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Amino Acids and Peptides. VII.[1] Phosphorus in Organic Synthesis. II.[2] A New Method for the Synthesis of Peptides using the Adducts of Phosphorus Compounds and Tetrahalomethanes[3]

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The adducts of phosphorus compounds and tetrahalomethanes were applied to peptide synthesis as condensation reagents. Several kinds of phosphorus compounds, tetrahalomethanes, bases, and solvents were investigated for the Young racemization test and the racemization-free reaction conditions were established.

There are many methods for the peptide synthesis by the coupling of carboxyl components with amino components. Very few methods, however, can be successfully applied to the racemization-free peptide synthesis in the fragment condensation, and the only reliable method which is still extensively used is the well-known azide method.[5]

Recently several interesting methods for the synthesis of peptides using phosphorus compounds have been reported. Kenner, *et al.*[6] synthesized some peptides using tosic anhydride in hexamethylphosphoramide. Mukaiyama, *et al.*[7] reported a new method for the peptide synthesis using triphenylphosphine and 2,2'-dipyridyldisulfide as condensation reagents. We also found that diphenylphosphoryl azide (DPPA)[8] and diethylphosphoryl cyanide (DEPC)[9] can be conveniently used for the peptide synthesis. There are also some reports concerning the peptide synthesis using phosphorus compounds such as triphenylphosphite[10] and cyclic phosphonitrilic chloride.[11] It is of interest that all these peptide syntheses using phosphorus compounds as condensation reagents are practically lack of racemization in common.

On the other hand, Lee[12] has found that carboxylic acids are converted to the corresponding acid halides via acyloxyphosphonium salts by treatment with the adducts of triphenylphosphine and carbon tetrachloride. This reaction is very attractive from a viewpoint of peptide synthesis since the acyloxyphosphonium salt which is an intermediate in the reaction is similar to that of the Kenner's method,[6] and its structure has an important role both in accelerating the peptide bond formation and in suppressing racemization.

We tried to apply this procedure to the peptide synthesis and found suitable reaction conditions for the racemization-free peptide synthesis which is represented by the general

1) Part VI: M. Wagatsuma, S. Terashima, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **21**, 422 (1973).
2) Part I: S. Yamada and Y. Takeuchi, *Chem. Pharm. Bull.* (Tokyo), **22**, 634 (1974).
3) Preliminary communication: S. Yamada and Y. Takeuchi, *Tetrahedron Letters*, **1971**, 3595; Presented in part at the 9th Symposium on Peptide Chemistry, Shizuoka, November 24, 1971, Abstracts, p. 10.
4) Location: *7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
5) E. Schröder and K. Lübke, "The Peptides," Vol. I and II, Academic Press, New York and London, 1965.
6) G. Gawne, G.W. Kenner, and R.C. Sheppard, *J. Am. Chem. Soc.*, **91**, 5669 (1969).
7) T. Mukaiyama, R. Matsueda, and M. Suzuki, *Tetrahedron Letters*, **1970**, 1901.
8) T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972).
9) S. Yamada, Y. Kasai, and T. Shioiri, *Tetrahedron Letters*, **1973**, 1595.
10) Y.V. Mitin and O.V. Glinskaya, *Tetrahedron Letters*, **1969**, 5267.
11) J. Martinez and F. Winternitz, *Bull. Soc. Chim. France*, **1972**, 4707.
12) J.B. Lee, *J. Am. Chem. Soc.*, **88**, 3440 (1966).

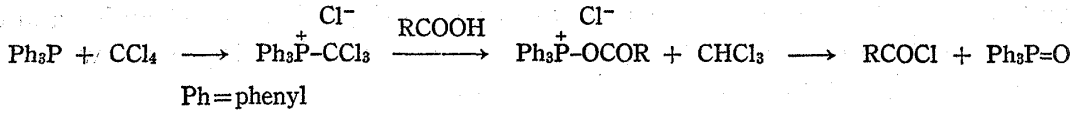

Fig. 1

scheme in Chart 1. To check the degree of racemization during the peptide bond formation, we adopted the stringent Young test[13] to our method which involves coupling of N-benzoyl-L-leucine with glycine ethyl ester.

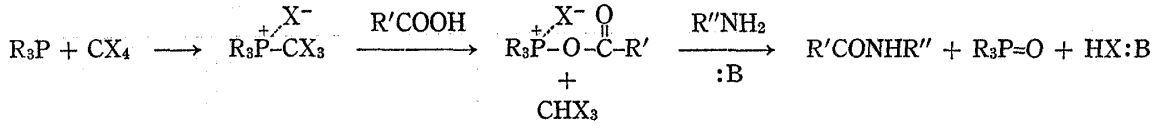

Chart 1

(I) Preliminary Test

Some preliminary experiments have first been done on the effects of the amounts of phosphorus compound, tetrahalomethane, and base on the yields of the products because it is rather difficult to isolate the intermediates (both the adduct of phosphorus compound and tetrahalomethane and the acyloxyphosphonium salt) in pure state. We also examined the effects of the reaction temperature, time, and the order of addition of phosphorus compound and tetrahalomethane.

1) Effects of the Amounts of Phosphorus Compound and Tetrahalomethane——The Young test was adopted to investigate the effects of the amounts of phosphorus compound and tetrahalomethane on the yield of the product. Reaction of N-benzoyl-L-leucine (3 mmoles) with glycine ethyl ester (3 mmoles) was performed at room temperature using tri-*n*-butylphosphine, carbon tetrabromide (each 3 mmoles or 4.5 mmoles), N-methylmorpholine (3 mmoles), and tetrahydrofuran (13 ml) as phosphorus compound, tetrahalomethane, base, and solvent, respectively.

TABLE I. Effects of the Amounts of Phosphorus Compound and Tetrahalomethane

Bz-L-Leu-OH + H-Gly-OEt ⟶ Bz-Leu-Gly-OEt[a]

Run	*n*-Bu_3P (mmoles)	CBr_4 (mmoles)	Bz-Leu-Gly-OEt		CBr_4 Recovery (%)
			Yield (%)	L-Isomer (%)	
1	3.0	3.0	77.1	23.1	22.7
2	4.5	3.0	85.1	22.4	—
3	3.0	4.5	77.6	22.6	49.0

a) Symbols and abbreviations are in accordance with the recommendation of the IUPAC-IUB Commission on Biocnemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1971).

As is shown in Table I, it is necessary to use tri-*n*-butylphosphine in excess to increase the yield of the product. It is not yet known, however, whether the other phosphorus compounds are also to be used in excess or not in this reaction. It is also shown that the optical yield of the product is independent on the amount of phosphorus compound or tetrahalomethane.

2) Effect of the Amount of Base on the Yield of the Product——Reaction of N-benzyloxycarbonylglycine (4 mmoles) with glycine ethyl ester (4—12 mmoles) at room temperature

13) M.W. Williams and G.T. Young, *J. Chem. Soc.*, **1963**, 881.

was adopted to check the effect of the amount of base, using triphenylphosphine and carbon tetrabromide (each 4 mmoles) as condensation reagents. Glycine ethyl ester was used also as a base to simplify the procedure of this reaction.

Table II. Effect of the Amount of Base

Z-Gly-OH + H-Gly-OEt ⟶ Z-Gly-Gly-OEt

Run	H-Gly-OEt (mmoles)	Yield (%)	
		Z-Gly-Gly-OEt	$Ph_3P=O$
1	4	40.0	81.3
2	8	76.6	91.5
3	12	75.9	92.6

Ph=phenyl

Results of the Table II suggest that two molar equiv. of glycine ethyl ester is required and it is also enough in this reaction. This fact means that one molar equiv. of base is necessary in our procedure to remove the hydrogen halide which is derived from tetrahalomethane.

3) Effect of the Reaction Temperature——When the reaction of N-benzyloxycarbonylglycine (4 mmoles) with glycine ethyl ester (8 mmoles) was performed at 0° in tetrahydrofuran using triphenylphosphine and carbon tetrabromide (each 4 mmoles) as condensation reagents, N-benzyloxycarbonylglycylglycine ethyl ester was obtained in 46% yield. When the reaction was performed under reflux, however, the dipeptide derivative was obtained in 82% yield (Table III). In general, the higher the temperature of the reaction, the higher the chemical yield of the peptide.

Table III. Effect of the Reaction Temperature

Z-Gly-OH + H-Gly-OEt ⟶ Z-Gly-Gly-OEt

Run	Reaction temperature (°C)	Yield (%)	
		Z-Gly-Gly-OEt	$Ph_3P=O$
1	0	46.1	92.5
2	18	76.6	91.5
3	66	82.2	87.8

Ph=phenyl

4) Effect of the Order of Addition of Phosphorus Compound and Tetrahalomethane——There were not significant differences obtained when the order of addition of phosphorus compound and tetrahalomethane were exchanged. The most important thing seemed to add phosphorus compound (or tetrahalomethane) into the solution of tetrahalomethane (or phosphorus compound) as slowly as possible, otherwise the unfavorable side reaction which produces dihalomethylenetriphenylphosphorane and triphenylphosphine dihalide[14] would occur if the attack of the carboxylate to the adduct of phosphorus compound and tetrahalomethane was very slow.

(II) Young Racemization Test

For the phosphorus compounds, triphenylphosphine, tri-*n*-butylphosphine, and tris-(diethylamino)phosphine were used mainly, but tris(N-methylpiperazino)phosphine and

14) R. Rabinowitz and R. Marcus, *J. Am. Chem. Soc.*, **84**, 1312 (1962); F. Ramirez, N.B. Desai, and N. McKelvie, *ibid.*, **84**, 1745 (1962).

tris(dimethylamino)phosphine were also tried for the racemization test. Both carbon tetrachloride and carbon tetrabromide were used as the halomethane in this test. For the base, N-methylmorpholine was used mainly which is proved to be the best one in the mixed anhydride method.[15] The other bases we tried were glycine ethyl ester and some phosphorous amides. For the solvents, both dimethylformamide and tetrahydrofuran which are the most popular solvents in peptide synthesis were adopted for the racemization test. The reaction

Table IV. Young Racemization Test

Bz-L-Leu-OH + H-Gly-OEt ⟶ Bz-Leu-Gly-OEt

Run	R_3P	CX_4	Temp.(°C)[a]	Solvent	Base	Bz-Leu-Gly-OEt Yield (%)	Bz-Leu-Gly-OEt L-Isomer (%)	Note[b]
1	Ph_3P	CCl_4	10	DMF	Me-N O	61.5	10.3	X
2	Ph_3P	CCl_4	−15	THF	Me-N O	54.1	12.4	X
3	Ph_3P	CCl_4	−20	THF	Me-N O	19.8	14.8	X
4	Ph_3P	CBr_4	0	DMF	H-Gly-OEt	38.5	52.1	X
5	Ph_3P	CBr_4	0	THF	H-Gly-OEt	61.7	51.2	X
6	*n*-Bu_3P	CBr_4	0	THF	H-Gly-OEt	61.4	85.0	X
7	*n*-Bu_3P	CBr_4	0	DMF	H-Gly-OEt	74.0	91.5	X
8	*n*-Bu_3P	CBr_4	0	THF	Me-N O	61.7	55.6	X
9	*n*-Bu_3P	CBr_4	0	DMF	Me-N O	57.2	61.5	X
10	*n*-Bu_3P	CBr_4	25	THF	Me-N O	60.4	40.3	X
11	*n*-Bu_3P	CBr_4	0	THF	Me-N O	52.0	58.5	X
12	*n*-Bu_3P	CBr_4	−15	DMF	Me-N O	45.8	94.4	X
13	*n*-Bu_3P	CBr_4	−15	THF	Me-N O	58.3	85.0	X
14	*n*-Bu_3P	CBr_4	−15	DMF	Me-N O	51.0	97.6	X
15	*n*-Bu_3P	CBr_4	−15	DMF	Me-N O	40.6	89.1	X, S
16	*n*-Bu_3P	CBr_4	−15	THF	Me-N O	47.0	83.8	P
17	$(Me_2N)_3P$	CBr_4	−20	DMF	Me-N O	26.0	95.6	X
18	$(Et_2N)_3P$	CCl_4	−20	THF	$(Et_2N)_3P$	46.2	94.4	P, S
19	$(Et_2N)_3P$	CBr_4	−20	THF	$(Et_2N)_3P$	56.1	92.7	P, S
20	$(Et_2N)_3P$	CCl_4	−20	THF	H-Gly-OEt	79.1	88.6	P
21	$(Et_2N)_3P$	CCl_4	−15	THF	$(Et_2N)_3P$	41.7	97.6	X
22	$(Et_2N)_3P$	CCl_4	0	THF	$(Et_2N)_3P$	34.3	46.2	X
23	$(Et_2N)_3P$	CBr_4	−20	THF	$(Et_2N)_3P$	36.4	92.6	X, S
24	(Me-N N)$_3$P	CCl_4	−20	THF	Me-N O	68.7	95.9	X, S
25	(Me-N N)$_3$P	CBr_4	−20	THF	Me-N O	70.8	96.5	X
26	(Me-N N)$_3$P	CCl_4	−15	THF	(Me-N N)$_3$P	66.5	97.1	X

a) Initial temperature of the reaction.

b) X: Reaction was performed by adding CX_4 to a solution of R_3P.
P: Reaction was performed by adding R_3P to a solution of CX_4.
S: Glycine ethyl ester hydrochloride was used together with one more equiv. of a base.

Ph=phenyl

15) G.W. Anderson, J.E. Zimmerman, and F.M. Callahan, *J. Am. Chem. Soc.*, **89**, 5012 (1967).

was performed between −20° and 25° to check the effect of temperature on the chemical and optical yields of the product.

The general procedure is as follows. To a solution of N-benzoyl-L-leucine, glycine ethyl ester (or its hydrochloride together with one more equiv. of base), phosphorus compound (or tetrahalomethane), and base (each 3 mmoles) in tetrahydrofuran or dimethylformamide (10—20 ml) is added in portions carbon tetrachloride (or phosphorus compound) (each 3 mmoles) sometimes as a tetrahydrofuran or dimethylformamide solution over 1—60 min with stirring at the proper temperature. The mixture is then stirred at room temperature overnight. After work-up as usual, N-benzoylleucylglycine ethyl ester is obtained directly or by the purification with column chromatography. Results are summarized in Table IV.

(III) Discussion

The results of the Table IV are briefly summarized as follows.

1) All phosphorus compounds except triphenylphosphine can be used in the fragment condensation for the peptide synthesis with suitable reaction conditions. Especially tri-*n*-butylphosphine and tris(N-methylpiperazino)phosphine give good yields and high optical purities probably because of their higher basicity.

2) When triphenylphosphine or tri-*n*-butylphosphine is used as the phosphorus compound in this reaction, triphenylphosphine oxide or tri-*n*-butylphosphine oxide is formed and it is difficult to remove them from the product because those phosphine oxides are neutral. When we use phosphorous amides as the phosphorus compound, corresponding oxides (phosphoric amides) could be removed from the products by washing with diluted acid.

3) Both carbon tetrachloride and carbon tetrabromide work very well as the tetrahalomethane. Carbon tetrachloride has the advantage that excess of it and its by-product (chloroform) are easily removed. Carbon tetrabromide is easy to handle because it is crystalline.

4) When carbon tetrachloride is used as the tetrahalomethane, hydrogen chloride is formed as a by-product. No chloride ion effect[16] is, however, observed since the result is almost the same as that of the case where carbon tetrabromide is used as tetrahalomethane. This fact suggests that amino acid or peptide esters can also be used as their hydrochlorides together with one more equiv. of base (see Run 15, 18, 19, 23, and 24).

5) Phosphorous amides are very useful as they work both as a base and a reactant, so the operation of the reaction can be simplified.

6) There are not significant differences when the order of addition of phosphorus compound and tetrahalomethane is exchanged.

7) Chemical and optical yields of the product are greatly influenced by the reaction temperature. In general, the higher the temperature of the reaction, the higher the chemical yield and the lower the optical yield of the Young product as is reported in the literature.[17]

8) Racemization is suppressed even in dimethylformamide which is known as a solvent that accelerates racemization.

9) Tris(N-methylpiperazino)phosphine is easily handled because it is a stable solid, and it may be the best phosphorus compound in all respects in this reaction.

Almost the same time as we published the preliminary communication[3] on this work, Hruby and Wieland independently reported about the trial of peptide synthesis using the similar reaction to our method. Hruby's report[18] is, however, mainly concerned with the synthesis of simple amides and they synthesized only one dipeptide, N-benzyloxycarbonylphenylalanylglycine ethyl ester, using triphenylphosphine and bromotrichloromethane as condensation reagents, and they did not refer anything about the problem of racemization

16) M.W. Williams and G.T. Young, *J. Chem. Soc.*, **1964**, 3701.
17) F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem.*, **75**, 282 (1963).
18) L.E. Barstow and V.J. Hruby, *J. Org. Chem.*, **36**, 1305 (1971).

in their procedure. On the other hand, Wieland and Seeliger[19] tried the coupling reaction of N-*tert*-butyloxycarbonylalanylphenylalanine with proline methyl ester using triphenylphosphine and carbon tetrachloride and they obtained racemized tripeptide derivative probably because unreactive triphenylphosphine was used as a phosphorus compound and the reaction temperature was too high.

Our results suggest that the racemization-free peptide synthesis may be achieved by the adducts of phosphorus compounds and tetrahalomethanes under suitable reaction conditions. The application of the method to the synthesis of other peptides and the comments on the mechanism will be reported shortly.

Experimental

All melting points were measured on a hot plate, and uncorrected. Wakogel C-200 was used for silica gel column chromatography.

Materials——N-Benzoyl-L-leucine,[13] glycine ethyl ester (or its hydrochloride[20]), N-benzyloxycarbonylglycine,[20] and tris(diethylamino)phosphine[21] were prepared according to the literatures. Tris-*n*-butylphosphine, triphenylphosphine, tris(dimethylamino)phosphine, carbon tetrachloride, and carbon tetrabromide were of commercial origin and all liquid compounds including solvents were used after distillation.

Tris(N-methylpiperazino)phosphine was prepared as follows: To a chilled (0°) solution of phosphorus trichloride (16.4 g, 0.12 mole) in anhyd. diethyl ether (500 ml) was added in portions a solution of N-methylpiperazine (85.1 g, 0.85 mole) in anhyd. diethyl ether (100 ml) over 2.5 hr maintaining the temperature at 0—10° under stirring. The mixture was stirred at 5° for 4 hr and then at room temperature overnight. The precipitates were filtered off, and the filtrate was concentrated to its one-fourth volume and filtered again and then kept in a refrigerator. Colorless prisms were collected by filtration. Yield 33.5 g (85.2%), mp 78°. IR ν_{max}^{KBr} cm^{-1}: 2940, 2795, 1463, 1448, 1285, 1163, 948, 930, 781, 699. *Anal.* Calcd. for $C_{15}H_{33}N_6P$: C, 54.85; H, 10.13; N, 25.59. Found: C, 54.41; H, 10.24; N, 24.88.

1) **Effects of the Amounts of Phosphorus Compound and Tetrahalomethane (Table I)**——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles), and carbon tetrabromide (1.00 g, 3 mmoles (Run 1 and 2), 1.49 g, 4.5 mmoles (Run 3)) in tetrahydrofuran (13 ml) was added in portions a solution of tri-*n*-butylphosphine (0.61 g, 3 mmoles (Run 1 and 3), 0.91 g, 4.5 mmoles (Run 2)) in tetrahydrofuran (2 ml) over 40 min maintaining the temperature below 18°. The mixture was stirred at room temperature for 12 hr. The precipitates were filtered off and the filtrate was concentrated *in vacuo* and the residue was added to ethyl acetate (80 ml) and washed with 5% hydrochloric acid, sat. aqueous sodium bicarbonate, and then sat. aqueous sodium chloride and dried over anhyd. magnesium sulfate. Evaporation of the solvent gave an oil which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (2: 1) and a colorless solid was obtained. Run 1: Yield 0.74 g (77.1%), mp 146—147°,[22] $[\alpha]_D^{20} = -7.87°$ (c=3.1 in EtOH),[22] L-Isomer=23.1%.[22] Carbon tetrabromide was recovered in 22.7% yield (0.23 g). Run 2: Yield 0.82 g (85.1%), mp 146°, $[\alpha]_D^{20} = -7.61°$ (c=3.1 in EtOH), L-Isomer=22.4%. No carbon tetrabromide was recovered. Run 3: Yield 0.75 g (77.6%), mp 145—146°, $[\alpha]_D^{20} = -7.68°$ (c=3.1 in EtOH), L-Isomer=22.6%. Carbon tetrabromide was recovered in 49.0% yield (0.73 g).

2) **Effect of the Amount of Base (Table II)**——To a solution of N-benzyloxycarbonylglycine (0.84 g, 4 mmoles), glycine ethyl ester (0.41 g, 4 mmoles (Run 1), 0.83 g, 8 mmoles (Run 2), 1.33 g, 12 mmoles (Run 3)), and carbon tetrabromide (1.33 g, 4 mmoles) in tetrahydrofuran (20 ml) was added in portions triphenylphosphine (1.05 g, 4 mmoles) over 10 min maintaining the temperature at 18° and the mixture was stirred at room temperature for 2 hr. The precipitates were filtered off and the filtrate was concentrated *in vacuo* which was added to ethyl acetate (80 ml) and washed with 5% hydrochloric acid, sat. aqueous sodium bicarbonate, and then sat. aqueous sodium chloride and dried over anhyd. magnesium sulfate. Evaporation of the solvent gave an oil which was chromatographed on silica gel (160 g) in *n*-hexane and chloroform (1: 1) and N-benzyloxycarbonylglycylglycine ethyl ester was obtained as a colorless solid. Run 1: Yield 0.47 g (40.0%), mp 81—82° (lit.[23] mp 80—81°). Triphenylphosphine oxide was obtained in 81.3% yield (0.91

19) T. Wieland and A. Seeliger, *Chem. Ber.*, **104**, 3992 (1971).
20) J.P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. II, John Whiley & Sons, Inc., New York and London, 1961.
21) D.N. Harpp and J.G. Gleason, *J. Org. Chem.*, **35**, 3262 (1970); V. Mark, G.A. Frank, and W.D. Emmons, *Organic Synthesis*, **46**, 42 (1966).
22) See 5) Young Racemization Test (Table IV), in experimental part.
23) S. Yamada, M. Wagatsuma, Y. Takeuchi, and S. Terashima, *Chem. Pharm. Bull.* (Tokyo), **19**, 2380 (1971).

g). Run 2: Yield 0.90 g (76.6%), mp 81—82°. Triphenylphosphine oxide was obtained in 91.5% yield (1.02 g). Run 3: Yield 0.89 g (75.9%), mp 80—81.5°. Triphenylphosphine oxide was obtained in 92.6% yield (1.03 g).

3) **Effect of the Reaction Temperature (Table III)**——To a solution of N-benzyloxycarbonylglycine (0.84 g, 4 mmoles), glycine ethyl ester (0.83 g, 8 mmoles), and carbon tetrabromide (1.33 g, 4 mmoles) in tetrahydrofuran (20 ml) was added in portions triphenylphosphine (1.05 g, 4 mmoles) over 10 min at 0° (Run 1), at 18° (Run 2), or under reflux (Run 3). The mixture was stirred at room temperature for 2 hr and the precipitates were filtered off. Concentration of the filtrate gave an oil which was added to ethyl acetate (80 ml) and washed with 5% hydrochloric acid, sat. aqueous sodium bicarbonate, and then sat. aqueous sodium chloride and dried over anhyd. magnesium sulfate. Evaporation of the solvent gave an oil which was chromatographed on silica gel (160 g) in *n*-hexane and chloroform (1: 1) and N-benzyloxycarbonylglycylglycine ethyl ester was obtained as a colorless solid. Run 1: Yield 0.54 g (46.1%), mp 81° (lit.[23] mp 80—81°). Triphenylphosphine oxide was obtained in 92.5% yield (1.03 g). Run 2: Yield 0.90 g (76.6%), mp 81—82°. Triphenylphosphine oxide was obtained in 91.5% yield (1.02 g). Run 3: Yield 0.97 g (82.2%), mp 81—82°. Triphenylphosphine oxide was obtained in 87.8% yield (0.98 g).

4) **Effect of the Order of Addition of Phosphorus Compound and Tetrahalomethane**——See 5) Young Racemization Test (Table IV, Run 1—26).

5) **Young Racemization Test (Table IV)**——Physical constants of the Young test product, N-benzoylleucylglycine ethyl ester were reported[13] as follows: L-isomer; mp 156.5—157°, $[\alpha]_D^{20} = -34.0°$ (c= 3.1 in EtOH). Racemate; mp 146°. Percentage of "L-Isomer" was calculated excluding the L-isomer present as racemate. All the Young test products obtained were also checked by infrared spectra.

In the case where tetrahydrofuran was used as the solvent, "work-up as usual" means the following procedures: After the reaction was completed, the solution was filtered and evaporated. To the residue was added ethyl acetate (50 ml) and the solution was washed with 5% hydrochloric acid, sat. aqueous sodium bicarbonate, and then sat. aqueous sodium chloride and dried over anhyd. magnesium sulfate. Evaporation of the solvent gave a residue which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (3: 1).

In the case where dimethylformamide was used as the solvent, however, "work-up as usual" means the following procedures: After the reaction was completed, the solution was added to water (30 ml) and extracted with four 20 ml portions of ethyl acetate. All the procedures followed were the same as those where tetrahydrofuran was used as the solvent.

Run 1——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), triphenylphosphine (0.79 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in dimethylformamide (8 ml) was added in portions carbon tetrachloride (5 ml) over 10 min maintaining the temperature below 10°. The mixture was stirred at 0° for 10 min and then at room temperature overnight. After work-up as usual, colorless crystals were obtained in 61.5% yield (0.59 g), mp 145—151°, $[\alpha]_D^{20} = -3.49°$ (c=3.1 in EtOH), L-Isomer=10.3%.

Run 2 and 3——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), triphenylphosphine (0.79 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (20 ml) (Run 2) or in tetrahydrofuran (20 ml) and dimethylformamide (4 ml) (Run 3) was added in portions carbon tetrachloride (5 ml) over 10 min maintaining the temperature at −15° (Run 2) or at −20° (Run 3). The mixture was stirred at −15° (Run 2) or at −20° (Run 3) for 3 hr and then at room temperature overnight. After work-up as usual, colorless crystals were obtained. Run 2: Yield 0.52 g (54.1%), mp 145—150°, $[\alpha]_D^{20} = -4.22°$ (c=3.1 in EtOH), L-Isomer=12.4%. Run 3: Yield 0.19 g (19.8%), mp 147—150°, $[\alpha]_D^{20} = -5.04°$ (c=2.7 in EtOH), L-Isomer=14.8%.

Run 4——To a chilled (0°) solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.62 g, 6 mmoles), triphenylphosphine (0.87 g, 3.3 mmoles) in dimethylformamide (10 ml) was added in portions carbon tetrabromide (1.09 g, 3.3 mmoles) over 10 min and the solution was stirred at 0° for 6 hr then at room temperature for 10 hr. After work-up as usual, colorless crystals were obtained in 38.5% yield (0.37 g), mp 147—148°, $[\alpha]_D^{20} = -17.7°$ (c=3.10 in EtOH), L-Isomer=52.1%.

Run 5——To a chilled (0°) solution of N-benzoyl-L-leucine (0.94 g, 4 mmoles), glycine ethyl ester (1.03 g, 10 mmoles), and triphenylphosphine (1.15 g, 4.4 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrabromide (1.46 g, 4.4 mmoles) over 10 min and the mixture was stirred at 0° for 8 hr and then at room temperature for 4 hr. After work-up as usual, colorless crystals were obtained in 61.7% yield (0.79 g), mp 148—150°, $[\alpha]_D^{20} = -17.4°$ (c=3.1 in EtOH), L-Isomer=51.2%.

Run 6——To a chilled (0°) solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.77 g, 7.5 mmoles), tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) in tetrahydrofuran (10 ml) was added in portions carbon tetrabromide (1.09 g, 3.3 mmoles) over 1 min and the mixture was stirred at 0° for 8 hr and then at room temperature for 8 hr. After work-up as usual, colorless crystals were obtained in 61.4% yield (0.59 g), mp 155—157°, $[\alpha]_D^{20} = -28.9°$ (c=3.1 in EtOH), L-Isomer=85.0%.

Run 7——To a chilled (0°) solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.93 g, 9 mmoles), and tri-*n*-butylphosphine (0.91 g, 4.5 mmoles) in dimethylformamide (15 ml) was added in portions carbon tetrabromide (1.49 g, 4.5 mmoles) over 20 min and the solution was stirred at 0° for 8 hr

and then at room temperature for 8 hr. After work-up as usual, colorless crystals were obtained in 74.0% yield (0.71 g), mp 154—156° $[\alpha]_D^{20}=-31.1°$ ($c=3.1$ in EtOH), L-Isomer=91.5%.

Run 8——To a chilled (0°) solution of N-benzoyl-L-leucine (0.94 g, 4 mmoles), glycine ethyl ester (0.41 g, 4 mmoles), N-methylmorpholine (0.40 g, 4 mmoles), and tri-*n*-butylphosphine (0.89 g, 4.4 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrabromide (1.46 g, 4.4 mmoles) over 5 min and the mixture was stirred at 0° for 8 hr and then at room temperature for 8 hr. After work-up as usual, colorless crystals were obtained in 61.7% yield (0.79 g), mp 146—147°, $[\alpha]_D^{20}=-18.8°$ ($c=3.1$ in EtOH), L-Isomer=55.6%.

Run 9 and 10——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles), and tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) in dimethylformamide (15 ml) (Run 9) or in tetrahydrofuran (15 ml) (Run 10) was added in portions carbon tetrabromide (1.09 g, 3.3 mmoles) over 7 min maintaining the temperature at 0° (Run 9) or at 25° (Run 10) and the mixture was stirred at 0° for 8 hr and then at room temperature for 8 hr (Run 9) or at room temperature for 8 hr (Run 10). After work-up as usual, colorless crystals were obtained. Run 9: Yield 0.55 g (57.2%), mp 146°, $[\alpha]_D^{20}=-20.9°$ ($c=3.1$ in EtOH), L-Isomer=61.5%. Run 10: Yield 0.58 g (60.4%), mp 145—146° $[\alpha]_D^{20}=-13.7°$ ($c=3.1$ in EtOH), L-Isomer=40.3%.

Run 11 and 12——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles), and tri-*n*-butylphosphine (1.21 g, 6 mmoles) in tetrahydrofuran (15 ml) (Run 11) or in dimethylformamide (15 ml) (Run 12) was added in portions carbon tetrabromide (1.99 g, 6 mmoles) over 7 min maintaining the temperature at 0° (Run 11) or at −15° (Run 12). The mixture was stirred at 0° for 8 hr and then at room temperature for 8 hr (Run 11) or at −15° for 3 hr, at 0° for 8 hr and then at room temperature for 5 hr (Run 12). After work-up as usual, colorless crystals were obtained. Run 11: Yield 0.50 g (52.0%), mp 146—148°, $[\alpha]_D^{20}=-19.9°$ ($c=3.1$ in EtOH), L-Isomer=58.5%. Run 12: Yield 0.44 g (45.8%), mp 156—158°, $[\alpha]_D^{20}=-32.1°$ ($c=3.1$ in EtOH), L-Isomer=94.4%.

Run 13 and 14——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles) and tri-*n*-butylphosphine (0.91 g, 4.5 mmoles) in tetrahydrofuran (15 ml) (Run 13) or in dimethylformamide (15 ml) (Run 14) was added in portions carbon tetrabromide (1.49 g, 4.5 mmoles) over 2 min maintaining the temperature below −15° and the mixture was stirred at −15° for 4 hr, at 0° for 8 hr and then at room temperature for 4 hr. After work-up as usual, colorless crystals were obtained. Run 13: Yield 0.56 g (58.3%), mp 152.5—154°, $[\alpha]_D^{20}=-28.9°$ ($c=3.1$ in EtOH), L-Isomer=85.0%. Run 14: Yield 0.49 g (51.0%), mp 157—159°, $[\alpha]_D^{20}=-33.2°$ ($c=3.1$ in EtOH), L-Isomer=97.6%.

Run 15——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester hydrochloride (0.42 g, 3 mmoles), N-methylmorpholine (0.61 g, 6 mmoles) and tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) in dimethylformamide (15 ml) was added in portions carbon tetrabromide (0.67 g, 3.3 mmoles) over 10 min maintaining the temperature below −15° and the solution was stirred at −15° for 2 hr, at 0° for 4 hr, and then at room temperature for 4 hr. After work-up as usual, colorless crystals were obtained in 40.6% yield (0.40 g), mp 154—155°, $[\alpha]_D^{20}=-30.3°$ ($c=3.1$ in EtOH), L-Isomer=89.1%.

Run 16——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles) and carbon tetrabromide (1.09 g, 3.3 mmoles) in tetrahydrofuran (6 ml) was added in portions a solution of glycine ethyl ester (0.31 g, 3 mmoles), *N*-methylmorpholine (0.30 g, 3 mmoles), and tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) in tetrahydrofuran (9 ml) over 9 min maintaining the temperature below −15°. The mixture was stirred at −15° for 4 hr and then at 0° for 4 hr. After work-up as usual, colorless crystals were obtained in 47.0% yield (0.40 g), mp 152—155°, $[\alpha]_D^{20}=-28.5°$ ($c=3.1$ in EtOH), L-Isomer=83.8%.

Run 17——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles), and tris(dimethylamino)phosphine (0.54 g, 3.3 mmoles) in dimethylformamide (15 ml) was added in portions carbon tetrabromide (1.09 g, 3.3 mmoles) over 5 min maintaining the temperature below −20° and the solution was stirred at −20° for 3 hr, at 0° for 3 hr, and then at room temperature for 2 hr. After work-up as usual, colorless crystals were obtained in 26.0% yield (0.25 g), mp 157—159.5°, $[\alpha]_D^{20}=-32.5°$ ($c=3.1$ in EtOH), L-Isomer=95.6%.

Run 18 and 19——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester hydrochloride (0.42 g, 3 mmoles), and carbon tetrachloride (0.46 g, 3 mmoles) (Run 18) or carbon tetrabromide (1.00 g, 3 mmoles) (Run 19) in tetrahydrofuran (10 ml) was added in portions a solution of tris(dimethylamino)phosphine (2.23 g, 9 mmoles) in tetrahydrofuran (5 ml) over 40 min maintaining the temperature at −20° and the mixture was stirred at −20° for 3 hr, at 0° for 3 hr, and then at room temperature overnight. After work-up as usual, colorless crystals were obtained. (In Run 18 N-benzoylleucylglycine ethyl ester was obtained directly without purification by column chromatography). Run 18: Yield 0.44 g (46.2%), mp 155—156°, $[\alpha]_D^{20}=-32.1°$ ($c=3.1$ in EtOH), L-Isomer=94.4%. Run 19: Yield 0.54 g (56.1%), mp 154—156°, $[\alpha]_D^{20}=-31.5°$ ($c=3.1$ in EtOH), L-Isomer=92.7%.

Run 20——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.93 g, 9 mmoles), and carbon tetrachloride (0.9 ml) in tetrahydrofuran (15 ml) was added in portions tris(diethylamino)phosphine over 10 min maintaining the temperature at −20°. The mixture was stirred at −20° for 2 hr, at 0° for 6 hr, and then at room temperature for 8 hr. After work-up as usual, colorless crystals

were obtained in 79.1% yield (0.76 g), mp 151—157°, $[\alpha]_D^{20}=-30.1°$ ($c=3.1$ in EtOH), L-Isomer=88.6%.

Run 21——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), and tris(diethylamino)phosphine (1.48 g, 6 mmoles) in tetrahydrofuran (15 ml) was added carbon tetrachloride (0.9 ml) over 10 min maintaining the temperature at −15° and the mixture was stirred at −15° for 16 hr. After work-up as usual, colorless crystals were obtained without purification by column chromatography. Yield 0.40 g (41.7%), mp 156—158°, $[\alpha]_D^{20}=-33.2\%$ ($c=3.1$ in EtOH), L-Isomer=97.6%.

Run 22——To a chilled (0°) solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), and carbon tetrachloride (0.46 g, 3 mmoles) in tetrahydrofuran (15 ml) was added in portions tris(diethylamino)phosphine over 13 min and the mixture was stirred at 0° for 1 hr. After work-up as usual, colorless crystals were obtained in 34.3% yield (0.33 g), mp 147—149°, $[\alpha]_D^{20}=-15.7°$ ($c=3.1$ in EtOH), L-Isomer=46.2%.

Run 23——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester hydrochloride (0.42 g, 3 mmoles), and tris(diethylamino)phosphine (2.23 g, 9 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrabromide (1.00 g, 3 mmoles) over 15 min maintaining the temperature at −20°. The mixture was stirred at −20° for 1 hr, at 0° for 5 hr, and then at room temperature for 12 hr. After work-up as usual, colorless crystals were obtained in 36.4% yield (0.35 g), mp 154—157°, $[\alpha]_D^{20}=-31.5°$ ($c=3.1$ in EtOH), L-Isomer=92.6%.

Run 24——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester hydrochloride (0.42 g, 3 mmoles), N-methylmorpholine (0.61 g, 6 mmoles), and tris(N-methylpiperazino)phosphine (0.99 g, 3 mmoles) in tetrahydrofuran (20 ml) was added in portions carbon tetrachloride (0.92 g, 6 mmoles) over 3 min maintaining the temperature at −20°. The mixture was stirred at −10° for 1 hr, at 0° for 7 hr, and then at room temperature for 8 hr. After work-up as usual, colorless crystals were obtained directly without purification by column chromatography. Yield 0.66 g, (68.7%), mp 156—158°, $[\alpha]_D^{20}=-32.6°$ ($c=3.0$ in EtOH), L-Isomer=95.9%.

Run 25——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), N-methylmorpholine (0.30 g, 3 mmoles), and tris(N-methylpiperazino)phosphine (0.99 g, 3 mmoles) in tetrahydrofuran (20 ml) was added in portions a solution of carbon tetrabromide (1.00 g, 3 mmoles) in tetrahydrofuran (5 ml) over 5 min maintaining the temperature at −20°. The mixture was stirred at −20° for 10 min, at −10° for 1 hr, at 0° for 7 hr, and then at room temperature for 8 hr. After work-up as usual, colorless crystals were obtained in 70.8% yield (0.68 g), mp 156—157°, $[\alpha]_D^{20}=-32.8°$ ($c=2.9$ in EtOH), L-Isomer=96.5%.

Run 26——To a solution of N-benzoyl-L-leucine (0.71 g, 3 mmoles) and tris(N-methylpiperazino)phosphine (1.97 g, 3 mmoles) in tetrahydrofuran (15 ml) was added in portions a solution of glycine ethyl ester (0.31 g, 3 mmoles) and carbon tetrachloride (0.46 g, 3 mmoles) in tetrahydrofuran (5 ml) over 1 hr maintaining the temperature at −15°. The mixture was stirred at −15° for 2 hr, at 0° for 3 hr, and then at room temperature for 12 hr. After work-up as usual, colorless crystals were obtained directly without purification by column chromatography. Yield 0.64 g (66.5%), mp 157—158°, $[\alpha]_D^{20}=-33.0°$ ($c=3.1$ in EtOH), L-Isomer=97.1%.