

SYNTHESIS OF CYCLIC PEPTIDES ON DUAL FUNCTION SUPPORTS\*

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There are two problems encountered in the synthesis of cyclic peptides <sup>1</sup>. The open chain must be built up from its constituent units. The linear chain is then activated, usually at the carboxyl end, and cyclization is achieved through intramolecular amide-bond formation. Yields in this step are frequently low due to the formation of linear oligomers even when conditions of high dilution are used.

Polymeric supports have been demonstrated to be extremely useful in peptide synthesis both for carboxyl protection as exemplified by the work of Merrifield <sup>2</sup> and for activation as shown by Fridkin <sup>3</sup> and Laufer <sup>4</sup>. Fridkin, using a 4-hydroxy-3-nitrostyrene polymer was able to cyclize peptides in high yields. The modification of a support during the synthesis to change its functional characteristics such as lability is now reported.

The 4-(methylthio)phenyl (MTP) group of Johnson and Jacobs <sup>5</sup> has been incorporated into two polymers, IA and IB, (see Fig. 1). Polymer IA was prepared by polymerization of 4-(methylthio)phenol with a 1.5 molar excess of formaldehyde. The mixture was refluxed 14 hours in the presence of a catalytic amount of 6N NaOH. The resulting brown amorphous solid showed characteristic bands in the infrared at 3450  $\text{cm}^{-1}$  (b) and 750  $\text{cm}^{-1}$  (s) due to the phenolic hydroxy moiety and aromatic ring, respectively. Polymer IB ( $\text{R}=\text{H}$ ,  $\text{R}_1=\text{C}_6\text{H}_4\text{-Polymer}$ ) was prepared <sup>6</sup> by the

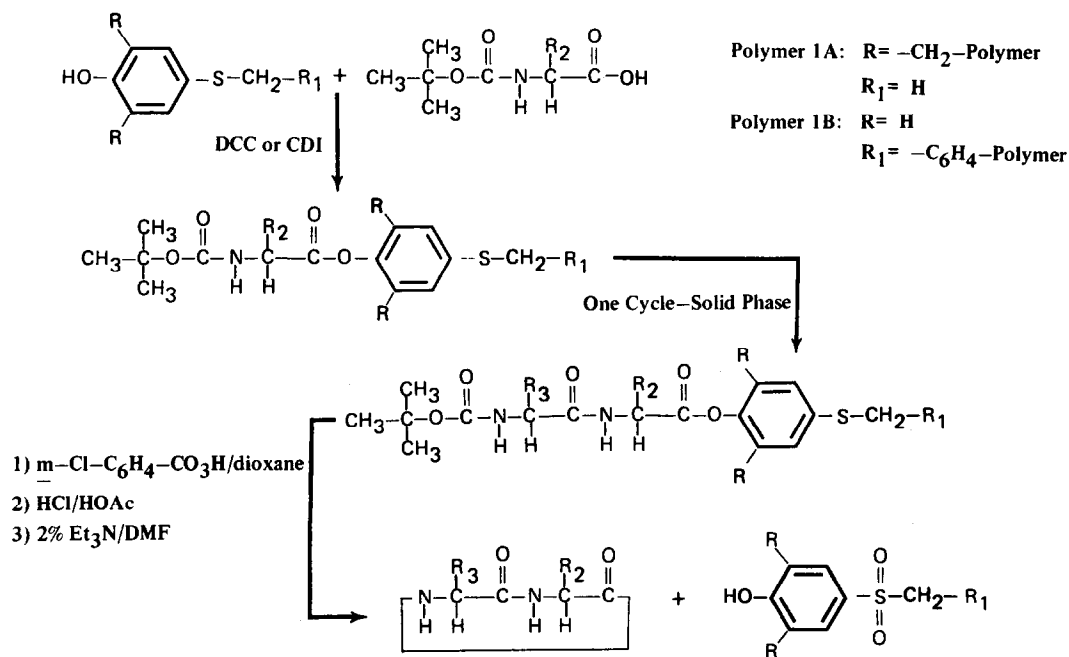
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reaction of 40 mmoles of p-mercaptophenol and an equivalent amount of KOH with 10 gms of chloromethylpolystyrene-2%-divinylbenzene (0.9 meq Cl/gm). The mixture was refluxed in dimethylformamide <sup>7</sup> (DMF) for 4 hours. Bands in the infrared were found at 3500  $\text{cm}^{-1}$  (s), 1600 (m) and 750  $\text{cm}^{-1}$  (s) due, again, to the phenolic hydroxy and aromatic moieties. Both polymers were insoluble in all solvents commonly used in peptide synthesis. The attractive feature of IA and IB is their facile conversion to the activated sulfonyl esters.

Polymeric MTP esters (see reaction 1) of Boc- $\alpha$ -amino acids were prepared by the dicyclohexylcarbodiimide (DCC), carbonyldiimidazole (CDI), mixed anhydride and N-ethoxycarboxyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) methods using  $\text{CH}_2\text{Cl}_2$  or tetrahydrofuran as solvents. When equimolar quantities of all reactants were used, the coupling efficiency was as follows: DCC>CDI>EEDQ> mixed anhydride.

Figure 1. Synthesis on MTP Supports



As shown in Fig. 1, chain elongation to desired lengths was carried out according to Merrifield<sup>2</sup> and Marshall and Merrifield<sup>8</sup>. The polymer was activated by oxidation to the sulfone using 3 equivalents of *m*-chloroperbenzoic acid<sup>9</sup> in dioxane. Complete conversion to the sulfone [I.R. bands at 1310  $\text{cm}^{-1}$  (s) and 1138  $\text{cm}^{-1}$  (s)] was accomplished in 24 hours. No loss of peptide was detected at this step. The terminal Boc-group was removed and the polymer was washed well with HOAc, EtOH and  $\text{MeCl}_2$ . The activated peptide ester was allowed to condense, intramolecularly, to the cyclic product by shaking the polymer 18 hours in 2%  $\text{Et}_3\text{N}/\text{DMF}$ . Evaporation of the solvent and re-crystallization of the solid from appropriate solvents yielded chromatographically pure cyclic peptides. The cyclic products gave ninhydrin negative tests in tlc, but were identified with hypochlorite-starch-KI<sup>10</sup>. Results of several preparations are shown in Table I. Low yields on IA may reflect the presence of the two ortho methylene groups or the degree of cross-linking in the polymer.

Table I. Cyclic Peptide Products\*

Product	mp. °C.	$R_f$ (I)	$R_f$ (II)	% Yield	
				IA	IB
c-(Ala-Gly)	238-239	.59	.46	23	52
c-(Gly-Gly)	308-309	.51	.35	40	63
c-(Gly-Phe)	261-265	.71	.66	21	42
c-(Ala-Ala-Ala-Gly)	279-280**	.57	.49	-	40
c-(Gly-Val-Ala-Phe-Ala-Gly)	209-210	.64	.58	-	50

\* All melting points are uncorrected. Thin layer chromatography was carried out on Silica Gel G. Solvent Systems: (I) *n*-Butanol:HOAc:HOH (4:1:1); (II) *sec*-Butanol:EtOAc:HOH (65:60:25). Yields are based on amino acids initially esterified to the polymer. Amino acid analyses and molecular ions from mass spectrometry are consistent with the proposed structures.

\*\* Decomposition

While the oxidative conditions of this method limits its application, a number of biologically-active cyclic peptides do not contain methionine, cysteine and cystine. The syntheses of valinomycin and antamanide are currently under investigation. The primary advantages of this system include higher overall yields, a minimization of racemization during activation and subsequent cyclization as well as the normal convenience of the solid phase method.

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