A New Protecting Group Combination for Solid Phase Synthesis of Protected Peptides

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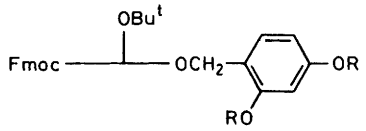
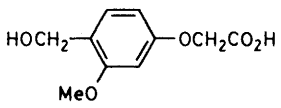
Use of N_α-fluorenylmethoxycarbonylamino-acids in combination with t-butyl-based side-chain protecting groups and a novel dialkoxybenzyl alcohol peptide–resin linkage agent allows solid phase synthesis of protected peptides suitable for use in fragment condensation strategies.

In conventional solid phase peptide synthesis,[1] individual t-butoxycarbonyl protected amino-acids are coupled sequentially to an insoluble gel support, usually chloromethylated polystyrene. Recent variations on this technique include use of polar polyamide supports,[2,3] individual peptide–resin linkage agents adjusted in lability to the needs of the protecting group strategy adopted,[2,4–6] and base-labile fluorenylmethoxycarbonylamino-acids enabling synthesis to be carried out under milder reaction conditions.[4,7] A limiting factor in this stepwise process is the need for near quantitative yields at every deprotection and acylation step to minimise the accumulation of resin-bound by-products. Such by-products may differ from the target sequence by omission of only a single amino-acid residue and thus be difficult or impossible to separate after cleavage from the resin support. An alternative strategy involves the sequential addition of separately prepared and purified protected peptides to the resin. The number of addition and deprotection steps is fewer and impurities arising from peptide omission differ more substantially in properties from the target sequence. They should thus be more easily separable. In the past such solid phase fragment condensation strategies have usually employed classically synthesised fragments,[8,9] largely because the solid phase procedure has not proved easy to adapt to the efficient preparation of protected peptides. Thus much of the advantage of the solid phase method is lost. We describe here a new protecting group combination which offers promise for the solid phase synthesis of such fragments.

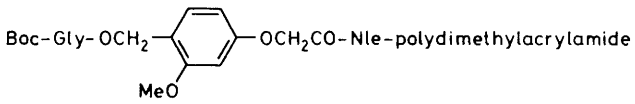
Strict protecting group orthogonality† is not required for the synthesis of amino and side-chain protected fragments. However, amino-protecting groups which are subject to repeated cleavage should not form part of any system of graded lability to the same reagent type. The requirements can be met by the combination depicted as (**1**). Independently cleavable base-labile fluorenylmethoxycarbonyl derivatives are used for amino-protection. Acid-labile t-butyl derivatives are used for



(1)



(2)



(3)

Fmoc-Glu(OBu^t)-Ala-Tyr(Bu^t)-Gly-OH

(4)

Fmoc-Leu-Glu(OBu^t)-Glu(OBu^t)-Glu(OBu^t)-Glu(OBu^t)-OH

(5)

† *i.e.* Combinations in which *N*-terminal, side-chain, and *C*-terminal protecting groups are independently cleavable.[10]

side-chain protection wherever possible,‡ and a more acid-labile 2,4-dialkoxybenzyl ester for carboxy-terminal protection and resin linkage (*cf.* ref. 11). Although the difference in acid-lability between such esters and some t-butyl based side-chain protecting groups is not large, adequate selectivity is possible by making use of the characteristics of the solid phase system (see below).

The new peptide–resin linkage agent (**2**) has been prepared by *C*-formylation of 3-methoxyphenol, conversion to the phenoxyacetic acid, and borohydride reduction of the formyl group.§

The preformed symmetrical anhydride of (**2**) was coupled with norleucylpoly(dimethylacrylamide) resin[3] and the product acylated with Boc-glycine anhydride in the presence of 4-dimethylaminopyridine. The resulting Boc-glycyl resin [(**3**), Gly: Nle = 0.85 : 1] was rapidly cleaved by 1% trifluoroacetic acid in dichloromethane (Gly: Nle = 0.45, 0.20, 0.08, and 0.02 : 1 after 5, 10, 15, and 70 min respectively, corresponding to 47, 77, 90, and 97% detachment). Boc-glycine was identified as the cleavage product by t.l.c. No free glycine was detected. In similar experiments in free solution, side-chain t-butyl derivatives of Fmoc-aspartic acid, glutamic acid, serine, and threonine were relatively stable (<3% cleaved by 1% trifluoroacetic acid in 30 min), but N_{ε}-Boc-lysine (7%) and *O*-t-butyltyrosine (12%) were more labile. Adequate selectivity could be achieved under the conditions pertaining to solid phase synthesis, however. Thus the *O*-t-butyltyrosine-containing peptide [(**4**), 95%] was cleaved from the 2,4-dialkoxybenzyl ester-linked resin by contact with 1% trifluoroacetic acid in dichloromethane for 30 min, the supernatant acid being removed and quenched in a chloroform–water mixture after 2, 5, 10, 15, 20, 25, and 30 min. The crude product contained only 2.4% (h.p.l.c.) of deprotected tyrosine derivative, separable by chromatography on silica and identified by comparison (h.p.l.c., t.l.c.) with an earlier sample,[13] characterised by 400 MHz n.m.r. spectroscopy. Another polar impurity (*ca.* 1.3%) was also present in the crude product.

(**3**) was stable to piperidine under the conditions of Fmoc-group cleavage (20% in dimethylformamide, 144 × 10 min), and to hydroxybenzotriazole (0.2 M in dimethylformamide, 16 h), used as catalyst in activated ester condensations.

Use of the new protecting group and resin linkage combination is illustrated by the preparation of the protected gastrin fragments (**4**) and (**5**). Both were obtained by chain extension of phenylalanylpolydimethylacrylamide resin, firstly with the linkage agent (**2**)¶ and then with the appropriate N_{α}-Fmoc-amino-acid anhydrides, side chains protected with t-butyl groups as necessary. The procedures used were essentially those of our stepwise 'Fmoc-t-butyl' polyamide solid phase method,[4] except that a high loading resin (*ca.* 1.2 mmol g^{-1}) was used. High initial concentrations and reaction rates could then be maintained using only 2.5 fold excesses of acylating species, and there were corresponding substantial economies in other reagent and solvent consumption. Amino-acid assembly was efficient apart from *ca.* 10% loss of peptide from the resin in both cases which may be due to dioxopiperazine formation at the dipeptide stage. Cleavage with 1% trifluoroacetic acid** gave crude (**4**) [95%, found: Glu, 0.98; Ala, 1.03; Tyr, 1.00; Gly, 1.00; t.l.c., single spot R_f 0.58 with traces

‡ Acetamidomethyl and trifluoroacetyl derivatives are preferred for the side chains of cysteine and lysine respectively. Both are stable to the conditions required for cleavage of the protecting groups depicted in (**1**).

§ Full details of this and a new preparation of the established acid labile 4-hydroxymethylphenoxyacetic acid linkage agent have been submitted for publication.[12]

¶ In these experiments, the benzyl alcohol (**2**) was generated immediately beforehand by sodium borohydride reduction of the corresponding aldehyde and isolated by solvent extraction, but the preparation of pure crystalline (**3**) has since been achieved.[12]

** Tryptophan-containing peptides require the presence of scavengers to prevent back reaction of the detached peptide with the reactive resin-bound cation from (**2**) (E. Atherton, unpublished results).

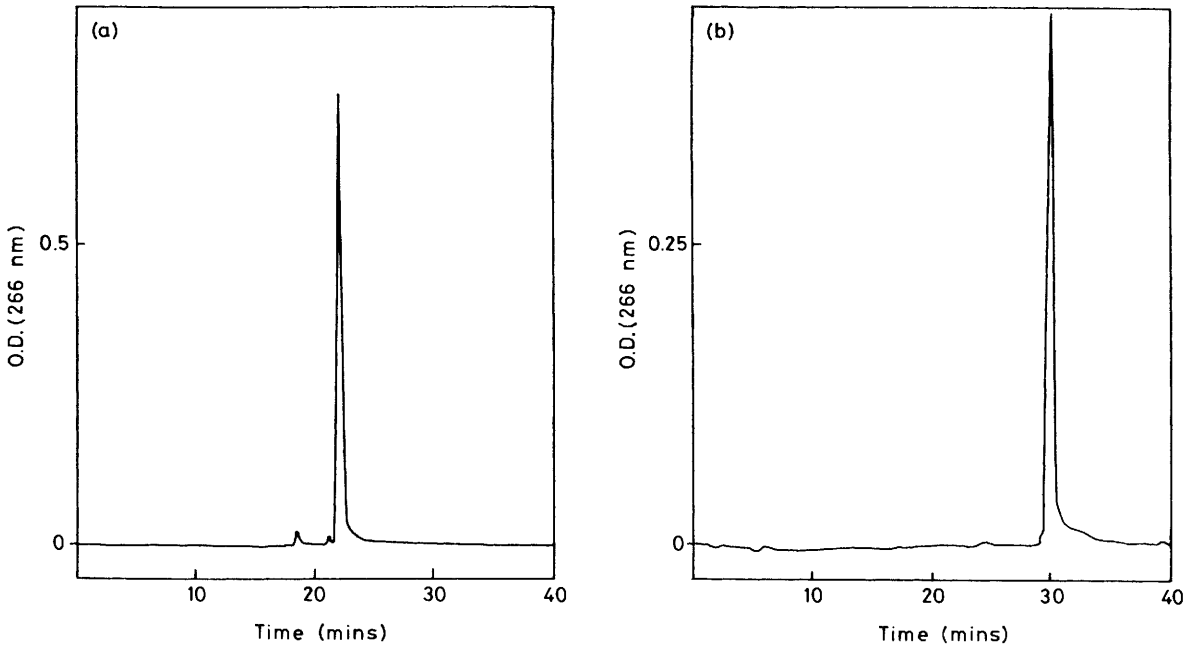


Figure 1. H.p.l.c. of (a) crude protected tetrapeptide (**4**), and (b) crude protected pentapeptide (**5**). Both chromatograms represent the total reaction products from cleavage of the respective peptide-resins with 1% CF_3CO_2H–CH_2Cl_2. H.p.l.c. conditions: μ-Bondapak C18 column eluted with a linear gradient of 18—90% MeCN in 0.01 M aq. NH_4OAc, pH 4.5 over 40 min at 1.5 ml min^{-1}.

at lower R_f in chloroform–methanol–acetic acid, 85:10:5; h.p.l.c., Figure 1(a)], and (**5**) [96%, found: Leu, 1.00; Glu, 4.40; t.l.c., single spot R_f 0.57; h.p.l.c., Figure 1(b)]. Preliminary results on the purification and combination of these protected peptide fragments with resin-bound tetrapeptide (**6**) have been discussed.[13]

The new peptide–resin linkage agent and protecting group combination offers promise for the efficient synthesis of amino and side-chain protected peptide fragments under mild reaction conditions. In our experience, much better cleavage yields are obtained than, for example, by procedures based upon photolysis.[13] Linkage agent (**2**) is equally applicable to stepwise solid phase synthesis using the Fmoc-t-butyl strategy[4] and, of course, to solid supports other than polyamides.

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