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A POLYMERIC REAGENT FOR PEPTIDE SYNTHESIS: O-HYDROXYNITROPHENYL DERIVATIVE OF POLYSTYRENE

Govind T. Panse and Daniel A. Laufer

Department of Chemistry, University of Massachusetts at Boston
Boston, Massachusetts 02116, U. S. A.

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It is known that the stepwise coupling of amino acids (eq 1) may be accelerated to com-

$$R\text{-}CO\text{-}X + NH_2\text{-}R' \longrightarrow R\text{-}CO\text{-}NH\text{-}R' + HX \qquad (1)$$

pletion by the use of excess molar quantities of either R-CO-X or R'-NH_2. The desired product may be isolated by filtration or centrifugation if either reagent is an insoluble polymer (eqs 2,3). Reaction 2 corresponds to the well known Merrifield solid phase method.[1]

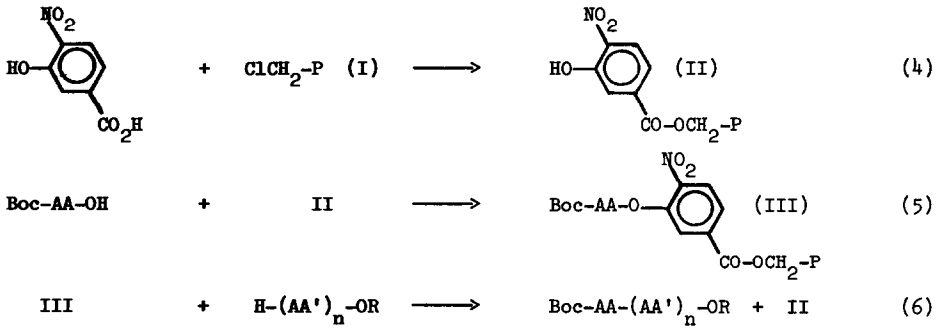
$$R\text{-}CO\text{-}X \text{ (excess)} + NH_2\text{-}R'\text{-}P \longrightarrow R\text{-}CO\text{-}NH\text{-}R'\text{-}P + HX \qquad (2)$$

$$R\text{-}CO\text{-}X\text{-}P \text{ (excess)} + NH_2\text{-}R' \longrightarrow R\text{-}CO\text{-}NH\text{-}R' + HX\text{-}P \qquad (3)$$

Reaction 3 has been successfully applied by Katchalski *et al.* in the preparation of cyclic[2] and linear[3] peptides, by Blout *et al.*,[4] and other groups.[5] The corresponding polymeric components of activated amino acids (P-XH, eq 3) which were previously used in peptide synthesis, include cross-linked poly-4-hydroxy-3-nitrostyrene,[2,3] branched copoly [DL-lysine-3-nitro-L-tyrosine],[2] linear and cross-linked copoly [ethylene-N-hydroxymaleimide],[4] and others.[5]

It appeared to us that in each of the above polymeric reagents (i) no substitutions in reactive functional groups are feasible, (ii) the distance between the reactive center and macromolecular backbone is fixed, (iii) there are no uniform methods of controlling the extent of reactive group substitution, and in some cases, (iv) no continuous controls of macromolecular cross-linking are available. In an attempt to provide access to these controls, and to demonstrate the feasibility of our approach, we wish to report (i) a general preparation of an insoluble polymeric reagent similar to the known[2,3] poly-4-hydroxy-3-nitrostyrene (eq 4), (ii) synthesis of corresponding polymeric amino acid active esters (eq 5), and (iii) stepwise

synthesis of a tetrapeptide using the above polymeric reagents as intermediates (eqs 3,6). Polymer I, cross-linked chloromethylated polystyrene, was selected as a starting material because (i) the physical and chemical properties of insoluble polystyrene are generally known (its use in solid phase peptide synthesis is an important example[1]), (ii) it is commercially



available[6] or may be readily prepared,[7] and (iii) it may be applied as a general starting material for the preparation of related polymeric active esters and coupling reagents.

Polymeric o-nitrophenol (II, eq 4) was prepared by treating chloromethylated copoly [styrene-2% divinyl benzene] (10.0 g, 1.8 m.mole Cl/g)[6] with a solution of 3-hydroxy-4-nitrobenzoic acid (10.8 g, 60 m.mole) and triethylamine (8 ml, 60 m.mole) in 75 ml ethanol.[1] The suspension was stirred at 75° for 65 hrs, and the polymeric product, II, was filtered off, washed with ethanol, water, methanol, and dried in vacuo (II, 10.9 g, ca. 0.6 m.mole o-nitrophenol/g). The infrared spectrum of II (KBr pellet, Perkin Elmer 225 spectrophotometer) included characteristic bands of H-bonded phenolic OH 3250 cm^{-1} (w), phenylbenzoate ester 1718 cm^{-1} (s), and NO_2 group 1582 cm^{-1} (s).

Polymeric o-nitrophenyl esters of Boc-D-Phe-OH, Boc-Leu-OH, and Boc-Ala-OH (III, eq 5) were prepared by the dicyclohexylcarbodiimide (DCC) method.[8,2,3] Polymer II (1.0 g, ca. 0.6 m.mole phenol/g) was suspended in a solution of Boc-D-Phe-OH (1.5 g, 5.4 m.mole) and DCC (1.1 g, 5.4 m.mole) in methylene chloride (25 ml). The suspension was stirred for 18 hrs at room temperature, and the polymeric active ester, III, was filtered off, washed with water, ethanol, methanol, methylene chloride, and dried in vacuo (III, 1.17 g, ca. 0.6 m.mole Boc-D-Phe-OH/g). The infrared spectrum of polymer III (KBr pellet) included characteristic bands of amino acid phenyl ester, 1770 cm^{-1} (m), and Boc group 1360, 1385 cm^{-1} (w). Polymeric esters of Boc-Leu-OH and Boc-Ala-OH were similarly prepared.

Use of polymeric o-nitrophenyl esters as intermediates in synthesis of peptides listed in Table I (eq 6) was made in the following way. Polymeric active ester of Boc-amino acid (ca. 1.5 equiv) and amino peptide hydrochloride (1 equiv) were treated with triethylamine (1 equiv) and stirred in dry methylene chloride for ca. 9 hrs at room temperature. The polymeric by-product, II, was filtered and washed with methylene chloride. The combined filtrates were extracted with water, dried and flash-evaporated. The chromatographically pure products were once crystallized from ethyl acetate-hexane. An improved yield was obtained in one instance when the reaction was carried out in dimethylformamide. Removal of the Boc- protecting group from intermediate peptides was carried out with anhydrous HCl in dimethoxyethane.[4] Table I summarizes the results of stepwise syntheses of the tetrapeptide Boc-Ala-Leu-D-Phe-Gly-OBzl with polymeric o-nitrophenyl esters, N-Hydroxysuccinimide (HOSu) esters,[9,10] and mixed anhydride[11] intermediates.

Table I - Peptides Synthesized with Polymeric and Conventional Methods[a]

Peptide	Method of Activation	mp[b] °c	Yield, % Crude	Cryst.	$[\alpha]_D^{25}$[c]
Boc-D-Phe-Gly-OBzl	Polymeric Ester	131-133	72	48	+25
"	HOSu Ester	"	73	60	+25
"	Mixed Anhydride	"	84	78	---
Boc-Leu-D-Phe-Gly-OBzl	Polymeric Ester	160-162	50	23	+12
"	Polymeric Ester[d]	161-162	62	58	---
"	HOSu Ester	"	64	54	+12
"	Mixed Anhydride	"	89	80	---
Boc-Ala-Leu-D-Phe-Gly-OBzl	Polymeric Ester	110-111	61	41	-14
"	HOSu Ester	"	76	59	-14
"	Mixed Anhydride	"	83	76	---

a. All peptides were authenticated with C,H,N analyses and infrared spectra.
b. Melting points were determined on a Kofler block and are uncorrected.
c. Specific rotations were measured in ethanol (c 1).
d. Coupling reaction was carried out in dimethylformamide.

The results reported above demonstrate the feasibility of our general approach. The use of these polymeric o-nitrophenyl esters may serve as a satisfactory alternative to conventional methods of peptide synthesis, particularly in reactions which require difficult work-up procedures. The advantages of using polymer I in preference to previously reported macromolecular starting materials are: (1) It may be attached to other potential coupling reagents which correspond to N-hydroxysuccinimide[9] and N-hydroxyphthalimide[12] esters, alkylchloroformates,[11] and dialkylcarbodiimides.[8] (2) Chloromethylated polystyrene may be coupled to reagents of

type x-$(CH_2)_n$-Y, thus providing a variable distance between the reactive center and the polymeric backbone, a potentially critical factor in coupling reactions.[13] (3) The extent of reactive group substitution on the macromolecule is controlled by chloromethylation and esterification, reactions which have been widely used in the Merrifield solid phase method[1] under a variety of conditions.

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