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Asparagine as a Masked Dehydroalanine Residue in Solid Phase Peptide Synthesis.

Carsten Blettner & Mark Bradley*

Department of Chemistry, University of Southampton, Highfield, Southampton, SO9 5NH (UK)

Abstract: Asparagine residues in peptides have been smoothly converted into dehydroalanine residues using solid phase techniques and the sequential application of a Hofmann type degradation reaction and a Hofmann elimination.

αβ-unsaturated amino acids such as dehydroalanine and dehydroaminobutyrate occur frequently in nature, as components of a variety of bacterially derived peptide based antibiotics and phytotoxins.[1] They are usually derived from serine or threonine residues by dehydration reactions and have been found to be intermediates in the biosynthesis of unusual or D-configured amino acids in several natural peptides.[2] They have the potential to introduce both side chain and backbone conformational constraints within peptides, a property which has led to them been studied intensively by a wide range of physical techniques[3] and encouraged their use in the synthesis of analogues of naturally occurring peptide hormones. In solid phase peptide synthesis they have been used as a device to generate C-terminal peptide amides upon cleavage of the peptide from the resin.[4]

Many different approaches have been utilised in the synthesis of dehydroamino acids[5], although there have been few reports of the synthesis of peptides containing dehydroamino acids using solid phase supports.[6] Here we report the synthesis of peptides containing dehydroalanines using solid phase techniques. The building blocks for the αβ-unsaturated amino acid are incorporated into the peptide as asparagine residues. These are converted to the dehydroamino acid by the sequential application of a Hofmann type degradation reaction using the reagent bis(trifluoracetoxy)iodobenzene (TIB)[7] followed by a Hofmann elimination reaction (see figure 1).[8] We have applied this technique to the preparation of a tripeptide (1) containing a single dehydroamino acid and to a pentapeptide (2) containing two dehydroalanine residues.

The peptides (3) and (4) were synthesised manually on the Merrifield resin by standard techniques using diisopropylcarbodiimide and hydroxybenzotriazole couplings in DMF[9] the success of which was followed using the Kaiser test.[10] The peptides attached to the resin were treated with TIB (1.5eq for each asparagine residue), in a mixture of DMF/THF/H_2O (1:1:1) followed by 1.5eq of pyridine after 10 mins, then stirred at room temperature for 2h. (This Hofmann degradation type of reaction was followed to completion using the quantitative ninhydrin reaction and small samples of resin).[11] The resin bound peptide amines were thoroughly washed (DMF) before being treated with 10eq of MeI and $KHCO_3$ in MeOH for 12h after which time the ninhydrin test was negative. The resin was again washed (DMF, MeOH) before being treated with 10% NEt_3 in

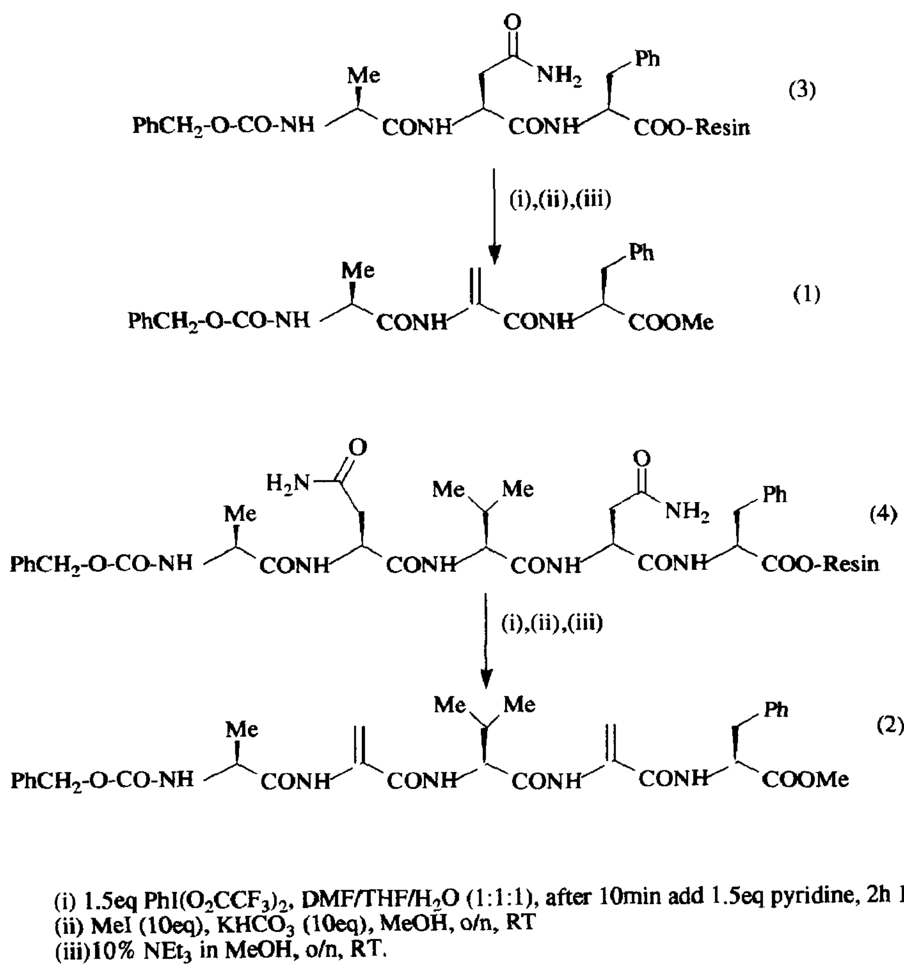

Figure 1.

MeOH to cleave the peptides from the resin as their methyl esters. These were purified by flash chromatography on silica gel to give isolated and purified yields for the tripeptide (1) and the pentapeptide (2) (both as gummy oils) of 23% and 9% respectively starting from the Merrifield resin (both unoptimised). The ^{1}H and ^{13}C nmr spectra were entirely consistent with the proposed structures, as were high resolution mass spectrometry measurements.[12]

This technique not only offers the potential for incorporating multiple dehydroalanines into peptides, but since asparagine and glutamine blocking groups are available the dehydroamino acid can be directed into the peptide at any point, additionally the use of TIB on a Resin linked peptide to produce a side chain primary amine from either asparagine or glutamine allows a simple avenue into the synthesis of branched peptides, without the use of exotically protected amino acids.

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12 data for pentapeptide, (tlc silica plate $R_f = 0.8$ (EtOAc); δ_H(500MHz, $CDCl_3$) 8.67 and 8.51 (2H, 2xs, 2xNH of dehydroalanine), 7.31-7.10 (10H, m, 2xPh), 7.08 (1H, d, J=1.5Hz, NH), 6.74 (1H, d,

J=7Hz, NH), 6.46 and 6.40 (2H, 2xs, 2xC$\underline{H}$H=C), 5.64 (1H, d, J=8Hz, NH), 5.42 and 5.24 (2H, 2xs, CH$\underline{H}$=C), 5.17-5.08 (2H, m, PhC$\underline{H}_2$O), 4.90, 4.53 and 4.44 (3H, 3xm, 3xC$\underline{H}\alpha$), 3.74 (3H, s, MeO), 3.14 (2H, ABX system, C$\underline{H}_2$Ph), 2.15 (1H, m, C$\underline{H}$Me$_2$), 1.41 (3H, d, J=7Hz, Me), 0.99 and 0.96 (6H, 2xd, J=3Hz,3Hz, Me$_2$CH), δ_C(126MHz, CDCl$_3$, DEPT analysis) 171.7, 170.3, 164.1 and 163.5 (5xs, 4x amides, 1x ester), 156.1 (s, urethane, O$\underline{C}$ONH), 136.4, 135.6, 134.1 and 133.9 (4xs, 2x Ar$\underline{C}$-1, 2x $\underline{C}$=CH$_2$), 129.4, 128.9, 128.7, 128.3, 128,2 and127.5 (6xd, Aromatic $\underline{C}$H's), 103.9 and 103.5 (2xt, 2xC=$\underline{C}$H$_2$), 67.2 (t, Ph$\underline{C}$H$_2$O), 59.7 (d, $\underline{C}$Hα), 53.9 (d, $\underline{C}$Hα), 52.7 (q, $\underline{Me}$O), 51.5 (d, $\underline{C}$Hα), 37.9 (t, Ph$\underline{C}$H$_2$CH), 31.7 (d, Me$_2\underline{C}$H), 19.3, 19.0 and 18.3 (3xq, $\underline{Me}$CH and $\underline{Me_2}$CH); m/z (+ve argon FAB), MH$^+$ (622); $C_{32}H_{40}O_8N_5$ expected 622.2877, found 622.2892

data for tripeptide (tlc silica plate R$_f$ = 0.5 (CH$_2$Cl$_2$/EtOAc (4:1)); δ_H(500MHz, CDCl$_3$) 8.51 (1H, s, NH of dehydroalanine), 7.25-7.15 and 7.03-7.01 (10H, m, 2xPh), 6.80 (1H, d, J=7Hz, NH), 6.32 (H, s, C$\underline{H}$H=C), 5.60 (1H, d, J=8Hz, NH), 5.14 (H, s, CH$\underline{H}$=C), 5.06-4.97 (2H, m, PhC$\underline{H}_2$O), 4.80 and 4.29 (2H, 2xm, 2xCHα), 3.65 (3H, s, MeO), 3.11-3.01 (2H, ABX system, C$\underline{H}_2$Ph), 1.30 (3H, d, J=7Hz, Me), δ_C(126MHz, CDCl$_3$, DEPT analysis) 171.8, 171.5 and 163.4 (3xs, 2x amides, 1x ester), 156.1 (s, urethane, O$\underline{C}$ONH), 136.3, 135.6 and 133.7 (3xs, 2x Ar$\underline{C}$-1, 1x $\underline{C}$=CH$_2$), 129.3, 128.8, 128.6, 128.1 and 127.4 (6xd, Aromatic $\underline{C}$H's), 103.1 (t, $\underline{C}$=CH$_2$), 67.2 (t, Ph$\underline{C}$H$_2$O), 53.7 (d, $\underline{C}$Hα), 52.6 (q, $\underline{Me}$O), 51.4 (d, $\underline{C}$Hα), 37.7 (t, Ph$\underline{C}$H$_2$CH), 18.7 (q, $\underline{Me}$CH); m/z (+ve argon FAB), MH$^+$ (454); $C_{24}H_{28}O_6N_3$ expected 454.1978, found 454.2000.

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