# Peptides Synthesis: Coupling of Pentachlorophenyl Active Esters of *N*-Carbobenzoxy Amino Acids and Peptides to the Pentachlorophenyl Active Ester Hydrochlorides of Amino Acids

*Sir:*

Pentachlorophenyl active esters afford a satisfactory method for the synthesis of peptides and polypeptides with the ordered sequence of amino acids (1–3). One of the main advantages in the use of pentachlorophenyl esters is their relatively higher melting points which lead to easy crystallization and purification. The other very significant advantage lies in the fact that pentachlorophenyl esters are stable to controlled hydrogenation conditions, thereby allowing the selective removal of the *N*-carbobenzoxy protecting group (3). During the course of our work for the synthesis of biologically active peptide hormones, we extended the use of pentachlorophenyl esters by directly coupling them with amino acids which were C-protected by suitable salt formation. The aim in mind was to develop a procedure whereby aqueous alkaline treatment and the problems brought about by it could be avoided. The alkaline hydrolysis which is usually employed for the removal of methyl or ethyl C-protection in peptides is associated with a number of problems, *e.g.*, racemization (4), transpeptidation (3, 5), and formation of urea and hydantoin derivatives from the carbobenzoxy protecting group (6). In addition, hydrolysis becomes more difficult as the number of amino acids increases in the peptide chain (7).

We recently reported that *N*-carbobenzoxy amino acid or peptide pentachlorophenyl esters couple in good yields with amino acids which are C-protected by salt formation with dicyclohexylamine (8). The salt protection from the resulting peptides was removed by mild acid treatment, thereby avoiding the alkaline treatment. Stepwise lengthening of the peptide chain was achieved by using the combination of mixed anhydride and pentachlorophenyl active ester methods.

We now wish to report an interesting reaction sequence which we have found to be very rewarding as a complementary approach to the method previously described (8). Using the new approach, we have been able to couple pentachlorophenyl active esters of amino acids or peptides with pentachlorophenyl active ester hydrochlorides of amino acids in satisfactory yields. The advantage of this approach is significant since all the amino acids or peptides used in the coupling reaction are in the form of their pentachlorophenyl esters; therefore, as mentioned above, they are crystalline derivatives and easily purified (3). The outlines of the reaction conditions for the synthesis of *N*-carbobenzoxy-glycyl-serine pentachlorophenyl ester[1] are given below.

To a solution of *N*-carbobenzoxy-glycine pentachlorophenyl ester, (0.02 mole) and a catalytic amount (0.2–0.3 Gm.) of 2-hydroxypyridine in 75 ml. of methylene chloride, was added one equivalent of triethylamine. A suspension of serine pentachlorophenyl ester hydrochloride (0.02 mole) in 10 ml. of methylene chloride was added to the above solution over a period of 1 hr. After 24 hr. of stirring at room temperature, the reaction mixture was freed from solvents under reduced pressure. The residue was taken up in ethyl acetate and extracted with 1 *N* hydrochloric acid, 5% aqueous sodium bicarbonate, and water. From the ethyl acetate solution Z-Gly-Ser-OPCP was isolated in 75% yield after crystallization from methanol, m.p. 160–161°.

*Anal.*—Calcd. for $C_{19}H_{15}Cl_5N_2O_6$: C, 41.87; H, 2.75; N, 5.15. Found: C, 41.73; H, 2.69; N, 5.21.

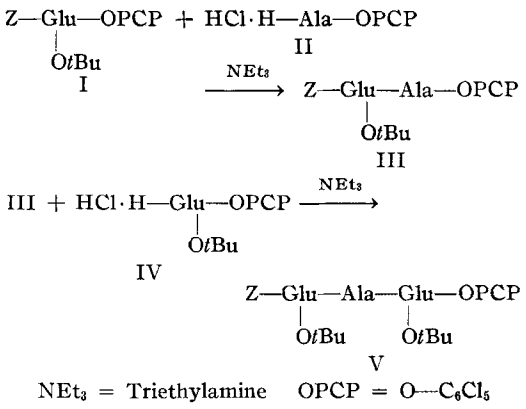
Z-Gly-Ser-OPCP was coupled following the above conditions with alanine pentachlorophenyl ester hydrochloride to afford Z-Gly-Ser-Ala-OPCP, in 69% yield, m.p. 169–170°.

*Anal.*—Calcd. for $C_{22}H_{20}Cl_5N_3O_7$: C, 42.91; H, 3.5; N, 6.83. Found: C, 43.12; H, 3.46; N, 6.78.

In order to further establish the usefulness of the above method, we selected a tripeptide active ester, *N*-carbobenzoxy-γ-*tert*-butyl-glutamyl-alanyl-γ-*tert*-butyl-glutamic acid pentachlorophenyl ester which was previously synthesized using the conventional methods (3). The yields for each step in Scheme I were above 62%.

The tripeptide (V) had melting point and ele-

[1] Abbreviations used in this paper for amino acids and peptides are those recommended in "Proceedings of the 5th European Peptide Symposium, Oxford, September, 1962," Young, G. T., ed., The Macmillan Co., New York, N. Y., 1963. Amino acids used in this work were all of L-configuration.




*Scheme I*

mental analysis identical to those previously reported (3).

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# Assay of Chlorpromazine Glucuronides in Human Urine

*Sir:*

Recently we reported a routine assay for conjugated and unconjugated chlorpromazine metabolites in human urine (1). The group of conjugated chlorpromazine metabolites was assayed by passing a small volume of urine through an ion-exchange resin to remove unconjugated drug metabolites and some contaminating endogenous urinary constituents. The eluate was then made up to 50% sulfuric acid content, and the conjugated drug metabolites were estimated spectroscopically. The procedure was satisfactory for conditions of chronic drug administration of 100 to 1400 mg. chlorpromazine per day. However, studying the excretion of chlorpromazine glucuronides in patients during the initial 4 days of low dosage chlorpromazine therapy (300 mg. or less per day), the method was found lacking in specificity and therefore inaccurate. In a typical determination, *i.e.*, with adequate amounts of chlorpromazine glucuronides, a certain amount of endogenous urinary chromogens can be compensated for by the background cancellation technique. At a starting level of 300 mg. chlorpromazine per day, an adequate urinary level of chlorpromazine glucuronides was reached only after about 1 week. A more selective assay was needed to measure the initial low levels of chlorpromazine glucuronides in the presence of excessive amounts of endogenous urinary chromogens. The following modification of the original procedure was found satisfactory.

Instead of adding 5 ml. of concentrated sulfuric acid to 5 ml. of effluent from the ion-exchange column, as previously specified, it was found practical to add 5 ml. of 50% sulfuric acid solution containing 75 mcg. $FeCl_3 \cdot 6H_2O$ per ml., in a 10-ml. volumetric flask immersed in an ice water bath. After thorough mixing under cooling, the solution was removed from the ice water bath, made up to volume with 25% $H_2SO_4$, and the absorption spectrum recorded between 400 and 700 mμ, when the mixture had reverted to room temperature. Maximum absorption was read as previously specified, at 550 mμ against a standard calibration curve prepared from 7-methoxychlorpromazine.

Sharper maxima, cleaner purple colors, and lower background readings were obtained by this modification of color development which lowered the over-all acidity from the previously specified 50% to 25%. Maximum color development was completed at 5 min., and colors remained stable for 60 min., the longest period tested.

The modification was therefore adopted for de-

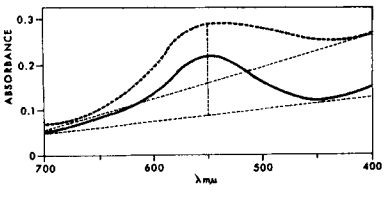


*Fig. 1—Comparison of two reagents. Determination of chlorpromazine O-glucuronides in 0.5 ml. urine of a patient dosed with 4.8 mg. chlorpromazine/Kg. per day. Key: - - -, previously reported determination in 50% sulfuric acid medium; ——, proposed modification using 25% sulfuric acid medium in the presence of ferric chloride.*