Keyphrases

Trifluoperazine metabolites—nonpolar
Urinary excretion, rats—trifluoperazine metabolites
TLC—separation, identity
Paper chromatography—separation, identity
UV spectrophotometry—analysis

Further Applications of Pentachlorophenyl Active Esters in Lengthening Peptide Chains from C- and N-Terminal Amino Acids Using Dicyclohexylamine C-Protection

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In order to eliminate the problems associated with the use of alkali during peptide synthesis, a systematic investigation was carried out to lengthen peptide chains by coupling N-protected pentachlorophenyl active esters of amino acids and peptides with amino acids and peptides, C-protected by dicyclohexylamine. There was an appreciable increase in yields when N-protected pentachlorophenyl active esters of amino acids were coupled with di- and tripeptides instead of single amino acid units C-protected by dicyclohexylamine. From this, it was concluded that peptide chains would be lengthened more profitably from C-terminal instead of N-terminal amino acid residues when the synthesis of peptides is carried out, using pentachlorophenyl active esters in combination with dicyclohexylamine C-protection. In addition to affording relatively better yields, this approach would further limit the degree of racemization as the active ester component used would always be a monomer.

THE SYSTEMATIC SEARCH for suitable "activated" esters, for the synthesis of peptides *via* the aminolysis of esters started with the historic paper of Wieland and Bernhard, when they reported the synthesis of peptides *via* the phenyl thiolesters (1). For more than a decade various active esters have been used extensively in the synthesis of peptides and polypeptides with known sequence of amino acids (2–5). The pentachlorophenyl active esters, which were first reported in the literature in 1961 (6), afford an excellent method for lengthening the peptide chain. The pentachlorophenyl active esters have the following advantages: (*a*) they are one of the most active esters (7), (*b*) they are generally higher melting compounds than other active esters, which leads to their easy crystallization and purification (8), (*c*) they are conveniently prepared without any significant racemization by *N*,*N'*-dicyclohexylcarbodiimide (DCC) method (9), (*d*) they are stable to controlled hydrogenation conditions and make an excellent combination with *N*-carbobenzoxy and *tert*-butyl protecting groups when the incorporation of trifunctional amino acids in the peptides is desired (8). Previously, the peptide chain was lengthened by coupling pentachlorophenyl active esters of *N*-carbobenzoxy amino acids or peptides with C-methyl protected amino acids or peptides. C-methyl protection at each activation stage and at the end of the synthesis was removed by saponification (8, 10–12). Alkali treatment of peptides is usually associated with a number of problems, *e.g.*, racemization (13), transpeptidation (8, 14), *etc.* In addition, removal of C-methyl protection by alkali becomes more difficult as the number of amino acids increases in

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the peptide chain (15). MacLaren reported the formation of urea or hydantoin derivatives when *N*-carbobenzoxy peptides were treated with alkali (16).

Avoiding racemization of optically active centers in the synthesis of peptides with biological activity is a major concern and new approaches for the synthesis which would limit the degree of racemization in synthetic peptides are under continual investigation. To overcome racemization and other problems associated with alkali treatment, the ideal approach would be to carry out the coupling of activated N-protected amino acids or peptides with amino acids or peptides C-protected by suitable salt formation, which can easily be removed by mild acid, instead of methyl or ethyl ester protection. Among the active esters, *p*-nitrophenyl esters have been frequently used for coupling reactions with amino acid salts. Various solvent mixtures and pH conditions have been reported; however, the yields were invariably low (17).

As the pentachlorophenyl active esters (OPCP) are among the most active esters, it was considered worthwhile to systematically study their coupling with amino acids or peptides, C-protected by suitable salt formation. The purpose of this paper is to report a general method which conveniently avoids the use of alkali during stepwise lengthening of the peptide chain, both from C- and N-terminal amino acid residues.

RESULTS AND DISCUSSION

C-protection by salt formation with three different bases was investigated as follows: (*a*) Amino acids were converted into their sodium salts by treatment with an equivalent amount of sodium bicarbonate in 5–6 ml. of water. (*b*) Triethylamine salts were prepared by making a slurry of amino acids with calculated amount of triethylamine (TEA) in 10–15 ml. of methylene chloride. (*c*) C-protection by dicyclohexylamine (DCA) was carried out in the same manner as described for TEA.

Z-Ala-OPCP[1] (8), was coupled separately with sodium salts of phenylalanine, alanine, and glycine. A mixture of eights parts of acetonitrile and two parts of water was used as a solvent with 2-hydroxypyridine (18) as a catalyst. After 24 hr. the salt protection from the resulting dipeptides was removed by mild acid (1 *N* hydrochloric acid) treatment and Z-Ala-Phe-OH, Z-Ala-Ala-OH, and Z-Ala-Gly-OH were isolated in 15, 18, and 17% yields, respectively. Increasing the coupling time up to 72 hr. and substituting various other solvents and solvent mixtures, *e.g.*, tetrahydrofuran, ethyl acetate, methylene chloride, dimethylformamide, and different proportions of water in acetone, did not afford any appreciable increase in the yields.

Z-Ala-OPCP was coupled separately in methylene chloride with triethylamine salts of phenylalanine, alanine, and glycine. 2-Hydroxypyridine was used as a catalyst and after 24 hr., removal of triethylamine protection by mild acid, afforded Z-Ala-Phe-OH, Z-Ala-Ala-OH, and Z-Ala-Gly-OH in 40, 45, and 38% yields, respectively; and no increase in yields was observed by increasing the time or changing to different solvents for reaction.

Under identical conditions, DCA salts of phenylalanine, alanine, and glycine were coupled with Z-Ala-OPCP (I) in better yields; Z-Ala-Phe-OH (IV), Z-Ala-Ala-OH (V), and Z-Ala-Gly-OH (VI) were isolated in 62, 61, and 60%, respectively. Z-Gly-OPCP (II) (8) and Z-Phe-OPCP (III) (19) were coupled with a number of other amino acids, C-protected by dicyclohexylamine, and the corresponding N-protected dipeptides (VII–XIII) were isolated in satisfactory yields as indicated in Table I. Since the yields were optimal with methylene chloride, the preceding coupling reactions were carried out in this solvent.

It would seem apparent that the hydrocarbon portion of the base used for C-protection, played an important role in the coupling reactions. This can be justified on the basis of better solubility of DCA salts as compared to sodium or TEA salts of amino acids in the reaction solvents, such as methylene chloride. If this was true, then N-protected OPCP esters of amino acids would be expected to couple better with relatively more soluble di- and tripeptides, C-protected by DCA. In order to establish the effect of solubility of the C-protected component, the coupling of N-protected, C-activated amino acids with di- and tripeptide DCA salts was undertaken. Z-Gly-OPCP(II) coupled with the DCA salt of H-Gly-Phe-OH (XVII) to afford Z-Gly-Gly-Phe-OH (XX) in 68% yield, and Z-Phe-Gly-Phe-OH (XXI) was prepared in 67% yield, by coupling Z-Phe-OPCP (III) with H-Gly-Phe-OH (XVII), C-protected by DCA. The yields were even better when the DCA salts of tripeptides were coupled with activated amino acids. H-Ala-Phe-Gly-OH (XIX) C-protected by DCA, coupled with Z-Gly-OPCP (II) in 75% yield, to afford Z-Gly-Ala-Phe-Gly-OH (XXIII). N-protected dipeptide-OPCP active esters were also coupled in satisfactory yields with amino acids or peptides, C-protected by DCA. In this case, the length of the C-protected components had the similar effects on the yields as noted above. Z-Gly-Ala-OPCP (XIV) coupled with DCA salt of H-Ala-OH to afford Z-Gly-Ala-Ala-OH (XXIV) in 43% yield. However, Z-Gly-Ala-OPCP (XIV) reacted with H-Phe-Gly-OH (XVIII) C-protected by DCA in 54% yield to afford Z-Gly-Ala-Phe-Gly-OH (XXIII). In line with the authors expectations, N-protected tripeptide OPCP active esters coupled in relatively lower yields with DCA salts of amino acids. Z-Gly-Gly-Phe-OPCP (XV) coupled with H-Phe-OH, C-protected by DCA in 38% yield to afford Z-Gly-Gly-Phe-Phe-OH (XXV). It is interesting to note that N-protected OPCP active esters of amino acids could not be coupled with glutamic or aspartic acids with DCA protection on both carboxyl groups. However, the coupling of N-protected-amino acid-OPCP active esters, was found to proceed in satis-

[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., Macmillan, New York, N. Y., 1963. Amino acids used in this work were all of L-configuration.

TABLE I—YIELDS AND MELTING POINTS OF N-PROTECTED DIPEPTIDES, MADE THROUGH COUPLING OF N-PROTECTED-OPCP ESTERS OF AMINO ACIDS WITH DCA, C-PROTECTED AMINO ACIDS

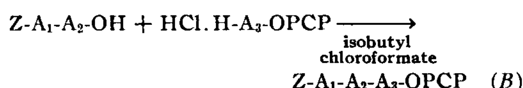
Compd.	OPCP Derivative		Amino Acid DCA Salt	Compd.	Dipeptide Formed	Yield, %	M.p., °C.	M.p., °C. (Lit)
I	Z-Ala-OPCP		H-Phe-OH	IV	Z-Ala-Phe-OH	62	121–122	122[a]
I	Z-Ala-OPCP		H-Ala-OH	V	Z-Ala-Ala-OH	61	152	152–153[b]
I	Z-Ala-OPCP		H-Gly-OH	VI	Z-Ala-Gly-OH	60	132–133	132[c]
II	Z-Gly-OPCP		H-Gly-OH	VII	Z-Gly-Gly-OH	55	178	178–179[d]
II	Z-Gly-OPCP		H-Ileu-OH	VIII	Z-Gly-Ileu-OH	59	115	114–115[e]
II	Z-Gly-OPCP		H-Ala-OH	IX	Z-Gly-Ala-OH	62	120	119.5[f]
II	Z-Gly-OPCP		H-Phe-OH	X	Z-Gly-Phe-OH	62	125–126	126[g]
II	Z-Gly-OPCP	XXVI	H-Asp(OBzl)-OH	XI	Z-Gly-Asp(OBzl)-OH	58	99–100	
III	Z-Phe-OPCP		H-Gly-OH	XII	Z-Phe-Gly-OH	61	154–155	154[g]
III	Z-Phe-OPCP		H-Pro-OH	XIII	Z-Phe-Pro-OH	63	110–111	109–110[h]

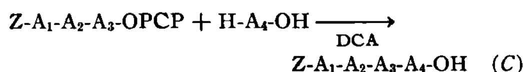
[a] *Reference 26.* [b] *Reference 27.* [c] *Reference 28.* [d] *Reference 29.* [e] *Reference 30.* [f] *Reference 31.* [g] *Reference 32.* [h] *Reference 33.*

factory yields with monoaminodicarboxylic acids, when one of the carboxyl groups was protected with DCA and the second carboxyl group was protected with a suitable ester, such as benzyl or *t*-butyl, which can be removed without alkali. Z-Gly-OPCP (II) was coupled with β-benzyl aspartate (XXVI) (20), C-protected with DCA, and Z-Gly-Asp(OBzl)-OH (XI) was isolated in 58% yield. From the above results, summarized in Table II, it can be concluded that, when OPCP esters are to be used for coupling with DCA salts of amino acids or peptides, the peptide chain should be lengthened from C-terminal instead of N-terminal amino acid residues.

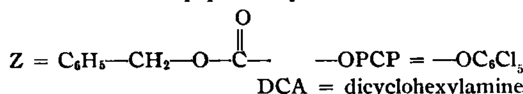
It was previously reported (21) that a combination of mixed anhydride (isobutyl chloroformate) and pentachlorophenyl active ester methods, provided a suitable approach for stepwise incorporation of amino acids into peptides with an ordered sequence. A complementary reaction sequence to this combination was also reported recently (22) in which the N-protected OPCP active esters of amino acids or peptides were coupled with OPCP active ester hydrochlorides of amino acids. The advantage of the latter approach is significant since all the amino acids or peptides used in the coupling reaction would be in the form of OPCP esters and, therefore, easily crystallized. A limitation of this approach is found in the very poor yields obtained when the coupling of N-protected OPCP active esters of amino acids or peptides is carried out with di- or tripeptide OPCP active ester hydrochlorides. This may be due to the formation of diketopiperazine derivatives in dipeptides and the formation of cyclic peptides or linear polypeptides in the case of tripeptides.

Since the C-activation by mixed anhydrides is stronger than the C-activation by OPCP esters, the yields for coupling with mixed anhydrides are better. However, problems involving cyclization and polymerization are synonymously encountered, though to a somewhat lesser degree when N-protected amino acid or peptides, C-activated by mixed anhydride, are coupled with di- or tripeptides OPCP ester hydrochlorides. It is concluded that, in the case of chain lengthening of peptides from the N-terminal residue, a combination of mixed anhydride and OPCP active ester methods would be most rewarding when single units of amino acid OPCP ester hydrochlorides are incorporated, as outlined in Scheme I. The disadvantage of this approach, as already discussed, is the lower yields at stage *C*.





A_1, A_2, A_3, and A_4 represent amino acid residues in a peptide sequence



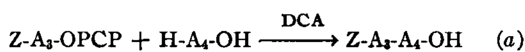
Scheme I

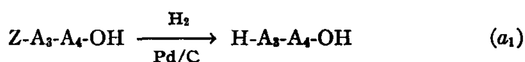
With respect to the above discussion, the following approach for extending the peptide chain from the C-terminal residue, as outlined in Scheme II, would be more suitable.

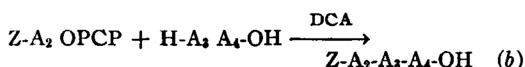
TABLE II—YIELDS AND MELTING POINTS OF N-PROTECTED TRI- AND TETRAPEPTIDES, MADE THROUGH COUPLING OF N-PROTECTED OPCP ESTERS OF AMINO ACIDS AND PEPTIDES, WITH DCA, C-PROTECTED AMINO ACIDS AND PEPTIDES

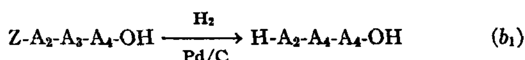
Compd.	OPCP Derivative	Compd.	DCA Protected Component	Compd.	Peptide Formed	Yield, %	M.p., °C.	M.p., °C. (Lit)
II	Z-Gly-OPCP	XVII	H-Gly-Phe-OH	XX	Z-Gly-Gly-Phe-OH	68	145–146	146[a]
III	Z-Phe-OPCP	XVII	H-Gly-Phe-OH	XXI	Z-Phe-Gly-Phe-OH	67	151–152	150–151[a]
I	Z-Ala-OPCP	XVIII	H-Phe-Gly-OH	XXII	Z-Ala-Phe-Gly-OH	68	171	
II	Z-Gly-OPCP	XIX	H-Ala-Phe-Gly-OH	XXIII	Z-Gly-Ala-Phe-Gly-OH	75	174–175	175[b]
XIV	Z-Gly-Ala-OPCP		H-Ala-OH	XXIV	Z-Gly-Ala-Ala-OH	43	173–174	172[c]
XV	Z-Gly-Gly-Phe-OPCP		H-Phe-OH	XXV	Z-Gly-Gly-Phe-Phe-OH	38	140–142	
XIV	Z-Gly-Ala-OPCP[d]	XVIII	H-Phe-Gly-OH	XXIII	Z-Gly-Ala-Phe-Gly-OH	54	175–176	175[b]
XVI	Z-Gly-Ala-Phe-OPCP		H-Gly-OH	XXIII	Z-Gly-Ala-Phe-Gly-OH	40	175	175[b]

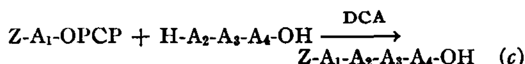
[a] *Reference 34.* [b] *Reference 32.* [c] *Reference 35.* [d] This compound was prepared by coupling through mixed anhydride, *N*-carbobenzoxy glycine with alanine pentachlorophenyl ester hydrochloride (XXVIII).











Scheme II

In Scheme II, there is a steady increase in the yields from stage $a \rightarrow b \rightarrow c$. The other advantage would be in further limiting the degree of racemization as the C-activated component used would always be a monomer. It has been reported that during coupling of N-protected, C-activated peptides, racemization of C-activated amino acid residue takes place, probably through an oxazolone (23–25). In order to compare the yields, tetrapeptide, Z-Gly-Ala-Phe-Gly-OH (XXIII) was synthesized as described below, both through Schemes I and II. As expected, the overall yield was about 10% more in the case of synthesis through Scheme II.

Synthesis of Z-Gly-Ala-Phe-Gly-OH (XXIII) Through Scheme I—Z-Gly-OPCP (II) was coupled with H-Ala-OH, C-protected by DCA, and after removal of DCA protection by mild acid, Z-Gly-Ala-OH (IX), was isolated in 62% yield. Coupling of N-protected dipeptide IX, with HCl.H-Phe-OPCP (XXVII) through mixed anhydride method, afforded Z-Gly-Ala-Phe-OPCP (XVI) in 74% yield. N-protected tripeptide active ester XVI was coupled with H-Gly-OH, C-protected by DCA, and after removal of DCA protection by mild acid, the desired tetrapeptide, Z-Gly-Ala-Phe-Gly-OH (XXIII) was isolated in 40% yield. Overall yield 18%.

Synthesis of Z-Gly-Ala-Phe-Gly-OH (XXIII) Through Scheme II—Z-Phe-OPCP(III) was coupled with H-Gly-OH, C-protected by DCA, and upon removal of DCA protection by mild acid, Z-Phe-Gly-OH (XII) was isolated in 61% yield. N-protected dipeptide XII, upon hydrogenation, afforded H-Phe-Gly-OH (XVIII) in 93% yield. Z-Ala-OPCP (I) was coupled with dipeptide XVIII, C-protected by DCA. After usual removal of DCA protection, Z-Ala-Phe-Gly-OH (XXII) was isolated in 68% yield. Removal of N-protection from tripeptide XXII by hydrogenation, afforded H-Ala-Phe-Gly-OH (XIX) in 95% yield. Z-Gly-OPCP (II) was coupled with tripeptide XIX, C-protected by DCA, and Z-Gly-Ala-Phe-Gly-OH (XXIII) was isolated in 75% yield. Overall yield 27.5%.

EXPERIMENTAL

All melting points are uncorrected and were taken on a Hoover Uni-Melt apparatus. The microanalyses were carried out by Drs. G. Weiler and F. B. Strauss, Oxford, England. Infrared spectra were determined in KBr pellets with a Beckman IR 8 spectrophotometer. Optical rotations were measured with a Rudolph precision ultraviolet polarimeter, model 200S-340-80Q3. *N*-Carbobenzoxy pentachlorophenyl active esters of amino acid used in this work were prepared by the DCC method, as previously reported (8).

Procedure A

This is the general method for the coupling of N-protected OPCP active ester derivatives with amino acids and peptides C-protected by DCA.

Preparation of N-Carbobenzoxy-alanyl-alanine (V)—To a stirred solution of 2.0 g. (4.24 mmoles) of *N*-carbobenzoxy alanine pentachlorophenyl ester-(I), and 0.1 g. of 2-hydroxypyridine, as a catalyst, in 80 ml. of methylene chloride, was added in 2-ml. portions over the period of 30 min. 10 ml. of a suspension containing 0.377 g. (4.24 mmoles) of alanine and 0.83 ml. (4.24 mmoles) of dicyclohexylamine (DCA). The reaction mixture, after magnetically stirring for 24 hr., at ambient temperature, was spin evaporated *in vacuo*. The residue was dissolved in 100 ml. of ethyl acetate and the resulting solution was treated with 40 ml. of 2 *N* HCl, then filtered to remove the insoluble dicyclohexylamine hydrochloride. The aqueous layer was drawn off and the ethyl acetate layer was washed with two 40-ml. portions of water. The ethyl acetate layer was extracted with three 15-ml. portions of 5% aqueous sodium bicarbonate. The bicarbonate extracts were combined and acidified with 1 *N* HCl; the clear solution became cloudy during the acid addition. The acidified suspension was extracted with two 50-ml. portions of ethyl acetate. The ethyl acetate layer was washed three times with 40-ml. portions of water and dried over anhydrous sodium sulfate. Spin evaporation of ethyl acetate solution *in vacuo* left a brown viscous oil which was crystallized from ethyl acetate–ether–petroleum ether (b.p. 30–60°). The mixture was kept at $-15°$ overnight, then the product, *N*-carbobenzoxy-alanyl-alanine (V) was collected on a filter; 0.76 g., (61%), m.p. 152° [lit. (27) m.p. 152–153°]. *N*-Carbobenzoxy peptides with free C-terminal, listed in Tables I and II were prepared using the above method.

Procedure B

This is a general method for the coupling of N-protected amino acids or peptides through mixed anhydride with pentachlorophenyl active ester hydrochlorides of amino acids.

Preparation of N-Carbobenzoxy Glycyl-alanine Pentachlorophenyl Ester (XIV)—Alanine pentachlorophenyl ester hydrochloride (XXVIII) was prepared by hydrogenation of *N*-carbobenzoxy alanine pentachlorophenyl ester (I) in the presence of anhydrous methanol containing hydrogen chloride, according to the method previously reported (8), in 91% yield, m.p. 216°, $[\alpha]_D^{25}$ +29.2° (c 1.0, methanol).

Anal.—Calcd. for $C_9H_7Cl_6NO_2$: C, 28.87; H, 1.87; N, 3.74. Found: C, 28.91; H, 1.79; N, 3.81.

To a stirred solution of 2.0 g. (9.57 mmoles) of *N*-carbobenzoxy glycine (11) in 50 ml. of tetrahydrofuran at $-10°$ was added 1.33 ml. (9.57 mmoles) of triethylamine and 1.3 ml.(9.57 mmoles) of isobutylchloroformate. After 30 min. 1.33 ml. (9.57 mmoles) of triethylamine was added to the reaction mixture followed by stepwise addition, over a 30-min. period, of a suspension of 3.59 g. (9.57 mmoles) of alanine pentachlorophenyl ester hydrochloride in

10 ml. of tetrahydrofuran. The stirred reaction mixture was left at −10° for 4 hr., then freed from the solvent by spin evaporation *in vacuo*. The residue was triturated with 100 ml. of hot ethyl acetate and filtered free from triethylamine hydrochloride. The filtrate was extracted three times with 30-ml. portions of 1 *N* hydrochloric acid, water, and 5% aqueous sodium bicarbonate and water. The ethyl acetate layer was dried over anhydrous sodium sulfate. The residue obtained after spin evaporation of ethyl acetate was crystallized from ethyl acetate–ether–petroleum ether to yield 4.40 g. (87%) of N-protected dipeptide active ester XIV, m.p. 158–159°, $[\alpha]_D^{19}$ −1.8° (c 1.0, DMF). The infrared 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 $C_{19}H_{15}Cl_5N_2O_5$: C, 43.21; H, 2.84; N, 5.30. Found: C, 43.15; H, 2.91; N, 5.41.

Procedure C

This consisted of removal of *N*-carbobenzoxy protecting group from peptides with free C-terminal residue.

Preparation of Glycyl-phenylalanine (XVII)—A suspension of 0.3 g. palladium-charcoal catalyst (10%) in a 20-ml. mixture of 8 parts of methanol and 2 parts of water, was hydrogenated at atmospheric pressure until no further uptake of hydrogen occurred. A solution of 2.0 g. (5.62 mmoles) of *N*-carbobenzoxy glycyl-phenylalanine(X), in 10 ml. of methanol was added to the reaction mixture and the hydrogenation was carried out until no further uptake of hydrogen occurred (55 ml. of hydrogen in 10 min.). The reaction mixture was filtered free from the catalyst and the filtrate was spin evaporated under reduced pressure. The residue was crystallized from ethanol–ethyl acetate to yield 1.17 g. (94%) of the free dipeptide XVII, m.p. 266–268° dec. [lit. (34) m.p. 264–268° dec.].

Preparation of Phenylalanyl-glycine (XVIII)—*N*-Carbobenzoxy phenylalanyl-glycine (XII) was hydrogenated according to Procedure C, in 93% yield to afford the dipeptide XVIII, m.p. 269–270° dec. [lit. (34) m.p. 270–271° dec.].

Preparation of N-Carbobenzoxy Glycyl-glycyl-phenylalanine (XX)—Using the appropriate amounts, *N*-carbobenzoxy glycine pentachlorophenyl ester (11) was coupled with glycyl-phenylalanine (XVII), C-protected by DCA in the manner described in Procedure A. The N-protected tripeptide XX was obtained in 68% yield, m.p. 145–146° [lit. (34) m.p. 146°].

Preparation of N-carbobenzoxy Phenylalanyl-glycyl-phenylalanine (XXI)—The coupling of N-protected phenylalanine active ester III with the free dipeptide XVII, C-protected by DCA according to Procedure A, afforded the N-protected tripeptide XXI in 67% yield, m.p. 151–152° [lit. (34) m.p. 150–151°].

Preparation of N-Carbobenzoxy Glycyl-alanyl-alanine (XXIV)—N-protected dipeptide active ester XIV was coupled with alanine, C-protected by DCA in the manner described in Procedure A and the desired tripeptide XXIV was isolated in 43% yield, m.p. 173–174° [lit. (35) m.p. 172°].

Preparation of N-Carbobenzoxy Glycyl-glycyl-phenylalanyl-phenylalanine (XXV)—*N*-Carbobenzoxy glycyl-glycyl-phenylalanine pentachlorophenyl ester (XV), was prepared in the manner described (8). N-Protected tripeptide active ester XV was coupled according to Procedure A with phenylalanine C-protected by DCA. The tetrapeptide XXV was isolated in 38% yield, m.p. 140–142°.

Anal.—Calcd. for $C_{30}H_{32}N_4O_7$: C, 64.29; H, 5.72; N, 10.00. Found: C, 64.38; H, 5.81; N, 9.92.

Preparation of N-Carbobenzoxy Glycyl-β-benzyl Aspartic Acid (XI)—β-Benzyl aspartate (XXVI) was prepared according to the method reported by Benoiton (20). From appropriate amounts of N-protected glycine active ester II, and compound XXVI, C-protected by DCA, coupling was carried out following Procedure A. The dipeptide XI was isolated in 58% yield after crystallization from ethyl acetate–ether, m.p. 99–100°, $[\alpha]_D^{20}$ +15.7° (c 1.0, methanol).

Anal.—Calcd. for $C_{21}H_{22}N_2O_7$: C, 60.8; H, 5.32; N, 6.77. Found: C, 60.88; H, 5.13; N, 6.76.

Preparation of N-Carbobenzoxy Glycyl-alanyl-phenylalanyl-glycine (XXIII)—*Method 1*—Coupling of N-protected dipeptide active ester XIV, to free dipeptide XVIII. From 2.0 g. (3.78 mmoles) of *N*-carbobenzoxy glycyl-alanine pentachlorophenyl ester (XIV), and 0.84 g. (3.78 mmoles) of phenylalanyl-glycine (XVIII), C-protected by appropriate amount of DCA, coupling was carried out according to Procedure A. The N-protected tetrapeptide XXIII, 0.99 g. (54%) was isolated after crystallization from methanol–ether, m.p. 175–176° [lit. (32) m.p. 175°].

Method 2—Stepwise chain lengthening of the tetrapeptide XXIII, from N-terminal residue (Scheme I). *N*-Carbobenzoxy glycyl-alanine (IX) was prepared by coupling 2.0 g. (4.38 mmoles) of N-protected glycine active ester II, with 0.39 g. (4.38 mmoles) of alanine C-protected by 0.86 ml. (4.38 mmoles) of DCA. Following the reaction conditions outlined in Procedure A, 0.76 g. (62%) of the N-protected dipeptide IX, was obtained, m.p. 120° [lit. (31) m.p. 119.5°]. Phenylalanine pentachlorophenyl ester hydrochloride (XXVII) was prepared by hydrogenation of *N*-carbobenzoxy phenylalanine pentachlorophenyl ester (III) in the presence of anhydrous methanol containing hydrogen chloride, according to the method previously reported (8), in 93% yield, m.p. 199–200°, $[\alpha]_D^{23}$ −23.4° (c 1.07, methanol).

Anal.—Calcd. for $C_{15}H_{11}Cl_6NO_2$: C, 40.00; H, 2.44; N, 3.11. Found: C, 39.95; H, 2.51; N, 3.08.

N-Carbobenzoxy glycyl-alanyl-phenylalanine pentachlorophenyl ester (XVI) was prepared by mixed anhydride method as outlined in Procedure B. From 1.0 g. (3.57 mmoles) of the N-protected dipeptide IX and 1.6 g. (3.57 mmoles) of the active ester hydrochloride XXVII, 1.78 g. (74%) of N-protected tripeptide active ester XVI was obtained, m.p. 187–189°. Recrystallization from ethyl acetate–ether raised the melting point to 191–192°, $[\alpha]_D^{22}$ −49.4° (c 1.0, DMF). Infrared spectrum showed peaks at 6.04 μ (amide I), 6.5 μ (amide II), and 5.6 μ (pentachlorophenyl ester).

Anal.—Calcd. for $C_{28}H_{24}Cl_5N_3O_6$: C, 49.74; H, 3.56; N, 6.22. Found: C, 50.03; H, 3.48; N, 6.15.

N-Protected tetrapeptide XXIII, was prepared according to Procedure A. From 1.5 g. (2.22 mmoles) of tripeptide active ester XVI, and 0.166 g. (2.22 mmoles) of glycine, C-protected by 0.44 ml.

(2.22 mmoles) of DCA, 0.43 g. (40%) of *N*-carbobenzoxy glycyl-alanyl-phenylalanyl-glycine (XXIII) was obtained, m.p. 175°.

Method 3—Stepwise chain lengthening of the tetrapeptide XXIII, from C-terminal residue (Scheme II). *N*-Carbobenzoxy phenylalanyl-glycine (XII) was prepared according to Procedure A. From 2.0 g. (3.65 mmoles) of N-protected phenylalanine active ester III, and 0.274 g. (3.65 mmoles) of glycine, C-protected by appropriate amount of DCA, 0.79 g. (61%) of the dipeptide XII, was obtained, m.p. 154–155° [lit. (32) m.p. 154°]. Removal of *N*-carbobenzoxy protecting group from XII, as described in Procedure C, afforded the free dipeptide XVIII, in 93% yield.

N-Carbobenzoxy alanyl-phenylalanyl-glycine (XXII), was prepared according to Procedure A, by coupling 2.0 g. (4.24 mmoles) of N-protected alanine active ester I, with 0.94 g. (4.24 mmoles) of the dipeptide XVIII, C-protected by appropriate amount of DCA, and 1.23 g. (68%) of N-protected tripeptide XXII, was obtained, m.p. 168–170°. Recrystallization from methanol–ether raised the melting point to 171°, $[\alpha]_D^{21}$ −46.5° (c 1.02, methanol).

Anal.—Calcd. for $C_{22}H_{25}N_3O_6$: C, 61.82; H, 5.85; N, 9.84. Found: C, 61.78; H, 5.73; N, 9.71.

N-Carbobenzoxy tripeptide XXII was hydrogenated in the manner described in Procedure C, and alanyl-phenylalanyl-glycine (XIX), was obtained in 95% yield upon crystallization from ethanol–ethyl acetate, m.p. 233–234° dec., $[\alpha]_D^{21}$ +17.7° (c 1.02, 1 *N* HCl).

Anal.—Calcd. for $C_{14}H_{19}N_3O_4$: C, 57.34; H, 6.48; N, 14.33. Found: C, 57.45; H, 6.57; N, 14.21.

N-Protected tetrapeptide XXIII, was prepared according to Procedure A. From 1.0 g. (2.18 mmoles) of *N*-carbobenzoxy glycine pentachlorophenyl ester II, and 0.64 g. (2.18 mmoles) of the tripeptide XIX, C-protected by 0.42 ml. (2.18 mmoles) of DCA, 0.79 g. (75%) of *N*-carbobenzoxy-glycyl-alanyl-phenylalanyl-glycine (XXIII) was obtained, m.p. 174–175°.

REFERENCES

(1) Wieland, T., and Bernhard, H., *Ann.*, 582, 218(1953).
(2) Bodanszky, M., *Acta Chim. Acad. Sci. Hung.*, **10**, 335(1956).
(3) Schwyzer, R., and Hurliman, C., *Helv. Chim. Acta*, **37**, 155(1954).
(4) Detar, D. F., "Proceedings of the 8th European Peptide Symposium, Noordwijk, Netherland, September 1966," Beyerman, H. C., Van De Linde, A., and Maassen Van Den Brink, W., Eds., Wiley, New York, N. Y., 1966, p. 125.
(5) Stewart, H. F., *Australian J. Chem.*, **18**, 887(1965).
(6) Kupryszewski, G., and Formela, M., *Rocsniki Chem.*, **35**, 1933(1961).
(7) Stick, K., and Leemann, G. H., *Helv. Chim. Acta*, **46**, 1887(1963).
(8) Kovacs, J., Gianotti, R., and Kapoor, A., *J. Am. Chem. Soc.*, **88**, 2282(1966).
(9) Kovacs, J., Kisfaludy, L., and Ceprini, Q. M., *ibid.*, **89**, 183(1967).
(10) Kovacs, J., and Kapoor, A., *ibid.*, **87**, 118(1965).
(11) Kovacs, J., Kovacs, N. H., Chakarabarti, J. K., and Kapoor, A., *Experientia*, **21**, 20(1965).
(12) Kovacs, J., Schmit, G. N., Gianotti, R., and Kapoor, A., Abstracts of 151st National Meeting of American Chemical Society, Pittsburgh, Pa., April 1966, p. K-20.
(13) Schroder, E., and Lubke, K., "The Peptides," vol. 1, Academic Press Inc., New York, N. Y., 1965, p. 56.
(14) Katchalski, E., and Sela, M., *Advan. Protein Chem.*, **13**, 243(1958).
(15) Erlanger, B. F., and Brand, E., *J. Am. Chem. Soc.*, **73**, 3508(1951).
(16) MacLaren, J. A., *Australian J. Chem.*, **11**, 360(1958).
(17) Guttman, S., Pless, J., Boissonnas, A. R., *Helv. Chim. Acta*, **45**, 170(1962).
(18) Beyerman, C. H., Vanden Brink, M. W., *Proc. Chem. Soc.*, **1963**, 266.
(19) Pless, J., and Boissonnas, A. R., *Helv. Chim. Acta*, **44**, 1609(1963).
(20) Benoiton, L., *Can. J. Chem.*, **40**, 570(1962).
(21) Kapoor, A., and Davis, E. J., *Experientia*, **23**, 253(1967).
(22) Kapoor, A., *J. Pharm. Sci.*, **56**, 1532(1967).
(23) Goodman, M., and Stueben, K., *J. Org. Chem.*, **27**, 3409(1962).
(24) Williams, W. M., and Young, G. T., *J. Chem. Soc.*, **1964**, 3701.
(25) Goodman, M., and McGahren, J. W., *J. Am. Chem. Soc.*, **87**, 3028(1965).
(26) Fu, S-C. J., Birnbaum, S. M., and Greenstein, N. P., *ibid.*, **76**, 6054(1954).
(27) Stein, W. H., Moor, S., and Bergmann, M., *J. Biol. Chem.*, **154**, 191(1944).
(28) Holland, G. F., and Cohen, L. A., *J. Am. Chem. Soc.*, **80**, 3765(1958).
(29) Bergmann, M., and Zervas, L., *Ber.*, **65**, 1192(1932).
(30) Stahmann, M., Furton, J. S., and Bergmann, M., *J. Biol. Chem.*, **164**, 753(1946).
(31) Erlanger, B. F., and Brand, E. J., *J. Am. Chem. Soc.*, **73**, 3508(1951).
(32) Clayton, D. W., Farrington, J. A., Kenner, G. W., and Turner, J. M., *J. Chem. Soc.*, **1957**, 1398.
(33) Neuman, R. E., and Smith, E. L., *J. Biol. Chem.*, **193**, 97 (1951).
(34) Ben-Ishai, D., *J. Org. Chem.*, **19**, 62(1954).
(35) Brand, E., Erlanger, B. F., Sachs, H., and Polatnick J., *J. Am. Chem. Soc.*, **73**, 3510(1951).

Keyphrases

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Alkali elimination—peptide chain lengthening
Optical rotation—identity
IR spectrophotometry—structure