Peptides Synthesis: Coupling of Pentachlorophenyl-Active Ester Hydrochlorides of Di- and Tripeptides with *N*-Carbobenzoxy Amino Acids Through Mixed Anhydride Method

Keyphrases ☐ Peptides—synthesis ☐ Pentachlorophenyl esters—peptide synthesis ☐ *N*-Carbobenzoxy amino acids-pentachlorophenyl esters—coupling

Sir:

Pentachlorophenyl esters, which are among the most active esters (1), afford an excellent method for the synthesis of peptides and polypeptides with an ordered sequence of amino acids (2–5). We recently reported that pentachlorophenyl active esters of *N*-carbobenzoxy amino acids couple in satisfactory yields with amino acids and peptides C-protected by dicyclohexylamine, which can be conveniently removed by mild acid treatment at the end of the synthesis (6, 7). It was observed that there was a definite increase in yields when N-protected pentachlorophenyl active esters of amino acids were coupled with di- or tripeptides instead of single amino acids C-protected by dicyclohexylamine. It was therefore concluded that in order to achieve better yields and to further limit the degree of racemization, the peptide chains should be lengthened from C-terminal instead of N-terminal residues of amino acids, when pentachlorophenyl active esters are used in combination with dicyclohexylamine C-protection (8, 9).

The above approach was extended in the synthesis of polypeptides with known sequence of amino acids by introducing C-terminal residues of amino acids as pentachlorophenyl-active ester hydrochlorides through mixed anhydride coupling. While the yields in the case of mixed anhydride coupling of N-protected amino acids with single amino acid pentachlorophenyl active ester hydrochlorides were quite satisfactory, an appreciable loss in yields was noted when the mixed anhydride coupling was attempted with di- or tripeptide pentachlorophenyl active ester hydrochlorides (9). This was attributed to the possible formation of diketopiperazine derivatives or cyclic and linear polypeptides. As in the case of the synthesis of peptides with free C-terminal amino acid residues, the ideal approach in the synthesis of polypeptides with known repeating sequence of amino acids would be, to extend the chain of desired peptide sequence from C-terminal residue of amino acids. Furthermore, C-terminal residues of amino acids must be suitably activated before they are incorporated into the peptide chain. In order to achieve this aim, mixed anhydride coupling of N-protected amino acids with di- or tripeptide pentachlorophenyl active ester hydrochlorides was reinvestigated; and the purpose of this communication is to report satisfactory reaction conditions which limit side reactions and would afford N-protected, C-activated tri- or tetrapeptides in good yields.

One of the most critical conditions in carrying out the mixed anhydride coupling in good yields is the controlled addition of di- or tripeptide pentachlorophenyl active ester hydrochlorides to the N-protected component. Reaction temperature between −5 and −10° further improves the yields. The outlines of the reaction conditions for the synthesis of Z-Gly-Gly-Gly-OPCP[1] are given below. To a solution of 2.0 g. (9.57 mmoles) of *N*-carbobenzoxy glycine in 75 ml. of ethyl acetate which was cooled to −10°, one equivalent amount each of isobutylchloroformate and triethylamine was added. After 5 min. one additional equivalent of triethylamine and a suspension of 4.0 g. (9.57 mmoles) of glycylglycine pentachlorophenyl ester hydrochloride, in 20 ml. of ethyl acetate were consecutively added over a period of 1 hr. in eight equivalent portions. During above addition the reaction temperature was maintained between −5 and −10° and after 15 min. additional stirring at −10°, triethylamine hydrochloride was removed by filtration. The filtrate was first extracted with 1 *N* hydrochloric acid and then with 5% aqueous sodium bicarbonate. The ethyl acetate solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue, a white solid, was crystallized from ethyl acetate–ether–petroleum ether to afford 4.7 g. (82%) of Z-Gly-Gly-Gly-OPCP, m.p. 189–190°. The IR spectrum showed peaks at 6.05 μ (Amide I), 6.5 μ (Amide II), and 5.6 μ (characteristic of pentachlorophenyl esters).

Anal.—Calcd. for $C_{12}H_{16}Cl_5N_3O_6$: C, 41.99; H, 2.79; N, 7.34. Found: C, 42.15; H, 2.82; N, 7.29.

Using the above procedure Z-Gly-Gly-Ala-OPCP and Z-Ala-Gly-Ala-Ala-OPCP were synthesized as shown below.

A. Synthesis of Z-Gly-Gly-Ala-OPCP

$$\text{Z-Gly-OH} + \text{HCl·H-Ala-OPCP} \xrightarrow[83\%]{\text{IBC}} \text{Z-Gly-Ala-OPCP}$$

II

$$\text{II} \xrightarrow[93\%]{\text{H}_2\text{Pd/C HCl}} \text{HCl·H-Gly-Ala-OPCP}$$

III

$$\text{Z-Gly-OH} + \text{III} \xrightarrow[81\%]{\text{IBC}} \text{Z-Gly-Gly-Ala-OPCP}$$

IV

B. Synthesis of Z-Ala-Gly-Ala-Ala-OPCP

$$\text{Z-Ala-OH} + \text{HCl·H-Ala-OPCP} \xrightarrow[84\%]{\text{IBC}} \text{Z-Ala-Ala-OPCP}$$

V

$$\text{V} \xrightarrow[91\%]{\text{H}_2\text{Pd/C HCl}} \text{HCl·H-Ala-Ala-OPCP}$$

VI

$$\text{Z-Gly-OH} + \text{VI} \xrightarrow[81\%]{\text{IBC}} \text{Z-Gly-Ala-Ala-OPCP}$$

VII

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

$$\text{VII} \xrightarrow[93\%]{H_2Pd/C\ HCl} \text{HCl} \cdot \text{H-Gly-Ala-Ala-OPCP}$$

VIII

$$\text{Z-Ala-OH} + \text{VIII} \xrightarrow[83\%]{IBC} \text{Z-Ala-Gly-Ala-Ala-OPCP}$$

IX

—OPCP = O—C_6Cl_5 IBC = isobutylchloroformate

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

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Books

REVIEWS

Molecular Orbital Theories of Bonding in Organic Molecules. By ROBERT L. FLURRY, JR. Marcel Dekker, Inc., 95 Madison Ave., New York, NY 10016, 1968. x + 334 pp. 16 × 23.5 cm. Price $17.75.

This first book in the Applied Quantum Chemical Series is intended to provide a conceptual understanding of the principles of chemical bonding as explained by molecular orbital theory. In addition, it has been written to provide a working knowledge of the methods in common usage for applying molecular orbital theory to moderately large molecules. These objectives have been met admirably by the author. The book develops the subject in a clear logical manner from a nonrigorous but understandable considerable of wave mechanics, through Hückel theory to more sophisticated extended Hückel theory and self-consistent field methods. Along the way, the author makes generous use of examples and illustrates applications. There are occasional exercises which the serious student will find advantageous to consider.

Of particular value is the chapter on sigma bonds, which is certainly a burgeoning area of activity by organic and medicinal chemists. The final chapter, dealing specifically with applications listed under physical and chemical phemomena is perhaps too short and sparse on examples; however, it does serve as a good review of significant recent work.

In my view, the book is an invaluable addition to the working library of the medicinal chemistry graduate student and a valuable guide to the older medicinal chemist, trained in classical chemistry, but desirous of learning something of this rapidly emerging chemical philosophy. This book in the hands of the graduate student, and along with other good texts in this area, should provide the basis for the conception of useful applications of molecular orbital theory to problems of drug action, drug-receptor interaction, and drug design.

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A Manual of the Penicillia. By KENNETH B. RAPER and CHARLES THOM. Hafner Publishing Co., 31 East 10th Street, New York, NY 10003, 1968. ix + 875 pp. 15 × 23.5 cm. Price $27.50.

A Manual of the Penicillia was an excellent thorough and authoritative treatise on an agriculturally and industrially most important group of fungi when it was first published in 1949. A review of that edition [*J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 59 (1950)] concluded "The authors state a twofold purpose in preparation of the manual: First 'to facilitate the identification of the Penicillia'; second 'to introduce the user to whatever information has accumulated regarding the physiology, biochemistry, pathogenicity, or other characteristics of individual species and groups.' These objectives they have achieved with consummate skill and in an interesting manner. This book should be a classic for mycologists for many years to come."

The present volume is not a new edition but, as indicated on the title page, a "facsimile of the 1949 edition," the only difference between the two being an increase of more than one hundred percent in price since the original printing which sold at $12.00—an interesting reflection on the economy of our time.

The book consists of three parts. Part I deals with Historical Aspects, Generic Diagnosis and Synonomy, Cultivation and Preservation of the Penicillia, and Penicillin. Part II is descriptive and taxonomic. The generous use of line drawings, black and white photographs, and color plates is a great asset. Part III contains two extensive bibliographies—one general, the other topical—and a useful check-list of organisms.

In view of the authors' second stated purpose, namely, "to introduce the user to . . . information that has accumulated regarding the physiology, biochemistry, pathogenicity . . . of individual species and groups," it is regrettable that cognizance has not been taken, even if only in the form of an addendum, of information that has accumulated in these areas during the 19 years since initial publication. The same comment applies also to the bibliographies in Part III, especially the topical one dealing with such subjects as allergy, antibiotics, deterioration and spoilage, culturing fungi, dermatomycoses, and enzymes—to mention only a few of the sixty categories covered.

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