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MULTI-DETACHABLE RESIN SUPPORTS FOR SOLID PHASE FRAGMENT SYNTHESIS

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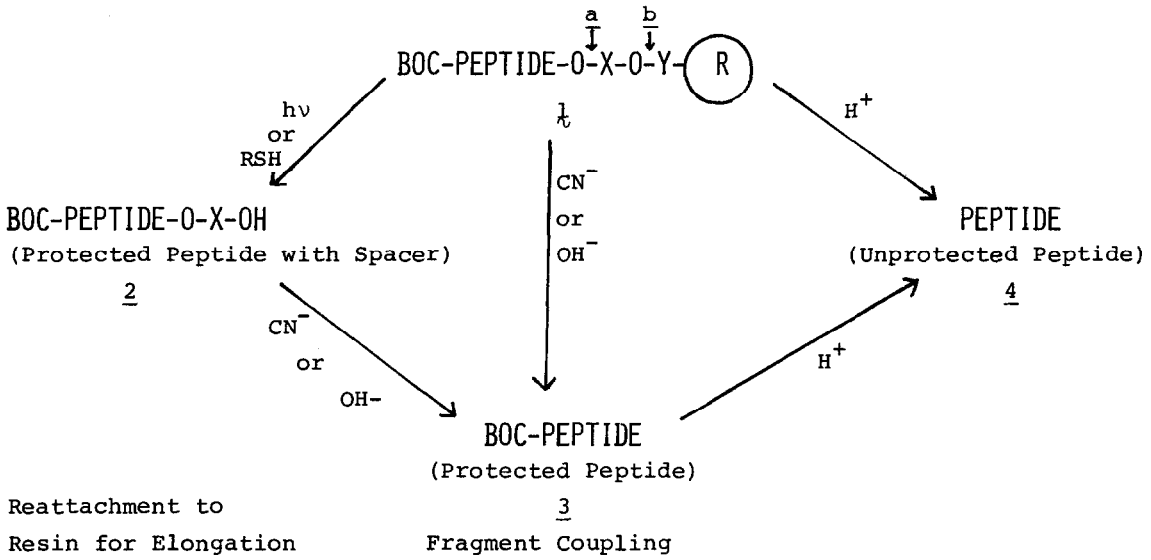
Summary: Two multi-detachable resin supports, each containing orthogonally removable benzyl and photolabile esters were prepared and applied to the synthesis of Leu-enkephalin and angiotensin. These resins, called Pop and Pon, could be cleaved by acid to give the free peptide, by nucleophiles to give protected peptides, and by photolysis to give the protected peptide still esterified to an oxymethylphenylacetic acid handle. Peptides of the latter type were readily attached to an aminomethyl-resin for further peptide elongation.

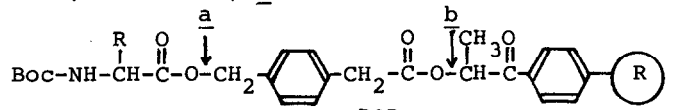
The merits of the fragment approach to the synthesis of polypeptides on solid supports have been well demonstrated[1]. Such an approach allows the purification and characterization of intermediates and minimizes the problems of insolubility often found when large protected fragments are coupled in solution. In our efforts to extend this general strategy to include the synthesis of the intermediate fragments by solid phase methods, we have encountered three main difficulties: (1) losses of α-amino groups during the first two cycles of synthesis on phenacyl-resin supports[2,3], (2) dependency on the C-terminal amino acid residue of the photolytic cleavage from α-methyl-phenacyl[4] and o-nitrobenzyl ester-resins[5]; (3) lack of satisfactory protocols to reattach the purified peptides to resin supports for extension of the chain by fragment couplings. To overcome these problems we have designed new types of resin supports, which provide more versatility than previous ones. They are termed multi-detachable resins because they can be cleaved at more than one site by more than one reagent to give peptides containing different degrees of protection.

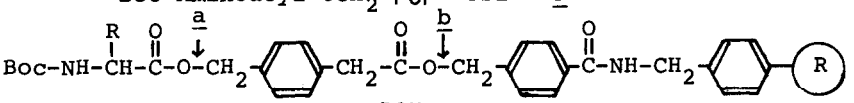
A generalized plan for the design of a multi-detachable resin is shown in Scheme 1. It illustrates a system in which a protected peptide, Boc-peptide-O-X-O-Y-resin 1, is anchored to a solid support through a linkage containing two selectively cleavable (orthogonal) ester bonds, peptide-O-X and X-O-Y-resin, separated by a spacer, X. The compound is designed so that it can be cleaved at bond b to give a protected peptide, 2, still esterified to the spacer, or at bond a to give a protected peptide, 3, containing its carboxyl group free. Under other conditions, cleavage at bond a can cause complete deprotection and give directly the free peptide. To achieve the full flexibility of such a scheme it is also important to be able to convert 2 to 3 and 3 to 4. Two resin supports have now been prepared which meet these requirements.



Scheme 1. A design of Multi-detachable Resin

The new multidetachable resins, containing a protected C-terminal amino acid, are Boc-aminoacyl-2-[4-(oxymethyl)phenylacetoxy]-propionyl-resin, Pop-resin, 5, and Boc-aminoacyl-4-[4-(oxymethyl)phenylacetoxymethyl]-3-nitro-benzamidomethyl-resin, Pon-resin, 6.

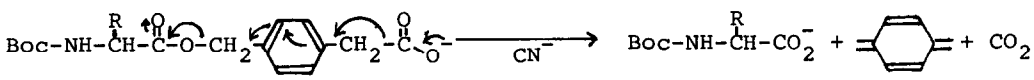




Boc-Aminoacyl-OCH_2-Pop- or Pon-resin were best prepared by esterification of Boc-aminoacyl-oxymethylphenylacetic acid[6,7] to either 2-(bromomethyl)-propionyl-resin[4] or 3-nitro-4-bromomethylbenzamidomethyl-resin[5] with potassium fluoride in N-methylpyrrolidone as solvent at 25 to 50° C for 8 to 24 h[8].

These compounds, 5 and 6, contain an acid-labile, hydrogenolyzable benzyl ester at a and an ester at b that can be cleaved by photolysis or by a variety of nucleophiles. Thus, the two cleavages can be orthogonal. The spacer, oxymethylphenylacetic acid (OMPA), imparts special properties to the resin support. First, it provides 35 to 200 times more acid stability to the peptide-O-X bond than is found for the ordinary p-alkylbenzyl esters. Thus, acidolytic loss during deprotection of Boc groups by trifluoroacetic acid was greatly reduced. Furthermore, when X is OMPA, we have found that 2 can be smoothly and selectively transformed to 3 by a decarboxylative 1,6-elimination.

In the presence of unsolvated cyanide ion (Scheme 2) this reaction was 1.5×10^4 faster for Boc-Val-OMPA than for its simple benzyl ester.



Scheme 2. Decarboxylative 1,6-Elimination of Boc-peptide-OMPA

The various cleavages of these multi-detachable resins that we have effected are illustrated with Boc-Val-OCH_2-Pop-resin and Boc-Val-OCH_2-Pon-resin as shown in Table I.

Table I. Orthogonal Cleavages of Multidetachable Resins.

	Cleavage Condition	Cleavage Point	Product	Yields (%)* Pop	Pon
1.	Strong Acid				
	a. HF	a	4, Valine	89	80
	b. methanesulfonic acid	a	4, Valine	88	81
2.	Hydrogenolysis	a	4, Valine (after TFA)	50	56
3.	Nucleophile and Base				
	a. cyanide	b+a	3, Boc-valine	71	-
	b. hydroxide	b+a	3, Boc-valine	71	71
	c. hydrazine	b+a	3, Boc-valine#	92	87
	d. thiophenoxide	b	2, Boc-valyl-OMPA	78	-
4.	Photolysis	b	2, Boc-valyl-OMPA	80	80

*For Boc-Val-OCH_2Pop-resin and Boc-Val-OCH_2-Pon-resin. #As hydrazide

It can be seen that the cleavage yields were generally good, ranging between 50 and 92%. Furthermore, depending on the reagents used, three different products could be obtained (see Scheme 1). Strong acids, HF or methanesulfonic acid, gave the free amino acid, as did catalytic hydrogenolysis Photolysis (>350 nm) or thiophenoxide produced the protected Boc-amino acid-OMPA ester 2. The nucleophiles tetrabutylammonium cyanide in DMF or benzyltrimethylammonium hydroxide (4 equiv, 0.1N) caused cleavage first at bond b to give the OMPA ester, followed by rapid cleavage of bond a to give Boc-amino acid, 3.

The multi-detachable resins, 5 and 6, were shown in several model syntheses to avoid formation of a dihydrooxazinone between the phenacyl carbonyl and the α-amino group of the C-terminal amino acid residue and to overcome the low photolytic cleavage yields with C-terminal residues other than glycine. These problems were both caused previously by direct esterification of the amino acid to the phenacyl group. In the new derivatives, it is the spacer, oxymethyl-phenylacetic acid, that is esterified to the phenacyl group. Since the first amino acid is thus separated from the phenacyl ester, it cannot form the

6-member oxazine ring and it also does not introduce the steric hindrance that caused the low cleavage yields.

The Pop-resin 5 was examined for its use in step-wise solid phase syntheses by the preparation of three model peptides; Leu-Ala-Gly-Val, Leu-enkephalin, and [Val^5]angiotensin. The HF cleavage yields were 91, 85 and 55%, respectively. The crude cleaved products were then shown chromatographically to contain 98.4, 98.5, and 88% of the total material in a single sharp peak, corresponding in each case to the desired target peptide.

Finally, it was demonstrated that products obtained from multidetachable resins 5 and 6 could be reattached to suitable solid supports for further fragment condensations. Thus, Boc-Leu-Ala-Gly-Val-OMPA, obtained by photolysis from a solid phase synthesis on a Pop-resin, was coupled to aminomethyl-resin[9] by dicyclohexylcarbodiimide/hydroxybenzotriazole activation and cleaved by HF to give the free tetrapeptide in 84% overall yield. Similarly, Boc-Leu-enkephalin-OMPA was coupled to the aminomethyl-resin in 95% yield and cleaved by HF in 82% yield. These products are Boc-peptide-oxymethyl-Pam-resins[7] and are expected to be especially suitable for the assembly of larger polypeptides by fragment condensation methods.

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