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BASIC POLYMERS FOR THE CLEAVAGE OF FLUORENYLMETHOXYCARBONYL AMINO-PROTECTING GROUPS IN PEPTIDE SYNTHESIS

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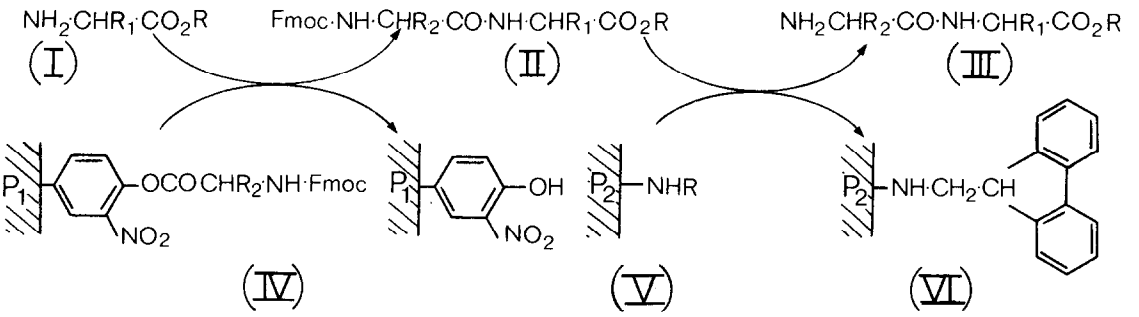
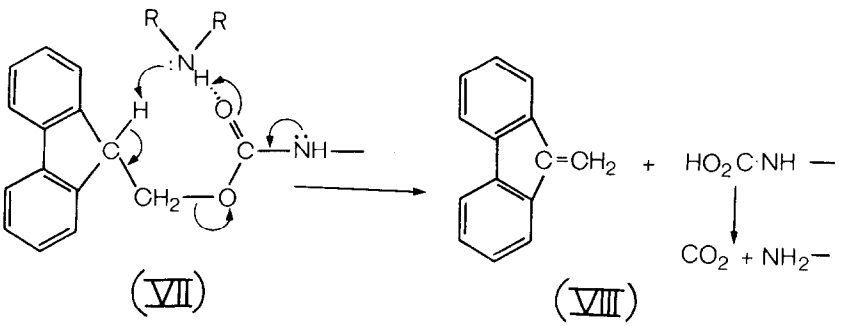
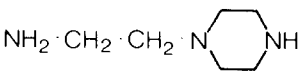
Summary: Increased efficiency in the cleavage of N-fluorenylmethoxycarbonyl derivatives by basic resins is obtained using polymer systems designed to take account of solvation and solute partitioning effects.

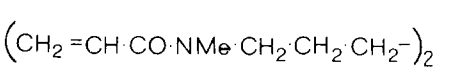
We described recently[1,2] the use of fluorenylmethoxycarbonyl (Fmoc) amino-acids[3] in solid phase peptide synthesis. In a concurrent study we have also investigated the use of polymer-bound Fmoc-derivatives (IV) in a reverse solid phase synthesis[4] procedure using both insoluble polymeric coupling and deprotection reagents. Such a procedure comprising a repeated cycle of the type I → II → III could be particularly favourable using the base (especially secondary amine[1,3]) sensitive Fmoc-protecting group. No neutralisation steps are required, the dibenzofulvene formed in the deprotection step may be scavenged by the polymeric base, and a simple flow scheme can be envisaged in which the protected or N-deprotected peptide is the only soluble component in the system. Three distinct steps are involved in this process. (1) Aminolysis of a polymer-supported activated ester, e.g. the o-nitrophenyl ester (IV). (2) Base-catalysed cleavage of the Fmoc-peptide derivative (II, or a higher homologue) by polymeric secondary amine (V). (3) Scavenging of the liberated dibenzofulvene, again by polymeric secondary amine. Our earlier work in more conventional solid phase peptide[5] and oligonucleotide[6] synthesis has shown that the nature of the polymeric support may have a substantial effect on the rate and efficiency of solid phase reactions. Although almost universally used, polystyrene is not necessarily the best support for polar reactants and intermediates. Optimisation of reaction conditions in the scheme discussed above will require careful consideration of the polymer matrices P_1 and P_2, particularly the latter because it is involved in the two chemically distinct steps of carboxylate elimination and amine addition. The recent report[7] by Carpino and his colleagues on the use of polystyrene-bound piperazine for the cleavage of Fmoc-derivatives prompts us to report now our own results for the deprotection step.



9-Fluorenylmethanol itself undergoes base catalysed β-elimination in water, methanol, and t-butanol by an ElcB or a related intermediary carbanion mechanism.[8] Although the secondary amine-catalysed decomposition of Fmoc-derivatives in aprotic solvents may have special mechanistic features, e.g. (VII, arrows), at least partial charge separation is presumably involved and the reaction should therefore be favoured in a polar environment. Our initial studies therefore concentrated on highly polar polymer matrices obtained by vigorous (24 hr, 215°C) treatment[9] of commercial poly(acryloylmorpholine) (Enzacryl Gel K2) with the complex amine (IX)* and other amines. Substitution levels of about 2 meq/g could be achieved before the polymer became soluble or too gelatinous to handle indicating that the primary site of attack by amine on the polymer are probably the methylene bisacrylamide cross links rather than the morpholide groups.







Simple neutral Fmoc-amino-acid or peptide derivatives (Fmoc.Ala.OBu^t, Fmoc.Gly.Ala.OBu^t, Fmoc.Gly.Ala.NHMe) were cleaved in dimethylformamide solution only slowly by this resin, with half lives of approximately 7, 2, and 1 h respectively.** In contrast, the polar, carboxy-containing substrates Fmoc-valine and Fmoc-glycylalanine were cleaved with $t_{\frac{1}{2}}$ of 7 and 5 min. Presumably these rate differences reflect unfavourable partition of the less polar neutral substrates between the solvent and the solvent-swollen basic polymer matrix. In agreement, this resin was a very inefficient scavenger for dibenzofulvene (VIII) compared with polystyrene-bound secondary amine.[7] Similar results were obtained using a more defined resin prepared by copolymerisation[†] of dimethylacrylamide, methyl acrylate, and ethylene bisacrylamide with subsequent replacement of the ester groups by amine (IX) under milder reaction conditions (10 hr, 100°C). A resin sample of functionality 2.6 meq/g and ∿2% cross-linking cleaved Fmoc.Gly.Ala.OBu^t in 10 - 12 hr; a similar resin of 3.7 meq/g functionality and 1.1% cross-linking gave complete reaction in 8 - 10 hr.

To achieve more favourable partition between the polymer matrix and solvent phases, a third resin type was prepared by co-polymerisation[††] of styrene, 2,4,5-trichlorophenyl acrylate, and N,N'-dimethyl-N,N'-bisacryloylhexamethylene diamine (X). The activated ester groups in the polymer were replaced rapidly (30 min, room temp.) by the amine (IX) giving a resin containing both polar (monosubstituted amide) and non-polar (phenyl) groups distributed along the polyethylene chains. The cross-linking agent (X) was chosen to provide greater mobility of the matrix than is possible with less flexible short chain cross links, particularly those from divinylbenzene.[10] Resin with functionality 3.3 meq/g derived from a nearly equimolar mixture of the two monomers and 2.1 mol.% cross linking agent cleaved Fmoc.Gly.Ala.OBu^t completely within 3 - 4 hr, reduced to 1 - 2 hr when the volume of solvent used was kept to the minimum needed to swell the resin. This compares favourably with the 80 -97% yields in 12 - 24 hr reported[7] for the cleavage of Fmoc-p-chloroaniline by 3.5 meq/g amine resin derived from commercial chloromethylated polystyrene. The scavenging of dibenzofulvene by the dimethylacrylamide-styrene copolymer was also much more effective than with the polar polyamides.

These results substantiate our view that polymer systems for solid phase reactions should be designed with careful regard for solvation and solute partitioning phenomena, and the internal topology of the resin matrix. These properties may not be immediately obvious simply from the apparent degree of cross-linking and solvent swellability of the resin.

We are grateful to Professor A. Ledwith for helpful discussion and the for gift of the polymerisation vessel.

* The residual tertiary amine group in resin bound (III) probably does not contribute appreciably to the rate of Fmoc cleavage.[1]

** Cleavage reactions were monitored by ion-exchange amino-acid or peptide analysis of the trifluoroacetic acid-deprotected product using a Jeol 5AH analyser, or latterly by direct t.l.c. examination of reaction mixtures. Times for complete cleavage obtained by this last simple procedure corresponded to about 8 - 10 half lives in a case where both methods were used, but the results should be regarded as only semi-quantitative.

† Polymerisation was carried out in a reverse suspension manner using a solution of 4% (w/v) cellulose acetate butyrate in 1,2-dichloroethane as suspension medium, ammonium persulfate as catalyst, water-DMF (2:1) as diluent, and a diluent to monomer ratio of 2:1. All quantities are based on the initial monomer mixtures.

†† Polymerisation was carried out according to the general procedure described[11] for styrene based resins, but in the presence of chlorobenzene as diluent with a diluent to monomer ratio of 2:3.

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