difference could be detected in the relative rates of decomposition of the enzymatic and synthetic nucleosides in 0.1 *N* potassium hydroxide as determined by the change in absorption at 280 mμ.

3. The various diastereoisomers, and the 5′-methylthioadenosine and L-homoserine arising from their decomposition products, had R_fs identical with those of authentic samples in the following solvent systems.

A. Ethanol (300), water (100), concd. hydrochloric acid (1). R_fs: S-adenosylmethionine, 0.12; 5′-methylthioadenosine, 0.62; L-homoserine, 0.80.

B. Methyl Cellosolve (112), water (12), acetic acid (1). R_fs: S-adenosylmethionine, 0.05; 5′-methylthioadenosine, 0.65; L-homoserine, 0.42.

C. 1-Propanol (6), ammonium hydroxide (3, d., 0.880), water (1). R_fs: 5′-methylthioadenosine, 0.88; L-homoserine, 0.76.

Configuration of S-Adenosylhomocysteine Isolated from Guanidinoacetate Methylpherase Reaction.—A reaction mixture containing potassium phosphate (30 μmoles, *p*H 7.4), guanidinoacetic acid (3.8 μmoles), freshly neutralized reduced glutathione (8.2 μmoles), (±)-S-adenosyl-D-methionine (4 μmoles, prepared by the methylation of S-adenosyl-D-homocysteine) and guanidinoacetate methylpherase (9 units) in a final volume of 1 ml. was incubated for 2 hr. at 37°. The reaction was terminated by the addition of 0.05 ml. of 30% perchloric acid. The precipitate was removed by centrifugation, the supernatant fluid brought to *p*H 6.7 and placed on a buffered Amberlite XE-64 column as for the separation of S-adenosylhomocysteine and S-adenosylmethionine. The control mixture contained all the components, but the enzyme was added after addition of perchloric acid. A similar pair of vessels was incubated with the corresponding chemically synthesized (±)-S-adenosyl-L-methionine (3.8 μmoles).

The material eluted from the column by 15 ml. of phosphate buffer 0.01 *M*, *p*H 6.7, was collected. The eluates from the incubated mixtures contained large amounts of ultraviolet absorbing material, while negligible amounts were found in the unincubated samples. It was shown by paper chromatography in three solvent systems that S-adenosylhomocysteine was the major component of this ultraviolet absorbing material. Aliquots of the eluates were assayed enzymatically for the presence of S-adenosyl-L-homocysteine using an enzyme which reversibly and specifically cleaves the L-form of this nucleoside.[9] Adenosine liberation was measured by means of adenosine deaminase (Table V).

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BETHESDA, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Peptide Syntheses *Via* Amino Acid Active Esters[1]

BY MURRAY GOODMAN AND KENNETH C. STUEBEN

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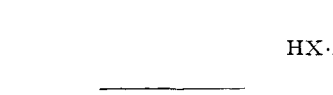
Amino acid active ester hydrobromides have been prepared. By use of these compounds, tripeptide derivatives have been synthesized in high over-all yield without isolation of a dipeptide intermediate. This has been accomplished by taking advantage of the difunctionality of the amino acid active ester as well as the difference in rate of reaction at the amino and active ester ends of the molecule.

Since 1950, several promising methods for peptide synthesis have been published. Among these are the mixed anhydride,[2-6] carbodiimide[7] and active ester[8] approaches. These new methods form amides at different rates. Thus, typical reaction times for peptide formation are 2-4 hours for mixed anhydrides, 5 hours for carbodiimides and 12–24 hours for the active esters. In this paper we wish to report the synthesis of difunctional amino acid derivatives of the type (where Act =



I

CH_2CN or $-p-C_6H_4NO_2$) and the general use of these compounds in peptide synthesis.

Intermediates of a similar nature, *i.e.*, peptide active ester hydrohalides, have been used to form cyclic peptides.[9-11] Schwyzer[9] employed the *p*-nitrophenyl ester of a decapeptide in his synthesis of gramicidin-S. In addition, Kenner[10] synthesized a cyclic pentapeptide utilizing a *p*-nitrophenyl thiol ester of the linear pentapeptide hydrobromide. While the methods just described have been designed expressly for cyclization, amino acid active esters afford a general method for the preparation of peptides.

By taking advantage of the difunctionality of the amino acid active esters as well as the difference in rate among certain acylation reactions, we have been able to synthesize tripeptides directly *via* two consecutive reactions. The first (more rapid) acylation reaction is brought about at the amino end of compound I by means of N,N′-dicyclohexylcarbodiimide or mixed anhydrides. The

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(2) (a) T. Wieland, W. Kern and R. Sehring, *Ann.*, **569**, 117 (1950); (b) T. Wieland and R. Sehring, *ibid.*, **569**, 122 (1950).

(3) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(4) (a) J. R. Vaughan, THIS JOURNAL, **73**, 3547 (1951); (b) J. R. Vaughan and R. L. Osato, *ibid.*, **74**, 676 (1952).

(5) T. Wieland and H. Bernhard, *Ann.*, **572**, 190 (1951).

(6) J. M. Kenner, *Chemistry & Industry*, 15 (1951).

(7) (a) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955); (b) J. C. Sheehan, M. Goodman and G. P. Hess, *ibid.*, **78**, 1367 (1956); (c) J. C. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **21**, 439 (1956).

(8) (a) R. Schwyzer, B. Iselin and M. Feurer, *Helv. Chim. Acta*, **38**, 69 (1955); (b) R. Schwyzer, M. Feurer, B. Iselin and H. Kägi, *ibid.*, **38**, 80 (1955); (c) R. Schwyzer, M. Feurer and B. Iselin, *ibid.*, **38**, 83 (1955); (d) B. Iselin, W. Rittel, P. Sieber and R. Schwyzer, *ibid.*, **40**, 373 (1957).

(9) R. Schwyzer and P. Sieber, *Angew. Chem.*, **68**, 518 (1956); *Helv. Chim. Acta*, **40**, 624 (1957).

(10) G. W. Kenner and J. M. Turner, *Chemistry & Industry*, 602 (1955).

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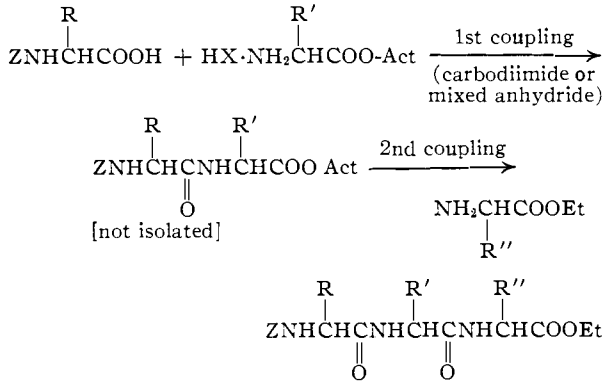
resulting product need not be isolated and can be used directly for the next step involving the active ester



where Z = the benzyloxycarbonyl group.

These intermediate amino acid active ester hydrohalides (I) are stable materials, easily prepared in high yields.

The technique of building up higher peptides by carrying out successive reactions in the same flask without isolation and purification of the lower intermediates often leads to intractable mixtures. For example the reaction

Z-amino acid + amino acid ester ⟶ Z dipeptide ester

will not usually proceed entirely to completion and if the product is not purified it will be contaminated with starting materials. If the dipeptide ester is then hydrolyzed to the free acid followed by coupling, a mixture of di- and tripeptides will result. However, with some coupling reagents side reactions rather than incompleteness of reaction are important. In our method the only neutral product which is likely is the desired tripeptide, as evidenced by the high yields obtained.

The most satisfactory route to the difunctional amino acid intermediates involves the removal of a benzyloxycarbonyl group from a blocked *p*-nitrophenyl ester by means of hydrogen bromide in acetic acid[12]

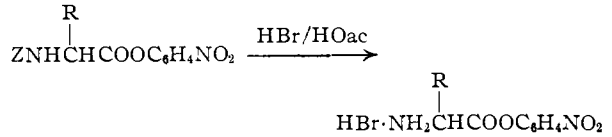
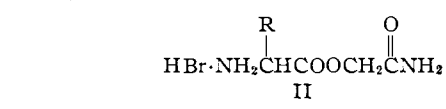
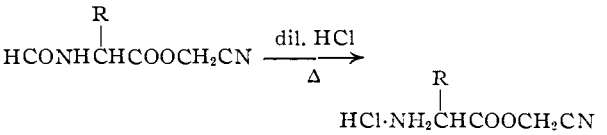


Table II contains a list of the benzyloxycarbonyl-amino acid active esters which we have prepared and Table III contains the amino acid active ester hydrobromides derived therefrom.

When the corresponding cyanomethyl ester was employed, this procedure gave rise to a carboxamido methyl ester II which was found to be a poor acylating agent. Hydrogenolysis of the benzyloxycarbonyl group was also unsuccessful for the preparation of the unblocked cyanomethyl ester. However, one successful approach to these compounds was found in the preferential hydrolysis of a formyl group



II



If too high a concentration of acid is used, the inactive carboxamido methyl ester (II) is the main product.

Table I contains a list of the tripeptide derivatives which were synthesized *via* amino acid active esters in high over-all yields.

An important advantage in our general scheme for the synthesis of tripeptides lies in the fact that there is no separate dipeptide stage which requires isolation and purification. Therefore in this tripeptide formation all the reactants are at the amino acid derivative stage. Although we did not usually isolate the intermediate dipeptide active ester, if desired, this could readily be accomplished, as shown by the preparation of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (XXIX).

A scheme related to our approach has been published by Sorm, Rudinger and co-workers.[13-16] In their method a blocked amino acid chloride, an N-carboxy-amino acid anhydride and an amino acid ester were used as the components in the tripeptide synthesis. In general, however, the yields were significantly below those obtained by our method.

Anderson and Callahan[17] have published a procedure for the determination of the extent of racemization during peptide synthesis. We employed their technique to examine the amount of racemization in our method. Using the Anderson standard, benzyloxycarbonylglycyl-L-phenylalanyl glycine ethyl ester, we have been unable to detect any measurable amount of the DL-isomer.

It is interesting to speculate on the extension of this method to higher peptides. If a benzyloxycarbonyl tripeptide ester is quantitatively hydrogenated, the resulting tripeptide ester may be coupled with a blocked amino acid and an amino acid active ester as in the general scheme above to yield the pentapeptide derivative. This extension is currently under investigation together with a study of other coupling agents.

Experimental[22]

Benzyloxycarbonyl-DL-phenylalanine cyanomethyl ester (IX) was prepared by the method of Schwyzer, *et al.*,[8a] in 95% crude yield. Colorless needles, m.p. 97–98°, were obtained from ether–acetone[23]; yield 82%.

(12) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

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(14) M. Zaoral, J. Rudinger and F. Šorm, *ibid.*, **18**, 530 (1953).

(15) M. Zaoral and J. Rudinger, *ibid.*, **20**, 1183 (1955).

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TABLE I

TRIPEPTIDE DERIVATIVES SYNTHESIZED USING AMINO ACID ACTIVE ESTERS

No.	Compound	Method	Yield, %[a]	M.p., °C.	$[\alpha]^{t}D$	t, °C.	,
III	Z-gly-L-phe-gly OEt	A	80	118–119[b]	−12.4°	23	2.0[i]
IV	Z-L-pro-L-leu-gly OEt	B	84	148–149.5[c]	−81.2	25.7	2.7[i]
V	Z-L-leu-gly-L-leu OMe	B	76	89–90.5[d]	−10.6	28	2.4[j]
VI	Z-L-ala-L-ala-gly OEt	B	50	174–176.6[e]	−40.2	25	0.7[i]
VII	Z-gly-gly-gly OEt	B	31[f]	165–166.5[g]	.....	..	....
VIII	Z-gly-L-leu-gly OEt	A	62	109–110[h]	−27.5	28	2.3[j]

[a] Over-all yield to tripeptide stage. [b] Lit.[7c] m.p. 117–118°, $[\alpha]^{26.0}D$ −12.4° (*c* 2.0, ethanol). [c] Lit.[18] m.p. 145–146°, $[\alpha]^{22.5}D$ −79.8° (*c* 2.5, ethanol). [d] Lit.[19] m.p. 89–81°. [e] Anal. sample crystallized from aqueous ethanol, m.p. 176.7–177.5°. *Anal.* Calcd. for $C_{18}H_{25}O_6N_3$: C, 56.98; H, 6.64; N, 11.08. Found: C, 57.03; H, 6.66; N, 11.28. [f] The low yield encountered here is most probably due to the side reaction (diacylamide formation) described by Kopple.[20] [g] Lit.[21] m.p. 166–167°. [h] Lit.[19] m.p. 109°. Crystallization from ethyl acetate–petroleum ether gave 120–125° with no change on further crystallization. When ethanol–water was used the m.p. fell to 109°. Apparently the latter is a hydrated form. [i] Ethanol. [j] Ethyl acetate.

TABLE II

BENZYLOXYCARBONYLAMINO ACID *p* NITROPHENYL ESTERS

No.	*p*-Nitrophenyl ester of	M.p.,[a] °C.	$[\alpha]^{25.0}D$	t, EtAc	Yield, %		Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XVI	Z-L-phe	126.5–127.5	−8.9	2.2[b]	75	$C_{23}H_{20}N_2O_6$	65.70	4.80	6.66	65.76	5.10	6.88
XVII	Z-L-pro	93.5–94.5	−72.0	2.3	65	$C_{19}H_{18}N_2O_6$	61.61	4.90	7.57	61.80	4.91	7.57
XVIII	Z-L-ala	79–79.5	−38.1	1.4	58	$C_{17}H_{16}N_2O_6$	59.29	4.68	8.14	59.26	4.70	8.16
XIX	Z-γ-OBz-L-glu	111	−20.4	3.2	95	$C_{26}H_{24}N_2O_8$	63.41	4.91	5.69	63.34	5.09	5.86
XX	Z-SBz-L-cys[8d]	91.5–92.5	−33.0	1.9	87	$C_{24}H_{22}N_2O_6S$	61.79	4.75	6.01	61.98	4.56	6.27

[a] Recrystallization solvents: XVIII, ethanol–petroleum ether; all others from ethanol. [b] Chloroform.

Anal. Calcd. for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: C, 67.18; H, 5.50; N, 8.57.

DL-Phenylalanine Carboxamido Methyl Ester Hydrobromide (X).—Treatment of the benzyloxycarbonyl derivative IX with a saturated solution of hydrogen bromide in acetic acid[12] afforded a 90% yield of the hydrobromide X, m.p. 189–189.5° dec. after recrystallization from dimethylformamide–ether.

Anal. Calcd. for $C_{11}H_{15}N_2O_3Br$: C, 43.57; H, 4.98; N, 9.24. Found: C, 43.63 H, 5.11; N, 9.41.

Benzyloxycarbonylglycyl-DL-phenylalanine Carboxamido Methyl Ester (XI).—To a stirred suspension of the hydrobromide X (4.0 g., 0.0132 mole) in 50 ml. of ethyl acetate was added dropwise over a 15-minute period 70 ml. of an ethereal solution of benzyloxycarbonylglycine acid chloride (prepared from 0.014 mole of benzyloxycarbonylglycine). The temperature was maintained at 0°. The gradual addition of 5% sodium bicarbonate kept the *p*H between 7 and 8 during the reaction. After this treatment the resultant solid was filtered and washed with 2 *N* hydrochloric acid, 5% sodium bicarbonate and water. On drying *in vacuo* the dipeptide derivative XI was obtained, m.p. 140.5–141°. An analytical sample was crystallized from acetone and then from ethanol, m.p. 141–142°, yield 3.6 g. (66%).

Anal. Calcd. for $C_{21}H_{23}N_3O_6$: C, 61.00; H, 5.61; N, 10.16. Found: C, 61.30; H, 5.78; N, 10.36.

Attempted Aminolysis of XI.—To a solution of XI (0.206 g., 0.0005 mole) in 10 ml. of acetonitrile was added glycine ethyl ester hydrochloride (0.14 g., 0.001 mole) followed by triethylamine (0.14 ml., 0.001 mole). The resultant clear solution was stored at 55° for 23 hours, diluted with ethyl acetate, washed with 2 *N* hydrochloric acid, 5% sodium bicarbonate, water and finally dried. Removal of the solvent *in vacuo* gave 0.199 g. of unreacted starting material (97% recovery), m.p. 140–142°. A mixed melting point determination with original III gave 140–141°.

Formyl-DL-phenylalanine cyanomethyl ester (XII) was obtained by refluxing a solution of the formylamino acid with chloroacetonitrile and triethylamine[8a] in ethyl acetate. The product was crystallized from ethyl acetate–petroleum ether, m.p. 80.5–81.5°, yield 83%.

Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.07. Found: C, 62.25; H, 5.19; N, 12.15.

Formyl-L-phenylalanine cyanomethyl ester (XIII) was prepared in similar fashion except that rather than refluxing, the reactants were warmed slowly over four hours to 80°. After storage at room temperature for two days the reaction mixture was worked up in the usual manner. Crystallization from ethyl acetate–petroleum ether gave a 76% yield of colorless needles, m.p. 91–93°. The analytical sample was crystallized from ethanol–petroleum ether in a 70% yield, m.p. 96.5–97.0°, $[\alpha]^{25.0}D$ −3.58° (*c* 2.3, ethyl acetate).

Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.07. Found: C, 62.32; H, 5.35; N, 11.91.

DL-Phenylalanine Cyanomethyl Ester Hydrochloride (XIV).—A suspension of the formyl compound XII (0.6 g., 0.0026 mole) in 24 ml. of *N* hydrochloric acid was heated at boiling water-bath temperature for 5 minutes with frequent swirling. The clear solution resulting was cooled and extracted with three 5-ml. portions of ethyl acetate. Freeze drying of the aqueous layer afforded 0.38 g. of solid, m.p. 150.5–152.5°. After repeated recrystallizations from ethanol–ether, an analytical sample (yield 20%) of the hydrochloride XIV, m.p. 165–165.5°, was obtained.

Anal. Calcd. for $C_{11}H_{12}N_2O_2Cl$: C, 54.89; H, 5.44; N, 11.64. Found: C, 55.22; H, 5.90; N, 11.45.

Optimum conditions for this hydrolysis have not been fully ascertained. Higher concentrations of acid and longer contact times lead to increasing quantities of the carboxamido methyl ester, the infrared spectrum of which was identical to X.

L-Phenylalanine Cyanomethyl Ester Hydrochloride (XV).—Similar treatment of XIII led to the L-isomer XV, m.p. 186.5–188.5° dec. The analytical sample (yield 56%) crystallized from ethanol had m.p. 185–185.5° dec.

Anal. Calcd. for $C_{11}H_{13}N_2O_2Cl$: C, 54.89; H, 5.44; N, 11.64. Found: C, 55.12; H, 5.34; N, 11.96.

Benzyloxycarbonylamino Acid-*p*-nitrophenyl Esters.—The intermediate *p*-nitrophenyl esters were prepared *via* reaction of the corresponding benzyloxycarbonylamino acids with tris-(*p*-nitrophenoxy)-phosphine in pyridine solution.[8d] Pertinent data are presented in Table II. All yields are for recrystallized materials.

Amino Acid-*p*-nitrophenyl Ester Hydrobromides.—Removal of the benzyloxycarbonyl group from the blocked amino acid *p*-nitrophenyl esters with saturated hydrogen bromide in acetic acid[12] gave the crystalline hydrobromides in high yield. These data are summarized in Table III. All yields are for recrystallized materials.

(22) All melting points are corrected. Analyses are by Schwarzkopf Laboratories, Woodside 77, N. Y.

(23) The infrared spectrum of this compound showed no nitrile peak. This is in agreement with previous observations that an oxygen-containing group attached to the same carbon as the nitrile may result in the complete "quenching" of this peak; L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 266.

TABLE III

AMINO ACID *p*-NITROPHENYL ESTER HYDROBROMIDES

No.	*p* Nitrophenyl ester of	M.p.,[a] °C.	$[\alpha]^{25.0}D$	*c*, EtOH	Yield, %		Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XXI	HBr-gly	213–213.5 d.	.....	...	96	$C_8H_9N_2O_4Br$	34.80	3.29	10.15	34.91	3.58	9.86
XXII	HBr-L-phe	215–216 d.	+46.8°	2.3	91	$C_{15}H_{15}N_2O_4Br$	49.06	4.12	7.63	48.97	4.22	7.77
XXIII	HBr-L-leu	198.5–199.5 d.	+11.4	2.2	92	$C_{12}H_{17}N_2O_4Br$	43.25	5.14	8.41	43.27	5.10	8.62
XXIV	HBr-L-ala	182–183.5 d.	− 2.4	2.1	97	$C_9H_{11}N_2O_4Br$	37.13	3.81	9.62	37.41	3.94	9.71
XXV	HBr-L-pro	198–199 d.	−18.6	2.2[d]	99	$C_{11}H_{13}N_2O_4Br$	41.66	4.13	8.87	41.94	4.13	9.05
XXVI	HBr-L-cys	155–155.5 d.	+14.6	2.1	97	$C_{16}H_{17}N_2O_4SBr$	46.49	4.15	6.78	46.29	4.02	6.87
XXVII	HBr-L-glu[b]	155.5–157 d.	....	..	100	$C_{11}H_{13}N_2O_6Br$	37.84	3.75	8.03	38.26	3.88	8.16
XXVIII	HBr-γ-OBz-L-glu[b]	120–120.5 d.	+26.9	2.0	84[c]	$C_{18}H_{19}N_2O_6Br$	49.21	4.36	6.38	48.82	4.62	6.39

[a] Recrystallization solvents: XXV, ethanol; XXVIII, chloroform–ether; all others from ethanol–ether. [b] Compound XXVII (the α-*p*-nitrophenyl ester) resulted from treatment of XIX with saturated hydrogen bromide in acetic acid. Compound XXVIII (γ-benzyl-α-nitrophenyl diester) was prepared by Mr. Edward Schnitt of our laboratories using 15% hydrogen bromide in acetic acid for 2 minutes. [c] This yield is based on unrecovered starting material. [d] Methanol.

Tripeptide Derivatives. Method A. Preparation of Benzyloxycarbonylglycyl-L-phenylalanylglycine Ethyl Ester (III).—To a solution of benzyloxycarbonylglycine (0.21 g., 0.001 mole) in 12 ml. of acetonitrile maintained at 0° was added L-phenylalanine *p*-nitrophenyl ester hydrobromide (XXII, 0.37 g., 0.001 mole), followed by triethylamine (0.14 ml., 0.001 mole). The mixture was stirred magnetically and when solution was complete, N,N′-dicyclohexylcarbodiimide (0.21 g., 0.001 mole) was added. The reaction was allowed to proceed for one hour at 0° and then for five hours at room temperature. Following this there were added glycine ethyl ester hydrochloride (0.14 g., 0.001 mole) and triethylamine (0.15 ml., 0.001 mole). The mixture was stored for 15 hours at room temperature. Work-up of the product involved replacing the solvent with ethyl acetate, washing with 2 *N* hydrochloric acid, 5% sodium carbonate,[24] water and finally drying over anhydrous magnesium sulfate. On crystallization of the crude product from ethyl acetate–petroleum ether 0.35 g. (80% over-all) of crystals was obtained, m.p. 118–119°, $[\alpha]^{23.0}D$ −12.40° (*c* 2, ethanol) (reported m.p. 117–118°, $[\alpha]^{26.0}D$ −12.4° (*c* 2, ethanol).

Method B. Preparation of Benzyloxycarbonyl-L-Prolyl-L-Leucyl-Glycine Ethyl Ester (IV).—A solution of benzyloxycarbonyl-L-proline (0.37 g., 0.0015 mole) and triethylamine (0.21 ml., 0.0015 mole) in 4 ml. of dry chloroform was cooled to −5°. To this was added isobutyl chloroformate (0.22 g., 0.0016 mole) and the mixture stirred for a half-hour. At this time, L-leucine *p*-nitrophenyl ester hydrobromide (XXIII, 0.5 g., 0.0015 mole) was added. This was followed by the gradual addition of a solution of triethylamine (0.25 ml., 0.0018 mole) in 0.8 ml. of chloroform over a 15-minute period. The resultant clear solution was maintained at 0 to 10° for 3 hours and for one hour at room temperature. Then glycine ethyl ester hydrochloride (0.42 g., 0.003 mole) and triethylamine (0.42 ml., 0.003 mole) were added with stirring. After storage at room temperature for a period of two days the solution was diluted with ethyl acetate, washed with 5% sodium carbonate solution, 2 *N* hydrochloric acid, water and dried with anhydrous magnesium sulfate. Evaporation gave the crude product. One crystallization from hot ethyl acetate yielded 0.56 g. (84%) of benzyloxycarbonyl-L-prolyl-L-leucylglycine ethyl ester (IV), m.p. 148–149.5°, $[\alpha]^{25.5}D$ −81.2° (*c* 2.7, ethanol) (reported[18] m.p. 145–146°, $[\alpha]^{22.5}D$ −79.8° (*c* 2.5, ethanol).

Table I contains a list of the peptide derivatives prepared by the methods described above.

Benzyloxycarbonylglycyl-L-phenylalanine-*p*-nitrophenyl Ester (XXIX).—To a solution of benzyloxycarbonylglycine (0.21 g., 0.001 mole) in 12 ml. of acetonitrile maintained at 0° was added L-phenylalanine *p*-nitrophenyl ester hydrobromide (XXII, 0.37 g., 0.001 mole) followed by triethylamine (0.14 ml., 0.001 mole). The mixture was stirred magnetically and when solution was complete, N,N′-dicyclohexylcarbodiimide (0.21 g., 0.001 mole) was added. The reaction was allowed to proceed for one hour at 0° and then for five hours at room temperature. The reaction mixture was worked up by replacing the solvent with ethyl acetate, washing with 5% sodium bicarbonate solution, 2 *N* hydrochloric acid, water, and finally by drying over anhydrous magnesium sulfate. Addition of petroleum ether gave 0.40 g. (84%) of crystals of benzyloxycarbonylglycyl-L-phenylalanine *p*-nitrophenyl ester (XXIX), m.p. 146–146.5°, $[\alpha]^{25.0}D$ −6.5° (*c* 2.0, chloroform).

Anal. Calcd. for $C_{25}H_{23}N_3O_7$: C, 62.88; H, 4.85; N, 8.8. Found: C, 63.17; H, 4.82; N, 9.05.

Test for Racemization in the Amino Acid Active Ester Method.—The preparation of benzyloxycarbonylglycyl-L-phenylalanylglycine ethyl ester was repeated to study the extent of racemization. Using the technique of Anderson and Callahan[17] the crude product (2.2 g., 90% yield) from a 0.00554-mole preparation was adjusted to a 2% solution in absolute ethanol. This solution was refrigerated at −4° for two days. No solid precipitated during this time even after the solution was seeded with the DL-isomer. Concentration of the solution to 55 ml. and refrigeration for 42 hours yielded about 5 mg. of a substance, m.p. 117.5–129°. Upon further evaporation to 25 ml. and seeding with the DL-isomer no crystals were obtained. However, after seeding with L-isomer, a crop of 0.15 g., m.p. 117–118.5°, $[\alpha]^{25}D$ −12.35° (*c* 1.34, ethanol), was removed. On additional storage at −4° only crops of the pure L-isomer were obtained, m.p. 116–118.5°, $[\alpha]^{24}D$ −12.2° (*c* 2.4, EtOH). The total yield of L-isomer was approximately 85%.

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(24) Extraction with sodium carbonate should be continued until removal of *p*-nitrophenol is complete.