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EFFECT OF TRIFLUOROACETIC ACID UPON BOC-AMINOACYL- AND BOC-PEPTIDYL-RESINS. DESCRIPTION OF A NEW POLYMERIC SUPPORT FOR SOLID PHASE PEPTIDE SYNTHESIS.

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The behavior of Boc-aminoacyl- and Boc-peptidyl-OCH_2-resins towards TFA/CH_2Cl_2 solutions has been studied. The results obtained point out the difficulty of finding an optimum TFA concentration which achieves total deprotection without substantial acidolytic losses. The synthesis of a new polymeric support which minimizes this problem is described.

The deprotection of the amino groups of a growing peptide chain before the coupling of a new amino acid residue is a required step in solid phase peptide synthesis. Whereas the yield of the coupling step can easily be assessed by any of the very sensitive methods available [1], there has been for some years a lack of adequate analytical procedures to measure the extent of deprotection, which has thus been traditionally effected under conditions in which the removal of the protecting group could be sufficiently ensured [2]. In the case of the most widely employed t-butyloxycarbonyl (Boc) group, deprotection is carried out with acidic reagents. However, the benzyl ester bond between the peptide and the commonly used oxymethylpoly(styrene-co-divinylbenzene) resin is not completely stable under these conditions and therefore some of the peptide chains esterified to it are lost by acidolysis [3]. In this paper we present a study of the behavior of aminoacyl- and peptidyl-resins against trifluoroacetic acid (TFA), which is currently employed as deprotecting agent.

Boc-glycyloxymethylpoly(styrene-co-divinylbenzene) was prepared by reaction of cesium Boc-glycynate with a Bio-Beads SX1 chloromethylated polymer (0.89 meq Cl/g) in DMF at 50 °C for 16 h [4]. Samples of ca. 500 mg of Boc-Gly-OCH_2-resin were suspended in solutions of TFA in CH_2Cl_2 (20 mL) of different concentrations (15%, 30%, 50% and 100% v/v) in screw-capped tubes and vortex-shaked at room temperature. Aliquots of ca. 50 mg were taken at different reaction times, treated with 10% (v/v) solution of Et_3N in CH_2Cl_2 to interrupt the action of TFA, thoroughly washed with CH_2Cl_2 and dried to constant weight. Evaluation of the free-amine contents after each treatment was made according to Gisin [5]. The following program was employed: i) CH_2Cl_2 (1 x 10 mL); ii) 10% Et_3N in CH_2Cl_2 (3 x 10 mL); iii) CH_2Cl_2 (3 x 10 mL); iv) 0.01 M picric acid in CH_2Cl_2 (2 x 10 mL); v) CH_2Cl_2 (6 x 10 mL); vi) 10% Et_3N in CH_2Cl_2 (3 x 10 mL); vii) CH_2Cl_2 (4 x 10 mL). Spectrophotometric determination of the triethylammonium picrate allows to know the free-amine contents of each aliquot.

As it can be seen in fig. 1, the evolution of the free-amine contents of a Boc-Gly-OCH_2-resin along the treatment time shows the simultaneous occurrence of two processes. On the one hand, a fast deprotection of the amino group provokes a rapid rise of the free-amine contents. On the other hand, acidolysis of the ester bond between the amino acid and the resin produces a slow decrease in the load of the polymer which becomes especially manifest at long treatment times.

The deprotection rate is shown to be very sensitive to the concentration of TFA, but in a rather different sense than it could be expected at first instance, i.e. the process becomes slower as the TFA concentration increases. Thus, whereas with 15% TFA solutions the highest value of the free-amine contents is attained within 30 min of treatment, the maximum value for 100% TFA is not reached until 2 h. A plausible interpretation of this slower deprotection rate may be that the increased TFA concentration provokes a poorer swelling of the resin, which diminishes the accessibility of the reacting sites. Thus, the wet volume of a Bio-Beads SX1 chloromethylated polymer ranges from 12 mL/g in 15% TFA to 3.4 mL/g in 100% TFA. The extent of the acidolysis reaction is illustrated in table 1, the decrease in amine contents being refered to the highest detected value in the whole set of experiments (0.66 meq/g). It can be seen that the resin load diminishes at long treatment times. In contrast with the deprotection reaction, the acidolysis rate increases, though slightly, with TFA concentration.

A further aspect to be considered is the influence upon the deprotection and acidolysis reaction rates of the amino acid side chain and of the eventual attachment of a new Boc-aminoacyl residue to the resin. In this sense, Boc-L-Phe-OCH_2-resin was prepared following a similar procedure than for Boc-Gly-OCH_2-resin and Boc-Gly-Gly-OCH_2-resin was prepared from the previous Boc-Gly-OCH_2-resin following standard solid phase synthesis procedures. Both resins were treated with 30% TFA/CH_2Cl_2 and ther amine contents quantitated as described above for Boc-Gly-OCH_2-resin. Comparative results are shown in fig. 2.

A striking feature of this series of experiments is the slow deprotection rate of Boc-Phe-OCH_2-resin, which seems likely to be due to the steric hindrance of the bulky benzyl side chain of phenylalanine. This anomalous behavior, not previously described in the literature, might be exclusive of those cases when the Boc-phenylalanine residue is at the C-terminal position and in which vicinity to the polymer may presumably play an important role. In any case, when a solid phase synthesis with phenylalanine as the C-terminal amino acid is devised, the difficult deprotection of the Boc group should be duly considered.

As shown in table 2, acidolysis rates are not sensibly affected by the nature of the amino acid side chain nor by the addition of a new glycine residue to the C-terminal amino acid. This fact seems to allow extrapolation of results from table 1 to the case of peptidyl-OCH_2-resins.

The previous results point out the difficulty of defining an optimum TFA concentration which reaches a compromise between desired deprotection and undesired acidolysis. Reid[6] showed, mainly from tlc results, that 40-45% TFA was the weakest possible acid concentration to remove quantitatively the Boc group in the course of a solid phase tetrapeptide synthesis. However, assuming that results from table 1 can be extended to Boc-peptidyl-OCH_2-resins, that would imply a substantial loss by acidolysis of at least 30% of the growing peptide chains in the synthesis of a 48-residue peptide (global deprotection time 24 h).

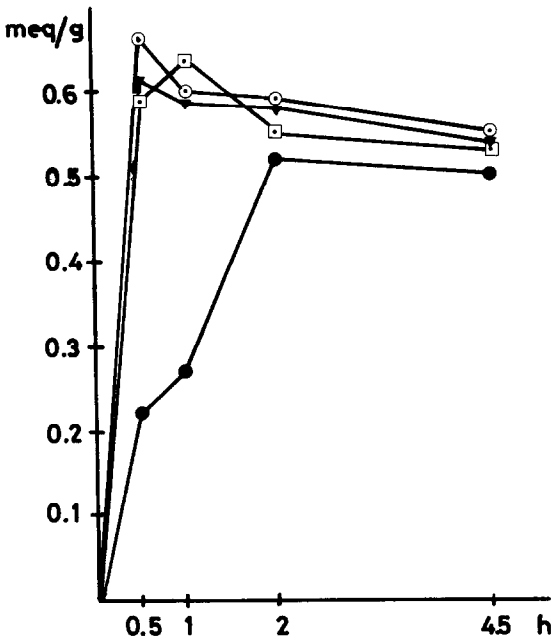

Fig.1 - Evolution of the free amine contents of a Boc-Gly-OCH_2-resin treated with 15% (⊙), 30% (▼), 50% (⊡), 100% (●) TFA/CH_2Cl_2 solutions.

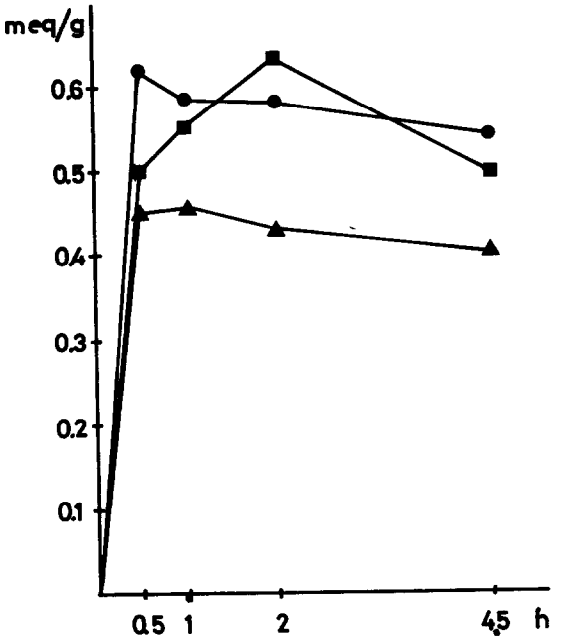

Fig.2 - Evolution of the free amine contents of Boc-Gly-OCH_2-resin (●), Boc-Phe-OCH_2-resin (■), and Boc-Gly-Gly-OCH_2-resin (▲) treated with 30% TFA.

Table 1 - Percentage amino acid loss from a Boc-Gly-OCH_2-resin treated with TFA/CH_2Cl_2 solutions.

TFA/CH_2Cl_2 concentrations (v/v)	time 2h	4.5h	24.5h
15%	11%	17%	24%
30%	12%	18%	27%
50%	17%	20%	32%
100%	21%	24%	36%

Table 2 - Percentage acidolytic losses from Boc-aminoacyl- or Boc-peptidyl-OCH_2-resins treated with 30% TFA/CH_2Cl_2. Comparison with a Boc-Gly-OCH_2-Pab-resin.

Resin	time 2h	4.5h	24.5h
Boc-Gly-OCH_2-resin	12%	18%	27%
Boc-Phe-OCH_2-resin	0%	21%	29%
Boc-Gly-Gly-OCH_2-resin	5%	12%	31%
Boc-Gly-OCH_2-Pab-resin	1%	1%	1%

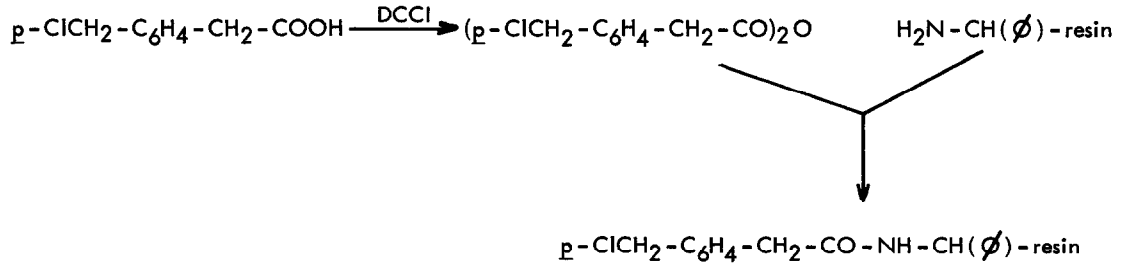

Fig.3 - Synthesis of 4-chloromethyl-Pab-resin (resin: 1% poly(styrene-co-divinylbenzene)).

An alternative to the search for optimum TFA concentrations has been the development of new solid supports whose peptide-resin linkage is more resistant to acidolysis. In this direction we have synthesized α-(4-chloromethylphenylacetamido)benzylpoly(styrene-co-divinylbenzene) (Pab resin) by condensation of 4-chloromethylphenylacetic acid[7] symmetric anhydride[8,9] and benzhydrylamine resin[10] (figure 3). This polymer is comparable to the Pam resins recently described by Merrifield et al.[11] but can be prepared by a much simpler procedure than that reported as the most convenient by these authors. The acid stability of the Boc-Gly-OCH_2-Pab resin has been tested as above and compared with that of conventional Boc-Gly-OCH_2-resin (table 2). No loss of functionalization was detected even after 24 h treatment. On the other hand, the yield of the HF cleavage of Boc-Gly-OCH_2-Pab resin, controlled by amino acid analysis and picric acid titration of the residual resin, was found to be comparable to that of a conventional Boc-Gly-OCH_2-resin. Thus, the introduction of the Pab group seems to protect the peptide-resin bond from TFA acidolysis during the deprotection steps without affecting its HF cleavage yields at the end of the synthesis.

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