Peptide Synthesis by Oxidation-Reduction Condensation. II.[1] The Use of Disulfide as an Oxidant

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A peptide synthesis starting from free *N*-protected amino acid and free amino acid ester by the use of triphenylphosphine, an oxygen acceptor, and disulfide, a hydrogen acceptor, was studied. The oxidation-reduction condensation reaction affords peptides with high optical purity in excellent yields by simple procedure.

In the preceding report,[2] it was shown that *N*-*o*-nitrophenylsulfenyl(NPS)-amino acid, in which NPS group is generally known as a protecting group of amino component in peptide synthesis, affords a new peptide linkage by one step procedure when it was treated with triphenylphosphine and carboxyl component. During this peptide forming reaction, phosphine is used as an oxygen acceptor and sulfenyl group of the amide is reduced to accept a hydrogen.

In the present experiment, a direct peptide synthesis by the oxidation-reduction condensation starting from free *N*-protected amino acid and free amino acid ester was tried by the use of disulfide as an oxidant.

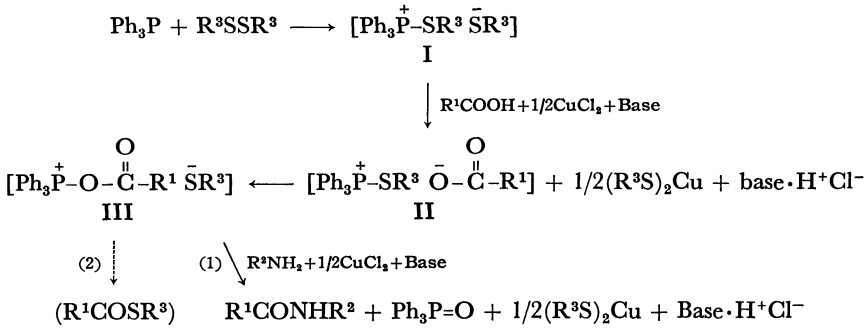
In the model reaction of carboxamide formation, *N*-*n*-butylhexanamide was obtained in 62% yield from hexanoic acid and *n*-butylamine by the use of *p*,*p*'-dichlorodiphenyl disulfide as an oxidant and cupric chloride as a mercaptan scavenger according to the following equation.

$$n\text{-}C_5H_{11}COOH + n\text{-}C_4H_9NH_2 + Ph_3P + (p\text{-}ClC_6H_4S)_2 + CuCl_2 + 2Et_3N \xrightarrow[\text{in } CH_2Cl_2]{\text{room temp., 3hr}} n\text{-}C_5H_{11}CONH(n\text{-}C_4H_9) + Ph_3P{=}O + (p\text{-}ClC_6H_4S)_2Cu + 2Et_3NH^+Cl^-$$

In this reaction, a small amount (2% yield) of thiohexanoic acid *S*-*p*-chlorophenyl ester was isolated along with *N*-*n*-butylhexanamide. The thiolester did not react with *n*-butylamine in methylene chloride when they were allowed to stand for 3 hr, indicating that the thiolester is not an intermediate of this reaction. The result shows that the reaction may proceed through an initial formation of phosphonium salt I, which in turn reacts with carboxylic acid to form the salt II; this subsequently, is transfomed into III, conceivably by way of an internal nucleophilic displacement. The active intermediate of acyloxyphosphonium mercaptide III has two possible pathways of decomposition: namely, (1) the formation of amide by nucleophilic attack of amine on carboxyl carbon and (2) the formation of thiolester by an intramolecular nucleophilic attack of mercapto anion on carboxyl carbon as shown in Scheme 1.

In order to minimize the undesirable formation of thiolester in the above experiment, the effects of various kinds of metal compounds were examined with the assumption that pathway (2) would be skipped when soft metallic compounds are used as mercaptan scavengers. The mercapto anion (soft base) in the intermediate of acyloxyphosphonium salt is expected to react readily with soft metallic cation (soft acid) as predicted by Pearson's principle.[3] Expectedly, the results showed a good agreement with the Pearson's principle and thiolester formation was completely prevented when mercuric chloride and silver chloride were used (see Table 1).

Then, the reaction was applied to peptide synthesis by using mercuric chloride as a metal component. First, the synthesis of benzyloxycarbonyl (Z)-L-phenylalanylglycine ethyl ester which has an optical active amino acid as a carboxyl component was tried and the dipeptide was obtained in 89% yield without racemization.



Scheme 1. Reaction pathway for the formation of amide from acid, amine, phosphine, and disulfide in the presence of $CuCl_2$ and base.

1) Preliminary communications: T. Mukaiyama, M. Ueki, H. Maruyama, and R. Matsueda, *J. Amer. Chem. Soc.*, **90**, 4490 (1968); T. Mukaiyama, R. Matsueda, H. Maruyama, and M. Ueki, *ibid.*, **91**, 1554 (1969); T. Mukaiyama, R. Matsueda, and H. Maruyama, This Bulletin, **43**, 1271 (1970).

2) M. Ueki, H. Maruyama, and T. Mukaiyama, Submitted for publication in This Bulletin.

3) R. G. Pearson, *J. Amer. Chem. Soc.*, **84**, 16 (1962); *ibid.*, **85**, 3533 (1963).

TABLE 1. EFFECTS OF METAL COMPONENTS ON THE PREVENTION OF THIOLESTER FORMATION

Metal compound	n-$C_5H_{11}CONH$-(n-C_4H_9) Yield, %	n-$C_5H_{11}COS$-C_6H_4Cl-p Yield, %
AgCl	72	0
$HgCl_2$	66	0
HgCl	62	0
CdI_2	57	0
$HgSO_4$	47	0
$CuCl_2$	62	2
$PdCl_2$	60	3
$PbCl_2$	54	2
$NiCl_2$	42	9
CuCl	43	8
$ZnCl_2$	38	6
$CoCl_2$	23	8

Z-L-Phe-OH + H-Gly-OEt + Ph_3P + (o-$NO_2C_6H_4S)_2$ + $HgCl_2$ + $2Et_3N$ ⟶ Z-L-Phe-Gly-OEt + Ph_3P=O (89% yield) + (o-$NO_2C_6H_4S)_2Hg$ + $2Et_3NH^+Cl^-$

Next, the synthesis of Z-Gly-L-Phe-OEt which has an optical active amino acid as an amino component was tried and the dipeptide was obtained in 91% yield as an oil by column chromatography on silica gel. This oily substance was identified to be an optical pure dipeptide by the subsequent saponification to Z-Gly-L-Phe-OH.

The results show that the peptide forming reaction is applicable for a stepwise elongation of peptide chain.

Next, the racemization was further studied in order to know the applicability of this reaction to the fragment condensation which is very sensitive to racemization.

In the synthesis of peptides, the complete retention of optical activity is an important though rarely achieved goal. Recent work[4] has established that oxazolone is an essential intermediate for the racemization of most of the activated species in the peptide synthesis. In order to check the racemization in the fragment condensation, the above mentioned new reaction was further examined by the Young test[5] (benzoyl(Bz)-L-leucylglycine ethyl ester synthesis) which is known to be the most sensitive racemization test. The authentic samples of the L and DL peptides were synthesized according to Young's report[5] and it was found that the infrared spectra of the two peptides were different to each other as shown in Table 2.

TABLE 2. INFRARED SPECTRA OF L AND DL Bz-Leu-Gly-OEt (KBr pellets)

	L Peptide (cm^{-1})	DL Peptide (cm^{-1})
NH stretching absorption	3300 (singlet)	3275, 3330 (doublet)
	1024 (singlet)	1039, 1024 (doublet)

Since the L peptide was observed to be more soluble in solvent than the DL peptide, the procedure of Young test in this reaction was determined to measure a specific rotation of an unrecrystallized crude product which is isolated by dry column chromatography[6] or a preparative layer chromatography on silica gel. By this procedure, azide method gave the value of $[\alpha]_D^{20}$ −32.6° (c 3.1 EtOH), L-isomer 96%.[5] While the oxidation-reduction condensation reaction carried out in methylene chloride at room temperature gave a poor result as shown in the following equation.

Bz-L-Leu-OH + 3H-Gly-OEt + Ph_3P + (o-$NO_2C_6H_4S)_2$ + $HgCl_2$ ⟶ Bz-Leu-Gly-OEt + Ph_3P=O (76% yield) + (o-$NO_2C_6H_4S)_2Hg$ + $2Cl^-H^+$·H-Gly-OEt

$[\alpha]_D^{20}$ −7.6° (c 3.1, EtOH), L isomer 22%[6]

As to the Young test, the racemization intermediate is known to be 4-isobutyl-2-phenyloxazolone whose formation is catalyzed by bases.[7] A base-catalyzed enolization mechanism which involves two proton abstraction steps from the amide hydrogen and from the oxazolone hydrogen was also reported by Kemp and his associates.[8]

In the above reaction, hydrochloric acid is produced along with the peptide by the reaction of the supposed key intermediate of acyloxyphosphonium salt, ($Ph_3\overset{+}{P}$-O-COR^1) $\bar{S}R^2$, ethyl glycinate and mercuric chloride. Therefore, tertiary amine must be used in order to scavenge hydrochloric acid produced at the same time.

In order to eliminate the basic substances as tertiary amine from the reaction system, the reactions were tried by using various kinds of organo-mercuric compounds. These mercuric compounds were classified into two groups through the mercuric mercaptide formation reaction: class a) mercuric salts of urea, succinimide, p-nitrophenol, *etc.* which yield mercuric mercaptide by the reference anion exchange of the phosphonium mercaptide, ($Ph_3\overset{+}{P}$-SR) $\bar{S}R$, with mercuric compounds; class b) di-p-methoxyphenylmercury and p-methoxyphenylmercuric bromide which yield mercuric mercaptide through the protonation of mercaptan to mercuric compounds, but not by the reference anion exchange. The results of the Young test by employing class a) compounds are shown in Table 3.

In these cases, the acyloxyphosphonium mercaptide readily reacts with the metal compounds (MX) to produce metal mercaptides and the second acyloxy phosphonium salts, ($Ph_3\overset{+}{P}$-O-COR^1)X^-, by the reference anion exchange. The X^- anion involved in the salt would be expected to have a significant effect on racemization and in accordance with this expectation, the optical purity increased as the X^- anion stability

4) M. Goodman and K. C. Steuben, *J. Org. Chem.*, **27**, 3409 (1962); M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, **86**, 2918 (1964); M. W. Williams and G. T. Young, *J. Chem. Soc.*, **1964**, 3701.

5) M. W. Williams and G. T. Young, *ibid.*, **1963**, 881. L-Isomer % was calcualted from Young's equation ($[\alpha]_D/-34\times 100$).

6) B. Loev and K. M. Snaper, *Chem. Ind.* (London), **1965**, 15; B. Loev and M. M. Goodman, *ibid.*, **1967**, 2026.

7) M. W. Williams and G. T. Young, *J. Chem. Soc.*, **1964**, 3701.

8) D. S. Kemp and S. W. Chien, *J. Amer. Chem. Soc.*, **89**, 2746 (1967).

Table 3. The Young test by the use of class a) compounds

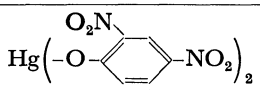
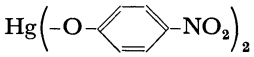
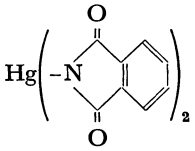
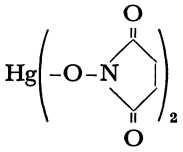
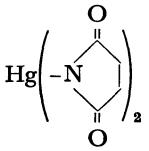
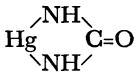
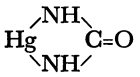
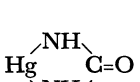
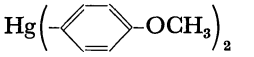
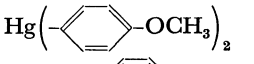
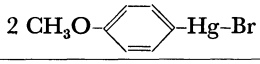
Metal compounds MX_2	pK_a value of HX	Additives	$(RS)_2$	Crude Bz-Leu-Gly-OEt	
				Yield, %	L isomer, %
	4.0	—	(p-$ClC_6H_4S)_2$	92	73
	7.2	—	(p-$ClC_6H_4S)_2$	82	51
	8.3	—	(p-$ClC_6H_4S)_2$	85	47
	9.4	—	(p-$ClC_6H_4S)_2$	89	59
	9.5	—	(p-$ClC_6H_4S)_2$	91	46
	13.8	—	(p-$ClC_6H_4S)_2$	81	23
	13.8	Pivalic acid (pKa 5.0)	(p-$ClC_6H_4S)_2$	76	60
	13.8	2 (pKa 9.4)	(p-$ClC_6H_4S)_2$	89	92

Table 4. The young test by the use of class b) compounds

Metal compounds MX_2	$(RS)_2$	Reaction Period, day	Crude Bz-Leu-Gly-OEt		
			Yield, %	$[\alpha]_D^{20}$ (c 3.1 EtOH)	L isomer, %
	(p-$ClC_6H_4S)_2$	3	88	−30.5°	90
	(o-$O_2NC_6H_4S)_2$	2	92	−32.4°	95
2 	(o-$O_2NC_6H_4S)_2$	2	92	−32.0°	94

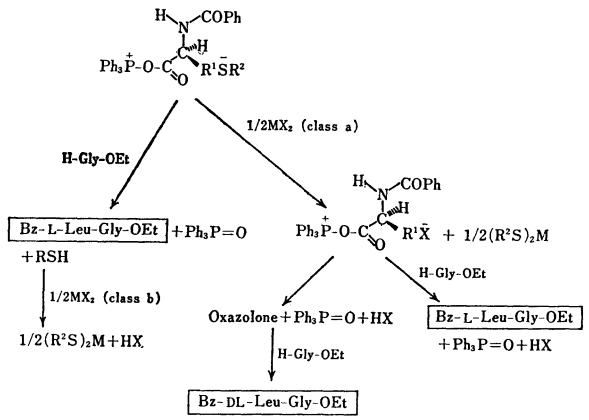
increased except in the case of *N*-hydroxysuccinimide. To investigate the characteristic effect of *N*-hydroxysuccinimide which is well known to give a favorable result in the dicyclohexylcarbodiimide method,[9] the reactions using mercuric salt of urea in the presence of acidic substances were studies. *N*-hydroxysuccinimide gave a better result than pivalic acid which has a smaller pK_a value. On the other hand, the more favorable results were obtained s shown in Table 4 when class b) compounds were used.

In these cases, the intermediate, acyloxyphosphonium salt, is attacked only by ethyl glycinate to produce the peptide and mercaptan. The mercaptan is in turn scavenged by the reaction with bis(p-methoxyphenyl)-mercury to yield mercuric mercaptide and anisole as shown in the following Scheme 2.

Since anisole is produced directly through the protonation of mercaptan to the mercuric compound, p-methoxyphenyl anion would not be produced during this mercaptide formation reaction and oxazolone formation can be prevented. The reaction by using bis(p-methoxyphenyl)mercury was further studied in various kinds of solvents and the results are shown in Table 5.

The reaction was found to proceed in all the solvents examined, but a partial racemization was observed in polar solvents as acetonitrile and *N*,*N*-dimethylformamide; while addition of *N*-hydroxysuccinimide also gave an improved result. As described above, peptides with high optical purity are produced in excellent

9) J. E. Zimmerman and G. W. Anderson, *J. Amer. Chem. Soc.*, **89**, 7151 (1967). Special character of preventing racemization of *N*-hydroxysuccinimide is also reported by H. Goodman, *Chem. Eng. News*, **1968**, 40.



Scheme 2. Reaction pathways for the formation of L-and DL-Bz-Leu-Gly-OEt from Bz-L-Leu-OH, H-Gly-OEt, Ph_3P, and $(o\text{-}NO_2C_6H_4S)_2$ in the presence of either class a) or class b) metallic compound.

TABLE 5. EFFECTS OF SOLVENTS ON RACEMIZATION BY THE YOUNG TEST

Solvent	Dielectric constant	Crude Bz-Leu-Gly-OEt Yield %	L isomer %
Benzene	2.2	91	95
Dioxane	2.3	89	89
$CHCl_3$	4.9	96	96
AcOEt	6.0	94	90
THF[1]	7.0	91	89
CH_2Cl_2	9.9	92	95
$ClCH_2CH_2Cl$	10.5	94	88
DMF[2]	37.0	86	37
CH_3CN	37.5	88	66
CH_3CN + HO-N(succinimide)	37.5	87	86

1) THF: tetrahydrofuran
2) DMF: *N*,*N*-dimethylformamide

yields in solution by stepwise synthesis but also by the model reaction of fragment condensation (Young test) when mercuric compounds, mercaptan scavenger, are used in this oxidation-reduction condensation.

In recent years, the potential utility of the solid phase method in peptide synthesis has been extended. With respect to this problem, insoluble metal mercaptides, produced at the same time in the above mentioned reaction, seem to be troublesome problem when it is applied to the solid phase method. Then, various kinds of non-metallic mercaptan scavengers were investigated in an attempt to purge the insoluble mercaptide from the reaction system.

First, the reaction was tried by the use of olefins as mercaptan scavengers since some olefins such as 2,3-dihydropyran is known to react readily with cysteine in the presence of Lewis acid,[10] and good results were obtained. When the Young test was carried out in methylene chloride by using 2,3-dihydropyran and zinc chloride as catalyst, Bz-L-Leu-Gly-OEt was obtained in 92% yield, $[\alpha]_D^{20}$ −32.9° (*c* 3.1 EtOH), L-isomer 97%. Good result was also obtained in the case when *n*-butyl vinyl ether is employed (90% yield, L-isomer 95%).

Next, the reaction was further examined by the Young test with sulfenate esters which can behave as both oxidant and mercaptan scavenger (see Table 6).

Bz-L-Leu-OH + H-Gly-OEt + Ph_3P + $2R^1S\text{-}OR^2$ ⟶ Bz-L-Leu-Gly-OEt + Ph_3P=O + $(R^1S)_2$ + $2R^2OH$

TABLE 6. THE YOUNG TEST BY THE USE OF SULFENATE ESTERS AS BOTH OXIDANT AND MERCAPTAN SCAVENGER

Sulfenate ester	Crude Bz-Leu-Gly-OEt Yield %	L isomer %
$o\text{-}O_2NC_6H_4SOCH_3$	84	55
$2,4\text{-}(O_2N)_2C_6H_3SO^tBu$	85	78
$2,4\text{-}(O_2N)_2C_6H_3SO^tBu$ + HO-N(succinimide)	84	95

Partial racemization observed in these reactions may be due to the basic character of alkoxy anion of the intermediate phosphonium salt which is produced by the reaction of triphenylphosphine and sulfenate ester, but a favorable result was also obtained in the presence of 1 equivalent of *N*-hydroxysuccinimide.

It is known that some of thiols such as mercaptopyridine, mercaptopurine, *etc.*, which bear a mercapto group α or γ with respect to a ring nitrogen exist in solution predominantly in the thione form.[11] This fact indicates that by employing 2,2′-dipyridyl disulfide as a disulfide component, the peptide forming reaction can be carried out in the absence of a mercaptan scavenger with the consideration that 2-mercaptopyridine produced along with peptide would isomerize to the stable thione form. The reaction by the Young test in methylene chloride was completed within 30 min at room temperature and the peptide was obtained in 91% yield, $[\alpha]_D^{20}$ −32.6° (*c* 3.1 EtOH), L-isomer 96%, according to the following equation.

Bz-L-Leu-OH + H-Gly-OEt + Ph_3P + $(N\text{=}C\text{-}S\text{-})_2$ ⟶ Bz-L-Leu-Gly-OEt + Ph_3P=O + 2 (N=C-S, H)

In conclusion, it is noted that peptide with high optical purity is produced in excellent yield from free *N*-protected amino acid, free amino acid ester, phosphine (an oxygen acceptor), and disulfide (a hydrogen acceptor) during oxidation-reduction process.

Experimental

All melting points are uncorrected. Infrared spectra were determined on a Hitachi Model EPI-G2 Spectrophotometer

10) G. F. Holland and L. A. Cohen, *J. Amer. Chem. Soc.*, **80**, 3765 (1958).

11) R. A. Jones and A. R. Katritzky, *J. Chem. Soc.*, **1958**, 3610.

and optical rotation was determined on a Perkin-Elmer Model 141 Polarimeter.

Reagents. Mercuric salts of urea,[12] succinimide and phthalimide, *p*-methoxyphenylmercuric bromide[13] and bis(*p*-methoxyphenyl)mercury[14] were prepared by the literature procedures. Mercuric salt of *N*-hydroxysuccinimide was prepared by the following procedure: A solution of 9.45 g(30 mmol) of mercuric acetate in ethanol (100 m*l*) was added with vigorous stirring to a solution of 6.09 g (60 mmol) of *N*-hydroxysuccinimide in ethanol. The white precipitate was collected by filtration, washed with ethanol and dried, 11.59 g (90%), mp 194—195°C (dec).

Found: C, 22.26; H, 2.18; N, 6.69%. Calcd for $C_8H_8N_2O_6Hg$: C, 22.41; H, 1.88; N, 6.69%. Mercuric salts of *p*-nitrophenol and 2,4-dinitrophenol were prepared by the same procedure in aqueous solution.

Methyl *o*-nitrobenzenesulfenate[15] and *t*-butyl 2,4-dinitrobenzenesulfenate[16] were prepared by the method described in the literature. 2-Mercaptopyridine was prepared by the method in the literature[11] with some modification: A solution of 2-chloropyridine 45.2 g (0.4 mol), thiourea 30.4 g (0.4 mol), and iodine 0.3 g in ethanol (120 m*l*) was refluxed for 6 hr. After cooling, 60 m*l* of concentrated aqueous ammonia (*d* 0.88) was added and the solution was kept standing for 5 days at room temperature. After evaporation of ethanol, the aqueous solution was acidified by acetic acid to pH 3 and extracted with chloroform. The chloroform layer was dried over sodium sulfate and evaporated to dryness. Crude material was recrystallized from benzene to give pure 2-mercaptopyridine, 27.7 g (62%), mp 124—126°C. 2,2′-Dipyridyl disulfide was obtained quantitatively by potassium ferricyanide oxidation of the 2-mercaptopyridine.[17]

Reaction of Hexanoic Acid, n-Butylamine, Triphenylphosphine (IV), and p,p′-Dichlorodiphenyl Disulfide in the Presence of Cupric Chloride and Triethylamine (TEA). To a mixture of hexanoic acid 1.16 g (10 mmol), cupric chloride 1.34 g (10 mmol), and *p,p′*-dichlorodiphenyl disulfide 2.87 g (10 mmol) in methylene chloride (20 m*l*) were added with ice cooling TEA 2.02 g (20 mmol), IV 2.62 g (10 mmol), and *n*-butylamine 0.73 g (10 mmol) in methylene chloride (10 m*l* each) in this order. The resultant solution was stirred for 4 hr at room temperature. After filtration off of the precipitated copper mercaptide, the solution was washed with water, N HCl, water, 5% $NaHCO_3$ solution and water and dried. The solvent was evaporated off and from the residue *N-n*-butylhexanamide was obtained by chromatography on silica gel, 1.06 g (62%), bp 115—118°C (2 mmHg).

Found: C, 69.94; H, 12.25; N, 8.08%. Calcd for $C_{10}H_{21}NO$: C, 70.12; H, 12.36; N, 8.18%. In addition, a small amount of thiohexanoic acid *S-p*-chlorophenyl ester was isolated, 0.05 g (2%), bp 138—140°C (2 mmHg).

Found: C, 59.67; H, 6.18; Cl, 14.52; S, 13.27%. Calcd for $C_{12}H_{15}ClOS$: C, 59.38; H, 6.19; Cl, 14.64; S, 13.20%.

The yields of amide obtained when various kinds of metallic compounds were used in place of cupric chloride in the above reaction are shown in Table 1.

Reaction of Z-L-Phe-OH, H-Gly-OEt, IV, and o,o′-Dinitrodiphenyl Disulfide(V) in the Presence of Mercuric Chloride and TEA. To a stirred mixture of Z-L-Phe-OH 2.99 g (10 mmol), mercuric chloride 2.71 g (10 mmol), and V 3.08 g (10 mmol) in methylene chloride (20 m*l*) was added successively with ice cooling TEA 2.02 g (20 mmol), IV 2.62 g (10 mmol), and H-Gly-OEt 1.03 g (10 mmol) in 10 m*l* each of the same solvent. The resultant mixture was stirred for 3 hr at room temperature and the precipitated mercury mercaptide was filtered off. The filtrate was washed with water, N HCl, water, 5% $NaHCO_3$ solution, water and dried over sodium sulfate. The solvent was evaporated *in vacuo* and from the residue Z-L-Phe-Gly-OEt was obtained by dry column chromatography[6] on silica gel using methylene chloride as solvent, 3.43 g (89%), mp 110—110.5°C, $[\alpha]_D^{20}$ −17.1° (*c* 2 EtOH). [lit,[18] mp 110—113°C, $[\alpha]_D^{25}$ −16.6° (*c* 2 EtOH)].

Found: C, 65.83; H, 6.53; N, 7.42%. Calcd for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29%.

Reaction of Z-Gly-OH, H-L-Phe-OEt, IV, and V in the Presence of Mercuric Chloride and TEA. Substitution of Z-L-Phe-OH for Z-Gly-OH and H-Gly-OEt for H-L-Phe-OEt in the above reaction gave Z-Gly-L-Phe-OEt in 91% yield as an oil [lit.[19] oil]. This oily material (0.97 g) was saponified by N NaOH in aqueous dioxane by the literature procedure[19] to yield Z-Gly-L-Phe-OH, 0.74 g (83%), mp 125—126°C, $[\alpha]_D^{25}$ 36.2° (*c* 2 acetone). [lit,[18] mp 125—126°C, $[\alpha]_D^{25}$ 37° (*c* 2 acetone)].

Found: C, 64.04; H, 5.64; N, 7.66%. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.03; H, 5.66; N, 7.86%.

The Test for the Racemization in This Type of Reactions by Young's Method.[5] Authentic samples of L- and DL-Bz-Leu-Gly-OEt were prepared by the azide method in the Young's report. Since the solubilities of L and DL peptides were observed to be different to each other, general isolation procedure was determined to be made by dry column chromatography or preparative layer chromatography on silica gel and $[\alpha]_D$ values of crude peptides were determined without recrystallization. By this procedure, Bz-L-Leu-Gly-OEt was obtained in 68% yield, $[\alpha]_D^{20}$ −32.6° (*c* 3.1 EtOH), L-isomer 96%,[5] according to the azide method carried out in ether.

Reaction of Bz-L-Leu-OH, H-Gly-OEt, IV, and V in the Presence of Mercuric Chloride and TEA. To an ice cooled mixture of Bz-L-Leu-OH 2.35 g (10 mmol), V 3.08 g (10 mmol), and mercuric chloride 2.71 g (10 mmol) in methylene chloride (30 m*l*) was added with strirring a solution of IV 2.62 g (10 mmol) and H-Gly-OEt 3.03 g (30 mmol) in 20 m*l* of the same solvent. Stirring was continued for 4 hr at room temperature and the precipitated mercury mercaptide was filtered off. The filtrate was washed with water, N HCl, water, 5% $NaHCO_3$ solution and water, dried and evaporated *in vacuo*. The residue was separated by preparative layer chromatography on silica gel by developing with ethyl ether. Zone corresponding to the peptide was scraped and eluted by methylene chloride and methanol (10:1). The eluate was evaporated to dryness and the residue was dissolved in methylene chloride, washed with water, and dried over sodium sulfate. After evaporation of the solvent, Bz-L-Leu-Gly-OEt was washed with petroleum ether (30—50°C) and collected by filtration, 2.58 g (81%), mp 144—147°C, $[\alpha]_D^{20}$ −7.9° (*c* 3.1 EtOH), L-isomer 23%.

Found: C, 63.99; H, 7.38; N, 8.98%. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74%.

Reactions of Bz-L-Leu-OH, H-Gly-OEt, IV, and V in the Presence of Organomercuric Compounds. (General Procedure) Each 10 mmol of H-Gly-OEt and IV in methylene chloride

12) N. V. Subba Rao and T. R. Seshadri, *Proc. Indian Acad. Sci., Sect. A*, **10**, 1 (1939).
13) F. Challenger and S. A. Miller, *J. Chem. Soc.*, **1938**, 894.
14) A. Michaels and J. Rabinerson, *Ber.*, **23**, 2343 (1890).
15) T. Zincke and F. Farr, *Ann. Chem.*, **391**, 57 (1912).
16) N. Kharasch, D. P. McQuarrie, and C. M. Buess, *J. Amer. Chem. Soc.*, **75**, 2658 (1953).
17) H. Kubota and T. Akita, *Yakugaku Zasshi*, **81**, 5111 (1961).
18) R. W. Young, K. H. Wood, R. T. Joyce, and G. W. Anderson, *J. Amer. Chem. Soc.*, **78**, 2126 (1956).
19) S. Goldschmidt and K. K. Gupta, *Chem. Ber.*, **98**, 2381 (1965).

(20 ml) was added at room temperature to vigorously stirred mixture of equimolar amounts of Bz-L-Leu-OH, V, and organomercuric compound in the same solvent (30 ml). The reaction mixture was stirred for a few hours in the case of class a) compounds, and for a few days in the case of class b) compounds. The peptide was isolated by dry column chromatography or preparative layer chromatography as described above. Results is are shown in Tables 3 and 4.

Reactions of Bz-L-Leu-OH, H-Gly-OEt, IV, and V in the Presence of Olefins. To a mixture of Bz-L-Leu-OH 1.17 g (5 mmol), V 1.54 g (5 mmol), 2,3-dihydropyran 1.68 g (20 mmol), and zinc chloride 0.68 g (5 mmol) in methylene chloride (30 ml) was added with ice cooling a solution of IV 1.31 g (5 mmol) and H-Gly-OEt 0.52 g (5 mmol) in the same solvent. The resultant mixture was stirred for 6 hr at room temperature, washed with 5% $NaHCO_3$ solution and water and dried. After evaporation of the solvent, the residue was chromatographed on silica gel. A fraction eluted by benzene gave *o*-nitrophenyl 2-tetrahydropyranyl sulfide, 2.04 g (85%), mp 60—61°C.

Found: C, 55.50; H, 5.49; N, 5.97; S, 13.58%. Calcd for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.47; N, 5.86; S, 13.40%.

Bz-L-Leu-Gly-OEt was obtained by elution with ether, 1.47 g (92%), $[\alpha]_D^{20}$ −32.9° (*c* 3.1 EtOH), L-isomer 97%.

Substitution of 2,3-dihydropyran for *n*-butyl vinyl ether gave the peptide in 90% yield with 95% L-isomer content.

Reactions of Bz-L-Leu-OH, H-Gly-OEt, IV, and Sulfenate Esters. A solution of H-Gly-OEt (5 mmol) and IV (5 mmol) in methylene chloride was added to a solution of Bz-L-Leu-OH (5 mmol) and sulfenate ester (10 mmol) and the mixture was stirred for a few hours. Work up was done as above and the results are shown in Table 6.

Reaction of Bz-L-Leu-OH, H-Gly-OEt, IV, and 2,2′-Dipyridyl Disulfide. A solution of IV 1.31 g (5 mmol) and H-Gly-OEt 0.52 g (5 mmol) in methylene chloride (20 ml) was added drop by drop at room temperature to a stirred solution of Bz-L-Leu-OH 1.17 g (5 mmol) and 2,2′-dipyridyl disulfide 1.10 g (5 mmol) in the same solvent (30 ml). Stirring was continued for 30 min and the solution was washed with N HCl, water, 5% $NaHCO_3$ solution and water, and dried over sodium sulfate. Work up as mentioned above gave Bz-L-Leu-Gly-OEt, 1.46 g (91%), mp 148—152°C, $[\alpha]_D^{20}$ −32.6° (*c* 3.1 EtOH), L-isomer 96%.