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Amino Acids and Peptides. III.[1] Synthesis of Some Glycine-Oligopeptides by Way of Pentachlorophenyl Esters[2,3]

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Some glycine-containing oligopeptides were prepared as model substrates. In the course of the synthesis, Goodman's one-step coupling method as well as some fragment condensation by means of PCPOH was applied.

In connection with our studies of proteolytic enzymes[5] and of chemical modification of peptides,[1,6] some glycine-containing oligopeptides were required as model substrates and their synthetic intermediates. This paper is concerned with the synthesis of these peptides employing pentachlorophenol (PCPOH) as an acitve ester component either by the adaptation of Goodman's one-step coupling method or by a fragment condensation.

A very interesting device with regard to the active ester method[7] involves certain difunctional amino acid derivatives **2** originally prepared and utilized by Goodman and Stueben.[8] By taking advantage of the difunctionality of **2** as well as the difference in rate among common acylating reaction in peptide formation, several tripeptides were synthesized directly *via* two consecutive reactions as outlined in Chart 1. The first and rapid acylating reaction

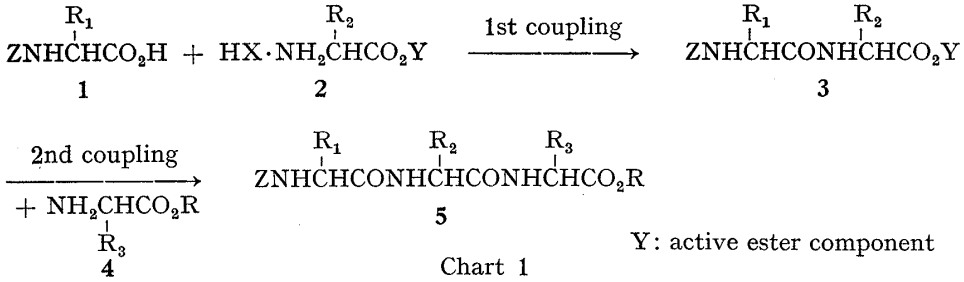

Chart 1

is made at the amino end of the active ester (**2**; Y=PNP) by means of dicyclohexylcarbodiimide (DCC) or mixed anhydride (MA) method. Without isolation of the resultant **3**, the second

1) Part II: Y. Kanaoka, E. Sato, and O. Yonemitsu, *Tetrahedron*, **24**, 2591 (1968).
2) Presented in part before the 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, Oct. 1966; Abstracts of Papers, p. 143.
3) Abbreviations of amino acids used are those recommended by IUPAC-IUB: *cf. Biochemistry*, **5**, 2485 (1966); Gly, glycine; Phe, phenylalanine; Trp, tryptophan; Tyr, tyrosine; Z, benzyloxycarbonyl; Pht, phthalyl; Bz, benzoyl; OEt, ethyl ester; OMe, methyl ester.
4) Location: *Kita-12, Nishi-6, Sapporo.*
5) K. Tanizawa, S. Ishii, and Y. Kanaoka, *Seikagaku*, **38**, 530 (1966) (Abs. Papers); Y. Kanaoka, *Biochem. Biophys. Res. Commun.*, **32**, *K.* Tanizawa, S. Ishii and 893 (1968).
6) Y. Kanaoka and O. Yonemitsu, to be published.
7) *a*) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N.Y., 1961, p. 1027; *b*) N.F. Albertson, "Organic Reactions," Vol. 12, John Wiley and Sons, Inc., New York, N.Y., 1962, p. 222; *c*) E. Schröder and K. Lubke, "The Reptides," Vol. 1., Academic Press, New York, N.Y., 1966, p. 97.
8) M. Goodman and K.C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).

step is brought about at the active ester end of **3** to form desired protected tripeptide **5**. This method has been conveniently used by Goodman, *et al.* for the synthesis of oligopeptides.[9]

In view of the fact that the utility of PCPOH in peptide synthesis has recently been established by the work of Kovacs, *et al.*,[10] it seemed advisable to employ PCPOH as an active ester component in an attempt to improve this procedure.

In order to reconfirm the reactivity of PCPOH in experiments on a preparative scale, a series of simple acylation reactions with pentachlorophenyl esters (PCP–ester) were compared with those of corresponding *p*–nitrophenyl esters (PNP–ester), a typical active ester. Table I gives sets of experiments including three classes of examples; *i.e.*, preparation of acid amides, amino acid amides and dipeptides. These paired data show that acylation with PCP–esters invariably gave better yield than that with PNP–esters indicating good reactivity of PCPOH in practical coupling reactions.

Table I. Comparison of PCP–ester with PNP–ester in Coupling Reactions

Active ester	Amine component	Product	Time (solvent refluxed) (min)	Yield (%)	mp (C°)
Bz·OPNP	benzylamine	N–benzylbenzamide	60 (AcOEt)	74	103—105 (EtOH)[a]
Bz·OPCP	benzylamine	N–benzylbenzamide	60 (AcOEt)	89	
Pht·Gly·OPNP **6**	benzylamine	Pht·Gly–benzylamide **8**	5 ($CHCl_3$)	86	213—214 (EtOH)[b]
			10 (C_6H_6)	63	
Pht·Gly·OPCP **7**	benzylamine	**8**	5 ($CHCl_3$)	91	
			10 (C_6H_6)	83	
6	**14-1**	Pht·Gly·Gly·OEt **9**	10 ($CHCl_3$)	42	190—193 (EtOH)[c]
7	**14-1**	**9**	10 ($CHCl_3$)	51	

a) lit.,[11] mp 105—107° *b*) lit.,[12a] mp 214—216°; lit.,[12b] mp 216—218° *c*) lit.,[12a] 192—194°; lit.,[13] mp 191—193°

A key compound in Goodman's scheme is an amino acid active ester **2**, which is to be prepared from N–blocked amino acid active ester **13-1** (m=1 in **13**) by removing the benzyloxycarbonyl group.

There is diversity in the described melting points of N–benzyloxycarbonylglycine pentachlorophenyl ester **13-1** as following: 186—187°[14a]; 133—134°[14b,d]; 134°[15]; 180—182°.[14c] The ester **13-1** having the lower melting point (133—134°) was obtained from N–benzyloxycarbonylglycine **1a** (R_1=H in **1**) and PCPOH by conventional DCC or MA method in fairly good yield. The structure of this compound was established by elemental analysis, IR spectrum (carbonyl band of PCP–ester at 1787 cm^{-1}), and finally, by conversion to a known compound. Coupling of **13-1** with glycine ethyl ester **14-1** (n=1 in **14**) yielded the known N–benzyloxycarbonylglycylglycine ethyl ester **15-2** ($m+n$=2 in **15**), which was identical with the authentic specimen prepared by independent synthesis.

In an experiment using MA method with ethyl chloroformate as a reagent, small amount of by–product having the melting point of 169—171° was isolated. The N–acylamide structure

9) M. Goodman and F. Boardman, *J. Am. Chem. Soc.*, **85**, 2483 (1963); M. Goodman, I.G. Rosen and M. Safdy, *Biopolymers*, **2**, 503 (1964); M. Goodman and M. Langsam, *ibid.*, **4**, 275 (1966).
10) J. Kovacs, L. Kisfaludy and M.Q. Ceprini, *J. Am. Chem. Soc.*, **89**, 183 (1967); *cf.* also papers cited therein.
11) C. Blacher, *Chem. Ber.*, **28**, 432 (1895).
12) *a*) Y. Kanaoka, M. Machida, O. Yonemitsu, and Y. Ban, *Chem. Pharm. Bull.* (Tokyo), **13**, 1065 (1965); b) J.C. Sheehan and V.S. Frank, *J. Am. Chem. Soc.*, **72**, 1312 (1950).
13) J.C. Sheehan and J.J. Hlavka, *J. Org. Chem.*, **23**, 635 (1958).
14) *a*) G. Kupryszewski and M. Formela, *Roczniki Chemi*, **35**, 1533 (1961) [*C.A.*, **57**, 7373 (1962)]; *b*) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, **38**, 1980 (1965); *c*) Y. Wolman, D. Ladkany, and M. Frankel, *J. Chem. Soc.*, (C), **1967**, 689; *d*) M. Fujino and T. Hatanaka, the 5th Symposium on Peptide Chemistry, Nov. 1967, Kyoto, Abs. p. 15.
15) J. Kovacs, R. Giannotti, and A. Kapoor, *J. Am. Chem. Soc.*, **88**, 2282 (1966).

Z–NHCH$_2$CO
Z–N–CH$_2$CO$_2$PCP
10

10 is tentatively assigned for this compound based on elemental analysis and IR data (imide carbonyl band at 1740 cm^{-1}), which was presumably produced by subsequent acylation of the initially formed **13-1** with the second acylating species such as **13-1** itself.[16] This occasional occurrence of **10** under the coupling conditions might explain the above diversity of the description of **13-1** in the literatures.

Treatment of **13-1** with hydrobromic acid in acetic acid smoothly gave the active ester with unprotected amino group **2a**, which was good crystallizing and could be stored unchanged for a long period.

Since it seemed helpful to examine each step of the synthesis separately, the first coupling was tried with **1a** and **2a** using isobutyl chloroformate as a reagent. Benzyloxycarbonylglycylglycine pentachlorophenyl ester **13-2** (m=2 in **13**; R_1=R_2=H, Y=PCP in **3**) was obtained as good crystallizing fine needles of mp 172—174° in 91% yield.[17] The second coupling, to combine **13-2** with the third component, glycine ethyl ester **14-1** (n=1 in **14**), gave the tripeptide **15-3** (m+n=3 in **15**) in only 36% yield. This poor result for the synthesis of the triglycine derivative may be ascribed to the side reaction as commented by Goodman in the case of PNP–ester.[8] However, when applied to several hydrochlorides of amino acid esters such as L–phenylalanine methyl ester **4a** or DL–tryptophan methyl ester **4b** in the presence of equivalent amount of base, the dipeptide active ester **13-2**, gave the tripeptides, **5a** and **5b**, in 93 and 84% yield, respectively.

Finally, **1a**, **2a** and then **4a** or **4b** were subjected to the one-step procedure without isolation of the intermediate **13-2**. Thus **5a** or **5b** was obtained in 83 or 78% yield. Similarly, **1a**, **2a** and L–tyrosine ester **4c** were coupled to give N–benzyloxycarbonyglycylglycine–L–tyrosine methyl ester **5c** in 82% yield. These examples thus demonstrate that PCP–esters can be employed satisfactorily in Goodman's scheme. This adaptation may provide, together with the good results in racemization tests with PCPOH esters by Kovacs, *et al.*,[10] a convenient technique for conventional oligopeptide synthesis. Some of the above tripeptides

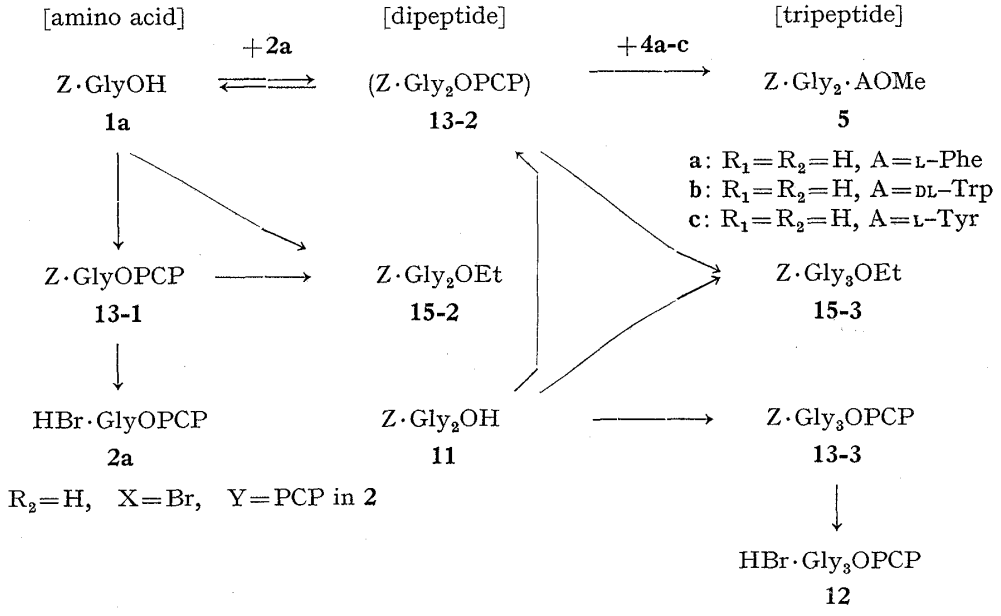

Chart 2

16) Wieland and Heinke isolated 10% of the acid (PCP=H in **10**) by treating Z·GlyOH with phosphoryl chloride; T. Wieland and B. Heinke, *Annal.*, **599**, 70 (1956).

17) Recently, Kapoor and Davis reported the synthesis of **13-2** in a similar manner; A. Kapoor and E.J. Davis, *Experientia*, **15**, 253 (1967). *cf.* also ref. 10*d*.

containing aromatic amino acids will be used as substrates in studies of chemical modification of peptides.[6]

Since it is well known that dipeptide esters in general can easily be cyclized to form diketopiperazines, no attempt was made to prepare diglycine PCP–ester from **13-2**. From N–benzyloxbcarbonylglycine **11** and **2a**, N–benzyloxycarbonyltriglycine PCP–ester **13-3** ($m=3$ in **13**) was prepared, which was further treated with hydrobromic acid in acetic acid to form the tripeptide active ester **12**. **12** may be useful both as an intermediate in stepwise coupling and as a monomer in polymerization reaction. Chart 2 summarizes the synthesis involving these PCP–esters.

The good reactivity of PCP–esters in aminolysis, as known kinetically,[18,19] and illustrated in preparative model experiments (Table I), together with their utility as reactive monomers in the synthesis of sequence peptide polymers,[15] suggests that they would be particularly effective in "fragment condensation" of peptides, in which two fragments of peptides are combined to form a long peptide chain.[20] As shown in Chart 3, several N–protected glycine PCP–esters **13** were thus coupled with some glycine–peptide esters **14** in order to examine their use in fragment condensation.

$$\underset{\textbf{13}}{Z\cdot Gly_mOPCP} + \underset{\textbf{14}}{Gly_nOEt} \longrightarrow \underset{\textbf{15}}{Z\cdot Gly_{m+n}OEt}$$

Chart 3

Table II contains a list of the glycine–oligopeptides which were synthesized using corresponding PCP–esters in good yields. Some of the peptides are synthetic intermediates of model substrates to form backbones of peptide chains.[5,6]

Table II. Synthesis of Glycine–oligopeptides

PCP–ester	Gly–ester	Product	Yield (%)	mp (°C)	IR (carbonyl)[a]		
					Z	peptide	ester
13-1	**14-3**	**15-4**	87	210—212[b]	1694	1650	1748
13-2	**14-3**	**15-5**	95	255—256[c]	1699	1645	1726
13-3	**14-3**	**15-6**	87	281—283[c]	1705[d]	1644	1725[d]

a) Nujol, cm^{-1} *b*) lit.,[21] mp 205°
c) crude yield and mp. The product was treated with hydrobromic acid to remove benzyloxycarbonyl group.
d) broad

Experimental[22]

Model Coupling Reaction with PCP-ester; General Procedure (*cf.* Table I)——Active ester (1.5 mmoles) and amino component (1.5 mmoles) were mixed with *ca.* 10 ml of solvent and the mixture was refluxed. After the reaction, the solution was washed consecutively with 10% HCl, water, 10% Na_2CO_3, water and dried with anhydrous sodium sulfate. A solvent was removed *in vacuo* and the residue was recrystallized to give the product.

N-Benzyloxycarbonylglycine Pentachlorophenyl Ester 13-1——a) MA Method: To a mixture of **1a** (2092 mg) and triethylamine (1214 mg) in tetrahydrofuran (120 ml) was added isobutyl chloroformate (1639 mg) with stirring and cooling. After stirring for 15 min, a solution of PCP–OH (2664 mg) in tetrahydrofuran was added and the mixture stirred for 4 hr with cooling. The precipitated triethylamine salt

18) J. Pless and R.A. Boissonnas, *Helv. Chem. Acta*, **46**, 1906 (1963).
19) K. Stich and H.G. Leeman, *Helv. Chem. Acta*, **46**, 1887 (1963).
20) Fujino and Hatanaka reported preparation of *t*–alkyl pentachlorophenyl carbonate and its use in peptides synthesis; ref. 14*d*.
21) S. Goldschmidt and H. Lautenschlagen, *Annal.*, **580**, 68 (1953).
22) All melting points are uncorrected.

was removed by filtration and the solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate, the solution washed with sodium bicarbonate solution, water, and dried with anhydrous sodium sulfate. Removal of a solvent *in vacuo* gave a solid residue, which was recrystallized from ethanol to form colorless needles of mp 133—134°; 3152 mg (69%). The second crop was obtained from the mother liquor to give total yield of 82%. IR ν_{max}^{Nujol} cm^{-1}: 1787 (C=O, PCP–ester), 1690 (C=O, Z). *Anal.* Calcd. for $C_{16}H_{10}O_4NCl_5$: C, 42.00; H, 2.20; N, 3.06. Found: C, 42.08; H, 2.12; N, 3.17.

b) DCC Method: The same amounts of **la** and PCPOH were reacted with DCC (2476 mg) as a reagent in ethyl acetate solution with cooling for 5 hr. After working-up as usual, **13-1** was obtained in 83% yield.

N-(N-Benzyloxycarbonylglycyl)glycine Pentachlorophenyl Ester 10——**1a** (4183 mg) and PCPOH (5327 mg) were dissolved in tetrahydrofuran (80 ml) and coupled by MA method as above with ethyl chloroformate (2387 mg) as a reagent. After evaporating the solvent *in vacuo*, the residue was recrystallized from ethyl acetate and the product, having broad melting point (110—123°), was dissolved in hot ethanol, from which **13-1** was obtained on cooling in 37% yield. The material which was undissolved in ethanol was recrystallized from ethyl acetate to give colorless needles of mp 168—170°; 881 mg. One more recrystallization gave an analytical sample of mp 169—171°. IR ν_{max}^{Nujol} cm^{-1}: 1785 (C=O, PCP–ester), 1740 (C=O, imide), 1695 (Z). *Anal.* Calcd. for $C_{26}H_{19}O_7N_2Cl_5$: C, 48.14; H, 2.95; N, 4.32. Found: C, 48.09; H, 3.07; N, 4.23.

Glycine Pentachlorophenyl Ester Hydrobromide 2a——To a suspension of **13-1** (18.3 g) in dichloromethane (50 ml) was added 25% hydrobromic acid in acetic acid (104 g) with stirring and the whole was stirred for 5 hr at room temp. Ether was added and the precipitate was collected, washed with ether, recrystallized from methanol–ethyl acetate to form colorless fine needles of mp 241—243° (decomp.); 14.8 g or 91%. IR ν_{max}^{Nujol} cm^{-1}: 1790 (C=O, PCP–ester). *Anal.* Calcd. for $C_8H_4O_2NCl_5 \cdot HBr$: C, 23.85; H, 1.25; N, 3.47. Found: C, 23.83; H, 1.52; N, 3.54.

N-Benzyloxycarbonylglycylglycine Ethyl Ester 15-2——To a solution of **13-1** (458 mg) in dioxane (10 ml) was added freshly distilled **14-1** (113 mg) and the mixture was stood at room temp overnight. The reaction mixture was worked up by replacing the solvent with ethyl acetate, washing with 10% hydrochloric acid, water, sodium carbonate solution, water, and dried with anhydrous sodium sulfate. Removal of the solvent *in vacuo* left a solid, which was recrystallized from ethyl acetate to give **15-2** as colorless needles of mp 79–80°, 270 mg or 92% (lit.,[23] mp 80—81°). IR ν_{max}^{Nujol} cm^{-1}: 1740 (C=O, ester), 1690 (Z), 1658 (C=O, peptide). This compound was identical with the authentic specimen prepared from **1a** and **14-1** by a conventional MA method.

N-Benzyloxycarbonylglycylglycine 11——Prepared from diketopiperazine and benzyloxycarbonyl chloride according to the procedure of Bergmann and Zervas.[24] **11** was obtained in 70% yield; colorless needles of mp 178—179° (lit.,[24] mp 178°).

N-Benzyloxycarbonylglycylglycylglycine Ethyl Ester 15-3——a) From **11**:**11** (0.04 mole) was coupled with **14-1** hydrochloride by means of isobutyl chloroformate in tetrahydrofuran. Colorless needles of mp 165—167° from water, 67% (lit., [25] mp 166—167°). IR ν_{max}^{Nujol} cm^{-1}: 1750 (ester), 1690 (Z), 1648 (peptide). On hydrolysis with methanol–sodium hydroxide, it gave N–benzyloxycarbonylglycylglycylglycine as colorless leaflets of mp 199—200° from water in 80% yield (lit.,[26] mp 196°).

b) From **13-2**: To a solution of **13-2** (1 mmole) in tetrahydrofuran (20 ml) was added freshly distilled **14-1** (1.1 mmoles) and the mixture was stood at room temp for 20 hr. The solution on evaporation *in vacuo* left a residue, which was washed with dil hydrochloric acid, water, sodium bicarbonate solution and water, and dried. This crude material was recrystallized twice from ethanol followed by washing with ether, and recrystallized from water to give **15-3** of mp 164—165° in 39% yield.

c) One-Step Procedure: **1a** (2 mmoles), **2a** (2 mmoles) and **14-1** hydrochloride (3 mmoles) were coupled as in the case of **5a**; 257 mg or 35%.

Glycylglycylglycine Ethyl Ester 14-3 Hydrobromide——25% Hydrobromic acid in acetic acid (20 g) was added to powdered **15-3** (3.51 g) and the suspension was stirred at room temp. **15-3** gradually dissolved and the whole became turbid again and almost solidified after 3 hr. Ether was added with stirring and the precipitate was collected, washed well with ether, dried in a desiccator, and recrystallized from ethanol–ethyl acetate to give colorless fine crystals of mp 190—191° (lit.,[27] mp 191°); 2.55 g or 86%.

N-Benzyloxycarbonylglycylglycine Pentachlorophenyl Ester 13-2——a) From **1a**: To a solution of **1a** (418 mg; 2 mmoles) and triethylamine (243 mg; 2.4 mmoles) in tetrahydrofuran (50 ml) was added isobutyl chloroformate (328 mg; 2.4 mmoles) and the mixture stirred for 15 min with cooling. Powdered **2a** (808 mg; 2 mmoles) was added followed by addition of triethylamine (202 mg; 2 mmoles) and stirring was continued for 4 hr with cooling. The solution was evaporated *in vacuo* and the residue was taken into

23) D.W. Clayton, J.A. Farrington, G.W. Kenner, and J.M. Turner, *J. Chem. Soc.*, **1957**, 1398.
24) M. Bergman and L. Zervas, *Chem. Ber.*, **65**, 1200 (1932).
25) G.W. Anderson and R.W. Young, *J. Am. Chem. Soc.*, **74**, 5307 (1952).
26) M. Bergman, L. Zervas and J.S. Fruton, *J. Biol. Chem.*, **111**, 237 (1935).
27) A.H. Cook and L. Levy, *J. Chem. Soc.*, **1950**, 646.

ethyl acetate, the extract washed successively with 10% hydrochloric acid, water, aqueous sodium bicarbonate and water, and dried with anhydrous sodium sulfate. The solvent was evaporated *in vacuo* and the residue was recrystallized from ethyl acetate to give **13-2** as colorless needles of mp 172—174°, 934 mg or 91%. IR ν_{max}^{Nujol} cm^{-1}: 1790 (PCP–ester), 1694 (Z), 1670 (peptide). *Anal.* Calcd. for $C_{18}H_{13}O_5N_2Cl_5$: C, 42.03; H, 2.55; N, 5.45. Found: C, 41.90; H, 2.58; N, 5.38.

b) From **11**: To a solution of **11** (266 mg) and PCPOH (320 mg) in DMF (8 ml) was added DCC (248 mg) with stirring and cooling. The reaction was allowed to proceed for 4 hr. After decomposition of excess of DCC with acetic acid, the precipitate was filtered off and the filtrate concentrated *in vacuo* to a semi–solid material which was triturated with ethyl acetate and the insoluble urea was removed. On standing the slightly concentrated solution, **13-2** was obtained as colorless needles, 420 mg or 82%. By the use of DCC–PCPOH complex, as described by Kovacs,[5] the yield was raised to 92%.

N-Benzyloxycarbonylglycylglycyl-L-phenylalanine Methyl Ester 5a——a) From **13-2** and **4a**: To a solution of **13-2** (1029 mg; 2 mmoles) in tetrahydrofuran (54 ml) was added powdered **4a** hydrochloride (431 mg; 2 mmoles) and triethylamine (202 mg; 2 mmoles) with stirring, and stirring continued for 22 hr at room temp. Precipitated salt was filtered and washed with tetrahydrofuran, and the filtrate, combined washing, was evaporated *in vacuo*. The residue was taken into ethyl acetate and the solution was washed with dil hydrochloric acid, and water, and dried with anhydrous sodium sulfate. The solvent was evaporated *in vacuo* to leave crude product, which was treated with ether to remove PCPOH and recrystallized from ethyl acetate. Colorless needles of mp 103—105°, 794 mg or 93% (lit.,[28] Z.Gly_2–L–PheOMe·$0.5H_2O$, mp 96—97°). $[\alpha]_D$ +5.3 (c=2, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1754 (ester), 1734 (Z), 1669, 1650 (peptide). *Anal.* Calcd. for $C_{22}H_{25}O_6N_3 \cdot H_2O$ (Z.Gly_2–L–PheOMe-H_2O): C, 59.32; H, 6.11; N, 9.43. Found: C, 59.49; H, 6.00; N, 9.27.

b) One–Step Procedure: **1a** (2mmoles), and **2a** (2mmoles) were coupled using isobutyl chlororoformate (2.4mmoles) as in the case of **13-2**. After stirring in the cold for 3 hr, stirring continued for additional 1 hr at room temp. To the mixture was added **4a** (2mmoles) and triethylamine (2mmoles), and stirring continued for 22 hr at room temp. Working–up as in the case of a), **5a** was obtained in 83% yield.

N-Benzyloxycarbonylglycylglycyl-DL-tryptophan Methyl Ester 5b——a) From **13-2** and **4b**: **13-2** (2 mmoles) and **4b** hydrochloride (2mmoles) were reacted as in the case of **5a**. **5b** formed colorless needles of mp 111–113° from ethyl acetate, 781 mg or 84%. IR ν_{max}^{Nujol} cm^{-1}: 1750 (ester), 1708 (Z), 1655 (peptide). *Anal.* Calcd. for $C_{24}H_{26}O_6N_4 \cdot H_2O$ (Z.Gly_2-DL-TrpOMe-H_2O): C, 59.28; H, 5.83; N, 11.56. Found: C, 59.29; H, 5.63; N, 11.63,

b) One–Step Procedure: **1a, 2a** and **4b** were coupled as in the case of **5a**. Yield, 78%.

N-Benzyloxycarbonylglycylglycyl-L-tyrosine Methyl Ester 5c——**1a, 2a,** and **4c** hydrochloride (2mmoles each) were coupled by the one–step procedure as above to give **5c** as colorless needles of mp 157—159° from ethyl acetate in 82% yield (lit.,[29] mp 159.5—161.5°). $[\alpha]_D$ +18 (c=2, MeoH). IR ν_{max}^{Nujol} cm^{-1}: 1750 (ester), 1705 (Z), 1680, 1658 (peptide). *Anal.* Calcd. for $C_{22}H_{25}O_7N_3$: C, 59.61; H, 5.68; N, 9.48. Found: C, 59.59; H, 5.52; N, 9.37.

N-Benzyloxycarbonylglycylglycylglycine Pentachlorophenyl Ester 13-3——**11** (0.02 mole) and **2a** (0.02 mole) were coupled with isobutyl chloroformate (0.024 mole) as in the case of **13-2**. After the reaction,the solvent (tetrahydrofuran) was evaporated *in vacuo* and the residue was washed with sodium bicarbonate solution, water, 10% hydrochloric acid, and water, dried and recrystallized from ethanol–water to form colorless fibers of mp 198—199° (decomp.), 63%. The analytical sample, obtained by one more recrystallization, had mp 201—203° (decomp.). IR ν_{max}^{Nujol} cm^{-1}: 1788 (PCP–ester), 1695 (Z), 1660 (peptide). *Anal.* Calcd. for $C_{20}H_{16}O_6N_3Cl_5$: C, 42.20;H,2.82; N, 7.35; Found: C, 41.90; H, 2. 93; N,7.41.

Glycylglycylglycine Pentachlorophenyl Ester Hydrobromide 12——**13-3** (3 mmoles) was treated with hydrobromic acid in acetic acid as in the case of **2a**. After the reaction, dichloromethane was added instead of ether, and the precipitate was collected, washed with ether and recrystallized from methanol–ethyl acetate to give colorless needles of mp 237—238° (decomp.); 75%. IR ν_{max}^{Nujol} cm^{-1}: 1790 (PCP–ester). *Anal.* Calcd. for $C_{12}H_{10}O_4N_3Cl_5 \cdot HBr$: C, 27.80; H, 2.14; N, 8.10. Found: C, 27.93; H, 2.25: N, 8.41.

Fragment Condensation with PCP-Ester 3; General Procedure (*cf.* Table II)——To a solution of 13 (2 mmoles) in DMF (8—25 ml) was added **14** hydrodromide (2mmoles) and triethylamine (2mmoles) and the mixture was stood at room temp overnight. Ice–water (100—200 ml) was added to the reaction mixture and the precipitate was collected, washed consecutively with 5% hydrochlorric acid, water, sodium bicarbonate solution, water, and finally with ether and dried to give a crude product, which was purified by recrystallization or subjected to further reaction.

Glycylglycylglycylglycylglycylglycine Ethyl Ester 14-5 Hydrobromide——A suspension of crude **15-5** (860 mg) in 25% hydrobromic acid in acetic acid (13 g) was stood at room temp for 5 hr, followed by addition of ether. The precipitate was decanted and washed repeatedly with ether until ether layer became colorless.

28) L.V. Ionova, E.A. Morozova, S.A. Pliner, and N.A. Drobinskaye, *Vestn. Mosk. Univ. Ser. II Khim.*, **19** (4), 85 (1964) [*C.A.*, **61**, 1279*e*(1964)].

29) R.B. Woodward, R.A. Olofson, and H. Meyer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

Methanol was added and the solution was concentrated *in vacuo* at room temp. To the concentrated solution was added a drop of water, followed by addition of ethyl acetate and the whole was stood to deposit colorless fine needles of mp 209—210° (decomp.); 644mg or 84%. IR ν_{max}^{Nujol} cm^{-1}: 1744 (ester). *Anal.* Calcd. for $C_{12}H_{21}O_6N_5 \cdot HBr$: C,34.97; H,5.38; N,16.99. Found: C, 34.94; H,5.45; N,16.88.

Glycylglycylglycylglycylglycylglycylglycine Ethyl Ester 14-6 Hydrobromide——15-6 (846 mg) was deblocked as above. Colorless fine needles of mp 231—232° (decomp.) from methanol–water, 654 mg or 86%. IR ν_{max}^{Nujol} cm^{-1}: 1745 (ester). *Anal.* Calcd. for $C_{14}H_{24}O_7N_6 \cdot HBr$:C, 36.14; H, 5.42; N, 18.06. Found: C, 36.25; H, 5.69; N, 17.64.

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