Registry No.—1, 22922-43-6.

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A Modified Support for Solid-Phase Peptide Synthesis Which Permits the Synthesis of Protected Peptide Fragments[1]

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Peptide products obtained by the solid-phase technique are normally not suited for use in subsequent coupling reactions owing to removal of N-terminal and side-chain protecting groups during the cleavage step. We have now developed a modified polymer support which retains the former advantages of the solid-phase technique but also permits removal of the peptide product with protecting groups intact.

The modified support is easily synthesized from the chloromethylated polystyrene resin (**1**) used in conventional solid-phase procedures by reaction with the O-carbonate ester of *p*-mercaptophenol[2] (**2**). The first N-protected amino acid is then coupled to the phenol–sulfide resin (**3**) by means of the mixed anhydride method or with dicyclohexylcarbodiimide. The remaining amino acids are introduced in the usual manner.[3] To remove the peptide from the support, the sulfide is oxidized to the sulfone (**4**) with hydrogen peroxide in acetic acid. This converts the anchoring ester linkage into an activated ester capable of acylating an amine. Thus the peptide, by acylating an amino acid (**5**), is released from the polymer support with the various side-chain and N-terminal protecting groups still in place and is lengthened by one amino acid at the C-terminal end (Scheme I).

The applicability of this modified support for the synthesis of peptides was first tested by the synthesis of N-benzoylglycine from a benzoylated phenol–sulfide polymer. Then additional peptides were prepared, including the sequence 180–184 of human growth hormone.[4] The model peptide, N-benzoyl-L-leucylglycine ethyl ester,[5] was prepared as a test for racemization which might occur during the acylation involving the active, insoluble ester. The optical rotation and melting point of the product prepared by this technique were in agreement with the values for the L-peptide indicating that little or no racemization had occurred.

The use of this type of convertible protecting group in conventional peptide synthesis was recently described by Johnson and Jacobs.[6] They report the peptide linkage to be stable during the H_2O_2 oxidation. However, cysteine, methionine, and tryptophan would be affected by this treatment. These amino acids could be incorporated by using them as the amino acid to be acylated by the activated, insoluble ester.

Experimental Section[7]

Preparation of Modified Polymer Support (3).—Four grams of chloromethylated polystyrene[8] was suspended in 25 ml of dimethylformamide (DMF) and refluxed for 3 hr with a methanol solution containing 1.2 g (6 mmol) of the O-carbonate ester of *p*-mercaptophenol (**2**) and 0.72 g (18 mmol) of NaOH. The resin was filtered and washed successively with DMF, methanol, acetic acid, 1 *N* HCl in acetic acid, and methanol and then dried. As judged by weight increase, the modified polymer contained *ca.* 0.91 mmol/g of phenol–sulfide groups.

N-Benzoylglycine.—The phenol–sulfide resin (**3**), suspended in DMF, was benzoylated with benzoyl chloride in the presence of pyridine to give a resin containing *ca.* 0.8 mmol/g of benzoyl groups as judged by weight increase. Oxidation with H_2O_2 in acetic acid at room temperature for 12 hr converted the benzoylated polymer into an active ester resin. Glycine (as the sodium salt) was added to a DMF–H_2O suspension of the resin and stirred for 24 hr, at which time a ninhydrin test on an aliquot indicated no free glycine. Filtration and acidification of the filtrate followed by evaporation of the DMF–H_2O gave a residue of N-benzoylglycine. Recrystallization from H_2O gave crystals, mp 189–190° (lit. mp 190°).

N-*p*-Nitrobenzyloxycarbonyl-L-leucyl-L-γ-benzylglutamylglycine.—In this procedure the first amino acid coupled to the polymer is the one which will be second from the C-terminal end in the final product. For this tripeptide, N-*t*-butyloxycarbonyl-L-glutamic acid γ-benzyl ester (0.53 g, 1.5 mmol) was dissolved in 15 ml of methylene chloride and added to 1 g of modified support (**3**) contained in a reaction vessel similar to that described by Merrifield.[3] Dicyclohexylcarbodiimide (0.31 g, 1.5 mmol) dissolved in 15 ml of methylene chloride was then added and stirred overnight with a mechanical stirring motor and rod at a rate just fast enough to keep the resin well suspended. The resin was filtered, washed with ethanol, acetic acid, and ethanol, and dried. The amount of glutamic acid coupled to the support was *ca.* 0.8 mmol/g. Following deprotection and neutralization of the glutamyl amino group, the next amino acid and N-terminal one for this product, N-*p*-nitrobenzyloxycarbonyl-L-leucine (0.52 g, 1.6 mmol), was added as a methylene chloride solution along with 0.33 g (1.6 mmol) of dicyclohexylcarbodiimide. The leucyl–glutamyl polymer was filtered and washed as before. The dipeptide polymer was treated with 2 ml of 30% H_2O_2 in 20 ml of acetic acid with stirring for 12 hr at room temperature. After filtering and washing with ethanol, removal of the peptide from the polymer was accomplished by stirring for 24 hr with 0.75 mmol of glycine (as the sodium salt) in a DMF–H_2O solvent. The mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in water, the pH was adjusted to 3.5, and the precipitate which formed was collected by decantation. The wet residue was dissolved in absolute ethanol and evaporated to dryness. A white, granular product was obtained from this residue by precipitation from ethyl acetate–petroleum ether; this was followed by trituration of the precipitate with petroleum ether. The protected tripeptide amounted

(1) Supported in part by Grant GM-12837 from the United States Public Health Service to I. E. L.

(2) T. Zincke and C. Ebel, *Chem. Ber.*, **47**, 1100 (1915).

(3) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

(4) C. H. Li, W. K. Liu, and J. S. Dixon, *J. Amer. Chem. Soc.*, **88**, 2050 (1966).

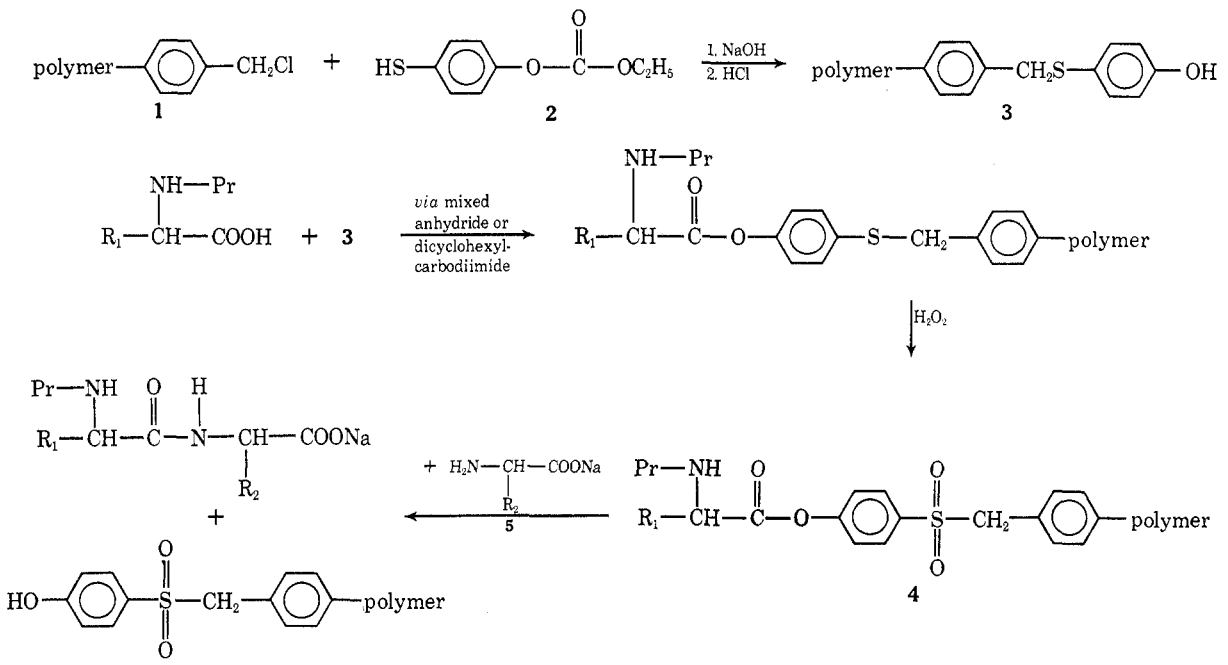
(5) N. A. Smart, G. T. Young, and M. W. Williams, *J. Chem. Soc.*, 3902 (1960).

(6) B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968).

(7) Melting points were determined using Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Rudolph polarimeter, Model 80.

(8) Supplied by Bio-Rad as Bio-Beads SX-2, 1.5 mequiv of Cl/g.

SCHEME I



Pr = protecting group

to 0.195 g (40% based on amount of glutamic acid attached to polymer). The product was purified by Sephadex G-15 chromatography using 50% acetic acid as solvent to give a ninhydrin-negative product which gave an amino acid analysis in agreement with the expected values. (amino acid ratios in acid hydrolyzate: Leu, 1.00; Glu, 1.06; Gly, 1.08.)

N-α-Carbobenzyloxycarbonyl-ω-nitro-L-arginyl-O-benzyl-L-seryl-L-valyl-L-γ-benzylglutamylglycine Ethyl Ester (Human Growth Hormone Sequence 180–184).—The general plan used for the previous tripeptide was used starting with the attachment of the penultimate C-terminal residue (N-*t*-BOC-L-glutamic acid γ-benzyl ester) to the support and followed by successive addition of the remaining residues. Oxidation with H_2O_2 converted the resin into an active, insoluble ester which, when treated with ethyl glycinate (free base), resulted in the formation of the desired protected pentapeptide removed from the polymer support. Chromatography on Sephadex G-25 using 50% acetic acid as solvent gave a product with the following amino acid analysis: Arg, 0.94; Ser, 1.00; Val, 1.10; Glu, 0.96; Gly, 1.06.

N-Benzoyl-L-leucylglycine Ethyl Ester.—N-*t*-BOC-L-leucine was coupled to the support with dicyclohexylcarbodiimide and the BOC group was removed by exposure to 50% trifluoroacetic acid–methylene chloride for 30 min. The amino group was neutralized with triethylamine, washed with DMF, and benzoylated by treatment with benzoyl chloride–pyridine in DMF at 5°. The benzoylated leucyl polymer was oxidized with H_2O_2 in acetic acid to give the active, insoluble ester. The resin was suspended in DMF and stirred for 24 hr with ethyl glycinate (free base). The dipeptide product was obtained by filtration and evaporation of the DMF. Crystallization of the residue from ethyl acetate–petroleum ether gave 0.18 g of product (38% yield based on amount of leucine attached to the polymer) whose optical activity and melting point agreed well with the reported values for the L isomer, $[\alpha]$ −34.1° (*c* 0.94, EtOH) (lit.[6] $[\alpha]$ −34.0°), mp 155–157° (lit.[6] mp 156–157°).

Registry No.—N-Benzoylglycine, 495-69-2; N-*p*-nitrobenzyloxycarbonyl-L-leucyl-L-γ-benzylglutamylglycine, 23025-41-4; N-α-carbobenzyloxycarbonyl-ω-nitro-L-arginyl-O-benzyl-L-seryl-L-valyl-L-γ-benzylglutamylglycine ethyl ester, 23025-42-5; N-benzoyl-L-leucylglycine ethyl ester, 2418-77-1.

Synthesis of a Diribonucleoside Monophosphate by the β-Cyanoethyl Phosphotriester Method[1]

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Since the β-cyanoethyl phosphotriester technique has proved useful for the synthesis of short-strand oligodeoxyribonucleotides in quantity,[1] it was of interest to see whether the technique could be extended to the synthesis of oligoribonucleotides. The major problem in the transition to the ribo series appeared to center on the steric effect of the substituent at the 2′ position. Specifically, would the condensation leading to a triester proceed satisfactorily with bulky substituents at the 2′ positions of the nucleosides? To answer this question a synthesis of uridylyl(3′-5′)-uridine *via* the β-cyanoethyl phosphotriester was attempted.

Nucleosides protected at the 2′-O position and at the 2′-O and 5′-O positions were prepared by utilizing the

(1) Part XVI in series on Nucleotide Chemistry. Part XV: R. L. Letsinger, K. K. Ogilvie, and P. S. Miller, *J. Amer. Chem. Soc.*, **91**, 3360 (1969). This research was supported by the Division of General Medical Sciences, National Institutes of Health (GM 10265).