Tetrahedron Letters No.27, pp. 2323-2327, 1965. Pergamon Press Ltd.
Printed in Great Britain.

SYNTHESIS OF PEPTIDES IN SOLUTION ON A POLYMERIC SUPPORT

I. SYNTHESIS OF GLYCYLGLYCYL-L-LEUCYLGLYCINE

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(Received 17 May 1965)

Despite the considerable achievements in the synthesis of polypeptides and the lower proteins (insulin, adrenocorticotropic hormone, etc.) many obstacles stand in the way of its further progress along classical lines, due mainly to the low solubility of polypeptides in organic solvents and to the difficulty of removing the side products in the process of the synthesis. Such complications, increasing with the number of amino acid residues in the molecule, set up a barrier to the establishment of standartized synthetic methods in peptide chemistry not to mention the mechanization of peptide synthesis.

A fundamentally new method which partly circumvents such difficulties is solid phase synthesis wherein the peptide chain which is being built up has been linked covalently (by an ester bond) to an insoluble polymer (a chloromethylated

three-dimensional styrene-divinylbenzene copolymer), allowing ready removal of excess reagents and side products (1-4). The possibilities of such a method are clearly seen in the synthesis of bradykinin (5,6).

However, this method, too, is not free of some essential shortcomings. In fact, it can be used only when all the reactions take place rapidly and in quantitative yields, otherwise the ultimate polypeptide, removed from its polymeric support would be contaminated by difficultly removable peptides of lower molecular weights. At the same time the rate and extent of formation of peptide bonds and the removal of the protecting groups depend on the permeability of the polymer. This property is very specific for each given case (method of formation of the peptide bond, the nature of the acylating amino acids, etc.) and depends on a number of factors which often can not be forseen. Thus, p-nitrophenyl esters which had been considered to be unsuitable for use in the solid phase synthesis of peptides were found to be quite satisfactory in ethyl acetate medium (7). On the other hand, acylation by proline derivatives proceeds at sufficient rates only in methylene chloride (6). Hence in the synthesis of higher peptides by this method it may turn out difficult or even impossible to follow a standart recipe.

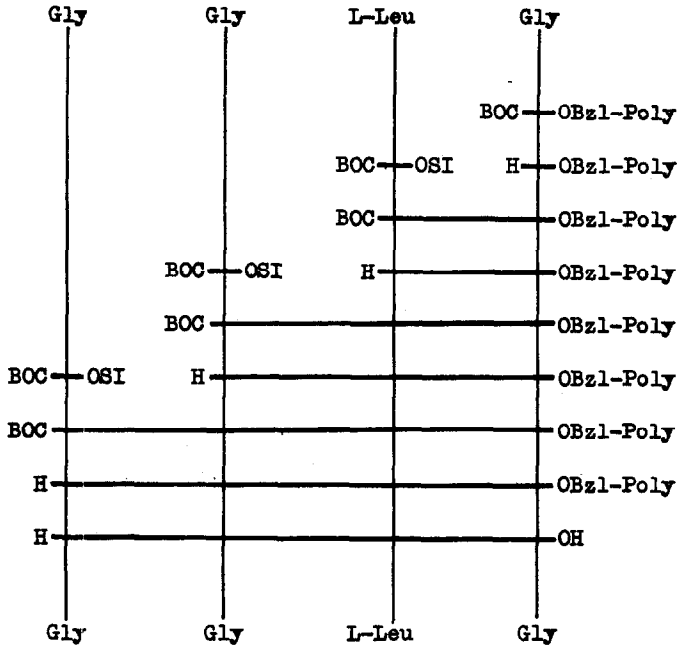
The above shortcomings could be overcome in a novel way by carrying out the peptide synthesis on a support (for instance polymer) not in the solid phase but in solution. To this end the polymer support must be so chosen that it will not only keep the substances in solution but also faci-

litate removal of excess reactants and side products. Such requirements could be met by binding the growing peptide chain to a soluble polymer support while maintaining the peptide-support ratio at a sufficiently low and little changing level throughout the synthesis, so as not to cause any considerable changes in the original properties of the support. Synthesis of the peptide chain can be carried out both in aqueous solution on a water soluble polymer, or in organic medium on a polymer soluble in the latter. The second pathway is the preferable one since it makes possible the utilization of the entire arsenal of synthetic techniques now at the disposal of the peptide chemist.

The present paper describes, as example, the synthesis of a peptide (glycylglycyl-L-leucylglycine) in an organic solvent. Emulsion polystyrene of average molecular weight 200,000 was selected as the polymer support. The aromatic nuclei of polystyrene can be substituted to various degrees by chloromethylation of the polymer with monochlorodimethyl ether in the presence of zinc chloride, depending on the amount of catalyst and the reaction time. In the present case we made use of a polymer with 5.9% Cl, which corresponds to 25% chloromethylated benzene rings. Obviously, for the synthesis of higher molecular weight peptides it is feasible to take a polymer with less chloromethyl groups in order to ensure complete solubility during the entire synthesis.

As starting point for the synthesis (see Scheme 1) served an glycyloxymethylpolystyrene hydrochloride containing 0.91 mmole glycine per g of resin, prepared by reaction of

SCHEME 1



chloromethylpolystyrene with the triethylammonium salt of tert.-butyloxycarbonylglycine in dioxane (100°, 12 hrs.) and subsequent treatment with HCl. The peptide chain was built up with the aid of N-hydroxysuccinimide esters of tert.-butyloxycarbonylamino acids (8). To a solution of the glycyloxymethylpolystyrene hydrochloride in dimethylformamide there is added 1 mole of Et_3N and 1.5 moles of N-hydroxysuccinimide ester of BOC-L-leucine. After some time gaseous HCl is bubbled through the reaction mixture at room tempera-

ture and the mixture is then poured into water. The peptide attached to the water-insoluble polymer support is filtered off, all the excess reagents and side products remaining in solution. The entire procedure is then repeated, but with the N-hydroxysuccinimide ester of another BOC-amino acid. Removal of the ultimate tetrapeptide from the resin was achieved by means of HBr in trifluoroacetic acid. The glycyl-glycyl-L-leucylglycine obtained in this way proved to be practically homogeneous chromatographically. Its yield following chromatography on Dowex 50x8 (pyridine-acetate buffer) was 65% referred to the glycyloxymethylpolystyrene hydrochloride.

Apparently this method permitting construction of a chain in solution by ordinary methods will prove to be very convenient not only in the synthesis of polypeptides, but also of other natural oligomers such as nucleotides.

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