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Solid Phase Synthesis of α-Hydroxyglycine Extended Peptides -Biological Precursors of Peptide Amides

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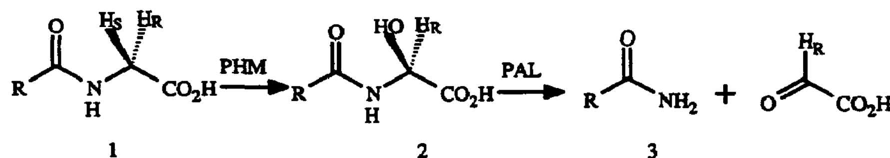
Abstract: Fmoc α-methoxyglycine can be incorporated into solid phase peptide synthesis (SPPS) methodology to give α-hydroxyglycine extended peptides on final deprotection and cleavage from the solid support with trifluoroacetic acid containing 5% H_2O.

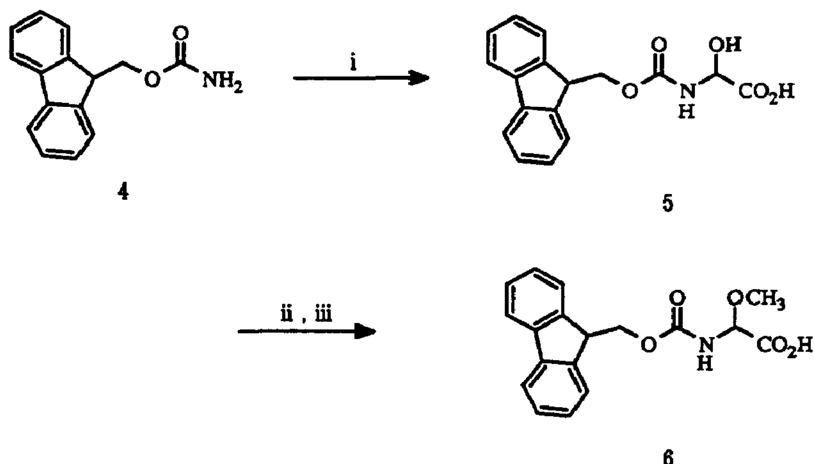
Many peptide hormones require a primary amide function at the C-terminus for full biological activity[1]. The biosynthesis of peptide amides involves a post translational two step[2] enzyme mediated oxidative cleavage of a glycine extended peptide **1** via an α-hydroxyglycine intermediate **2** (Scheme 1)[3].

The first step is catalysed by peptidyl α-hydroxyglycine monooxygenase (PHM, EC 1.4.17.3), which selectively removes the *pro*-S hydrogen from the C-terminal glycine residue and adds a hydroxy group with retention of configuration, to give the S-α-hydroxyglycine residue **2**[4]. The second enzyme, peptidyl amidoglycolate lyase (PAL, EC 4.3.2.5), then catalyses the decomposition of **2** to the peptide amide **3** and glyoxylic acid. This biosynthetic pathway suggests that sequences containing C-terminal α-hydroxyglycine could be useful for the design of inhibitors of C-terminally amidated hormones.



Scheme 1

Whilst the synthesis of α-hydroxyglycine extended peptides has previously been reported both by condensation of peptide amides with glyoxylic acid[2b,3b] and by the reaction of glycine extended peptides with recombinant α-amidating enzyme[5], we sought to find a general synthetic route to these compounds using solid phase peptide synthesis in order to investigate whether these α-hydroxyglycine extended peptides have a biological function. We now report the first solid phase synthesis of α-hydroxyglycine extended peptides.



Scheme 2. i, glyoxylic acid; ii, MeOH, sulphuric acid; iii, LiOH

Fmoc carbamate[6] 4 (Scheme 2) was treated with glyoxylic acid (5 equiv.) in gently refluxing ethyl acetate for 6 hours to give racemic 9-fluorenylmethoxycarbonyl-α-hydroxyglycine 5 in 73% yield. The secondary alcohol was protected as the methyl ether 6 by O-methylation of the α-hydroxyglycine derivative[7] 5 followed by ester hydrolysis using LiOH in aqueous acetone to give 6 in 68% overall yield. Activation of 6 (DIC/HOBT/DMAP) was used to functionalise Wang resin[8] which was employed for the solid phase synthesis of the gastrin derivative 7 on an Applied Biosystems 430A peptide synthesiser.

H.Trp.Met.Asp.Phe.α-OH Gly.OH

7

H.Glu.Glu.Ala.Tyr.Gly.Trp.Met.Asp.Phe.α-OH Gly.OH

8

Fmoc deprotection monitoring[9] during synthesis showed low incorporation of Phe due to the instability of the O-methylated α-hydroxyglycine residue[10]. Coupling of Asp to Phe also showed a low incorporation which is characteristic of diketopiperazine (DKP) formation[11]. This resulted in the isolation of only small amounts of the diastereomeric peptide 7 after cleavage (TFA/H_2O/EDT) and RP-HPLC. The diastereomers of 7, due to the R and S configuration of the terminal α-hydroxyglycine system, were separable by HPLC and were shown to be identical by FAB mass spectrometry and amino acid analysis. Transformation of the α-OMe substituent to the α-OH during acid treatment probably proceeds via the corresponding imine intermediate.

Synthesis of the decapeptide gastrin analogue 8 was then carried out using 2-chlorotrityl chloride resin[12] in order to prevent DKP formation. Fmoc deprotection monitoring during synthesis of 8 showed no evidence of DKP formation and the target peptide was isolated in 3.8% overall yield (13mg) after cleavage and HPLC purification.

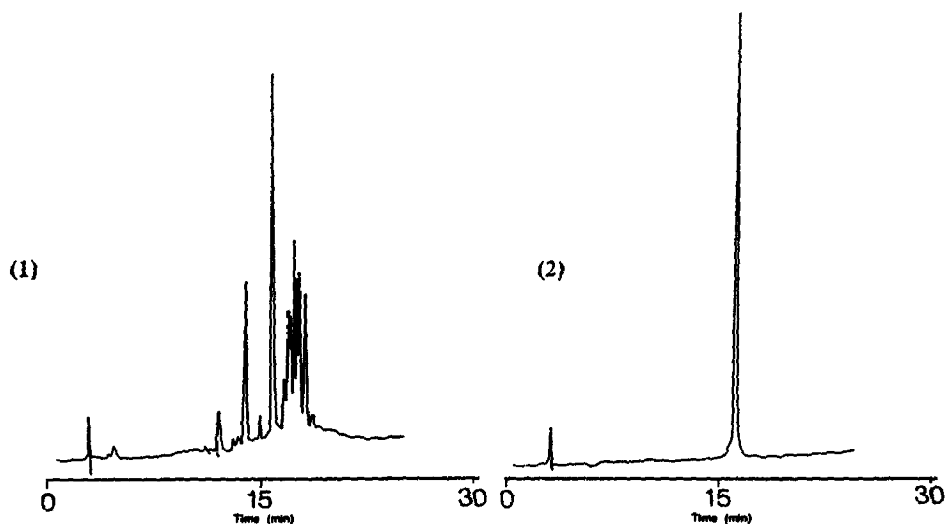


Figure 1. HPLC of crude (1) and pure (2) peptide 8

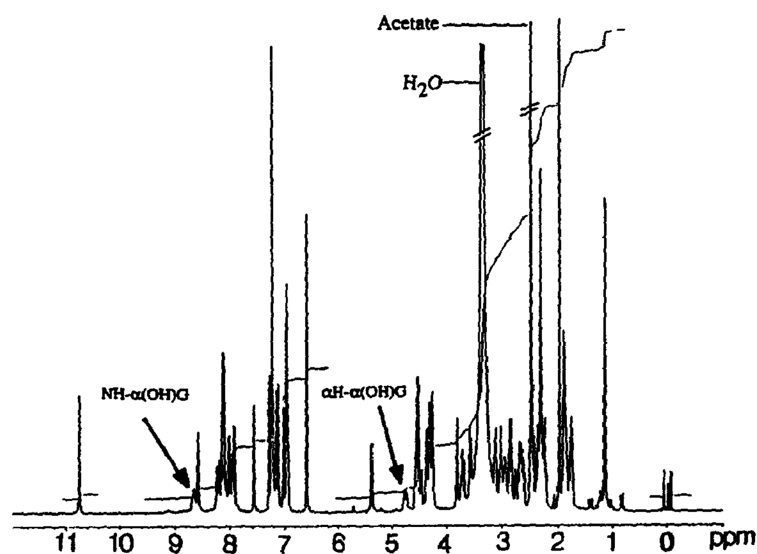


Figure 2. 600Mhz ^1H NMR of peptide 8

The diastereomeric decapeptides 8 were inseparable by RP-HPLC (Figure 1) but ^1H NMR (600Mhz,D^6 DMSO) showed a 1:1 ratio of diastereomers with the resonances at 4.79 ppm and 4.75 ppm (Figures 2 and 3) assigned to the αH of an α-hydroxyglycine residue. DQF-COSY (Double Quantum Filtered Chemical Shift Correlation Spectroscopy) allowed a complete assignment to be made of the ^1H NMR (Figure 2) and showed that the αH signals of the diastereoisomeric α-hydroxyglycine did not have any other crosspeaks except with the NH signal at 8.66 ppm and the resonance of these αH's showed a downfield shift with respect to the other αH's.

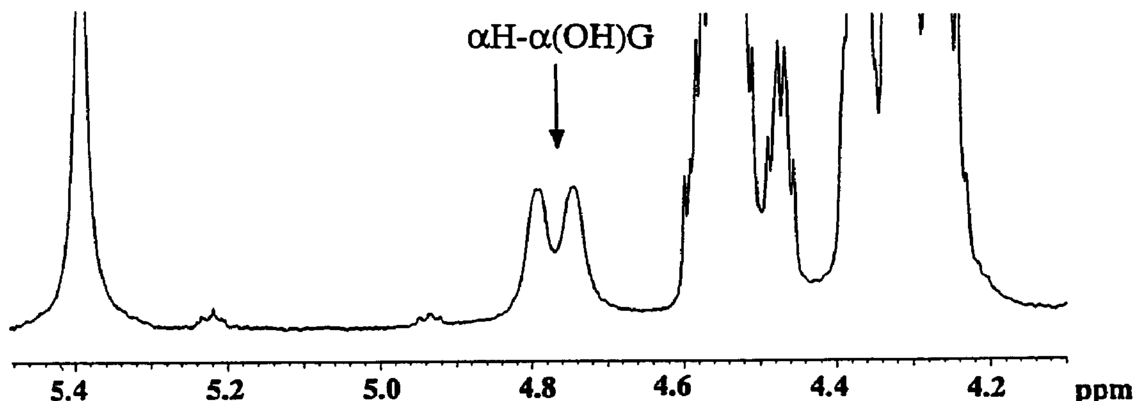


Figure 3. Expansion of Figure 2 to show α-hydroxyglycine αH signals

In conclusion,we have shown that it is possible to introduce α-methoxyglycine as the C-terminal residue in the assembly phase of Fmoc solid phase peptide synthesis. Subsequent acid treatment not only causes side chain deprotection and cleavage of the peptide from the resin but also converts the C-terminal residue to α-hydroxyglycine. The biological function of the α-hydroxyglycine peptides with respect to C-terminal amidation is currently under investigation by Professor G.J. Dockray (Physiological Laboratory, University of Liverpool).

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