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Amino Acids and Peptides. VIII.[1] Phosphorus in Organic Synthesis. III.[2] Synthesis of Some Peptides containing Various Kinds of α-Amino Acids using the Adducts of Phosphorus Compounds and Tetrahalomethanes[3]

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Some di and tripeptide derivatives containing various kinds of α-amino acids were synthesized with our new method using the adducts of phosphorus compounds and tetrahalomethanes to investigate the effects of the reaction on the side chains of amino acids. Mechanisms of this novel reaction were also discussed.

We have already reported that the adducts of some phosphorus compounds (R_3P) and tetrahalomethanes (CX_4) can be used as condensation reagents for peptide synthesis.[3] We also investigated about the problem on racemization in this method.[1] General scheme of the reaction is shown in Chart 1.

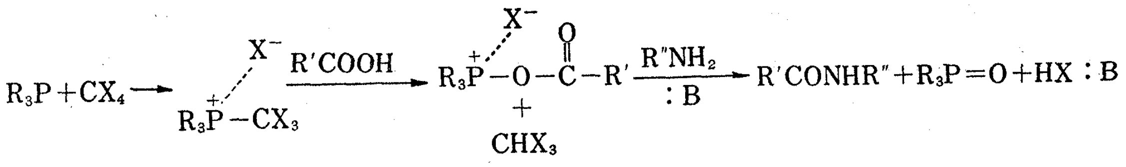

Chart 1

I) Synthesis of Some Di and Tripeptides

Having obtained good results in the Young racemization test with our method,[1] we tried to synthesize several di and tripeptides containing various kinds of α-amino acids to investigate the effects of this reaction on the side chains of amino acids and peptides.

For the phosphorus compound (R_3P), the rather reactive tri-*n*-butylphosphine and tris(N-methylpiperazino)phosphine were used mainly for the peptide synthesis, but tris(dimethylamino)phosphine and tris(diethylamino)phosphine were also tried. Both carbon tetrachloride and carbon tetrabromide were used as the halomethane (CX_4) in this reaction. The reaction temperature was dependent on the kinds of reactions and the kinds of amino acids used. For example, reactions were performed at 0—18° when there was no risk of racemization. But when asparagine was used in the reaction (Run 14), a lower temperature was adopted to prevent nitrile formation from the amide of asparagine, and in the case of fragment condensation (Run 16, 17) lower temperatures were also adopted to suppress the racemization. Both tetrahydrofuran and dimethylformamide were used for the solvent since they are very popular in peptide synthesis. For the base N-methylmorpholine was used mainly which was proved to be the best base in the mixed anhydride method.[5] When tris(dialkylamino)phosphines (phosphorous amides) were used as the phosphorus compound, two equiv. of them were

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[1] Part VII: Y. Takeuchi and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), 22, 832 (1974).

[2] Part II: Lit. 1).

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

sometimes used so that one equiv. of them might work as the base. Results are summarized in Table I.

Table I. Synthesis of Some Di and Tripeptides

Run	Product	Yield (%)	R_3P	CX_4	Temp.(°C)[a]	Solvent	Base	Note[b]
1	Z-Gly-Gly-OEt[c]	84.9	$(Et_2N)_3P$	CCl_4	0	THF	$(Et_2N)_3P$	X
2	Z-Ala-Gly-OEt	63.0	$(Me_2N)_3P$	CCl_4	0	DMF	$(Me_2N)_3P$	X
3	Z-Phe-Gly-OEt	63.5	(Me-N N)$_3$P	CCl_4	10	THF	(Me-N N)$_3$P	X
4	Z-Ala-Phe-OMe	83.5	$(Et_2N)_3P$	CCl_4	15	THF	$(Et_2N)_3P$	X, S
5	Z-Phe-Leu-OMe	57.8	n-Bu_3P	CBr_4	10	DMF	Me-N O	X, S
6	Z-Ala-Val-OMe	50.5	(Me-N N)$_3$P	CCl_4	7	THF	(Me-N N)$_3$P	X, S
7	Z-Ser-Gly-OEt	66.8	n-Bu_3P	CBr_4	10	THF	Me-N O	X
8	Z-Thr-Phe-OMe	53.3	n-Bu_3P	CBr_4	17	THF	Me-N O	X
9	Z-Val-Tyr-OMe	44.5	(Me-N N)$_3$P	CCl_4	4	THF	Me-N O	X, S
10	Z-Cys(Bzl)-Gly-OEt	41.1	(Me-N N)$_3$P	CBr_4	5	THF	Me-N O	X
11	Z-Pro-Phe-OMe	60.2	n-Bu_3P	CBr_4	15	THF	Me-N O	X
12	Z-Met-Gly-OEt	51.6	n-Bu_3P	CBr_4	0	THF DMF	Me-N O	P
13	Z-Glu(OEt)-Gly-OEt	43.1	(Me-N N)$_3$P	CCl_4	5	THF	Me-N O	X, S
14	Z-Asn-Gly-OEt	56.9	n-Bu_3P	CBr_4	−20	DMF	Me-N O	P
15	Boc-Trp-Gly-OEt	60.8	n-Bu_3P	CBr_4	18	THF	Me-N O	P
16	Z-Ala-Phe-Met-OMe	37.5	(Me-N N)$_3$P	CCl_4	−20	THF	Me-N O	X, S
17	Z-Leu-Leu-Val-OMe	21.8	(Me-N N)$_3$P	CBr_4	−15	THF	(Me-N N)$_3$P	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: Amino components were used as their hydrochloride together with one more equiv. of a base.
c) Symbols and abbreviations are in accordance with the recommendation of the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1971).

All peptide derivatives synthesized in our method were identified through infrared spectra, melting points, optical rotations, and elementary analyses by comparison with those of authentic samples or literatures.

II) Discussion

1) Usually the hydroxyl groups of serine and threonine derivatives have to be protected when the mixed anhydride method is used, since side reactions occur, otherwise.[6] There are also some reports[7,8] which show that hydroxyl groups are converted to the corresponding halides by a similar reaction to the one used in this procedure. Serine and threonine can be used, however, without any protection of their hydroxyl groups as far as our method is adopted probably because there might be much differences between the reactivity of carbonyl

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6) E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York and London, 1965, p. 207, 226.
7) J.B. Lee and I.M. Downie, *Tetrahedron*, **23**, 359 (1967); J. Hooz and S.S.H. Gilani, *Can. J. Chem.*, **46**, 86 (1968).
8) A.J. Burn and J.I.G. Cadogan, *J. Chem. Soc.*, **1963**, 5788.

group and hydroxyl group against the trihalomethylphosphonium halide. It seems better to carry out the reaction at lower temperature when serine and threonine derivatives are used (Run 7, 8). It is not necessry to protect the hydroxyl group of tyrosine[9] either in our method (Run 9).

2) If a valine derivative is used as a carboxyl component, it is sometimes difficult to activate it because of its steric hindrance and therefore the yield of coupling product is occasionally low.[6] But it is possible by this procedure to obtain this kind of peptide in moderarte yield (Run 9).

3) When S-benzylcysteine derivative is activated, β-elimination and therefore racemization sometimes occur easily.[6,10] We could get N-benzyloxycarbonyl-S-benzylcysteinylglycine ethyl ester, however, without racemization (Run 10).

4) It is well known that amide groups in the side chains of asparagine and glutamine are dehydrated to the corresponding nitriles when N,N′-dicyclohexylcarbodiimide is used as a coupling reagent.[11] There is also a report that amides are converted to nitriles by the reaction of triphenylphosphine and carbon tetrachloride.[12] No nitrile formation could be detected, however, when an asparagine derivative was used as a reagent in our reaction (Run 14).

5) We also tried to synthesize a few tripeptides as examples of the fragment condensation. The points of coupling are shown by the vertical lines (Run 16, 17). Both tripeptides were found to be formed without any amount of racemization as determined by their melting points and optical rotations.

As shown above, it has been found that there are no effects from this reaction on the side chains of the amino acids or peptides. Considering the fact that side reactions occur to some extent with any method for peptide synthesis, all the facts mentioned above are advantages of our method.

III) Plausible Mechanisms of This Novel Reaction

There are some reports[7,13] on the reaction of phosphine or phosphite with polyhalomethane, and it is already well known that the reaction produces phosphonium halide according to the scheme shown below. Though it is still not yet clear whether the character of the phosphorus atom in the phosphonium salt is pentacovalent or completely ionized (tetracovalent), it seems to be an intermediate situation, considering the reports of Rabinowitz,[14] Ramirez,[15] and Cadogan.[16]

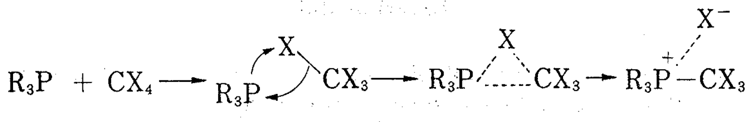

Fig. 1

Once the phosphonium salt is formed, the carboxylate anion will attack the phosphorus atom which is electron-deficient and the phosphorus-carbon bond is cleaved with electrons

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9) *cf.* J.P. Schaefer and J. Higgins, *J. Org. Chem.*, 32, 1607 (1967).

10) M. Bodanszky and M.A. Ondetti, "Peptide Synthesis," Interscience Publishers, New York, London, and Sydney, 1966, p. 142.

11) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959); D.T. Gish, P.G. Katsoyannis, G.P. Hess, and R.J. Stedman, *ibid.*, **78**, 5954 (1956).

12) E. Yamato and S. Sugasawa, *Tetrahedron Letters*, **1970**, 4383.

13) P.C. Crofts and I.M. Downie, *J. Chem. Soc.*, **1963**, 2559; J.B. Lee, *J. Am. Chem. Soc.*, **88**, 3440 (1966).

14) R. Rabinowitz and R. Marcus, *J. Am. Chem. Soc.*, **84**, 1312 (1962).

15) F. Ramirez, N.B. Desai, and N. McKelvie, *J. Am. Chem. Soc.*, **84**, 1745 (1962).

16) J.I.G. Cadogan and J.T. Sharp, *Tetrahedron Letters*, **1966**, 2733.

of the bond going to the carbon atom because of the strong inductive effect of three halogens attached to it. As a result the acyloxyphosphonium salt is formed accompanying the haloform. This acyloxyphosphonium salt must be fairly stable because of the existence of pπ—dπ resonance if R is a dialkylamino group as those in tris(N-methylpiperazino)phosphine.[17]

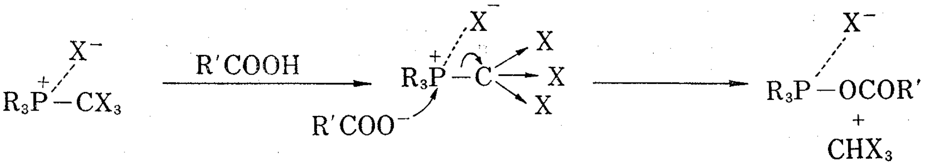

Fig. 2

The next step might be the attack on the carbonyl group of the acyloxyphosphonium salt by the amine. There is also a possibility that the halide anion might intramolecularly attack the carbonyl group to produce an acid halide as was mentioned before.[1] But any method for peptide synthesis where an acid halide is formed as an intermediate gives a racemized product, hence it is improbable to assume an acid halide as an intermediate in this reaction because the Young racemization test by this procedure gave good results.[1]

Therefore a plausible mechanism is as follows: The amino component attacks directly on the carbonyl group of the acyloxyphosphonium salt which is a kind of active ester, resulting in the formation of the peptide and phosphine oxide. The reason why aminolysis reaction is catalyzed without racemization in spite of the stabilized acyloxyphosphonium salt could be explained by the "concerted cyclic mechanism" which accelerates the abstraction of the proton on amino group by the halogen ion or nitrogen atom of the dialkylamino group.[17]

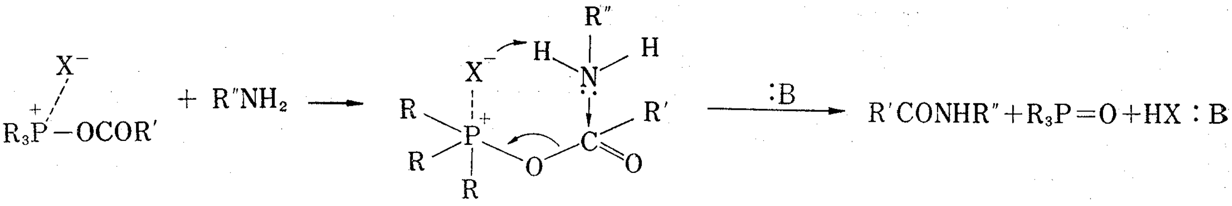

Fig. 3

Experimental

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

Synthesis of Some Di and Tripeptides (Table I)

All optically active amino acid derivatives which we used in this procedure were those of L-configuration and they were prepared according to the literature[18] or of commercial origin.

In the case where tetrahydrofuran was used as the solvent, "usual work-up" means the following procedures: After the reaction was completed, the solution was filtered and evaporated. To the residue was added ethyl acetate (100 ml) and the solution was washed with 10% hydrochloric acid, 5% aqueous sodium carbonate, and then sat. aqueous sodium chloride and dried over anhyd. magnesium sulfate. Magnesium sulfate was removed by filtration and the filtrate was concentrated *in vacuo*.

However, in the case where dimethylformamide was used as the solvent, "usual work-up" means the following procedures: After the reaction was completed, the solution was added to water (100 ml) and extracted with four 50 ml portions of ethyl acetate. All the procedures followed were the same as those where tetrahydrofuran was used as the solvent.

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[17] *cf.* G. Gawne, G.W. Kenner, and R.C. Sheppard, *J. Am. Chem. Soc.*, **91**, 5669 (1969).

[18] J.P. Greenstein and M. Winits, "Chemistry of the Amino Acids," Vol. II, John Wiley & Sons, Inc., New York and London, 1961, p. 887, 925.

N-Benzyloxycarbonylglycylglycine Ethyl Ester (Run 1)——To a chilled (0°) solution of N-benzyloxycarbonylglycine (0.63 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), and tris(diethylamino)phosphine (1.48 g, 6 mmoles) in tetrahydrofuran (10 ml) was added in portions a solution of carbon tetrachloride (0.46 g, 3 mmoles) in tetrahydrofuran (5 ml) over 30 min. The mixture was stirred at 10° for 4 hr and then at room temperature for 8 hr. After work-up as usual, a colorless solid was obtained in 84.9% yield (0.75 g), mp 82° (lit.[19] mp 80—81°). IR $\nu_{\max}\mathrm{cm}^{-1}$: 3300, 3250, 1740, 1687, 1660, 1550, 1215, 1030, 735, 690. Recrystallization from ethyl acetate and ligroin gave an analytical sample as colorless needles, mp 82°. *Anal.* Calcd. for $C_{14}H_{18}O_5N_2$: C, 57.13; H, 6.17; N, 9.52. Found: C, 57.45; H, 6.31; N, 9.19.

N-Benzyloxycarbonylalanylglycine Ethyl Ester (Run 2)——To a chilled (0°) solution of N-benzyloxycarbonylalanine (0.67 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), and tris(dimethylamino)phosphine (0.98 g, 6 mmoles) in dimethylformamide (10 ml) was added in portions a solution of carbon tetrachloride (0.92 g, 6 mmoles) in dimethylformamide (5 ml). The solution was stirred at 0° for 4 hr and then at room temperature for 8 hr. After work-up as usual, a semisolid was obtained which was chromatographed on silica gel (120 g) in *n*-hexane and ethyl acetate (3: 1). Yield 0.58 g (63.0%), mp 97—98° (lit.[20] mp 97.5—98°), $[\alpha]_D^{20}$ $-24.0°$ ($c=1.0$ in EtOH) (lit.[20] $[\alpha]_D^{20}$ $-24.4°$ ($c=1.0$ in EtOH)). IR $\nu_{\max}\ \mathrm{cm}^{-1}$: 3270, 1765, 1695, 1657, 1550, 1192, 1023, 737, 690. Recrystallization from benzene and petroleum ether gave colorless needles. mp 98°, $[\alpha]_D^{20}$ $-24.2°$ ($c=1.0$ in EtOH). *Anal.* Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.38; H, 6.54; N, 9.13.

N-Benzyloxycarbonylphenylalanylglycine Ethyl Ester (Run 3)——To a chilled (10°) solution of N-benzyloxycarbonylphenylalanine (0.90 g, 3 mmoles), glycine ethyl ester (0.61 g, 6 mmoles), and tris(N-methylpiperazino)phosphine (1.97 g, 6 mmoles) in tetrahydrofuran (10 ml) was added in portions carbon tetrachloride (0.92 g, 6 mmoles) over 1 hr and the solution was stirred at 10° for 4 hr and then at room temperature for 8 hr. After work-up as usual, a colorless solid was obtained in 63.5% yield (0.73 g), mp 112° (lit.[21] mp 110—111°), $[\alpha]_D^{20}$ $-16.7°$ ($c=5.1$ in EtOH) (lit.[21] $[\alpha]_D^{20}$ $-16.9°$ ($c=5$ in EtOH)). IR $\nu_{\max}\ \mathrm{cm}^{-1}$: 3275, 1769, 1692, 1653, 1537, 1189, 1045, 1024, 710, 692. Recrystallization from ethyl acetate and petroleum ether gave colorless needles, mp 112.5°, $[\alpha]_D^{20}$ $-17.0°$ ($c=5.0$ in EtOH). *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.41; H, 6.46; N, 7.01.

N-Benzyloxycarbonylalanylphenylalanine Methyl Ester (Run 4)——To a solution of N-benzyloxycarbonylalanine (0.67 g, 3 mmoles), phenylalanine methyl ester hydrochloride (0.65 g, 3 mmoles), and tris(diethylamino)phosphine (2.23 g, 9 mmoles) in tetrahydrofuran (15 ml) was added in portions a solution of carbon tetrachloride (0.9 ml) in tetrahydrofuran (2 ml) over 10 min maintaining the temperature at 10—15°, and the mixture was stirred at room temperature overnight. Usual work-up gave a yellow oil which was chromatographed on silica gel (120 g) in *n*-hexane and ethyl acetate (2: 1) and a colorless solid was obtained in 83.5% yield (0.96 g), mp 99—100° (lit.[22] mp 99—102°), $[\alpha]_D^{22}$ $-14.2°$ ($c=1.1$ in MeOH) (lit.[22] $[\alpha]_D^{22}$ $-14.9°$ ($c=1$ in MeOH)). IR $\nu_{\max}\ \mathrm{cm}^{-1}$: 3290, 1750, 1695, 1663, 1539, 1264, 1218, 1070, 989, 740, 688. Recrystallization from ether and petroleum ether gave colorless needles, mp 102—102.5°, $[\alpha]_D^{22}$ $-15.2°$ ($c=1.0$ in MeOH). *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.73; H, 6.32; N, 7.37.

N-Benzyloxycarbonylphenylalanylleucine Methyl Ester (Run 5)——To a solution of N-benzyloxycarbonylphenylalanine (0.90 g, 3 mmoles), leucine methyl ester hydrochloride (0.55 g, 3 mmoles), tri-*n*-butylphosphine (0.67 g, 3.3 mmoles), and N-methylmorpholine (0.61 g, 6 mmoles) in dimethylformamide (15 ml) was added in portions carbon tetrabromide (1.09 g, 3.3 mmoles) over 10 min maintaining the temperature at 5—10°. The solution was stirred at 5° for 2 hr and then at room temperature overnight. Usual work-up gave an oil which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (5: 1) and a colorless solid was obtained in 57.8% yield (0.74 g), mp 110—111° (lit.[23] mp 110°), $[\alpha]_D^{20}$ $-24.7°$ ($c=3.1$ in MeOH) (lit.[23] $[\alpha]_D^{25.5}$ $-24°$ ($c=3.0$ in MeOH)). IR $\nu_{\max}\ \mathrm{cm}^{-1}$: 3260, 1747, 1692, 1657, 1549, 1263, 1148, 1051, 1023, 731, 692. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 111—112°, $[\alpha]_D^{20}$ $-25.3°$ ($c=3.0$ in MeOH). *Anal.* Calcd. for $C_{24}H_{30}O_5N_2$: C, 67.58; H, 7.09; N, 6.57. Found: C, 67.49; H, 7.18; N, 6.82.

N-Benzyloxycarbonylalanylvaline Methyl Ester (Run 6)——To a solution of N-benzyloxycarbonylalanine (0.67 g, 3 mmoles), valine methyl ester hydrochloride (0.50 g, 3 mmoles), and tris(N-methylpiperazino)phosphine (3.00 g, 9 mmoles) in tetrahydrofuran (20 ml) was added in portions a solution of carbon tetrachloride (0.46 g, 3 mmoles) in tetrahydrofuran (1 ml) over 20 min maintaining the temperature below 7° and the mixture was stirred at room temperature overnight. After work-up as usual, a colorless solid was obtained in 50.5% yield (0.51 g), mp 84° (lit.[23] mp 84°), $[\alpha]_D^{20}$ $-38.3°$ ($c=1.0$ in MeOH) (lit.[23] $[\alpha]_D^{21}$ $-36.3°$ ($c=0.95$ in MeOH)). IR $\nu_{\max}\ \mathrm{cm}^{-1}$: 3280, 1742, 1683, 1653, 1529, 1260, 1211, 1048, 751, 696. Recrys-

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19) S. Yamada, M. Wagatsuma, Y. Takeuchi, and S. Terashima, *Chem. Pharm. Bull.* (Tokyo), **19**, 2380 (1971).

20) L. Heslinga and J.F. Arens, *Rec. Trav. Chim.*, **76**, 982 (1957).

21) R.W. Young, K.H. Wood, R.J. Joyce, and G.W. Anderson, *J. Am. Chem. Soc.*, **78**, 2126 (1956).

22) J.H. Seu, R.R. Smeby, and F.M. Bumpus, *J. Am. Chem. Soc.*, **84**, 3883 (1962).

23) T. Tomida, J. Ohashi, T. Tokuda, and M. Nakajima, *Nippon Nogeikagaku Kaishi*, **39**, 378 (1965).

tallization from ether and petroleum ether gave colorless prisms, mp 85°, $[\alpha]_D^{20}$ $-39.6°$ ($c=1.0$ in MeOH). *Anal.* Calcd. for $C_{17}H_{24}O_5N_2$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.79; H, 7.32; N, 8.59.

N-Benzyloxycarbonylserylglycine Ethyl Ester (Run 7)——To a solution of N-benzyloxycarbonylserine (0.72 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), tri-*n*-butylphosphine (0.61 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (10 ml) was added in portions carbon tetrabromide (1.00 g, 3 mmoles) over 30 min maintaining the temperature at 10°. The mixture was stirred at 10° for 2 hr and then at room temperature for 2 hr. After work-up as usual, a yellow oil was obtained which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (1: 1) and a colorless solid was obtained in 66.8% yield (0.65 g), mp 106—107° (lit.[24] mp 106—107°), $[\alpha]_D^{20}$ $-5.8°$ ($c=1.0$ in EtOH) (lit.[25] $[\alpha]_D^{20}$ $-5.5°$ ($c=1$ in EtOH)). IR ν_{max} cm^{-1}: 3280, 1763, 1696, 1659, 1542, 1200, 1059, 1028, 735, 691. Recrystallization from ethyl acetate and petroleum ether gave colorless needles, mp 107°, $[\alpha]_D^{20}$ -6.0 ($c=1.0$ in EtOH). *Anal.* Calcd. for $C_{15}H_{20}O_6N_2$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.84; H, 6.41; N, 8.57.

N-Benzyloxycarbonylthreonylphenylalanine Methyl Ester (Run 8)——To a solution of N-benzyloxycarbonylthreonine (0.76 g, 3 mmoles), phenylalanine methyl ester (0.54 g, 3 mmoles), tri-*n*-butylphosphine (0.61 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (10 ml) was added in portions a solution of carbon tetrachloride (1.00 g, 3 mmoles) in tetrahydrofuran (5 ml) at room temperature (17°). The mixture was stirred at room temperature overnight. After work-up as usual, a yellow oil was obtained which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (4: 1). A colorless solid was obtained in 53.3% yield (0.66 g), mp 107—108° (lit.[26] mp 105—106°), $[\alpha]_D^{20}$ $+6.3$ ($c=1.0$ in AcOH) (lit.[26] $[\alpha]_D^{22}$ $+5.0°$ ($c=1$ in AcOH)). IR ν_{max} cm^{-1}: 3270, 1741, 1698, 1649, 1540, 1245, 1118, 1045, 731, 692. Recrystallization from ethyl acetate and petroleum ether gave colorless needles, mp 107.5—108°, $[\alpha]_D^{20}$ $+6.5°$ ($c=1.0$ in AcOH). *Anal.* Calcd. for $C_{22}H_{26}O_6N_2$: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.44; H, 6.19; N, 6.86.

N-Benzyloxycarbonylvalyltyrosine Methyl Ester (Run 9)——To a solution of N-benzyloxycarbonylvaline (0.75 g, 3 mmoles), tyrosine methyl ester hydrochloride (0.70 g, 3 mmoles), tris(N-methylpiperazino)-phosphine (0.99 g, 3 mmoles), and N-methylmorpholine (0.61 g, 6 mmoles) in tetrahydrofuran (18 ml) was added in portions a solution of carbon tetrachloride (0.46 g, 3 mmoles) in tetrahydrofuran (2 ml) maintaining the temperature at 2—4°. The mixture was stirred at 0° for 4 hr and then at room temperature for 8 hr. After work-up as usual, a pale yellow oil was obtained which was chromatographed on silica gel (85 g) in *n*-hexane and ethyl acetate (3: 1). A colorless solid was obtained in 44.5% yield (0.57 g), mp 157—158° (lit.[27] mp 155.5—156°), $[\alpha]_D^{25}$ $+13.8°$ ($c=4.2$ in pyridine) (lit.[27] $[\alpha]_D^{25}$ $+10.2°$ ($c=4.8$ in pyridine)). IR ν_{max} cm^{-1}: 3240, 1753, 1686, 1645, 1550, 1532, 1246, 1213, 1169, 1034, 754, 694. Recrystallization from ethyl acetate and *n*-hexane gave colorless needles, mp 157—158°, $[\alpha]_D^{25}$ $+14.8°$ ($c=5.0$ in pyridine). *Anal.* Calcd. for $C_{23}H_{28}O_6N_2$: C, 64.47; H, 6.59; N, 6.54. Found: C, 64.31; H, 6.66; N, 6.71.

N-Benzyloxycarbonyl-S-benzylcysteinylglycine Ethyl Ester (Run 10)——To a solution of N-benzyloxycarbonyl-S-benzylcysteine (1.04 g, 3 mmoles) glycine ethyl ester (0.31 g, 3 mmoles), tris(N-methylpiperazino)phosphine (0.99 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrabromide (1.00 g, 3 mmoles) in tetrahydrofuran (5 ml) over 15 min maintaining the temperature at 3—5°. The mixture was stirred at 0° for 4 hr and then at room temperature for 12 hr. After work-up as usual, a pale brown oil was obtained which was chromatographed on silica gel (90 g) in *n*-hexane and ethyl acetate (3: 1). A colorless solid was obtained in 41.1% yield (0.53 g), mp 98—99° (lit.[28] mp 98—99°), $[\alpha]_D^{20}$ $-28.2°$ ($c=6.1$ in AcOH) (lit.[28] $[\alpha]_D^{20}$ $-26.8°$ ($c=6$ in AcOH)). IR ν_{max} cm^{-1}: 3250, 1742, 1689, 1676, 1642, 1531, 1270, 1215, 1047, 765, 705, 690. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 99.5—100°, $[\alpha]_D^{20}$ $-29.3°$ ($c=6.0$ in AcOH)). *Anal.* Calcd. for $C_{22}H_{26}O_5N_2S$: C, 61.38; H, 6.09; N, 6.51. Found: C, 61.40; H, 6.13; N, 6.64.

N-Benzyloxycarbonylprolylphenylalanine Methyl Ester (Run 11)——To a solution of N-benzyloxycarbonylproline (0.75 g, 3 mmoles), phenylalanine methyl ester (0.54 g, 3 mmoles), tri-*n*-butylphosphine (0.67 g, 3.3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrachloride (1.09 g, 3.3 mmoles) over 20 min at room temperature (15°) and the mixture was stirred at room temperature overnight. After work-up as usual, a yellow oil was obtained which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (3: 1). A colorless solid was obtained in 60.2% yield (0.74 g), mp 73—75°, $[\alpha]_D^{20}$ $-37.5°$ ($c=2.0$ in MeOH)). IR ν_{max} cm^{-1}: 3300, 1740, 1704, 1640, 1532, 1203, 1110, 1075, 1023, 760, 699, 692. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 74—75.5°, $[\alpha]_D^{20}$ $-37.7°$ ($c=2.0$ in MeOH). *Anal.* Calcd. for $C_{23}H_{26}O_5N_2$: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.30; H, 6.53; N, 7.03.

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N-Benzyloxycarbonylmethionylglycine Ethyl Ester (Run 12)——To a solution of N-benzyloxycarbonylmethionine (0.85 g, 3 mmoles), glycine ethyl ester hydrochloride (0.65 g, 3 mmoles), carbon tetrabromide (1.09 g, 3.3 mmoles), and N-methylmorpholine (0.91 g, 3 mmoles) in tetrahydrofuran (15 ml) and dimethylformamide (10 ml) was added in portions tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) over 15 min at 0°. The solution was stirred at 0° for 8 hr and then at room temperature for 8 hr. After work-up as usual, a yellow oil was obtained which was chromatographed on silica gel (180 g) (solvent; ethyl acetate: *n*-hexane: chloroform: ethanol=30: 50: 10: 1). A colorless solid was obtained in 51.6% yield (0.57 g), mp 96—97° (lit.[29] mp 95—96°, $[\alpha]_D^{27}$ $-18.6°$ (c=4.5 in EtOH) (lit.[29] $[\alpha]_D^{27}$ $-20.0°$ (c=4.6 in EtOH)). IR ν_{max} cm^{-1}: 3260, 1727, 1687, 1649, 1545, 1231, 1035, 752, 720, 693. Recrystallization from ether and petroleum ether gave colorless needles, mp 96—97°, $[\alpha]_D^{27}$ $-18.9°$ (c=4.8 in EtOH). *Anal.* Calcd. for $C_{17}H_{24}O_5N_2S$: C, 55.41; H, 6.57; N, 7.88. Found: C, 55.52; H, 6.67; N, 7.70.

N-Benzyloxycarbonyl-α-glutamylglycine Diethyl Ester (Run 13)——To a solution of N-benzyloxycarbonylglutamic acid γ-ethyl ester (0.92 g, 3 mmoles), glycine ethyl ester hydrochloride (0.42 g, 3 mmoles), tris(N-methylpiperazino)phosphine (0.99 g, 3 mmoles), and N-methylmorpholine (0.61 g, 6 mmoles) in tetrahydrofuran (15 ml) was added in portions carbon tetrachloride (0.46 g, 3 mmoles) over 1 hr maintaining the temperature at 5° and the mixture was stirred at room temperature overnight. After work-up as usual, a colorless semisolid was obtained which was chromatographed on silica gel (120 g) in *n*-hexane and ethyl acetate (4: 1). Yield 0.51 g (43.1%), mp 97—99° (lit.[30] mp 94—94.5°), $[\alpha]_D^{25}$ $-15.8°$ (c=2.0 in EtOH) (lit.[31] $[\alpha]_D^{25}$ $-12.5°$ (c=2.55 in 95% EtOH)). IR ν_{max} cm^{-1}: 3270, 1760, 1736, 1689, 1648, 1560, 1536, 1198, 1049, 1021, 738, 693. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 100°, $[\alpha]_D^{25}$ $-16.5°$ (c=2.0 in EtOH). *Anal.* Calcd. for $C_{19}H_{26}O_7N_2$: C, 57.86; H, 6.64; N, 7.10. Found: C, 57.64; H, 6.64; N, 7.25.

N-Beozyloxycarbonylasparaginylglycine Ethyl Ester (Run 14)——To a solution of N-benzyloxycarbonylasparagine (0.80 g, 3 mmoles), glycine ethyl ester (0.31 g, 3 mmoles), *N*-methylmorpholine (0.30 g, 3 mmoles), and carbon tetrachloride (1.00 g, 3 mmoles) in dimethylformamide (10 ml) was added in portions a solution of tri-*n*-butylphosphine (0.67 g, 3.3 mmoles) in tetrahydrofuran (5 ml) over 1 hr maintaining the temperature at $-20°$. The solution was stirred at $-20°$ for 2 hr, at 0° for 4 hr, and then at room temperature for 12 hr. After work-up as usual, a pale yellow oil was obtained which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (3: 2). Colorless crystals were obtained in 56.9% yield (0.60 g), mp 187—188° (lit.[32] mp 184—186°), $[\alpha]_D^{20}$ $-4.5°$ (c=1.0 in DMF) (lit.[32] $[\alpha]_D^{25}$ $-3.8°$ (c=1 in DMF)). IR ν_{max} cm^{-1}: 3360, 3250, 3180, 1717, 1695, 1667, 1649, 1622, 1558, 1539, 1262, 1053, 1023, 750. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 188.5—189°, $[\alpha]_D^{20}$ $-4.5°$ (c=1.1 in DMF). *Anal.* Calcd. for $C_{16}H_{21}O_6N_3$: C, 54.69; H, 6.02; N, 11.96. Found: C, 54.51; H, 6.33; N, 11.94.

N-*tert*-Butyloxycarbonyltryptophanylglycine Ethyl Ester (Run 15)——N-*tert*-Butyloxycarbonyltryptophan (0.91 g, 3 mmoles), glycine ethyl ester (0.62 g, 6 mmoles), carbon tetrabromide (1.00 g, 3 mmoles), and N-methylmorpholine (0.30 g, 3 mmoles) in tetrahydrofuran (15 ml) was added in portions a solution of tri-*n*-butylphosphine (0.91 g, 4.5 mmoles) in tetrahydrofuran (5 ml) over 1 hr at room temperature (18°) and the mixture was stirred at room temperature for 18 hr. After work-up as usual, a pale yellow oil was obtained which was chromatographed on silica gel (150 g) in *n*-hexane and ethyl acetate (3: 1). Colorless crystals were obtained in 60.8% yield (0.71 g), mp 117—118° (lit.[33] mp 118—119°), $[\alpha]_D^{20}$ $-12.0°$ (c=2.1 in EtOH) (lit.[33] $[\alpha]_D^{20}$ $-12.8°$ (c=1 in EtOH). IR ν_{max} cm^{-1}: 3350, 3275, 1730, 1687, 1642, 1535, 1529, 1170, 1020, 860, 740, 719. Recrystallization from ethyl acetate and petroleum ether gave colorless fine needles, mp 117.5—118°, $[\alpha]_D^{20}$ $-12.9°$ (c=2.5 in EtOH)). *Anal.* Calcd. for $C_{20}H_{27}O_5N_3$: C, 61.68; H, 6.99; N, 10.79. Found: C, 61.77; H, 7.20; N, 10.87.

N-Benzyloxycarbonylalanylphenylalanylmethionine Methyl Ester (Run 16)——N-Benzyloxycarbonylalanylphenylalanine, the starting material, was prepared as follows: N-Benzyloxycarbonylalanylphenylalanine methyl ester (Run 4) was dissolved in 1N aqueous solution of sodium hydroxide (30 ml) and stirred at room temperature for 1.5 hr. The solution was filtered and acidified with 10% hydrochloric acid and extracted with three 30 ml portions of ethyl acetate. The organic layer was washed with sat. aqueous solution of sodium chloride and dried over anhyd. magnesium sulfate. Evaporation of the solvent gave colorless crystals which were recrystallized from ethyl acetate and petroleum ether. Colorless needles were obtained in 75.5% yield (1.68 g), mp 129—129.5° (lit.[34] mp 122°). IR ν_{max} cm^{-1}: 3300, 1735, 1695, 1658, 1544, 1238, 731. *Anal.* Calcd. for $C_{20}H_{22}O_5N_2$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.58; H, 5.73; N, 7.60.

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To a solution of N-benzyloxycarbonylalanylphenylalanine (0.56 g, 1.5 mmoles), methionine methyl ester hydrochloride (0.30 g, 1.5 mmoles), tris(N-methylpiperazino)phosphine (0.59 g, 1.8 mmoles), and N-methylmorpholine (0.33 g, 3.3 mmoles) in tetrahydrofuran (20 ml) was added in portions a solution of carbon tetrachloride (0.25 g, 1.65 mmoles) in tetrahydrofuran (2 ml) over 5 min maintaining the temperature at −20°. The mixture was stirred at −15° for 1 hr, at 0° for 8 hr, and then at room temperature for 7 hr. After work-up as usual, a colorless semisolid was obtained which was chromatographed on silica gel (50 g) in *n*-hexane and ethyl acetate (3: 1). Colorless crystals were obtained in 37.5% yield (0.29 g), mp 162—163° (lit.[35] mp 156—157°), $[\alpha]_D^{20}$ −53.2° (c=1.0 in MeOH), $[\alpha]_{546}^{20}$ −57.2° (c=1.0 in MeOH) (lit.[35] $[\alpha]_{546}^{20}$ −62.1° (c=1 in MeOH)). IR $\nu_{\max}$ cm^{-1}: 3250, 1740, 1693, 1648, 1555, 1538, 1238, 1048, 740, 693. Recrystallization from ethyl acetate and ligroin gave colorless needles, mp 163—164°, $[\alpha]_D^{20}$ −54.6° (c=1.0 in MeOH), $[\alpha]_{546}^{20}$ −59.8° (c=1.0 in MeOH). *Anal.* Calcd. for $C_{26}H_{33}O_6N_3S$: C, 60.56; H, 6.45; N, 8.15. Found: C, 60.54; H, 6.31; N, 8.40.

N-Benzyloxycarbonylleucylleucylvaline Methyl Ester (Run 17)——To a solution of N-benzyloxycarbonylleucylleucine[36] (0.64 g, 1.7 mmoles), valine methyl ester (0.45 g, 3.4 mmoles), and tris(N-methylpiperazino)phosphine (1.12 g, 3.4 mmoles) in tetrahydrofuran (15 ml) was added in portions a solution of carbon tetrabromide (0.56 g, 1.7 mmoles) in tetrahydrofuran (5 ml) over 40 min maintaining the temperature at −15°. The mixture was stirred at −15° for 3 hr, at 0° for 3 hr, and then at room temperature for 2 hr. After work-up as usual, a pale yellow oil was obtained which was chromatographed on silica gel (120 g) in *n*-hexane and ethyl acetate (4: 1). Colorless crystals were obtained in 21.8% yield (0.32 g), mp 127—129° (Lit.[36] mp 123—124°) $[\alpha]_D^{20}$ −53.7° (c=1.0 in MeOH) (lit.[36] $[\alpha]_D^{19.5}$ −54.0° (c=1.0 in MeOH)). IR $\nu_{\max}$ cm^{-1}: 3260, 1747, 1704, 1669, 1645, 1540, 1234, 1207, 1155, 1117, 1040, 725, 690. Recrystallization from ethyl acetate and petroleum ether gave colorless needles, mp 129°, $[\alpha]_D^{20}$ −55.8° (c=1.0 in MeOH). *Anal.* Calcd. for $C_{26}H_{41}O_6N_3$: C, 63.54; H, 8.41; N, 8.55. Found: C, 63.26; H, 8.34; N, 8.60.

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