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Reversible Modification of the Acid Labile 2-Hydroxy-4-methoxybenzyl(Hmb) Amide Protecting Group: A simple scheme yielding Backbone Substituted Free Peptides

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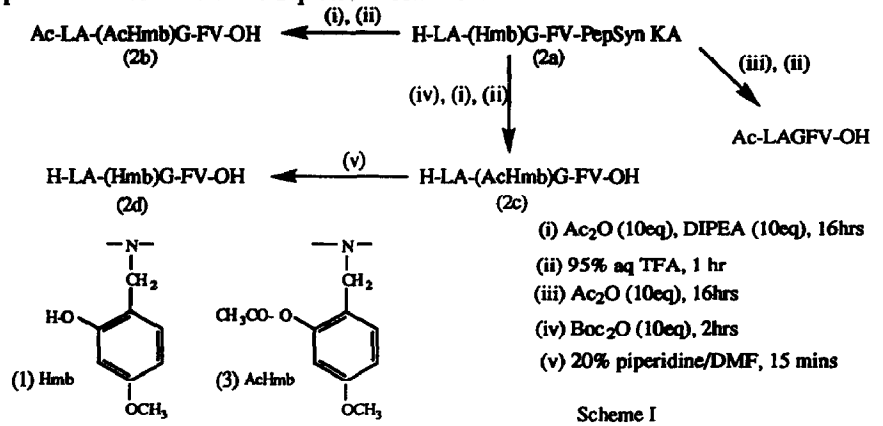
Abstract: Acetylation of the trifluoroacetic acid labile 2-hydroxy-4-methoxybenzyl (Hmb) amide protecting group (1) gave a dramatic increase in acid stability. This enabled peptides prepared by the Fmoc / t-butyl solid phase strategy to be deprotected and cleaved whilst retaining backbone amide protection. Acid lability was easily restored by piperidine mediated de-O-acetylation.

Peptide insolubility arises when the total intra- and interpeptide ionic and hydrophobic interactions overcome peptide-solvent interactions. Amide backbone hydrogen bonding contributes the major interaction leading to insolubility, probably through the formation of protein-like extended β-sheet type structures[1]. This situation is encountered in both solid phase peptide synthesis, the occurance of so-called "difficult sequences"[2,3] and in solution where certain peptides exhibit gross insolubility. We have previously described the TFA labile 2-hydroxy-4-methoxybenzyl (Hmb) backbone amide bond protecting group[4] (1), compatible with Fmoc based solid phase peptide synthesis. Incorporation of backbone amide protection prevented chain association during synthesis, giving improved crude products from a number of difficult sequences[5].

The objective of the current studies was the improvement of peptide solubility in solution, with a view to aiding the purification of low solubility peptides. Here we report a reversible modification of the Hmb group, imparting temporary TFA stability through acetylation of the 2-hydroxyl moiety. This allows peptides prepared by the Fmoc protocol to be deprotected and cleaved whilst retaining backbone protection . The free peptide may then be generated in solution as a final step, after advantage of the improved solubility has been taken.

The chemistry was developed using a test pentapeptide 2a. Initially peptide 2a was treated with acetic anhydride in DMF overnight. Upon cleavage, the characteristic pink colouration indicative of the carbocation derived from the Hmb group was immediately observed. The product was analysed by RP-HPLC, giving a major peak with retention time 14.48 mins, and the required positive FAB-MS m/z = 548.5 for Ac-LAGFV-OH. The acetylation reaction was repeated with the addition of the base diisopropylethylamine (DIPEA). Upon cleavage, no colouration was observed and HPLC gave a major peak with retention time 17.79 mins (no peak at 14.48 mins was observed). FAB-MS gave a top mass peak at m/z = 726.8, corresponding to the fully acetylated pentapeptide 2b. Peptide 2b was treated with 95% aq TFA for 16hrs, after which HPLC analysis showed no observable change. This confirmed a dramatically increased stability towards TFA mediated cleavage for the 2-acetoxy-4-methoxybenzyl (AcHmb) amide protecting group (3). Initial reaction of 2a with di-t-butyldicarbonate (no added base) followed by acetylation in the presence of DIPEA gave upon cleavage, a major peak by HPLC with retention time 16.34 mins, which exhibited FAB-MS m/z = 684.6, corresponding to 2c. Once in solution,

the TFA lability of the amide protecting group on 2c could be regenerated through de-O-acetylation of the AcHmb group by treatment with 20% piperidine in DMF (or 5% hydrazine in DMF) for 15 mins. Subsequent analysis by HPLC gave a major peak with retention time 15.50 mins (2c absent), and FAB-MS m/z = 642.4, corresponding to pentapeptide 2d. These results are depicted in scheme I.



Scheme I

The improved solubility introduced by backbone amide protection was demonstrated by the synthesis of the βA4 (34-42) amyloid related fragment (H-LMVGGVVIA-OH), with (4a) and without (4b) amide protection. The (Hmb)Gly^{37} amide protected peptide (4a), was prepared via the AcHmb protected derivative using the methods depicted in scheme I. After de-O-acetylation with 20% piperidine/DMF for 15 mins, HPLC analysis gave a major peak with retention time 17.22 mins by HPLC, and FAB-MS m/z = 995.6 (theory required 995.1). Peptide 4a exhibited a 7-fold increase in solubility compared to the non backbone protected derivative 4b, in 50% trifluoroethanol/0.1% aq TFA, and a four-fold increase in 50% acetonitrile/0.1% aq TFA. The N$^{\alpha}$-Fmoc derivatives of 4a and 4b showed a substantial solubility difference in DMF. The backbone protected peptide was soluble at >25mg/ml, at least a fourteen-fold increase over the non backbone protected analogue.

The simple protocols described herein allow peptides to be prepared that contain an acid-labile, solubilising backbone protecting group. The increased solubility afforded by amide protection should aid the characterization and purification of low solubility peptides[6] using established chromatographic techniques.

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6) We have recently prepared a backbone protected βA4 (1-43) that exibited a solubility of >150 mg/ml in aqueous acetonitrile (M. Quibell, W.G. Turnell and T. Johnson, submitted for publication).

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