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Solid phase synthesis of azapeptides utilising reversible amide bond protection to prevent hydantoin formation

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Abstract

An efficient method for the solid phase assembly of 'aza-peptides' is described using Fmoc/*tert*-butyl chemistry. Elimination of unwanted hydantoin side product is achieved with the use of Hmb reversible amide bond protection.

Keywords: aza-peptides; Hmb amide bond protection.

Peptides can be used as tools in the design and development of protease inhibitors. However, due to their poor metabolic stability their alteration to pseudopeptides or peptidomimetics is often favoured. One such conversion is seen in the replacement of one or more α-CH groups with a nitrogen bearing an appropriate side chain to generate 'aza-peptides'. These have been shown to provide resistance to enzymic cleavage and several groups have recently reported their use as inhibitors of serine, cysteine and aspartyl proteases.[1–3]

Aza-peptides can be prepared via formation of a resin-bound isocyanate[4,5] or activated carbamate intermediate,[6] followed by nucleophilic attack with a substituted amino acid acyl hydrazide. Unfortunately, this methodology also leads to formation of peptide-chain terminated hydantoin derivatives and gives rise to impure products and reduced yields. Hydantoin formation arises as a result of intramolecular nucleophilic attack on the activated intermediate by a secondary nitrogen from the preceding C-terminal peptide backbone chain. Reversible *N*-substitution of this secondary amide would appear to be a solution for eliminating this side reaction.

The solid phase synthesis of aza-peptides using fluoren-9-ylmethoxycarbonyl (Fmoc) amino acid carbazates and hydrazides has been reported previously.[6] Described below is an adaptation of this technique which utilises the *N*-2-hydroxy-4-methoxybenzyl (Hmb) reversible amide bond protecting group[7] in the preparation of hydantoin free aza-peptides.

The target azaGly-peptide was assembled as shown in Scheme 1. RPHPLC analysis (Fig. 1a) of the deprotected peptide revealed two peaks. ESMS analysis of these showed peak 1 (t_R=9.0 min;

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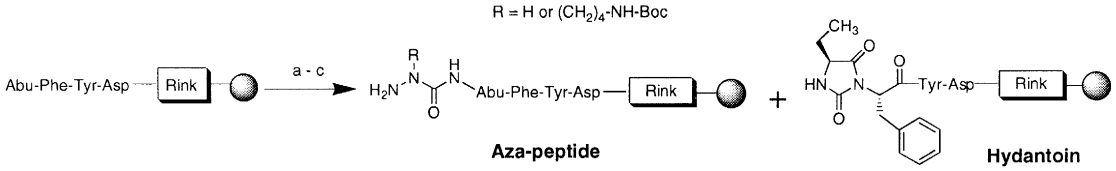
MH+=585.9) to be the target peptide and peak 2 (t_R=13.5 min; MH+=553.9) to be the anticipated hydantoin side product. Formation of the hydantoin side product was also evident during the synthesis of the azaLys analogue (data not shown).



Scheme 1. Synthesis of aza pentapeptide. Reagents and conditions: (a) 2,4-dinitrophenyl carbonate, DMF, 2 h; (b) Fmoc-NH-NH(R), DMF, 16 h; (c) 20% piperidine in DMF

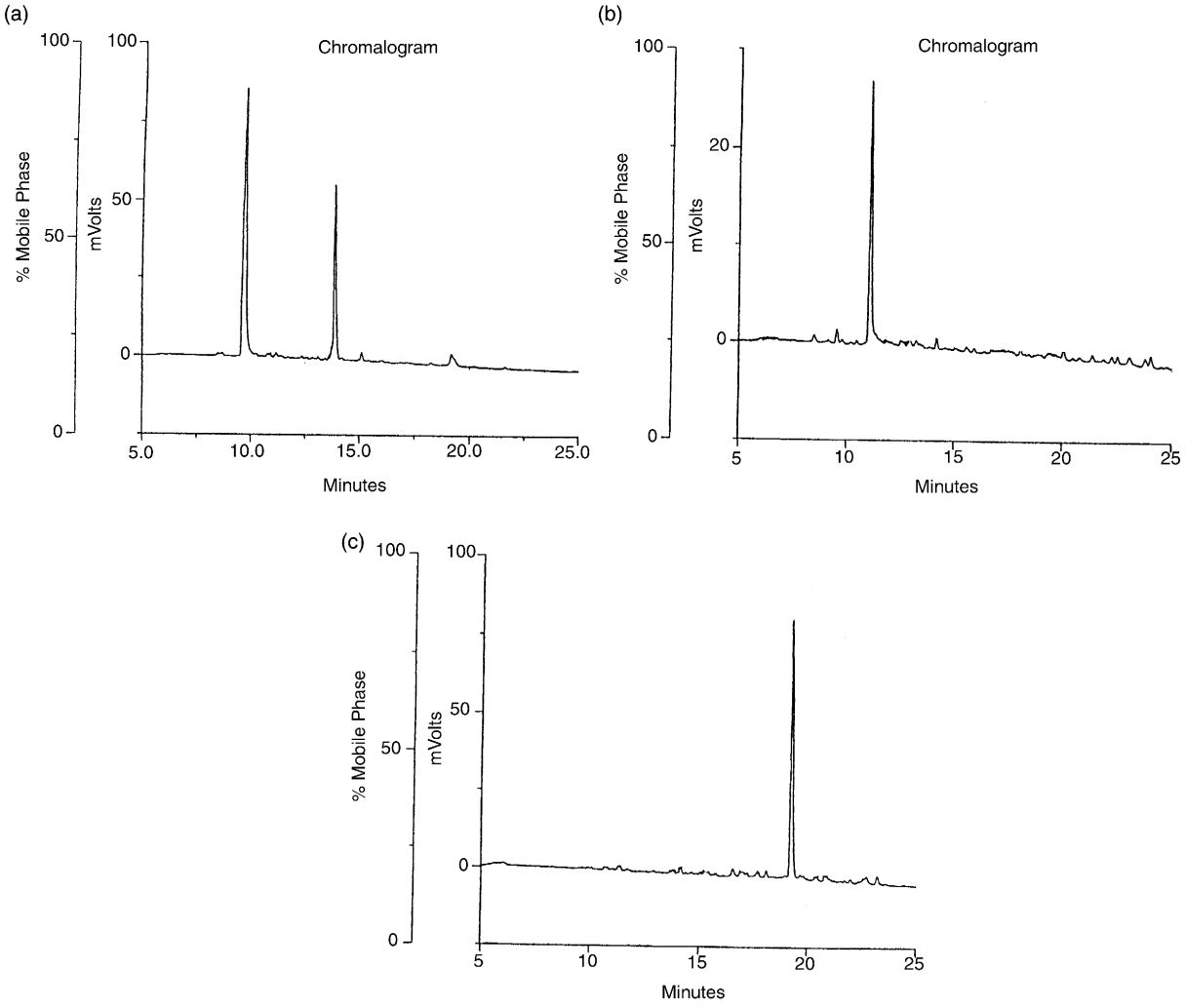
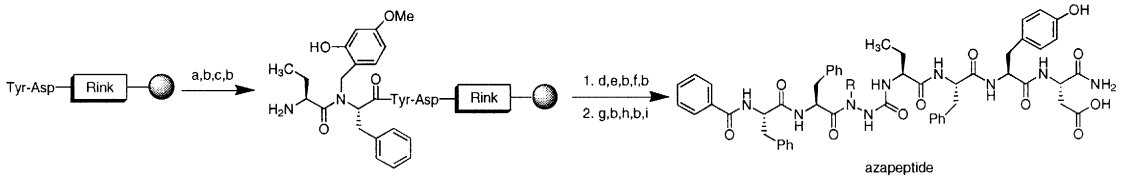


Fig. 1.

The synthesis was repeated (Scheme 2) except that $Fmoc_2$(Hmb)Phe-OPfp was utilized. A purity check at the pentapeptide stage showed only a single species on RPHPLC (Fig. 1b) that gave the anticipated ESMS analysis 1 (t_R=9.0 min; MH+=585.8). There was no evidence of the hydantoin side product.

Continuation of the synthesis to the target heptapeptide proceeded smoothly and yielded the desired aza-peptide in excellent purity (Fig. 1c) (t_R=19.5 min; MH+=985.2). Thus, the simple application of reversible amide bond substitution has been shown to completely suppress the formation of hydantoin derived side products in the synthesis of aza-peptides.



Scheme 2. Synthesis of aza-peptide with incorporation of Phe(Hmb). Reagents and conditions: (a) Bis-Fmoc-Phe(Hmb)-OPfp/HOBt, DMF, 2 h; (b) 20% piperidine/DMF; (c) Fmoc-Abu symmetrical anhydride, 16 h; (d) 2,4-dinitrophenyl carbonate, DMF, 2 h; (e) Fmoc-azaGly/Lys, DMF, 16 h; (f) Fmoc-Phe-OH/HBTU/HOBt/NMM, DMF, 2×3 h; (g) Fmoc-Phe-OPfp/HOBt, DMF, 1 h; (h) Benzoic anhydride, DMF, 2 h; (i) TFA/TES

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