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SOLUBLE POLYMERS IN ORGANIC SYNTHESIS: II. USE OF POLYETHYLENE GLYCOL-BOUND REAGENTS FOR PEPTIDE SYNTHESIS

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Insoluble polymer reagents on the basis of crosslinked polystyrene have been proposed for peptide synthesis (1-3). The main objective of this strategy is the simplification of the synthesis cycle for the stepwise building-up of peptide sequences. This is achieved by applying the polymer-bound carboxylic component in large excess in the peptide forming step in order to obtain quantitative coupling yields and by removing excess reagents simply by filtration. This elegant method suffers mainly from the tedious and ineffective preparation of the polymer reagents and the objections brought forward against heterogeneous reaction conditions in peptide synthesis (4).

When soluble polymer reagents are used instead, the strategy of building-up peptides is similar to that of the classical stepwise procedure (5), because all reactions proceed under homogeneous conditions. However, the use of soluble polymer reagents facilitates the reaction cycle considerably due to the ease of removal of excess reagents. This is exemplified for the case of the synthesis of several model peptides using polyethylene glycol (PEG)-bound reagents described in the preceding paper, hereafter designated I.

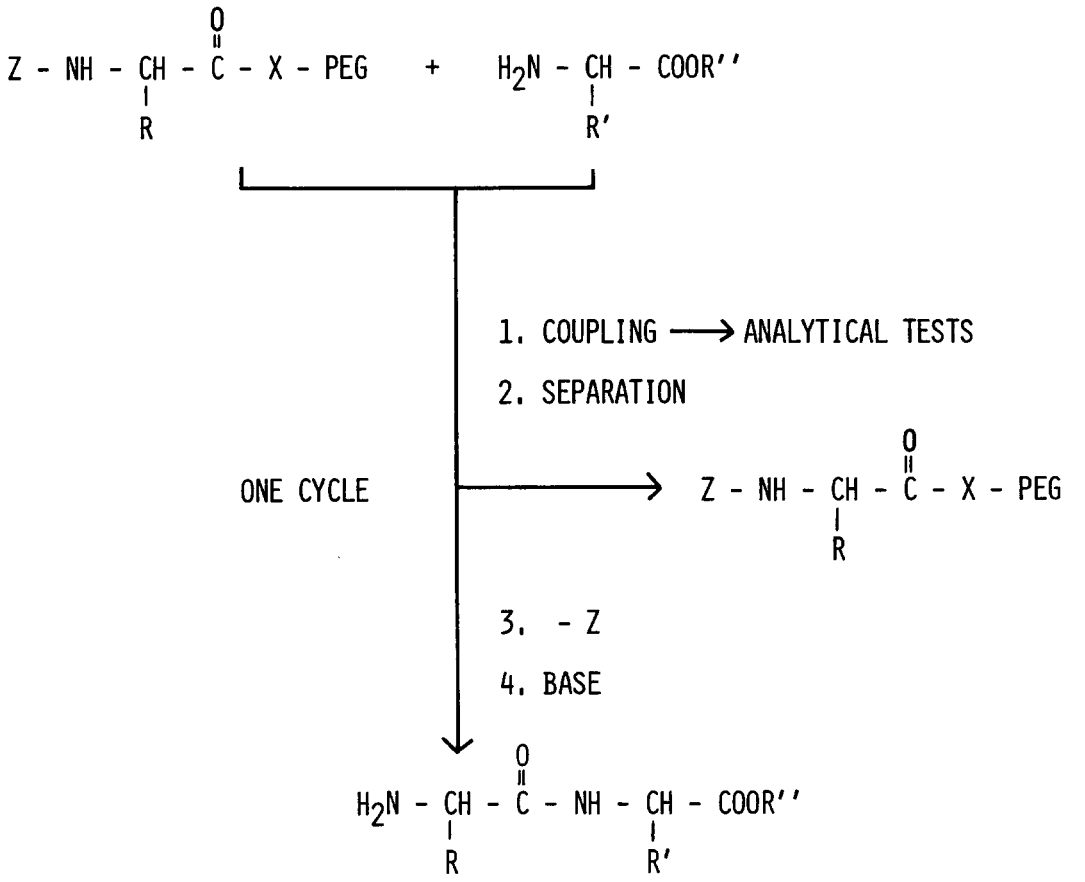
The following general synthesis cycle applies when active PEG esters of type $\underset{\sim}{7}$ in I are used for coupling:

A C-terminal protected (e.g. tert.-butyl)amino acid (or peptide) is reacted with excess (2-4 eq) of $\underset{\sim}{7}$ in CH_2Cl_2, DMF, DMSO or mixtures depending upon the solubility of the amino component. Samples of the homogeneous coupling mixture are taken to ensure quantitative reaction. After coupling, the excess of PEG-bound reagent is removed by one of the following procedures:

1. Precipitation: to the coupling mixture (30 % w/v) ether is added until the PEG-components are precipitated quantitatively; the soluble fully protected peptide is separated by filtration.

2. In cases, where the peptide is insoluble in the mixtures above, the precipitate is recrystallized from EtOH or MeOH (5-10 % (w/v) solution, cooling to 0°).

3. Separation by ultrafiltration, gelchromatography or adsorption chromatography by making use of the difference in molecular weight or polarity.



For all procedures, good solubility of the fully protected peptides is necessary for obtaining high yields of recovery in each step. When the coupling step has proceded to quantitative yield and no impurities can be detected, further purification steps can be omitted. After splitting off the N-terminal protecting group and neutralization of the amino component the next PEG-bound amino acid derivative is coupled. According to this synthesis cycle the following model peptides have been synthesized:

BOC-Val-Pro-Gly-Gly-OMe (A)

Z-Leu-Ala-Gly-Val-OBut (B)

For the synthesis of A, PEG-bound N-BOC protected amino acid o-nitrophenyl-esters (4 in I) were used in 4-fold excess (24 h reaction time). Sequence B was built up by the more reactive N-Z protected amino acid hydroxybenzotriazol esters (5 in I). In this case, 3-fold excess was sufficient to obtain quantitative coupling yields (12 h reaction time). The model peptides A and B which proved identical with products obtained by classical methods were obtained in overall yields of 60-70 % related to the starting C-terminal amino acid ester.

The following observations were made during synthesis:

- Excess polymer reagent can be removed quantitatively by precipitation according to procedure 1 or 2 described above.
- The isolated PEG-bound reagents (compounds 4 and 5 in I) could be transfered to active esters (7 in I) and were reused for synthesis.
- The loss of main product during precipitation by inclusion was negligible owing to the highly crystalline character of the PEG-derivatives.
- A comparison of the reaction rates between PEG-bound and low molecular weight active ester derivatives in the peptide forming step indicated that the soluble polymeric group did not influence the reaction rates. The same observation was made previously for PEG-bound amino components (6).

A problem often encountered in peptide synthesis is the quantitative removal of the urea derivative resulting from activation of the carboxylic component by carbodiimide. Several attempts have been made to solve this problem by attaching the carbodiimide to polymers, mainly based on polystyrene (7-9). PEG-bound carbodiimides as described in I were used for the preparation of several dipeptides. To this end, N-BOC protected amino acids were reacted in equimolar amounts with compound 8 in I and mixed with C-protected amino acids. In contrast to the case of insoluble carbodiimides, the PEG-bound derivative had not to be applied in large excess to the carboxylic component, indicating homogeneous reaction sites. The resulting PEG-urea derivative, which was readily soluble in CH_2Cl_2 or DMF, was removed after coupling by adding ether to the coupling mixture. The isolated polymeric urea derivative could be converted to active carbodiimide by reaction with TsCl and Et_3N. No difference in the activity of PEG-bound and free carbodiimide could be detected. For example, the same coupling yields (as determined by quantitative ninhydrine test (10)) were obtained for both DCCI and PEG-carbodiimide. However, the dipeptides were free

of urea-derivative using the polymeric carbodiimide after a single precipitation step. So far, the following conclusions can be drawn:

PEG-bound reagents are useful for the synthesis of peptides which exhibit good solubility. In this case, the addition of one amino acid residue to the growing peptide chain can be accomplished much more effectively than in classical procedures. The strategy can be easily combined with the classical procedure. A major advantage over insoluble polymer reagents is the fact that the kinetics proceeds analogously to low molecular weight reactions and that the course of the reaction can be monitored more readily in homogeneous solution.

The expense of synthesizing the polymer reagents prior to synthesis seems to be justified in view of the simplification of the synthesis cycle and the higher yields of reaction. However, the availability of a large stock of polymer reagents and their repeated use are important factors from an economical point of view. The ease of accessibility of PEG-bound reagents may render these compounds more attractive for a more widespread application in organic synthesis.

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