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β-Nitro-α-Amino Acids as Latent α,β-Dehydro-α-Amino Acid Residues in Peptides

Phillip A. Coghlan and Christopher J. Easton*‡

Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia

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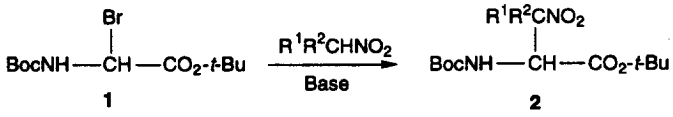
Abstract: Peptides containing α,β-dehydro-α-amino acid residues are readily prepared by incorporating β-nitro-α-amino acids at the corresponding positions, followed by elimination of nitrous acid.

Keywords: Amino acids and derivatives; Nitro compounds; Elimination Reactions; Enamides

α,β-Dehydro-α-amino acid residues are common components of naturally occurring peptides,[1] and they markedly affect the physiological activity of peptides,[2] as well as their conformations[3] and resistance to enzyme-catalysed degradation.[4] Consequently there is considerable interest in methods for the synthesis of α,β-unsaturated amino acids incorporated in peptides.[5-7] Free dehydro amino acids are generally unsuitable for direct peptide synthesis because, being enamines, they are unstable[8] and only poor nucleophiles.[9] Instead, a peptide containing such a residue can be prepared by incorporation of a β-hydroxy amino acid. Subsequent conversion of the hydroxy group to a mesylate or other suitable leaving group, followed by elimination, unmasks the dehydro amino acid residue already within the peptide.[6] The utility of this approach is restricted by the lack of ready access to β-hydroxy amino acids other than serine and threonine, although some of these can be obtained through synthesis.[10] Variations of this methodology involve substituting the β-hydroxy amino acid with *S*-methyl cysteine and other β-functionalised amino acids,[7] but again these are constrained to the synthesis of



a) $R^1 = R^2 = Me$
b) $R^1 = R^2 = H$
c) $R^1 = CH_2CO_2Me$, $R^2 = H$

Scheme 1

‡email: easton@rsc.anu.edu.au

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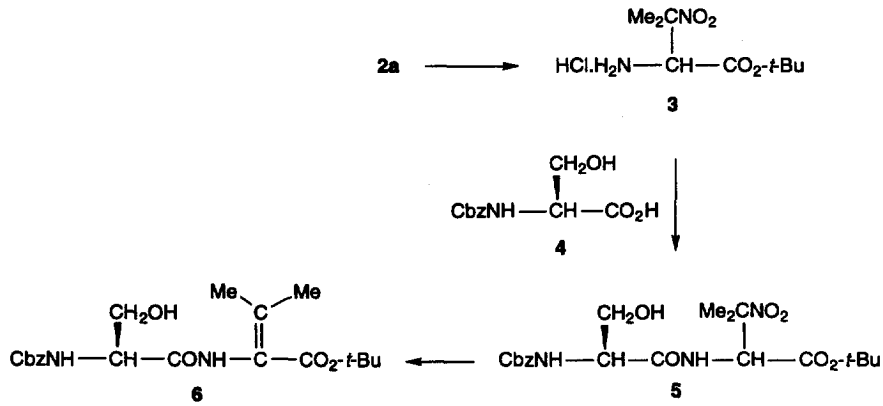
limited types of dehydro amino acid residues, because of the small range of readily available precursors. In this report we present a complementary method for the synthesis of peptides containing α,β-unsaturated residues and discuss some of the advantages of this approach.

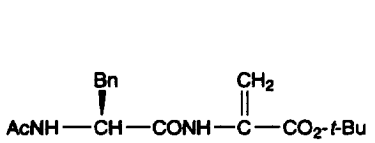
The procedure involves the use of β-nitro amino acid derivatives, which are readily accessible with a wide variety of side chains through reaction of α-bromoglycine derivatives with alkyl nitronates,[11] as shown in Scheme 1 for the examples used in this work. The amino group of these compounds is selectively deprotected, and the products are incorporated into peptides and then elaborated to dehydro amino acid residues, under mild conditions.



Scheme 2

The following protocols for the synthesis of the seryl-α,β-dehydrovaline derivative **6** (Scheme 2)[12] exemplify the procedure. *N*-(*tert*-Butoxycarbonyl)-β-nitrovaline *tert*-butyl ester **2a** was obtained through reaction of the α-bromoglycine derivative **1** with the anion of 2-nitropropane. Selective deprotection of the amino group was then accomplished by treatment with hydrogen chloride in ethyl acetate.[13] Accordingly, acetyl chloride (0.58 ml) was added carefully to a solution of methanol (0.33 ml) in ethyl acetate (6.5 ml) and the mixture was stirred at room temperature for 0.75 h, then it was cooled to 4 °C, and a solution of the protected β-nitro amino acid **2a** (0.47 mmol, 150 mg) in ethyl acetate (4.2 ml) was added. This mixture was allowed to warm to room temperature and was stirred at room temperature for 10.5 h. The solid white precipitate which formed was isolated by filtration. Additional product was obtained by adding diethyl ether to the filtrate and isolating the resultant precipitate. The solids were combined to give β-nitrovaline *tert*-butyl ester hydrochloride **3** (88 mg, 73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.86 (s, 3H), 1.92 (s, 3H), 4.71 (s, 1H), 9.25 (br s, 3H). The amine hydrochloride **3** (0.2 mmol, 50 mg) was then converted to the free amine, by treatment with potassium carbonate, and peptide bond formation was then accomplished in a standard fashion. This involved dissolving the amine in dichloromethane (2 ml) and adding *N*-benzyloxycarbonyl-(*S*)-serine **4** (0.2 mmol, 47 mg), *N,N'*-dicyclohexylcarbodiimide (0.2 mmol, 40.5 mg) and 1-hydroxybenzotriazole (0.2 mmol, 26.5 mg).

The mixture was stirred at room temperature for 2 h, then at reflux for a further 11 h, before it was cooled to room temperature and filtered. HPLC [YMC ODS-AQ 250 x 10 mm column, eluting with 70/30 (v/v) MeOH/H_2O] of the residue obtained by concentration of the filtrate under reduced pressure afforded a *ca.* 1:1 mixture of the diastereomers of *N*-benzyloxycarbonyl-(*S*)-seryl-β-nitrovaline *tert*-butyl ester **5** (47 mg, 47%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 and 1.42 (s and s, total 9H), 1.50 (s, 3H), 1.67 and 1.71 (s and s, total 3H), 3.71 (m, 1H), 4.09 (m, 1H), 4.31 (m, 1H), 5.10 (m, 3H), 5.88 (m, 1H), 7.34 (m, 5H). Finally, treatment of the peptide **5** with base unmasked the dehydro amino acid residue. Thus, to a solution of the dipeptide **5** (0.01 mmol, 5 mg) in chloroform (0.2 ml), diisopropylamine (0.46 mmol, 0.06 ml) was added, and the mixture was stirred at reflux for 48 h, then it was cooled and concentrated under reduced pressure. HPLC [65/35 (v/v) MeOH/H_2O] of the residue gave a quantitative yield of *N*-benzyloxycarbonyl-(*S*)-seryl-α,β-dehydrovaline *tert*-butyl ester **6**; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.78 (s, 3H), 2.11 (s, 3H), 3.67 (dd, *J* 5.5 and 11 Hz, 1H), 4.13 (m, 1H), 4.26 (m, 1H), 5.13 (s, 2H), 5.80 (m, 1H), 7.35 (m, 5H).



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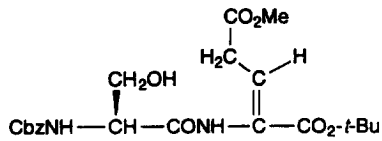

8

The generality of the procedure is illustrated by the synthesis of the peptides **7** and **8**. The derivatives of β-nitroalanine **2b** and β-nitroglutamate **2c** were obtained through reactions of the bromide **1** with the anions of nitromethane and methyl 3-nitropropanoate, respectively (Scheme 1). Removal of the *N*-protecting group with hydrogen chloride in ethyl actetate gave the corresponding amine hydrochlorides, which were converted to the free amines and then coupled with *N*-acetyl-(*S*)-phenylalanine and *N*-benzyloxycarbonyl-(*S*)-serine **4** to produce the corresponding dipeptides. Treatment of these with base gave *N*-acetyl-(*S*)-phenylalanyl-α,β-dehydroalanine *tert*-butyl ester **7** [^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.93 (s, 3H), 3.01 (dd, *J* 7 and 14 Hz, 1H), 3.07 (dd, *J* 7 and 14 Hz, 1H), 4.66 (apparent q, *J* 7 Hz, 1H), 5.73 (d, *J* 1 Hz, 1H), 5.93 (br d, *J* 7 Hz, 1H), 6.41 (s, 1H), 7.08-7.25 (m, 5H), 7.89 (br s, 1H)] and *N*-benzyloxycarbonyl-(*S*)-seryl-α,β-dehydroglutamate α-*tert*-butyl ester γ-methyl ester **8** [^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 3.20 (d, *J* 7 Hz, 2H), 3.70 (s, 3H), 3.73 (m, 1H), 4.10 (m, 2H), 4.35 (m, 1H), 5.13 (s, 2H), 5.87 (d, *J* 7 Hz, 1H), 6.77 (t, *J* 7 Hz, 1H), 7.35 (m, 5H), 8.09 (br s, 1H)]. The assignment of *Z*-stereochemistry to the dehydroglutamate residue in the peptide derivative **8** is made on the basis of the tendency of dehydro amino acid derivatives to favour this configuration.[14]

These procedures for the synthesis of dehydro amino acid residues in peptides are quite versatile. Many β-nitro amino acid derivatives are easily prepared and they are compatible with the conditions required for peptide bond formation. After incorporation in peptides, they readily eliminate nitrous acid to unmask the corresponding unsaturated amino acids. This method is likely to offer particular advantages in the synthesis of peptides

containing both hydroxy and dehydro amino acid residues, as is the case with the peptides **6** and **8**, where synthesis of the unsaturated amino acid residues from the corresponding β-hydroxy amino acids would require the use of protecting groups to achieve selective reaction of either a secondary or tertiary hydroxy group in the presence of the primary hydroxy group of the serine residue. It will also be particularly useful where the required β-functionalised amino acid precursor of the dehydroamino acid residue is not otherwise readily available, as is the case with the dehydroglutamate residue of the peptide **8**.

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