reflux (R = Boc/Fmoc), (88%/84%).

To extend the methodology the pentapeptide **4** was synthesised. Oxidation was again performed using sodium metaperiodate. Mono-oxidation was observed to take place rapidly but mild heating (40°C) was required for the reaction to go cleanly to completion.[9] Elimination of the *bis*-sulfoxide of **4** was also observed to occur in a stepwise manner (as seen by TLC and electrospray mass spectrometry) with mono-elimination complete within one hour and *bis*-elimination after a 12 hour exposure to DBU. More conveniently, treatment of the *bis*-sulfoxide with NaOH (5eq)[10] gave a very clean stepwise elimination to give **5b** in excellent yield (93%) and allowing further elaboration of the *bis*-αβ-unsaturated peptide at the C-terminus.



Scheme 2. (i) $NaIO_4$, H_2O, Dioxane, (99%); (ii) DBU, MeOH, (56%), or NaOH, MeOH, (93%).

In summary we have developed a highly efficient route to the synthesis of peptides containing dehydroalanine residues. The αβ unsaturated amino acids are obtained from commercially available S-methyl cysteine in excellent yield and can be accessed at any time during the synthesis. The method does have a limitation in that it cannot be used with oxidation labile residues for example, we have demonstrated that it is not possible to oxidise S-methyl cysteine in the presence of S-trityl cysteine. However the ease of synthesis of peptides containing multiple dehydroamino acid residues such as **5b** has allowed us access to peptides suitable for the study of lantibiotic biomimetic cyclisations.

ACKNOWLEDGEMENTS

We thank the Royal Society for a University Research Fellowship to MB and Roche Discovery Welwyn and the EPSRC for a CASE studentship to SB.

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9. Typical Oxidation Protocol. Sodium metaperiodate (265mg; 2.2eq) was dissolved in water (10ml) and cooled on an ice bath. Boc-Lys(Boc)-Cys(SMe)-Glu-(O^{t}Bu)-Cys(SMe)-Leu-OMe (**4**, 500mg; 0.56mmol; 1eq) was dissolved in dioxane (20ml) and added dropwise to the oxidant. The reaction was stirred on ice for 1 hour and at 40°C for 4 hours. The reaction mixture was concentrated to ~10ml, water (20ml) was added and the product extracted into DCM (2x30ml). The combined organics were washed with water (50ml), brine (50ml), dried ($MgSO_4$) and concentrated *in vacuo* to give a colourless glass. Yield: 510mg; 99%; R_f:0.53 (10%MeOH / DCM); δ_H (400MHz; DMSO) (complicated by sulfoxide diastereoisomers) 0.93 (6H; d + m; *J* 7; Leu-CH(C$\underline{H}_3$)$_2$); 1.17 - 1.28 (2H; m; Lys-$\underline{H}_\gamma$); 1.42 (27H; 3s;

$C(C\underline{H}_3)_3$); 1.52 - 1.64 (7H; m; Leu-C<u>H</u>+C<u>H</u>$_2$ + Lys-<u>H</u>$_\beta$+<u>H</u>$_\delta$); 1.84 + 1.93 (2H; m; Glu-<u>H</u>$_\beta$); 2.22 (2H; m; Glu-<u>H</u>$_\gamma$); 2.61 (6H; 2s; $2SOC\underline{H}_3$); 2.94 (2H; q; *J* 8; Lys-<u>H</u>$_\delta$); 3.14 - 3.35 (4H; m; 2Cys-C<u>H</u>$_2$); 3.62 + 3.67 (3H; 2s; $CO_2C\underline{H}_3$); 3.98 (1H; m; Lys-<u>H</u>$_\alpha$); 4.33 - 4.46 (2H; m; Leu+Glu-<u>H</u>$_\alpha$); 4.71 - 4.78 (2H; m; 2Cys-<u>H</u>$_\alpha$); 6.75 (1H; bs; OCONH); 6.92 (1H; m; Lys-CONH); 8.21 - 8.27 (3H; m; 3CONH); δ_C (75.5MHz; $CDCl_3$): 21.3 + 21.4 (Leu-CH($\underline{C}H_3$)$_2$); 22.8 (Lys-<u>C</u>$_\gamma$); 24.2 (Leu-<u>C</u>H(CH_3)$_2$); 26.9 (Lys-<u>C</u>$_\beta$); 27.8 + 28.3 + 28.4 ($C(\underline{C}H_3)_3$); 29.3 (Glu-<u>C</u>$_\beta$); 30.9 (Lys-<u>C</u>$_\delta$); 31.4 (Glu-<u>C</u>$_\gamma$); 46.1 + 47.6 ($SO\underline{C}H_3$); 48.0 (Lys-<u>C</u>$_\varepsilon$); 50.6 (<u>C</u>$_\alpha$); 52.2 (<u>C</u>$_\alpha$); 52.4 (<u>C</u>$_\alpha$); 54.5 (<u>C</u>$_\alpha$); 55.0 ($CO_2\underline{C}H_3$); 55.4 (Cys-<u>C</u>H$_2$); 55.9 (<u>C</u>$_\alpha$); 77.4 + 78.3 + 79.9 ($\underline{C}(CH_3)_3$); 155.7 (2O<u>C</u>ONH); 169.4 + 169.9 + 170.0 + 170.0 (<u>C</u>ONH); 171.7 ($\underline{C}O_2CH_3$); 172.6 ($\underline{C}O_2{}^t$Bu); m/z (ES^+): 925.4 (100%) $[M+H]^+$; 947.4 (20%) $[M+Na]^+$ HRMS: $[M+H]^+$ $C_{40}H_{73}N_6O_{14}S_2$ Calc. 925.4626, found 925.4709.

10. Elimination protocols: (a). The peptide **2a** (2.9g; 6.1mmol) was dissolved in methanol (15ml), DBU (2.22g; 2.18ml; 2 equivalents) was added and the mixture stirred for one hour before being concentrated *in vacuo* over a cold water bath. The residue was dissolved in ethyl acetate (5ml), filtered through a silica plug and concentrated *in vacuo* to give the desired product as a colourless glass. Yield: 2.41g; 98%.

(b). The peptide **2a** or **b** (100mg) was dissolved in dioxane (10ml) and refluxed for 15 hours before being concentrated *in vacuo*. The residue was dissolved in ethyl acetate (2ml), filtered through a silica plug and concentrated *in vacuo* to give the desired product as a colourless glass. Yields **3a**: 75mg; 88%; **3b**: 69mg; 84%

(c). Boc-Lys(Boc)-Cys(SOMe)-Glu(O^{t}Bu)-Cys(SOMe)-Leu-OMe (475mg; 0.51mmol; 1eq) was dissolved in MeOH (22ml) and cooled on an ice-salt bath. 1M aqueous NaOH (2.5ml; 5eq) was added dropwise and the reaction mixture allowed to warm to room temperature over 1.5 hours. The reaction mixture was concentrated *in vacuo* to approx. 3ml, acidified to pH 4 with 2M $KHSO_4$ and the product extracted into DCM (3 x 40ml) washed with water (100ml) and brine (100ml), dried ($MgSO_4$) and concentrated *in vacuo* to give a white foam. Yield: 370mg; 93%; R_f:0.29 (10% MeOH / EtOAc. δ_H (400MHz; DMSO): 0.91 (6H; 2d; *J* 7, 7; Leu-C<u>H</u>$_3$); 1.17 - 1.26 (2H; m; Lys-<u>H</u>$_\gamma$); 1.42 (27H; bs; $C(C\underline{H}_3)_3$); 1.52 - 1.64 (7H; m; Leu-C<u>H</u>+C<u>H</u>$_2$ + Lys-<u>H</u>$_\beta$+<u>H</u>$_\delta$); 1.92 + 2.03 (2H; 2m; Glu-<u>H</u>$_\beta$); 2.21 (2H; m; Glu-<u>H</u>$_\gamma$); 2.88 (2H; m; Lys-<u>H</u>$_\varepsilon$); 3.98 (1H; m; Lys-<u>H</u>$_\alpha$); 4.34 (1H; m; Leu-<u>H</u>$_\alpha$); 4.42 (1H; m; Glu-<u>H</u>$_\alpha$); 5.61 (2H; 2s; C=C<u>H</u>$_2$); 6.20 (2H; bs; C=C<u>H</u>$_2$); 6.83 (1H; bt; *J* 6;OCONH); 7.31 (1H; bd; *J* 7; Lys-CONH); 8.45 (1H; bd; *J* 7; Glu-CONH); 8.62 (1H; bd; *J* 8; Leu-CONH); 9.06 + 9.10 (2H; 2s; Dha-CONH); δ_C (75.5MHz; $CDCl_3$): 21.3 (Leu-CH($\underline{C}H_3$)$_2$); 23.0 (Lys-<u>C</u>$_\gamma$); 24.6 (Leu-<u>C</u>H(CH_3)$_2$); 26.5 (Lys-<u>C</u>$_\beta$); 27.9 + 28.3 + 28.4 ($C(\underline{C}H_3)_3$); 29.3 (Glu-<u>C</u>$_\beta$); 30.9 (Lys-<u>C</u>$_\delta$); 31.7 (Glu-<u>C</u>$_\gamma$); 48.7 (Lys-<u>C</u>$_\varepsilon$); 51.1 (<u>C</u>$_\alpha$); 53.8 (<u>C</u>$_\alpha$); 55.5 (<u>C</u>$_\alpha$); 77.5 + 78.6 + 79.9 ($\underline{C}(CH_3)_3$); 103.5 (C=<u>C</u>H$_2$); 134.6 (<u>C</u>=CH$_2$); 155.7 (O<u>C</u>ONH); 163.8 (<u>C</u>ONH); 164.3 (<u>C</u>ONH); 170.5 (<u>C</u>ONH); 171.7 (<u>C</u>ONH); 171.9 ($\underline{C}O_2{}^t$Bu); 173.8 ($\underline{C}O_2H$); m/z (ES^+): 683.3 (25%) $[M-BOC+H]^+$; 783.3 (100%) $[M+H]^+$; HRMS: $[M+H]^+$ $C_{37}H_{63}N_6O_{12}$ Calc. 783.4504, found 783.4559.