Benzotriazol-1-yl Diethyl Phosphate and Benzotriazol-1-yl Diphenyl Phosphate. New Convenient Reagents for the Peptide Synthesis

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Benzotriazol-1-yl Diethyl phosphate and benzotriazol-1-yl diphenyl phosphate were conveniently prepared in essentially quantitative yields by the reaction of diethyl chlorophosphate and diphenyl chlorophosphate with equal amounts of 1-hydroxybenzotriazole and triethylamine in tetrahedrofuran at room temperature, respectively. Benzotriazol-1-yl diethylphosphate was effective for the preparation of amides from carboxylic acids amines. Young test and Anderson test for racemization studies using benzotriazol-1-yl diethyl phosphate were investigated and practically no racemization occurred. However, racemization occurred to some extent during coupling of Z-Phe-Val-OH with Pro-OBu.^{t} Several dipeptides and tripeptides were prepared without little racemization using benzotriazol-1-yl diethyl phosphate. Benzotriazol-1-yl diphenyl phosphate was less effective than benzotriazol-1-yl diethyl phosphate in terms of the degree of racemization.

Introduction

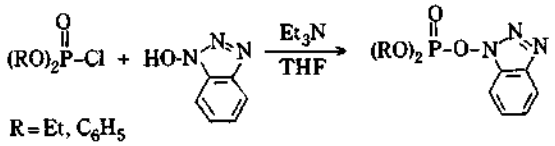
One of the popular methods for coupling two fragments in peptide synthesis involves activation of N-protected amino acids or N-protected peptide acids by the formation of the mixed carboxylic-carbonic anhydrides with alkyl chloroformates, especially isobutyl chloroformate in the presence of a tertiary amine base.[1] However, the mixed carboxylic-carbonic anhydride methods have certain limitations such as the dependence of the degree of recemization on the reaction conditions and urethane formation by attack of an amino acid ester at carbonic acid carbonyl of the mixed anhydrides.[1,2] Therefore, mixed anhydrides of carboxylic acids with phosphoric acids have attracted a great deal of recent attention in peptide synthesis. Among a number of phosphate types of coupling reagents developed for this purpose,[3] diphenylphosphoroazidate is the most well known and frequently utilized in racemization-free peptide synthesis.[4] Recently developed phosphate types of coupling reagents include diethyl phosphorobromidate,[5] N-succinimidyl diphenyl phosphate,[6] norborn-5-ene-2,3-dicarboximido diphenyl phosphate,[7] and diethyl 2-(3-oxo-2,3-dihydro-1,2-benzisosulfoazolyl)phosphate.[8] The use of phosphate types of coupling reagents provides several advantages over other coupling methods as follows. First, coupling reaction generally proceeds without appreciable recemization. Second, the reaction can be carried out by one step procedure, indicating that phosphate types of coupling reagents selectively react with carboxylate anions to form mixed anhydrides in the presence of an amino group. Thirdly, the byproducts from the reaction are normally removable with aqueous workup. Finally, the reagents are easily prepared in high yields.

Synthetic usefulness of coupling reagents containing 1-benzotriazolyl moiety has been previously demonstrated. It has been reported that 1,1′-(carbonyldioxy)dibenzotriazole[9] and 1,1′-bis [6-(trifluoromethyl) benzotriazolyl]oxalate[10] are new reactive coupling reagents for the synthesis of esters, amides, and dipeptides. We have recently reported t-butoxycarbonylation of amino acids with t-butyl benzotriazol-1-yl carbonate[11] and the preparation of isothiocyanates from amines with 1,1′-(thiocarbonyldioxy) dibenzotriazole.[12] Concerning 1-benzotriazolyl related organophosphorous coupling reagents, bis (benzotriazol-1-yl) phenyl phosphate as a versatile phosphorylating reagent in the synthesis of nucleotides[13] and benzotriazoltris (dimethylamino)phosphonium hexafluorophosphate as a peptide coupling reagent[14] have been reported.

In connection with our continuous efforts toward the development of new useful coupling reagents,[15] we have previously reported that benzotriazol-1-yl diethyl phosphate is a new convenient coupling reagent for the synthesis of amides and peptides.[16] The present paper describes a full detail of the preparation of benzotriazol-1-yl diethyl phosphate and benzotriazol-1-yl diphenyl phosphate, their use for the synthesis of amides and peptides, and recemization study during peptide sythesis.

Results and Discussion

Preparation of Benzotriazol-1-yl Diethyl Phosphate and Benzotriazol-1-yl Diphenyl Phosphate. Benzotriazol-1-yl diethyl phosphate (BDP) was prepared by mixing equimolar amounts of diethyl chlorophosphate, 1-hydroxybenzotriazole, and triethylamine in tetrahydrofuran and subsequent stirring at room temperature for 30 min. When we attempted to purify the crude product by Kugelrohr distillation, it was converted into an unidentified product. The unknown product was stable to aqueous workup and it did not react with amines. Therefore, the crude product was purified by the filtration through a short column of silica gel or cellulose. Using this procedure, BDP was obtained as an oil in essentially quantitative yields (90-95%) and could be stored in refrigerator for several weeks without little decomposition.



Similarly, benzotriazol-1-yl diphenyl phosphate was prepared by the reaction of diphenyl chlorophosphate with equimolar amounts of 1-hydroxybenzotriazole and triethylamine in tetrahydrofuran at room temperature for 30 min. The reagent was obtained in 85-95% yield after purification. It is noteworthy that the reagent was crystallized from methylene

Table 1. Preparation of Amides with BDP at Room Temperature

$RCOOH + R'NH_2 + BDP + Et_3N \rightarrow RCONHR'$

R	R′	solvent	time, h	isolated yield, %[a]
$C_6H_5CH_2$	C_6H_5	DMF	0.2	96
	$C_6H_5CH_2$	CH_3CN	0.2	94
CH_3	$C_6H_5CH_2$	CH_3CN	0.2	92
	c-C_6H_{11}	CH_3CN	0.3	95
	t-C_4H_9	CH_3CN	0.5	89
$CH_3CH=CH$	C_6H_5	CN_3CN	0.2	93
	$C_6H_5CH_2$	CH_3CN	0.2	93
C_6H_5	c-C_6H_{11}	DMF	0.2	93
		CH_3CN	0.2	93
		CH_2Cl_2	0.8	92
		THF	0.3	91
	$C_6H_5CH_2$	DMF	0.2	95
	C_6H_5	DMF	0.2	96

[a]The isolated yield was determined by Kugelrohr distillation in vacuum.

chloride-hexane below 0°C, but it rapidly melted at room temperature.

Preparation of Amides. First, we have briefly studied solvent effects using benzoic acid and cyclohexylamine. Reaction of benzoic acid with equimolar amounts of cyclohexylamine, BDP, and triethylamine in dimethylformamide proceeded rapidly and cleanly, yielding N-cyclohexylbenzamide in 92% yield within 10 min at room temperature. Acetonitrile and tetrahydrofuran were equally effective, though the use of methylene chloride required 1 h for completion of the reaction. In all cases N-cyclohexylbenzamide was obtained in essentially quantitative yields without contamination of byproducts, indicating that BDP reacted selectively with a carboxylate anion. Furthermore, it is noteworthy that the use of N,N′-dicyclohexylcarbodiimide did not give any trace of N-cyclohexylbenzamide from benzoic acid and cyclohexylamine,[17] demonstrating the effectiveness of BDP as a coupling reagent.

$$RCOOH + R'NH_2 + BDP + Et_3N \rightarrow RCONHR'$$

In order to determine limitations and the scope of BDP, the preparation of amides has been studied with several structurally different amines. Table 1 summarizes some experimental results obtained in this study. In general, the reaction was carried out with an equimolar ratio of BDP, and acid, an amine, and triethylamine in acetonitrile and/or dimethylformamide at room temperature. Benzoic acid was readily converted to the corresponding amides by the reaction of BDP with benzylamine or aniline within 20 min. Similarly, reaction of acetic acid with sterically hindered t-butylamine occurred smoothly to yield t-butyl acetamide in 89% yield within 30 min. Crotonic acid was cleanly converted to the corresponding amides in high yields without 1,4-addition side products.

Recemization Studies. In order to investigate the synthetic usefulness of BDP and benzotrizol-1-yl diphenyl phosphate in the peptide synthesis, we have performed several racemization tests. Young found that the racemization occurred during the condensation of acetyl-L-leucine or benzoyl-L-leucine with glycine ethyl ester hydrochloride when various coupling methods were employed.[18] The Young test have been widely used in measuring the degree of racemization and in proving the effectiveness of the new coupling method. The Young test indicated that the acid azide method gave products of high optical yields without little racemization, whereas racemizations were observed to some extent when dicyclohexylcarbodiimide method, the mixed anhydride method, and the active ester method were employed.

Table 2. Effect of Solvent on the Young Test Using BDP[a]

$$Bz\text{-}L\text{-}Leu\text{-}OH + Gly\text{-}OEt\cdot HCl \xrightarrow[Et_3N]{BDP} Bz\text{-}Leu\text{-}Gly\text{-}OEt$$

solvent	isolated yield, %	$[\alpha]_D$	L-isomer, %
DMF	89	−33.0	97
	90	−32.6	96[b]
CH_3CN	77	−32.1	95
THF	60	−31.0	91
EtOAc	79	−30.4	89
CH_2Cl_2	80	−27.8	82

[a]The reaction was carried out with 2.1 equiv of Et_3N at room temperature for 4 h. [b]With 1.1 equiv of Et_3N for 12 h.

$$Bz\text{-}L\text{-}Leu + Gly\text{-}OEt\cdot HCl \longrightarrow Bz\text{-}L\text{-}Leu\text{-}Gly\text{-}OEt$$

In order to find out an optimum condition which could minimize the degree of racemization during the peptide synthesis, we performed the Young test using BDP and benzotriazol-1-yl diphenyl phosphate under various conditions. First, the solvent effects were investigated and the result is shown in Table 2. The Young test was carried out with 2.1 equiv of triethylamine by the known procedure. The coupling of Bz-L-Leu-OH with Gly-OEt HCl and BDP in dimethylformamide gave Bz-L-Leu-Gly-OEt in 97% optical yield. The crude product was crystallized from methylene chloride and hexane to give white needle crystals (mp, 156.5-157°C; $[\alpha]_D$ −33.0°. lit.[18] mp, 156.5-157°C; $[\alpha]_D$ −34.0°). When the reaction was carried out in acetonitrile, tetrahydrofuran, ethyl acetate, and methylene chloride, Bz-L-Leu-Gly-OEt was obtained in 95%, 91%, 89%, and 82% optical yields, respectively, indicating that dimethylformamide is the most effective in terms of chemical and optical yields Furthermore, the use of the highly polar solvent, dimethylformamide, in which even large peptides might be soluble, widens the scope of the present coupling method.

We have briefly studied the effect of bases in dimethylformamide as a solvent. When the Young test was carried out with 2.1 equiv of N-methylmorpholine, the optical yield was 93%($[\alpha]_D$ −37.1°), whereas the use of triethylamine, imidazole, and pyridine gave 97%($[\alpha]_D$ −33.0°), 87%($[\alpha]_D$ −29.5°), and 40%($[\alpha]_D$ −13.7°) optical yield, respectively. Furthermore, we performed the Young test by the two step sequence. First, Bz-L-Leu-OH was converted to the corresponding 1-benzotriazolyl active ester with BDP and triethylamine in dimethylformamide at room temperature and then 1-benzotriazolyl ester was treated with glycine ethyl ester hydrochloride to afford Bz-L-Leu-Gly-OEt. Surprisingly, $[\alpha]_D$ value of the product was zero, indicating that complete racemization had occurred during coupling and the Young test under the standard condition did not proceed via the intermediacy of 1-benzotriazolyl ester.

Table 3. Synthesis of Dipeptides Using BDP As a Coupling Agent in Dimethylformamide[a]

peptides[b]	isolated yield, %	mp, °C	$[\alpha]_D$ (c, solvent, °C)	Lit mp, °C	Lit $[\alpha]_D$ (c, solvent, °C)
Z-Phe-Gly-OEt	95	109-110	-17.0 (1.7, EtOH, 16)	109	-17.0 (2.0, EtOH, 20)[22]
Z-Phe-Ser-OMe	96	121-122	-5.5 (1.0, DMF, 20)	122-123	-5.7 (1.0 DMF, 23)[23]
Z-Phe-Leu-OMe	96	110-111	-25.5 (2.0, MeOH, 20)	110-111	-24.7 (3.1, MeOH, 20)[21]
Z-Phe-Tyr-OMe	93	155-157	-10.3 (2.1, EtOH, 15)	155-157	-9.4 (1.0, EtOH, 24)[21]
Z-Pro-Leu-OMe	77	76.5-77	-69.4 (1.3, EtOH, 18)	75.5-78	-69.0 (1.0, EtOH, 21)[25]
Z-Val-Tyr-OMe	90	155-156	+14.6 (1.8, pyridine, 16)	157-158	+14.8 (5.0, pyridine, 25)[21]
Z-Val-Gly-OEt	94	166-167	-30.2 (1.5, MeOH, 16)	166-167	-32.0 (1.0, MeOH, 22)[22]
Z-Met-Gly-OEt	90	94-95	-19.2 (1.2, EtOH, 17)	96-97	-18.9 (4.8, EtOH, 27)[21]
Z-Met-Val-OMe	92	103-104	-26.3 (0.8, MeOH, 17)	103	-26.0 (1.0, MeOH, 20)[21]
Z-Ala-Gly-OEt	93	97-98	-21.3 (2.7, EtOH, 18)	97.8	-24.0 (1.0, EtOH, 20)[21]
Boc-Ile-Val-OMe	76	167-168	-15.4 (1.2, EtOAc, 15)	177-167	-15.0 (1.0, EtOAc, 20)[23]
Boc-Ile-Gly-OEt	89	104	-28.5 (1.4, EtOH, 15)	104	-28.2 (1.0, EtOH, 20)[21]
Boc-Tyr-Gly-OEt	90	117-118	-12.7 (1.0, EtOH, 18)	117	-12.9 (2.5, EtOH, 20)[21]
Boc-Val-Val-OMe	96	166-167	-9.3 (2.0, EtOAc, 18)	165-166	-9.0 (1.0, EtOAc, 20)[26]
Boc-Phe-Gly-OEt	93	91-92	-5.1 (1.2, EtOH, 16)	88-89.5	-4.2 (5.0, EtOH, 25)[27]
Boc-Phe-Met-OMe	94	90-91	-21.9 (1.0, MeOH, 15)	80	-22.3 (1.0, MeOH, 20)[28]
Boc-Leu-Leu-OMe	96	140-141	-49.5 (1.8, MeOH, 20)	140-141	-50.4 (1.0, MeOH, 25)[29]
Z-Val-*Phe-Gly-OEt	94	190	-20.9 (1.7, AcOH, 18)	191.5-192	-21.0 (2.1, AcOH, 24)[24]
Boc-Gly-Phe-*Met-OMe	92	oil	-24.0 (1.5, MeOH, 16)	68	-24.35 (1.5, MeOH, 20)[28]
Z-Phe-*Val-Pro-OBu^t	92	115-116	-76.6 (0.8, MeOH, 16)	115-116	-76.1 (1.0, MeOH, 20)[20]

[a]The reaction was carried out with 1.1 equiv of BDP and 2.1 equiv of triethylamine for 4 h. [b]The CO-NH bond formed in the peptide synthesis was indicated by asterisk.

We have briefly studied the Young test using benzotriazol-1-yl diphenyl phosphate. The Young test was carried out with 2.1 equiv of triethylamine in dimethylformamide under the standard condition and 61% of L-isomer ($[\alpha]_D$ -20.8°) was obtained. When the reaction was carried out at room temperature, 38% of L-isomer was obtained. The present study indicates that the degree of racemization depends critically on the nature of organophosphate coupling reagents and reaction conditions such as solvents, bases, and temperature.

Anderson test involves the coupling of Z-Gly-Phe-OH and Gly-OEt HCl and has been commonly used in the racemization test.[19] Coupling of Z-Gly-Phe-OH with Gly-OEt HCl using BDP in dimethylformamide gave Z-Gly-Phe-Gly-OEt in 97% yield. The mp and $[\alpha]_D$ value were almost identical with those of reported data,[19] indicating that the reaction proceeded without little racemization.

We have further examined a recently developed racemization test by the use of high performance liquid chromatography (HPLC). Shioiri extensively studied the extent of racemization during coupling of Z-Phe-Val-OH with Pro-OBu^t with various coupling methods.[20] Therefore, Z-Phe-Val-OH was prepared by coupling of Z-Phe-OH and Val-O-Bu^t HCl with BDP in dimethylformamide and

$$\text{Z-Phe-Val-OH} + \text{Pro-OBu}^t \xrightarrow[\text{BDP}]{\text{DMF}} \text{Z-Phe-L/D-Val-Pro-OBu}^t \xrightarrow{\text{TFA}} \text{Z-Phe-L/D-Val-Pro-OH}$$

following hydrolysis of t-butyl group with trifluoroacetic acid. Treatment of Z-Phe-Val-OH with Pro-OBu^t and BDP followed by hydrolysis of t-butyl ester with trifluoroacetic acid gave Z-Phe-L/D-Val-Pro-OH. A standard sample of Z-Phe-L-Val-Pro-OBu^t was similarly prepared by coupling of Z-Phe-OH and L-Val-Pro-OBu^t with BDP in dimethylformamide. HPLC analysis of the product from coupling of Z-Phe-Val-OH with Pro-OBu^t indicates the presence of L-and D-isomer in a ratio of 82:18, showing that racemization occurred to some extent during coupling reaction with BDP, though the standard sample was obtained as a pure L-isomer.

Peptide Synthesis. In order to demonstrate the coupling efficiency of BDP in the peptide synthesis, we have prepared several dipeptides and tripeptides. The coupling reaction was normally carried out with equimolar amounts of N-protected amino acids, amino acid, esters hydrochloride, and BDP in the presence of 2.1 equiv of triethylamine in dimethylformamide at room temperature and the reaction was generally complete within 4 h. The identities of the products obtain in this study were determined by the comparison of mp, $[\alpha]_D$ value, and nmr data with those of reported data. Table 3 summarizes some experimental results and illustrates the efficiency and applicability of the present method. As shown in Table 3, various peptides were prepared in high yields without little racemization. Furthermore, it is noteworthy that the present method can be successfully applied to the coupling of two fragments having hydroxyl or amino group without its protection. For instance, coupling of Z-Phe-OH with Ser-OMe HCl and Try-OEt HCl occurred cleanly, yielding Z-Phe-Ser-OMe and Z-Phe-Try-OEt in 96% and 93% yield, respectively. When a valine derivative is used as a carboxyl component, the present method consistently gives the high yield of coupling product, though it has been reported that other coupling methods give relatively the low yield of coupling product because of the difficulty of activating a valine derivative due to its steric hindrance.[21]

We have briefly studied the effectiveness of benzotriazol-

Table 4. Synthesis of Dipeptides Using Benzotriazol-1-yl Diphenyl Phosphate in Dimethylformamide at Room Temperature[a]

dipeptide	islated yield, %	mp, °C	$[\alpha]_D$ (c, solvent, °C)[b]
Z-Phe-Gly-OEt	97	109-110	-16.9 (1.3, EtOH, 17)
Z-PHe-Leu-OMe	91	110-111	-24.8 (1.8, MeOH, 20)
Z-Val-Tyr-OMe	95	155-157	+14.7 (1.5, pyridien, 18)
Boc-Ile-Val-OMe	85	167-168	-15.5 (1.8, EtOAc, 20)
Boc-Ile-Gly-OEt	95	104	-28.4 (1.2, EtOH, 16)
Boc-Leu-Leu-OMe	86	140-141	-48.5 (1.8, MeOH, 20)

[a]The reaction was generally carried out with 1.1 equiv of the reagent and 2.1 equiv of triethylamine at room temperature for 4 h. [b]mp and $[\alpha]_D$ values are in accord with reported values and literatures are shown in Table 3.

1-yl diphenyl phosphate in the peptide synthesis, though racemization during Young test with this reagent has been observed to some extent. We adopted the similar conditions employed in the peptide synthesis using BDP and the results are summarized in Table 4. The reaction was complete within 4 h at room temperature and several dipeptides were prepared in high yields without appreciable racemization as determined by their melting points and optical rotations.

Experimental

^{1}HNMR spectra were recorded with Varian T-60A or FT-80A spectrometer and chemical shifts are expressed as units relative to tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 267 spectrophotometer and frequences are given in reciprocal centimeter and optical rotations were recorded on Autopol III automatic polarimeter. High performance liquid chromatography was obtained on an Waters Associates Model 244 equipped with Model 6000 A solvent delivery system and Model 440 absorbance detector. Melting points were determined on a electrothermal melting point apparatus and reported boiling points are those observed during distillation with Kugelrohr apparatus. Elemental analysis were performed by Korea Institute of Chemical Technology. Analytical thin layer chromatography was performed on precoated silica gel plate (0.25 mm, 60F-254, E. Merck) and silica gel (0.063-0.020 mm, E. Merck) was used for column chromatography.

All the reagents purchased from Aldrich and Sigma Chemical companies were used without further purification. Dimethylformamide and acetonitrile were distilled over calcium hydride. Tetrahydrofuran was refluxed over sodium and benzophenone for 12 h under nitrogen and distilled prior to use.

Preparation of Benzotriazol-1-yl Diethyl Phosphate (BDP). To a solution of 1-hydroxybenzotriazole (1.35 g, 10.0 mmol) and triethylamine (1.01 g, 10.0 mmol) in tetrahydrofuran (40 m*l*) at 0°C was slowly added diethyl chlorophosphate (1.73 g, 1.0 mmol) with stirring. After 30 min, the formed salt was filtered off by using sintered filter, and the solution was evaporated to dryness. The crude product was purified by column chromatography (Cellulose, Avicel, E. Merck) gave BDP (2.58 g) in 95% yield. ^{1}HNMR ($CDCl_3$)δ 1.56 (tt, J = 8.0, 1.3 Hz, 6H), 4.10-4.65 (m, 4H), 7.28-7.75 (m, 4H). IR(film) 3000, 1450, 1400, 1380, 1280 cm^{-1}. Anal. Calcd for $C_{10}H_{14}N_3O_4P$; C, 43.51; H, 5.20; N, 14.64. Found: C, 43.29; H, 5.20; N, 14.49.

Preparation of Benzotriazol-1-yl Diphenyl Phosphate. To a solution of 1-hydroxybenzotriazole (1.35 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) in tetrahydrofuran (40 m*l*) at room temperature was slowly added diphenyl chlorophosphate (2.69 g, 10 mmol). The reaction mixture was stirred for 30 min and the formed salt was filtered off and the resulting solution was evaporated to dryness and purified by column chromatography (Cellulose, Avicel, E. Merck) to yield benzotriazol-1-yl diphenyl phosphate (3.42 g) in 93% yield. IR(film) 3100, 1600, 1500, 1470, 1460, 1380, 1345 cm^{-1}. Anal. Calcd for $C_{18}H_{14}N_3O_4P$: C, 58.80; H, 3.84; N, 11.45. Found: C, 58.53; H, 4.35; N, 11.05.

General Procedure for the Preparation of Amides. To a solution of a carboxylic acid (2.0 mmol), an amine (2.0 mmol), and triethylamine (2.0 mmol) at room temperature was added BDP (2.1 mmol) in acetonitrile (10 m*l*) or dimethylformamide (10 m*l*) and the resulting solution was stirred at room temperature until completion of the reaction. The reaction mixture was diluted with diethyl ether (50 m*l*) and washed with saturated sodium carbonate solution, water, and brine. The ether extracts were evaporated to dryness and the crude product was purified by distillation with a Kugelrohr. The products obtained in this study were previously known compounds and their spectral and physical data were identical with those of reported data.

Typical Procedure for the Young Test. To a stirred solution of benzoyl-L-leucine (235 mg, 1.0 mmol) and glycine ethyl ester hydrochloride (154 mg, 1.1 mmol) in dimethylformamide (2.5 m*l*) was added BDP (300 mg, 1.1 mmol) in dimethylformamide (2.5 m*l*) at 0°C, followed by the addition of triethylamine (213 mg, 2.1 mmol). The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate (30 m*l*) and washed with 5% aqueous HCl solution, water, 10% sodium bicarbonate solution, water, and brine. Drying over anhydrous magnesium sulfate and the following evaporation gave colorless crystals. The crude product was purified by passing through a short column of silica gel using ethyl acetate-hexane (1:6) as an eluant to yield colorless crystals of benzoyl-L-leucine ethyl ester (285 mg) in 89% yield. mp; 156.5-157°C, Lit.[18] 156.5-157°C. $[\alpha]_D$ -33.0 (2.0, EtOH, 18). Lit.[18] $[\alpha]_D$ -34.0 (2.0, EtOH, 18). IR(KBr) 3320, 1770, 1670, 1645 cm^{-1}.

Preparation of Z-Gly-Phe-Gly-OEt for Anderson Test. To a stirred mixture of Z-Gly-Phe-OH (357 mg, 1.0 mmol) and glycine ethyl ester hydrochloride (1400 mg, 1.0 mmol) in dimethylformamide (5 m*l*) at 0°C was added a solution of BDP (302 mg, 1.1 mmol) and triethylamine (215 mg, 2.1 mmol) in dimethylformamide (2 m*l*). The reaction mixture was stirred at 0°C for 1 h and then at room temperature for 6 h, diluted with ethyl acetate (50 m*l*) and washed with 5% aqueous HCl solution, 10% sodium carbonate solution, water and brine. The organic phase was dried and evaporated to dryness and the crude product was purified by passing through a short column of silica gel to yield Z-Gly-Phe-Gly-OEt (395 mg) in 90% yield. mp, 117-118°C, $[\alpha]_D$ -12.3 (2.0, EtOH, 16). Lit.[19] mp, 117-118°C, $[\alpha]_D$ -12.0 (2.0, EtOH, 25).

Preparation of Z-Phe-Val-Pro-OBu^t for Racemization Test with HPLC. To a solution of Z-Phe-Val-OH (260 mg,

0.8 mmol), which was prepared by the known procedure, and commercially available Pro-OBu^t (150 mg, 0.9 mmol) in dimethylformamide (5 m*l*) at 0°C was slowly added a solution of BDP (244 mg, 0.9 mmol) in dimethylformamide and then triethylamine (100 mg, 10 mmol) was slowly added to the solution. After being stirred at room temperature for 5 h, the reaction mixture was diluted with ethyl acetate (50 m*l*) and washed with 5% aqueous HCl solution, 10% sodium carbonate solution, water, and brine, dried, evaporated to dryness. The crude product was purified by passing through a short column of silica gel to give Z-Phe-Val-Pro-OBu^t (340 mg) in 89% yield. mp, 114°C, $[\alpha]_D$ -80.4 (1.3, MeOH, 16). Lit.[20] mp, 115-116°C, $[\alpha]_D$ -76.11 (1.0, MeOH, 20). Z-Phe-Val-Pro-OBu^t (300 mg, 0.63 mmol) was dissolved in trifluoroacetic acid (1 m*l*) and stirred at room temperature for 2 h. Trifluoroacetic acid was removed in vacuo to give a residue, which was dissolved in benzene and evaporated to dryness. Without further purification of the crude product, it was subjected to HPLC analysis under the following conditions. Column; P/N 84040 -Bondapak NH_2; temperature, room temperature; eluant, 0.02M ammonium formate in methanol-water (95:5); flow rate, 0.6 m*l* per min; range, 0.05 absorbance unit; detector, 254 nm; retention time, L-D-L form, 19.10 min, L-L-L form, 22.30 min; the ratio of L-D-L/L-L-L, 18/82.

Typical procedure for Peptide Synthesis. To a solution of Z-Phe-OH (300 mg, 1.0 mmol) and HCl Gly-OEt (154 mg, 1.1 mmol) in dimethylformamide (4 m*l*) at room temperature was added a solution of BDP (300 mg, 1.1 mmol) in dimethylformamide (3 m*l*) and then triethylamine (210 mg, 2.1 mmol) was slowly added to the solution. The reaction mixture was stirred at room temperature for 4 h, diluted with ethyl acetate (50 m*l*), washed with 5% aqueous HCl solution, 10% sodium carbonate solution, water and brine, dried over anhydrous $MgSO_4$, and evaporated to dryness. The crude product was purified by passing through a short column of silica gel to yield Z-Phe-Gly-OEt (365 mg) in 95% yield. mp, 109-110°C, $[\alpha]_D$ -17.0 (1.7, EtOH, 16). Lit.[22] mp, 109°C, $[\alpha]_D$ -17.0 (2.0, EtOH).

Acknowledgement. This research was financially supported by a grant from Korea Science and Engineering Foundation.

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