Further Applications of Pentachlorophenyl Active Esters in the Synthesis of Peptides

It was recently reported[1-3] that pentachlorophenyl active esters afford a very satisfactory method for the synthesis of peptides and polypeptides with an ordered sequence of amino acids. The peptide chain was lengthened by coupling pentachlorophenyl esters of N-carbobenzoxy amino acids or peptides with C-methyl protected amino acids or peptides. Resulting N-protected peptide methyl esters were subjected to hydrolysis to afford the N-protected free peptides.

Alkali treatment of the C-protected peptides is usually associated with a number of problems, e.g. racemization[4], transpeptidation[1,5] etc. In addition, hydrolysis becomes more difficult as the number of amino acids increases in the peptide chain[6]. It was pointed out by MACLAREN[7] that the carbobenzoxy group, on treatment with alkali, may lead to the formation of urea or hydantoin derivatives. In order to overcome these difficulties, various methods have been used for the coupling with the amino acids where the carboxyl groups were protected by suitable salt formation. Not much attention has been given to this approach because of the preparative difficulties. Among the activated esters, the *p*-nitrophenyl ester has been most frequently used for coupling reactions with amino acid salts. Various solvent mixtures and suitable pH conditions[8] have been reported; however, the yields were invariably low.

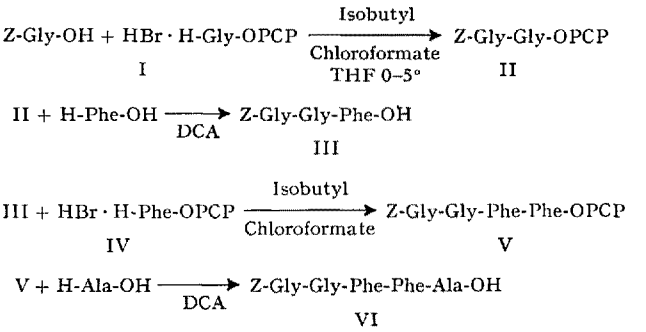
During the course of our present work for the synthesis of biologically active peptide hormones, we have employed various conditions in an attempt to couple pentachlorophenyl active esters of N-carbobenzoxy amino acids or peptides, directly with the amino acids with suitable C-protection by salt formation. We now wish to report a method for lengthening the peptide chain, by which the hydrolysis step is conveniently avoided. Dicyclohexylamine affords a satisfactory C-protection of the amino acids. These amino acid salts couple in good yields, with pentachlorophenyl esters of N-carbobenzoxy amino acids or peptides in one solvent system. From the reaction products, dicyclohexylamine is removed by mild acid treatment. The outlines of the reaction conditions for the synthesis of Z-Ala-Phe-OH are given below[9,10].

To a solution of N-carbobenzoxy-alanine pentachlorophenyl ester[1], and a catalytic amount (200–300 mg) of 2-hydroxypyridine in methylene chloride, a suspension of phenylalanine with one equivalent of dicyclohexylamine in methylene chloride was added over a period of 1 h. After 24 h stirring at room temperature, the reaction mixture was first extracted with 1N hydrochloric acid and then with 5% aqueous sodium bicarbonate. The bicarbonate extract was acidified with 3N hydrochloric acid, and immediately extracted with ethyl acetate. From the ethyl acetate solution, Z-Ala-Phe-OH[11] was isolated in 76% yield.

Using the same conditions, Z-Ala-OPCP was coupled separately with alanine and glycine to afford Z-Ala-Ala-OH[12] and Z-Ala-Gly-OH[13]. The yields in each of these reactions were 75–76%. The dipeptides so synthesized gave satisfactory elemental analysis and had identical melting points, as reported in the literature.

For stepwise lengthening of the peptide chain, we have found the combination of mixed anhydride and pentachlorophenyl active ester methods to be very rewarding. The first amino acid of the desired peptide is N-carbobenzoxylated and coupled with the C-activated pentachlorophenyl ester hydrobromide of the second amino acid, using mixed anhydride method. The N-protected dipeptide active ester so obtained is coupled with the dicyclohexylamine salt of the third amino acid. The above reaction sequence is repeated to incorporate additional amino acids. By this approach, the N-protected peptides with an odd number of amino acids will have the terminal amino acid without any C-protection. For the synthesis of even numbered peptides, the first amino acid after N-protection is converted into the pentachlorophenyl ester and coupled with the second amino acid as the dicyclohexylamine salt. Further extension of the peptide chain is carried out as described above.

To cite a typical example, Z-Gly-Gly-Phe-Phe-Ala-OH (VI), was synthesized in our laboratories. The yields for each step, described below, were above 65%.



-OPCP = -O-C_6Cl_5. DCA = Dicyclohexylamine. THF = Tetrahydrofuran.

The elemental analysis of the above compounds were within experimental tolerance.

Zusammenfassung. Pentachlorophenylaktivierte Ester von N-Carbobenzoxy-aminosäuren verbinden sich in guten Ausbeuten mit Dicyclohexylaminsalzen von Aminosäuren. Um die Hydrolyse zu vermeiden, wurde eine Kombination der Methoden der gemischten Anhydriden und pentachlorophenylaktivierten Estern zur Verlängerung der Peptidenketten benützt.

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[1] J. KOVACS, R. GIANNOTI and A. KAPOOR, J. Am. chem. Soc. *8*, 2282 (1966).
[2] J. KOVACS and A. KAPOOR, J. Am. chem. Soc. *87*, 118 (1965).
[3] J. KOVACS, H. NAGY KOVACS, J. K. CHAKRABARTI and A. KAPOOR, Experientia *21*, 20 (1965).
[4] E. SCHRÖDER and K. LÜBKE, *The Peptides* (Academic Press Inc., New York 1965), vol. 1, p. 56.
[5] E. KATCHALSKI and M. SELA, Adv. Protein Chem. *13*, 243 (1958).
[6] B. F. ERLANGER and E. BRAND, J. Am. chem. Soc. *73*, 3508 (1951).
[7] J. A. MACLAREN, Aust. J. Chem. *11*, 360 (1958).
[8] S. GUTTMANN, J. PLESS and R. A. BOISSONNAS, Helv. chim. Acta *45*, 170 (1962).
[9] Abbreviations for amino acids and peptides used in this paper are those recommended in *Proceedings of the 5th European Peptide Symposium on Peptides, Oxford, Sept. 1962* (Ed. G. T. YOUNG; The Macmillan Co., New York 1963).
[10] Amino acids used in this work were all of L configuration.
[11] J. S.-C. FU, S. M. BIRNBAUM and J. P. GREENSTEIN, J. Am. chem. Soc. *76*, 6054 (1954).
[12] W. H. STEIN, S. MOORE and M. BERGMAN, J. biol. Chem. *154*, 191 (1944).
[13] G. F. HOLLAND and L. A. COHEN, J. Am. chem. Soc. *80*, 3765 (1958).