TOWARD MECHANIZED PEPTIDE SYNTHESIS VIA POLYMERIC REAGENTS APPROACH

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Simultaneous application of high-molecular-weight active esters and polyvinyl N,N-diethylbenzylamine was used as a basis for a continuous peptide synthesis via the polymeric reagents approach. Using the synthetic procedure developed, the hexapeptide Boc-L-Pro-L-Val-L-Lys[Z(p-NO2)]-L-Val-L-Tyr(Dnp)-L-Pro-OBzl1 and the tetrapeptide Boc-L-Lys[Z(p-NO2)]-L-Lys[Z(p-NO2)]-L-Arg(NO2)-L-Arg(NO2)-OBzl1, corresponding to residues 19–24 and 15–18 of human ACTH, were synthesized in 63 and 70% overall yields, respectively.

INTRODUCTION

Peptide synthesis via the polymeric reagents approach involves the step-wise elongation of a soluble peptide at its α-amino terminal, using insoluble polymeric active esters (1–4). Such reagents, which can be readily separated from the reaction mixture by filtration, are generally employed in excess and provide rapid reaction rates, high coupling yields, and high product purity. Although many peptides have been successfully synthesized using this technology (e.g., 5–9) one of the method’s major advantages, the possibility of its being adopted for automated peptide synthesis, has not yet been realized.

In the present study, we investigate one possible approach to mechanized peptide synthesis with polymeric reagents. It encompasses three operative steps which are performed consecutively without isolation of intermediate compounds: (I) coupling of the free α-amino group of an amino acid ester with a polymeric active ester to yield N- and C-blocked dipeptide; (II) selective removal by acidolysis of the N-protecting group to afford the corresponding ammonium salt of the dipeptide ester; and (III)

neutralization of ammonium salt with a weakly basic polymer, concomitantly with coupling of the newly exposed $\alpha$-amino function with a desired polymeric reagent to produce $N$- and $C$-blocked tripeptide. Further repetitions of these reactions will finally lead to the designed peptide. The principal features of this method and its application to the synthesis of Boc-Pro-Val-Lys[\textit{Z}(p-\textit{NO}2)]-Val-Tyr(Dnp)-Pro-OBzl, a peptide corresponding to residues 19–24 of human ACTH, and of Boc-Lys[\textit{Z}(p-\textit{NO}2)]-Lys[\textit{Z}(p-\textit{NO}2)]-Arg(NO2)-Arg(NO2)-OBzl, a peptide corresponding to sequence 15–18 of human ACTH, are described.

**MATERIALS AND METHODS**

Macroporous polyvinyl $N,N$-diethylbenzylamine (20–50 mesh; bead form; type A-21) was obtained from Rohm and Haas. Thin-layer chromatography was performed on fluorescent silica gel plates (Riedel-De Haen, Hanover) using the solvent systems: chloroform–methanol (9:1 and 3:1, v/v) and acetonitrile–water (9:1, v/v). Peptides were detected by iodine vapors, by charring the plates over a flame, or by fluorescence under an ultraviolet lamp. Peptide derivatives with free $\alpha$-amino groups were also detected by ninhydrin. Amino acid analyses were performed on a Spinco-Beckman Model 120C amino acid analyzer. The assayed blocked peptide derivatives were hydrolyzed with 6 N hydrochloric acid for 48 h at 110$^\circ$C in evacuated, sealed tubes. Melting points were determined on a capillary melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Insoluble polymeric active esters derived from polystyrene-bound 1-hydroxybezotriazole (PHBT esters) (8) and from (4-hydroxy-3-nitro)-benzylated polystyrene (PHNB esters) (10) were prepared as previously described.

**Regeneration of Polyvinyl $N,N$-Diethylbenzylamine**

Polymer A-21 (100 g) was suspended in 1 N NaOH (600 ml), and the mixture was mildly agitated for 15 min at room temperature. The polymer was then filtered, washed with distilled water to neutrality, washed with methanol, ether, and finally dried under high vacuum. To evaluate the content of tertiary amine groups in the polymer, a sample (~25 mg) was suspended in a solution of 0.2 M trifluoroacetic acid (TFA) in dichloromethane (1–2 ml) and stirred for 15 min at room temperature. Excess trifluoroacetic acid in the solution was then backtitrated with sodium methoxide using thymol blue as an indicator (11). The polymer was found to contain about 4 mmol amino groups/g.