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Cleavage of Resin-Bound Peptide Esters with Ammonia Vapour. Simultaneous Multiple Synthesis of Peptide Amides.

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Abstract: *Peptides prepared on Pepsyn KB resin were cleaved with ammonia/tetrahydrofuran vapour and then eluted from the resin in a two step process. The method is a general one, applicable to simultaneous multiple peptide synthesis on resin supports.*

Simultaneous multiple peptide synthesis (SMPS)[1-6] on resin supports[4,5] is generally limited by the need for individual peptide handling at the side-chain deprotection and cleavage stages of preparation. Even the use of specialized apparatus for multiple side-chain deprotection/cleavage[7] only partially addresses the problem as the cleaved peptides must be freed from potentially toxic scavengers prior to use in bio-screening work. This applies equally to peptides prepared by either the Boc or Fmoc strategies. Multiple peptide separation has been explored, but the approach can only deal with small numbers of peptides, and only if these are separable by HPLC[8].

By interposing a washing protocol in between side-chain deprotection (SCD) and cleavage, by-products of SCD can be removed from the target peptides prior to cleavage[3,9] Two step cleavage strategies are better suited to parallel handling, hence SMPS, particularly when cleaved peptides are liberated into solutions which may only need to be evaporated in order to afford peptides of acceptable purity.

SMPS by the multipin method is well suited to the simultaneous preparation of hundreds to thousands of discrete peptides[1-3] This is due to the modular nature of the system and to the use of two step cleavage chemistries which avoid the need for individual peptide handling[3,9,10]. Peptide amides have been prepared by treating pin-bound peptide esters with ammonia/THF vapour[10]. The cleaved peptides are eluted from the hydrophilic graft polymer pin surface with a solvent of choice. As SMPS on beaded resin supports would also benefit by a reduction in peptide handling, the vapour-phase ammonolysis technique has been applied to resin-bound peptides **1** prepared on polydimethylacrylamide-Kieselguhr resin[11,12] (Pepsyn KB, MilliGen) as outline in Fig. 1.

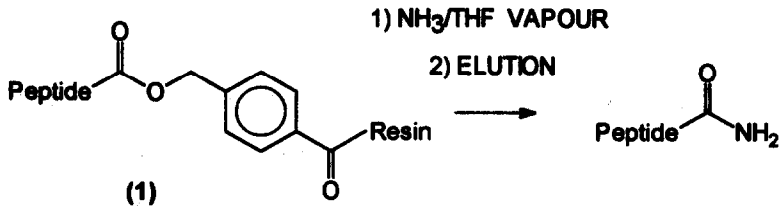

Fig. 1. Cleavage of peptides from Pepsyn KB resin with NH_3/THF vapour.

Peptides 2 and 3 (Table 1) were assembled on Pepsyn KB resin by an Fmoc synthesis protocol. Following SCD, the resins were washed and dried. The resin-bound peptides were then stored over a 30% solution of ammonia in THF in a laboratory desiccator for 20 h[10]. The peptides were then eluted from the resin with AcOH/MeCN/H_2O (3:4:3) and these solutions evaporated. The resulting unpurified peptides 2 and 3 were examined by reverse phase HPLC (Fig. 2) and ion spray MS (Table 1, Fig. 3). MS confirmed the identity of the products, with the expected $[M+H]^+$ signals being observed. Amino acid analysis[13,14] was performed on the resins before and after cleavage, and on the cleaved peptide solutions. As shown in Table I, acceptable yields were obtained.

Studies[15] on model peptides prepared by the multipin method demonstrated that vapour phase ammonolysis cleaved most oxymethylbenzamido esters[12] with 70-90% efficiency. However, esters of Ile and Val cleaved with less than 10% efficiency[15]. In this study, a similar result was obtained when a Pepsyn KB resin-bound C-terminal Ile peptide (Ile-Tyr-Ser-Tyr-Phe-Pro-Ser-Val-Ile) was treated with ammonia/THF vapour to give target peptide amide 4 in 5% yield. In the case of peptides prepared on pins[15], greatly improved yields (ca. 90%) were obtained when peptides with Ile or Val C-termini were assembled on the relatively labile glycolamido ester handle[16].

Table 1. Test Peptides Cleaved From Pepsyn KB Resin with NH_3/THF Vapour.

	Peptide Sequence	Yield μmol (%)	$[M+H]^+$ Ion Spray MS Observed (expected)
2	Ac-Ser-Thr-Asp-Asp-Tyr-Ala-Ser-Phe-Ser-Arg-Ala-Leu-NH_2	7.4 (82%)	1374 (1373.6)
3	H-Lys-Glu-Gln-Leu-Ala-Lys-Ser-Glu-Glu-Gln-Leu-Ala-Lys--Ser-Glu-Glu-Gln-Leu-Ala-Lys-Ser-Glu-Glu-Gln-Leu-Ala--Lys-Ser-Glu-Glu-Leu-Ala-NH_2	4.4 (59%)	3730 (3728.9)

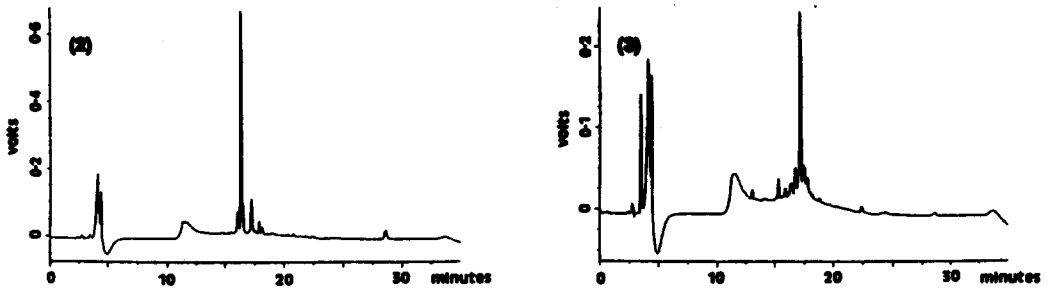

Fig. 2. HPLC traces of peptides 2 and 3. Detection at 214 nm. Solvent A: H_2O (0.1% TFA); Solvent B: 60% MeCN (0.1% TFA). Linear gradient A to B (5 min to 20 min). Column: Merck LiChrosphere^R 100 RP-18, 5 μm.

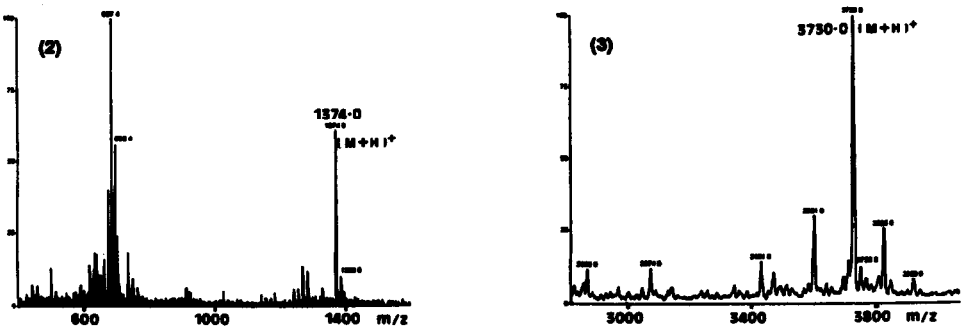

Fig. 3. Ion spray mass spectra of peptides 2 and 3.

When peptides 2, 3 and 4 were cleaved with the vapour from a 30% ammonia/MeOH, the peptide products were found to be contaminated with their corresponding methyl esters. Peptide 4 was particularly prone to this problem. Ester contamination was also encountered with solution phase ammonolysis. Even when 4 was cleaved in 30% ammonia/MeOH solution[11] (18 h) methyl ester was the major product. In contrast, ammonia/THF only gave amide products.

Vapour phase ammonolysis is a technique intended for the simultaneous cleavage of large numbers of discrete peptide-functionalized supports. The results of this study indicate that the method is applicable to resin-based SMPS strategies. As most of the emphasis in SMPS development has focussed on simultaneous assembly strategies, there is a need to devise complementary multiple cleavage strategies. The approach presented here addresses the shortfall in post-synthesis SMPS techniques for the mass production of peptide amides.

Peptides were synthesized on a MilliGen 9050 Peptide Synthesizer using standard

cycles and pentafluorophenol esters of Fmoc-protected amino acids. Side-chain protection was as follows: Arg(Pmc), Asp(OBu^t), Glu(OBu^t), Lys(Boc), Ser(Bu^t), Thr(Bu^t), and Tyr(Bu^t). The resin-bound peptides were side-chain deprotected with TFA/H_2O (95:5) (90 min) and washed sequentially with CH_2Cl_2, DMF and CH_2Cl_2 (2x), and air dried. Open glass vials containing dried resin (130 mg/vial) were placed in a desiccator together with NH_3/THF (3:7, 50 mL), which had been cooled to -78°C. The desiccator was clamped shut, evacuated (10 sec) and then left to stand at 20°C for 20 h. Cleaved peptides were eluted from the resin with AcOH/MeCN/H_2O (3:4:3).

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14. Amino acid ratios with expected values given in paretheses. Note: Gln analyses as Glu. Peptide **2** [Ala: 5.3 (5); Glu: 13.7 (14); Leu: 5.4 (5); Lys: 4.3 (5); Ser: 4.2 (4)]. Peptide **3** [Ala: 2.1 (2); Arg: 1.0 (1); Asp: 2.0 (2); Leu: 1.1 (1); Phe: 1.0 (1); Ser: 2.8 (3); Thr: 0.9 (1); Tyr: 1.0 (1)]. Peptide **4** [Ile: 1.7 (2); Phe: 1.1 (1); Pro: 1.0 (1); Ser: 2.2 (2); Tyr: 2.3 (2); Val: 0.7 (1)].
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