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A new tag reagent for efficient capping and easy separation of deletion peptides

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Abstract—*N*-(Biphenyl-4-carbonyl)-L-proline was tested as a new capping reagent. The reagent was successfully used in the synthesis of the classic difficult sequence, acyl carrier protein (65–74). Analytical RP-HPLC with on line ESI-MS detection was used to analyse the unpurified cleavage products. The crude peptide was eluted as a single peak at 16.7 min. In the ESI mass spectrum of this peak a major ion is observed at m/z 1063.9 that corresponds to the complete protonated molecular ion $[M+H]^+$. The molecular mass of the truncated *des*-Val^{65} nonapeptide contaminant is not present. Due to both its reactivity and its lipophilic nature, *N*-(biphenyl-4-carbonyl)-L-proline can be used as a tag for the deletion peptides in the sequence dependent difficult synthesis and in the automated synthesis of a longer peptide, facilitating the purification of the target peptide. © 2002 Elsevier Science Ltd. All rights reserved.

The synthesis of a great variety of peptides is now possible due to rapid progress in solid-phase methodology. Despite the methodological improvements, solid phase peptide synthesis is still accompanied by various problems that decrease the yields and interfere with the purification of the desired products. One of the major difficulties in the synthesis of large peptides is due to incomplete aminoacylation during the coupling step. In order to overcome this problem, recoupling is usually recommended. If unreacted sites still remain, then capping is necessary, using an appropriate terminating agent.

The terminating agent is used to permanently block any N-terminal amino group, which has not been acylated in the coupling step. Merrifield[1] described the use of a mixture of acetic anhydride and triethylamine in DMF as terminating agent, in the solid phase synthesis of H-LeuAlaGlyValOH.

Generally, a terminating reagent must (a) have a high reactivity towards the amino group being terminated, yet no effect on the peptide being synthesised, (b) form a covalent bond with the amino group being terminated, that will be stable to all the reaction conditions used in the subsequent synthetic procedure and (c) facilitate separation, with the commonly used techniques, from the desired peptide being synthesised.

Many reagents have been used as terminating agents, including 3-nitrophthalic anhydride,[2] methanesulfenyl chloride,[3] stearoyl chloride,[4] *N*-acetyl imidazole,[5] and *N*-(2-chlorobenzyloxycarbonyloxy)succinimide.[6] Acetic anhydride–triethylamine and *N*-acetyl imidazole are the most popular capping reagents but their use in the synthesis of longer peptides in combination with an automated peptide synthesiser has not proved to be altogether satisfactory.

Moreover, the above reagents fulfil criteria (a) and (b), but not (c), since the terminated deletion peptides usually elute very close to the desired product. Therefore, purification using RP-HPLC is a laborious task involving considerable losses of the target peptide.

To overcome this problem several lipophilic or affinity probes were introduced to the resin-bound peptide in the last coupling step, facilitating the separation of the product from the accompanying impurities.[6,7] However, in order to obtain the native molecule, an additional step is required for probe removal, after cleavage from the resin and purification of the derivatised peptide.

An alternative route is to render the deletion peptides lipophilic by using a suitable terminating agent, which

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enables them to be strongly retained by RP-HPLC chromatography and facilitates the purification of the target peptide. To this end we examined several reagents and we found that when 2-naphthoic and 4-biphenylcarboxylic acids were used to terminate a peptide, they rendered it lipophilic enough to elute far away from the peptide been synthesised. Unfortunately all attempts to make these reagents reactive enough to terminate the remaining amino groups failed, so the criterion (a) was not fulfilled. In order to increase the reactivity, several amino acid derivatives were synthesised. *N*-(Biphenyl-4-carbonyl)-L-proline was proved to be the most efficient (Fig. 1).[8]

The solid-phase synthesis of the classic difficult sequence, acyl carrier protein (65–74)[9] was selected as the model system for the evaluation of the suggested capping reagent. The synthesis was conducted utilising a reaction vessel clamped to a manually operated mechanical shaker. The trityl-type resin Fmoc-Gly-(2ClTrt)-CO-$NHCH_2$-PS-DVB was used[10] (0.3 mmol g^{-1}). In all steps DMF was the solvent of choice. Stepwise elongation of the resin bound-peptide proceeded using 4 equiv. of each amino acid activated by the HOBt/DIC method for 1 h. After coupling, terminations of the free amino groups were done with the preactivated *N*-(biphenyl-4-carbonyl)-L-proline using HOBt/DIC/DMAP (0.25 M). Piperidine/DMF 20% solution washes (4 min intervals) were used for the removal of the Fmoc protecting groups until the completion of the deprotection was confirmed by UV absorption at 301 nm. After the completion of the ten coupling cycles, the peptide was cleaved from the support using TFA/H_2O (90:10). Analytical RP-HPLC (Waters Alliance equipped with a Merck LiChrosper 100 RP-18 analytical column) with on line ESI-MS detection (AQA Navigator, Finnigan) was used to analyse the unpurified cleavage products. As shown in Fig. 2, the chromatogram of the crude product contains a major peak eluted at 16.7 min and a minor one eluted at 27.5 min. In the ESI mass spectrum of the first peak, a major ion was observed at m/z 1063.9 that corresponds to the complete protonated molecular ion $[M+H]^+$. The molecular mass of the truncated *des*-Val^{65} nonapeptide (MW = 963) contaminant is not present.

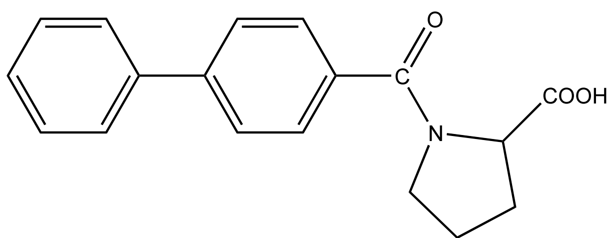

Figure 1. *N*-(Biphenyl-4-carbonyl)-L-proline.

In conclusion, we propose the use of *N*-(biphenyl-4-carbonyl)-L-proline as a new capping reagent. Due to both its reactivity and its lipophilic nature, *N*-(biphenyl-4-carbonyl)-L-proline can be used as a tag for the deletion peptides in the sequence dependent difficult synthesis or in the automated synthesis of a longer peptide, facilitating the purification of the target peptide. The reagent was successfully used in the synthesis of the classic difficult sequence, acyl carrier protein (65–74). Further investigation of the utility of *N*-(biphenyl-4-carbonyl)-L-proline in notoriously difficult cases of peptide synthesis will follow.

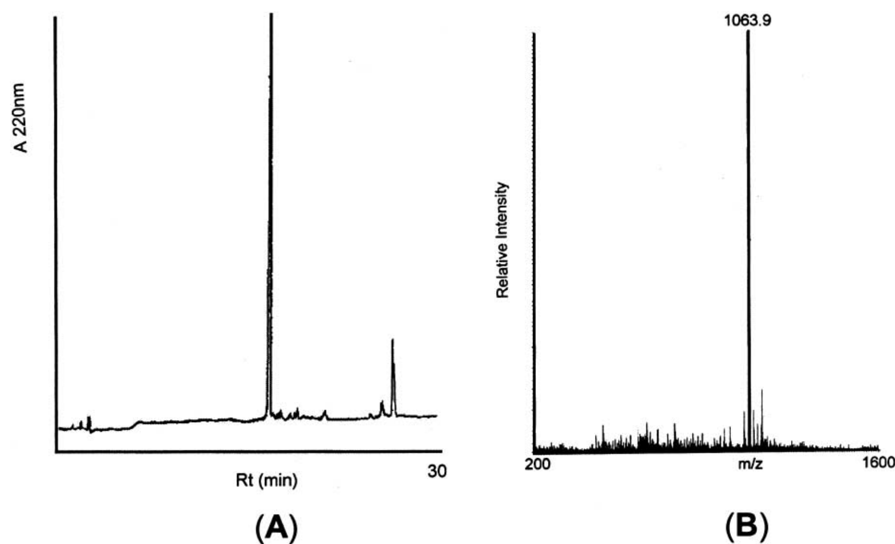

Figure 2. (A) Analytical RP-HPLC and (B) ESI-MS analysis of unpurified cleavage products. For HPLC analysis, elution of injected material was by a gradient of 0–67% B over 30 min (solvent A, 0.1% TFA in water; solvent B, 0.09 TFA in 90% acetonitrile/10% water). For ESI mass spectra, hot nitrogen gas (Dominic-Hunter UHPLCMS-10) was used for desolvation. In the electrospray source the spray needle was grounded. Voltages of +4.5, +3.5 and +3.0 kV were applied to the capillary, plate and cylindrical electrodes, respectively.

Acknowledgements

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8. Synthesis of *N*-(biphenyl-4-carbonyl)-L-proline: L-proline (18 mmol) in sodium hydroxide 1 M (40 mL) was cooled to 0°C. Biphenyl-4-carbonyl chloride (Aldrich, 18 mmol) in dichloromethane (15 mL) was added over 30 min with stirring. Stirring was continued for 2 h at room temperature and then water (40 mL) was added. The aqueous phase was separated and acidified to pH 2 with sulphuric acid 1.5 M. The resulting precipitate was separated by filtration, washed with water and dried. Recrystallisation from ethyl acetate/petroleum ether (40/60) afforded 4.5 g of solid product. Yield 80%. Mp 188–190°C. ^{1}H NMR ($CDCl_3$): δ 1.8–2.2 (2H, m, CH_2), 2.6–2.8 (2H, m, CH_2), 3.4–3.6 (2H, m, CH_2), 4.8 (1H, t, CH), 7.4–7.6 (9H, m, CH_{Ar}).
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