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Boc SPPS of Two Hydrophobic Peptides using a "Solubilising Tail" Strategy: Dodecaalanine and Chemotactic Protein 10^{42-55}

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Abstract: The solid phase syntheses of the hydrophobic peptides dodecaalanine and chemotactic protein-10^{42-55} were achieved using a "solubilising tail" strategy. Peptide constructs of the form H-hydrophobic peptide-glycolamide ester-(Gly-Arg)$_4$-Gly-OH were synthesised by Boc SPPS. The peptide-constructs were soluble in water, which allowed their purification by HPLC. Treatment of the purified H-hydrophobic peptide-glycolamide ester-(Gly-Arg)$_4$Gly-OH constructs with mild aqueous base gave the pure hydrophobic peptides. Copyright © 1996 Elsevier Science Ltd

The purification and characterisation of hydrophobic synthetic peptides is often difficult due to their poor solubility in the solvents commonly used for HPLC purification. We report here a simple Boc SPPS strategy for rendering hydrophobic peptides soluble for subsequent purification and characterisation. The strategy is illustrated by syntheses of the hydrophobic peptides dodecaalanine (1) and chemotactic protein 10^{42-55} H-(CP-10^{42-55})-OH (2). Our synthetic strategy, using dodecaalanine as an example, is shown in Scheme 1.

Scheme 1

Boc-(Gly-Arg(Tos))$_4$-Gly-PAM-resin

↓ i-iv

Boc-Ala-O-CH_2-CO-(Gly-Arg(Tos))$_4$-Gly-PAM-resin

↓ v,i

H-Ala_{12}-O-CH_2-CO-(Gly-Arg(Tos))$_4$-Gly-PAM-resin

↓ vi

H-Ala_{12}-O-CH_2-CO-(Gly-Arg)$_4$-Gly-OH

↓ vii

H-Ala_{12}-OH + HO-CH_2-CO-(Gly-Arg)$_4$-Gly-OH

i: TFA, ii: 20% DIEA/DMF, iii: $(BrCH_2CO)_2O$, iv: Boc-Ala Cs, v: 11 cycles Boc SPPS, vi: HF:p-cresol, vii: OH^-

Boc-(Gly-Arg(Tos))$_4$-Gly-PAM-resin was synthesised using Boc methodology with *in-situ* neutralisation (3,4). Boc-Ala was anchored to the peptide-resin via an acid-stable, base-labile glycolamide ester (5,6). Subsequent SPPS (7) gave H-Ala_{12}-glycolamide ester-(Gly-Arg(Tos))$_4$-Gly-PAM-resin, which was cleaved with HF (8) to give an H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH peptide construct. The crude, ether precipitated H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH was initially insoluble in water. It was therefore dissolved in TFA, diluted with water, and lyophilised. After lyophilisation the peptide-construct dissolved readily in water. In a similar manner H-(CP-10^{42-55})-glycolamide ester-(GR)$_4$-G-OH [H-PQFVQNINIENLFR-glycolamide ester-(GR)$_4$-G-OH] was synthesised. Purified H(CP-10^{42-55})-OH (2) has not previously been obtainable in high purity owing to its tendency to form intermolecular aggregates.

An HPLC chromatogram of crude H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH is shown in Figure 1a (9). Analysis of peak 1 by electrospray mass spectroscopy (ES-MS) showed a mass of 1767 Da (calc. for H-Ala_{11}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH: 1767 Da). Peak 2 had a mass of 1838.1 Da, corresponding to that expected for H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH (calc. 1839.0 Da). The des-Ala construct was resolved cleanly by HPLC which allowed pure H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH to be isolated (10). H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH was treated with mild base to cleave the glycolamide ester (11). A white precipitate formed. ES-MS analysis of the supernatant showed a single ion of mass 986.7 Da, which corresponded to HO-CH_2-CO-(Gly-Arg)$_4$-Gly-OH (calc. 986.5 Da) derived by cleavage of the glycolamide ester. No ions corresponding to uncleaved H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH or H-Ala_{12}-OH were found in the filtrate. ES-MS analysis of the precipitate (Figure 1b) confirmed the presence of an H-Ala_{12}-OH pseudomolecular ion MH^+ with mass 871.6 Da (calc. for H-Ala_{12}-OH: 871.5 Da).

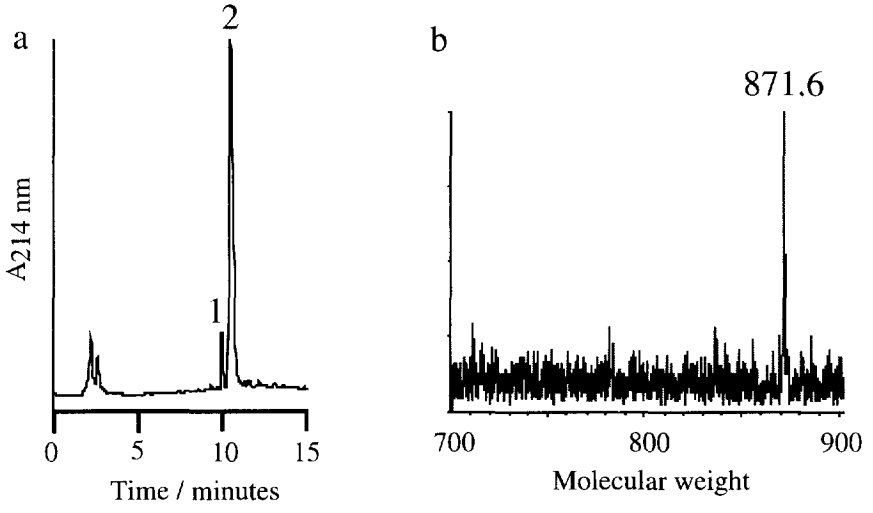

Figure 1. a) HPLC chromatogram of crude cleaved H-Ala_{12}-glycolamide ester-(Gly-Arg)$_4$-Gly-OH, (peaks 1 and 2 identified in text); b) ES-MS spectrum of H-Ala_{12} -OH after base cleavage.

This simple strategy allowed a straightforward synthesis of dodecaalanine, a notoriously difficult peptide (1). A control synthesis of H-Ala_8-Gly-PAM-resin showed an onset of difficult couplings beginning at alanine residue 6 (12). Our strategy placed the first difficult Ala, usually the sixth (1a), 15-16 residues out from the peptide-resin. While it has been noted that difficult couplings in SPPS usually occur within the first 5-15 residues of a synthesis (13), the molecular mechanism for the dramatic improvement in the synthesis of dodecaalanine by spacing the peptide away from the resin is unclear.

Figure 2a is an HPLC chromatogram of crude cleaved H-(CP-$10^{42\text{-}55}$)-glycolamide ester-(GR)$_4$-G-OH. ES-MS of the major peak from Figure 2a gave a mass of 2699 Da (calc. 2700 Da). HPLC purified H-(CP-$10^{42\text{-}55}$)-glycolamide ester-(GR)$_4$-G-OH was also cleaved with base (11). ES-MS analysis of the precipitate (Figure 2b) showed the expected mass for H-(CP-$10^{42\text{-}55}$)-OH (calc. 1732.0 Da, found 1731.5 Da).

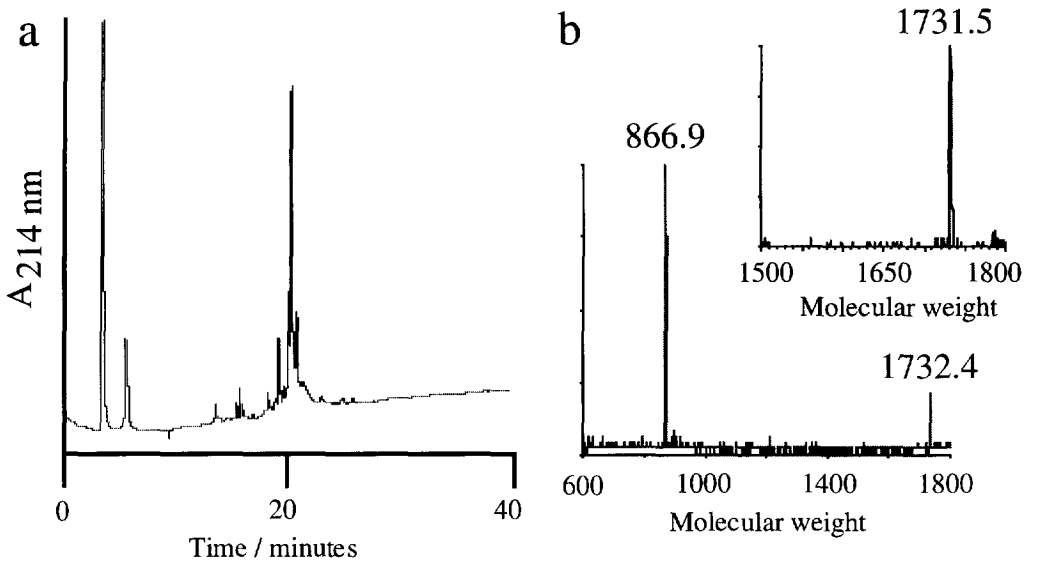

Figure 2. a) HPLC chromatogram of crude cleaved H-(CP-10^{42-55})-glycolamide ester-$(GR)_4$-G-OH; b) ES-MS spectrum of H-(CP-10^{42-55})-OH after base cleavage (a reconstructed mass spectrum is inset).

In summary, we have demonstrated a simple Boc SPPS procedure which links hydrophobic peptides to a solubilising peptide tail via an acid-stable base-labile glycolamide ester. The resulting water-soluble peptide constructs were purified readily using standard HPLC methods. The positive charges on the tail peptide aided in the characterisation of the peptide constructs by ES-MS. The glycolamide ester was stable to all conditions of synthesis and purification, but was cleaved with mild aqueous base to give the hydrophobic peptides as precipitates. ES-MS analysis of the base-cleaved hydrophobic peptides showed them to be of good purity. We expect that this procedure should be applicable to the synthesis of many other hydrophobic peptides.

By suitable modification this basic strategy should be applicable to Fmoc SPPS of hydrophobic peptides, and also to the synthesis and purification of protected peptide segments. The facile synthesis of the difficult peptide dodecaalanine showed that the strategy may prove advantageous in the synthesis of other difficult peptides. Finally, the amino acids of the tail segment may be chosen to modify the HPLC properties of the attached target peptide in any desired manner. As a simple example, hydrophobic amino acids could be used to retard the elution of extremely hydrophilic synthetic peptides on HPLC in order to aid in their purification.

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4. Boc-Gly-PAM polystyrene resin (0.80 mmole/g, ABI, Foster City, CA) was used to synthesise Boc-(Gly-Arg(Tos))$_4$Gly-PAM-resin on a 1 mmole scale. Boc-L-amino acids (2eq) were activated with HBTU and coupled for 10 minutes (average coupling by ninhydrin assay: 99.8%, all couplings performed once) using the *in-situ* neutralisation method described in Reference 3.
5. Boc-(Gly-Arg(Tos))$_4$Gly-PAM-resin was treated with TFA (2 x 1 min), washed with DMF, neutralised with 20% DIEA/DMF, washed with DMF, and reacted with 1 mmole of the anhydride of bromoacetic acid for 10 minutes. After washing with DMF the N-bromoacetyl-(Gly-Arg(Tos))$_4$-Gly-PAM-resin was reacted with Boc-Ala cesium salt (1 mmole in 5 ml DMF) for 14 hours. Displacement of bromine by Boc-Ala was quantitative: ES-MS analysis (performed using a Perkin Elmer-Sciex API III instrument operated in positive ion mode) of H-Ala$_6$-glycolamide ester-(Gly-Arg)$_4$-Gly-OH, cleaved at an intermediate stage of the synthesis of dodecaalanine, showed that no peptides attributable to incomplete displacement of bromine by Boc-Ala cesium salt were present.
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7. Boc-Ala (2 mmole, 6 eq) was coupled to 0.33 mmole of Boc-Ala-glycolamide ester-(Gly-Arg(Tos))$_4$-Gly-PAM-resin for 10 minutes using the *in-situ* neutralisation method described in Reference 3. All couplings were performed once. The average stepwise Ala coupling (ninhydrin assay) was 99.7%.
8. Dry H-Ala$_{12}$-glycolamide ester-(Gly-Arg(Tos))$_4$-Gly-PAM-resin was cleaved with HF:p-cresol (9:1 v:v) at 0°C for one hour. HF was removed *in-vacuo* and the peptide construct was precipitated with ether. The water-insoluble precipitate was dissolved in 3 ml TFA, diluted with water, and lyophilised. The peptide-construct dissolved readily in water after lyophilisation.
9. HPLC analyses: Vydac (Hesperia, CA) C4 4.6 x 150 mm column, 5-85%B over 40 minutes at 1 ml/min. Solvent A: 0.1% TFA; Solvent B: 0.1% TFA, 10% water, 90% acetonitrile.
10. Preparative HPLC: Vydac C4 25 x 250 mm column, 0-60%B over 60 minutes at 8 ml/min (solvents as above).
11. H-Ala$_{12}$-glycolamide ester-(Gly-Arg)$_4$-Gly-OH and H-(CP-10$^{42-55}$)-glycolamide ester-(GR)$_4$-G-OH (2.0 mg in 900 µl water, a clear solution) were treated with 100 µl of triethylamine for 10 minutes (pH 12.2). The resultant peptide precipitates were collected by centrifugation. After washing six times with 1 ml water the precipitates were shaken with a mixture of 20 µl TFA and 980 µl acetic acid. The suspensions of peptides which resulted were filtered for analysis by ES-MS.
12. A control synthesis of H-Ala$_8$-Gly-resin (0.33 mmole scale, 2 mmole (6 eq) Boc-Ala per coupling), using the same batch of Boc-Gly-PAM resin and SPPS protocols used for the previous syntheses, showed coupling yields (ninhydrin assay) of: Ala$_{1-4}$ 99.96% (average), Ala$_5$ 99.48%, Ala$_6$ 98.80%, Ala$_7$ 98.60%, Ala$_8$ 97.54%.
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