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Amino Acids and Peptides. XXX.[1-3] Phosphorus in Organic Synthesis. XVII.[4] Application of Diphenyl Phosphorazidate (DPPA) and Diethyl Phosphorocyanidate (DEPC) to Solid-phase Peptide Synthesis

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Two coupling reagents for peptide synthesis, diphenyl phosphorazidate (DPPA) and diethyl phosphorocyanidate (DEPC), were tested using the solid-phase method. The reactivities of DPPA and DEPC were examined by coupling Boc-Ile with Gly-resin. In a comparison of these reagents with DCCD, these reagents showed higher reactivity than DCCD in DMF. The N-Boc derivative of melanocyte release inhibiting hormone was synthesized in good yield by the solid-phase method using these reagents. Racemization during fragment condensation on the polymer support was examined by coupling Boc–Gly–L-Ala with Leu-resin (Izumiya test).

Keywords——amino acid; peptide; melanocyte release inhibiting hormone; racemization; fragment condensation; Izumiya test

Although many reagents are available for peptide synthesis, N,N′-dicyclohexylcarbodiimide (DCCD) is the sole reagent widely used for the solid-phase method.[6] We have already reported that diphenyl phosphorazidate (DPPA, $(PhO)_2P(O)N_3$)[7] and diethyl phosphorocyanidate (DEPC, $(EtO)_2P(O)CN$)[8] are very efficient coupling reagents for racemization-free conventional peptide synthesis, and they have been satisfactorily applied[9] to the syntheses of some fragments of gastrointestinal peptide hormones using the conventional solution method. This paper is concerned with their application to solid-phase peptide synthesis in both stepwise and fragment condensation approaches.

First, the reactivities of DPPA and DEPC on a solid support were examined using a suitable peptide model. Reaction of Boc-Ile with Boc-Gly resin was chosen as a model for this study, because the reaction rate of isoleucine was considered to be lower than those of other

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2) Unless otherwise stated, all optically active amino acids are of L-configuration. Symbols and abbreviations are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, **40**, 315 (1974). Other abbreviations used are: DCCD, N,N′-dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; CH_2Cl_2, methylene chloride; TEA, triethylamine; DMF, dimethylformamide; EtOH, ethanol; $CHCl_3$, chloroform.
3) Part of this work was the subject of a preliminary report: S. Yamada, N. Ikota, T. Shioiri, and S. Tachibana, *J. Am. Chem. Soc.*, **97**, 7174 (1975).
4) Part XVI: Y. Yokoyama, T. Shioiri, and S. Yamada, *Chem. Pharm. Bull.*, **25**, 2423 (1977).
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7) *a*) T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972); T. Shioiri and S. Yamada, *Chem. Pharm. Bull.*, **22**, 849, 855, and 859 (1974); *b*) Review: T. Shioiri and S. Yamada, *Yuki Gosei Kagaku Kyokai Shi*, **31**, 666 (1973).
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Table I. General Procedure for DPPA and DEPC Coupling in Solid-phase Peptide Synthesis

Step	Reagents and operations[a]	Mix times (min)
1	CH_2Cl_2 wash (3 times)	2
2	33% or 50% TFA in CH_2Cl_2 (1 time)	30
3	CH_2Cl_2 wash (3 times)	2
4	EtOH wash (3 times)	2
5	DMF wash (3 times)	2
6	TEA (10 eq) in DMF (1 time)	10
7	DMF wash (6 times)	2
8	Boc-amino acid in DMF	5
9	DPPA or DEPC in DMF	2
10	TEA in DMF	x
11	DMF wash (3 times)	2
12	EtOH wash (3 times)	2

a) 5—7 ml of solvent to 1 g of resin was used for washing.

amino acids due to steric hindrance. Comparison of these reagents with DCCD was also carried out. The general procedure[10] for the solid-phase method is shown in Table I; it is slightly modified from that described by Stewart and Young.[11] Incorporation of Boc-Gly into the polymer support was performed by reaction of the cesium salt of Boc-Gly with chloromethylated copolystyrene–2% divinylbenzene (chloromethyl resin) in DMF.[12] The Boc group was removed by treatment with trifluoroacetic acid (TFA) in CH_2Cl_2, and neutralization of the TFA salt was carried out with triethylamine (TEA) in DMF. When DPPA and DEPC were used as coupling reagents, they were added after addition of Boc-Ile, followed by the addition of TEA. In this study, 1.5 equivalents of Boc-Ile·$1/2H_2O$, coupling reagents, and TEA (in the case of DCCD, no TEA was added) were used in DMF or CH_2Cl_2 as solvents. The extent of the coupling reaction was determined according to the Porath method,[13] in which unreacted amino groups in the polymer are converted to the stable Schiff base with 2-hydroxy-1-naphthalenecarbaldehyde, the chromophore is then displaced from the polymer with benzylamine and the amount of the soluble aldimine newly formed is determined by measuring the absorption at 420 nm.

Table II. Comparison of the Reactivities of DPPA, DEPC, and DCCD for Coupling Boc–Ile·$1/2H_2O$ with Gly-resin

Reagent	Rate of coupling[a] (%)			
	5 min	30 min	60 min	24 hr
DCCD	12(95)	20(98.5)	32(99)	71(99)
DPPA	40(3)	72(12)	80(21)	84(75)
DEPC	98(55)	98(70.5)	99(82)	99(82)

a) Rates of coupling in dimethylformamide. Numbers in parentheses represent rates of coupling in methylene chloride.

10) Solid-phase peptide synthesis was carried out using a reaction apparatus with a thermostated jacket, designed by the Tanabe research group: T. Mizoguchi, K. Shigezane, and N. Takamura, *Chem. Pharm. Bull.*, **18**, 1465 (1970).
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The results are summarized in Table II. When CH_2Cl_2 was used as a solvent, the reactivity of DCCD was higher than that of DPPA or DEPC. However, in DMF, DPPA and DEPC were much more reactive than DCCD.

An application of these reagents to solid-phase peptide synthesis was then attempted in the synthesis of Boc–Pro–Leu–Gly–NH_2, which is the N-Boc derivative of the melanocyte release inhibiting hormone.[14] The synthesis was carried out in a stepwise manner from Boc–Gly-resin, which was prepared from Boc–Gly·TEA salt and chloromethyl resin in EtOH–$CHCl_3$. Removal of the Boc group and neutralization of the TFA salt were performed according to the general procedure. A three-fold excess of each Boc-amino acid, DPPA or DEPC, and TEA in DMF were used in the coupling step. Every coupling reaction, which was checked by the Porath method, was almost complete (about 98%) at room temperature for 2 hr. The completed peptide resin was then treated with ammonia in methanol to afford Boc–Pro–Leu–Gly–NH_2 in 70% (DPPA) or 76% (DEPC) yield after recrystallization from EtOH–water. This sample of Boc–Pro–Leu–Gly–$NH_2 \cdot 1/2H_2O$ (mp 136—137°, $[\alpha]_D^{25}$ −72° (c=1.64, MeOH)) was identical with an authentic sample, which was similarly prepared in good yield from Gly–OMe·HCl by the conventional method using DEPC as a coupling reagent (Chart 1).

In the solid phase method, any by-product or an unreacted amino component attached to the resin cannot be removed by simple washing procedures, and in the case of stepwise elongation, many peptides of similar molecular size and properties might be formed. This can be a serious problem in the purification of the final products. However, this drawback of the solid-phase method might be overcome by fragment condensation on the polymer support,[15] because the properties and the molecular size of the desired product and the incomplete reaction product are quite different, so separation of the product might not be difficult.

Since racemization is another problem during fragment condensation, racemization on the polymer support was examined. The coupling of Boc–Gly–L–Ala with H–L–Leu-resin was studied; this is one of the racemization tests reported by Izumiya and co-workers.[16] The

Table III. Racemization Test by Coupling Boc–Gly–L-Ala with H-L-Leu-resin

Run	Reagent[a]	Reaction temperature	Racemization[b] (%)
1	DPPA	20°	2.5
2	DPPA	0°	2
3	DEPC	20°	1
4	DEPC	0°	<0.5

a) 3 Eq of reagent was used in the presence of TEA (3×0.95 eq) in DMF.
b) Racemization=[D.L/(D.L+L.L)]×100.

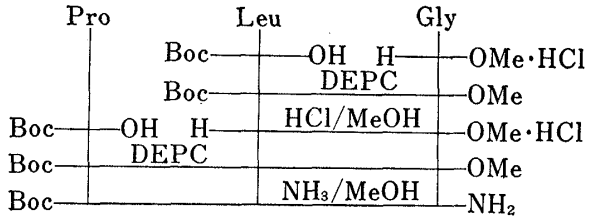

Chart 1. Synthetic Scheme for Boc–Pro–Leu–Gly–NH_2

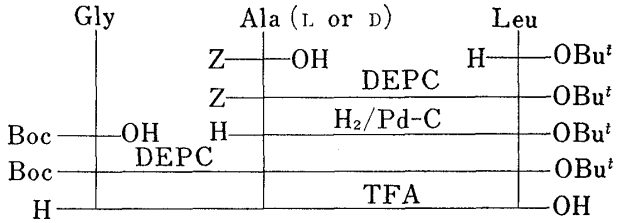

Chart 2. Synthetic Scheme for Boc–Gly–Ala–Leu–OBu^t

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two diastereomeric tripeptides, Gly–L-Ala–L-Leu and Gly–D-Ala–L-Leu were separated by ion-exchange chromatography using an amino acid analyzer. Three equivalents of Boc–Gly–L–Ala were coupled with H-L-Leu-resin using DPPA or DEPC (3 eq) in the presence of TEA (3× 0.95 eq) in DMF. The reaction was almost complete (about 98%, as checked by the Porath method) at room temperature or at 0° for 2 hr. After the coupling, the resin was treated with hydrogen bromide in TFA to afford Gly–Ala–Leu, which was analyzed in an amino acid analyzer, and the extent of racemization was calculated. The diastereomers of Gly–Ala–Leu were identical in retention volume with authentic samples, prepared from Leu–OBu^t in a stepwise manner using DEPC by the conventional method (Chart 2).

As shown in Table III, the extent of racemization seems to be small compared with other reagents.[17] In particular, almost no racemization was detected with DEPC at 0° in DMF (Table III, run 4).

Thus, DPPA and DEPC should be useful coupling reagents for solid-phase peptide synthesis both in stepwise and fragment condensation.

Experimental[18]

The Reactivities of DPPA, DEPC, and DCCD in the Reaction of Boc-Ile with Gly-resin. Attachment of Boc-Gly to Resin——Boc-Gly (0.875 g, 5 mM) was dissolved in 8 ml of EtOH and diluted with 2 ml of water. The pH of this solution was adjusted to 7.0 (pH-meter) by adding aqueous cesium carbonate. The neutral solution was then evaporated. After repeated evaporation to dryness with benzene, the Boc-Gly cesium salt was obtained as a solid. It was dried over P_2O_5 *in vacuo* for 5 hr and used without further purification. The Boc-Gly cesium salt was dissolved in DMF (50 ml). Chloromethyl resin[19] (9.0 g, Cl: 1.7 mM/g) was added to this solution and the mixture was stirred slowly at 35° for 20 hr. The resin was filtered, washed with DMF, DMF–H_2O (9: 1) and EtOH, and then dried. Yield, 9.5 g (Boc–Gly: 0.37 mM/g, determined by the Porath method described below).

Determination of Boc-Gly Residue on Resin——A portion (20—50 mg) of the foregoing Boc–Gly-resin was placed in a glass filter funnel with cock and deprotected by treatment with TFA–CH_2Cl_2, then neutralized with TEA in DMF according to the general procedure. After washing with DMF (Table I, step 7), the resin was washed with CH_2Cl_2 (×3), then allowed to react with 2-hydroxy-1-naphthalenecarbaldehyde (large excess) in CH_2Cl_2–EtOH (1: 1) at room temperature overnight. After washing the resin with CH_2Cl_2–EtOH (1: 1) and CH_2Cl_2, a solution of benzylamine (150 mg) in CH_2Cl_2 was added and the mixture was allowed to react at room temperature for 1 hr. The resin was then filtered and washed with CH_2Cl_2. The filtrate and washings were concentrated *in vacuo* to give a yellow oil, which was diluted with 95% EtOH. The absorption at 420 nm was measured spectrophotometrically and the amount of N-(2-hydroxy-1-naphthylmethylene)-benzylamine calculated [authentic N-(2-hydroxy-1-naphthylmethylene)benzylamine was prepared according to the reported procedure,[13b] mp 93—94°, $\varepsilon_{420\,nm}=10800$ (95% EtOH), lit.[13b] mp 93—94°, $\varepsilon_{420\,nm}=10040$ (95% EtOH)].

Coupling of Boc-Ile·1/2H_2O with Gly-resin——Boc–Gly-resin (1.0 g, Boc–Gly: 0.37 mM/g) was deprotected by treatment with TFA–CH_2Cl_2 (1: 1, 6 ml, at room temperature for 30 min) and converted to the glycyl resin as usual. A solution of Boc–Ile·1/2H_2O (133 mg, 0.56 mM) in DMF or CH_2Cl_2 (6 ml) was added. After stirring for 5 min, a solution of DPPA (153 mg, 0.56 mM) or DEPC (91 mg, 0.56 mM), or DCCD (114 mg, 0.56 mM) in DMF or CH_2Cl_2 (1 ml; in the case of DCCD, 2 ml of solvent was used) was added, followed by the addition of TEA (56 mg, 0.56 mM) in DMF or CH_2Cl_2 (1 ml; in the case of DCCD, no TEA was added) during a period of 2 min, and after specified times (5 min, 30 min, 60 min, 24 hr), aliquots of about 50 mg of polymer were removed and washed thoroughly with DMF, EtOH and CH_2Cl_2, and dried *in vacuo*. The content of residual amino groups was then determined by the Porath method as described above. The results are summarized in Table II.

17) *a*) M.A. Barton, R.U. Lemieux, and J.Y. Savoie, *J. Am. Chem. Soc.*, **95**, 4501 (1973); *b*) M. Gondo, M. Waki, T. Kato, and N. Izumiya, Proc. 8th. Symp. Peptide Chem., Protein Research Found., 1970, p. 1.

18) All melting points are uncorrected. IR spectral measurements were performed with a JASCO DS-402G infrared spectrometer and a JASCO IRA-1 grating infrared spectrometer. NMR spectra were measured with JNM-PS 100 and Hitachi R-24 high resolution NMR spectrometers. All signals are given as ppm downfield from tetramethylsilane used as an internal standard. Optical rotations were measured with a Yanaco OR-50 automatic polarimeter. UV spectra were measured with Hitachi 124 and EPS-3T spectrophotometers.

19) Chloromethyl resin (100—200 mesh) was purchased from the Protein Research Foundation.

Boc-Pro-Leu-Gly-NH_2——a) Boc–Gly–resin (Boc–Gly: 0.3 mM/g) was prepared from Boc–Gly (4.0 g, 22.9 mM), TEA (2.3 g, 22.9 mM) and chloromethyl resin[19] (8.0 g, Cl: 1.14 mM/g) in $CHCl_3$–EtOH (1: 2, 33 ml) at room temperature for 1 hr, and then under reflux for 48 hr.

Boc–Gly-resin (2.0 g, Boc–Gly: 0.6 mM) was deprotected by treatment with TFA–CH_2Cl_2 (1: 2, 10 ml, 30 min), then converted to the glycyl resin with TEA (0.6 g) in DMF (10 ml) (room temperature, 10 min). Next, Boc–Leu·H_2O (0.45 g, 1.8 mM) and Boc–Pro (0.385 g, 1.8 mM) were successively coupled using DPPA (0.495 g, 1.8 mM) in the presence of TEA (0.18 g, 1.8 mM) in DMF (10 ml) at room temperature for 2 hr. The resin was suspended in 50 ml of MeOH, and gaseous NH_3 was bubbled for 100 min with ice-cooling, then the mixture was stirred at room temperature for 21 hr. The resin was filtered off and washed with MeOH. The filtrate and washings were concentrated *in vacuo* to give a slightly yellow oil (205 mg), which was crystallized from a small volume of water to afford a white solid. Recrystallization from water and EtOH gave colorless needles (0.165 g, 70%), mp 136—138° (lit.[10] mp 137—138°), $[\alpha]_D^{25}$ −72° (c=1.64, MeOH) (lit.[10] $[\alpha]_D^{25}$ −72.3° (c=1.8, MeOH)). *Anal.* Calcd for $C_{18}H_{32}N_4O_5 \cdot 1/2H_2O$: C, 54.87; H, 8.44; N, 14.22. Found: C, 54.86; H, 8.59; N, 14.11.

b) Boc–Pro–Leu–Gly–NH_2 was also synthesized by the solid-phase method from Boc–Gly-resin (2.0 g, Boc–Gly: 0.6 mM) using DEPC (0.295 g, 1.8 mM) and TEA (0.18 g, 1.8 mM) by the procedure described above (method a), yield 0.18 g (76%), mp 136—138°, $[\alpha]_D^{25}$ −71° (c=1.2, MeOH).

c) Synthesis by the Solution Method Using DEPC: Boc–Leu–Gly–OMe: To a stirred mixture of Gly–OMe·HCl (0.3 g, 2.4 mM), Boc–Leu·H_2O (0.495 g, 2 mM) in DMF (10 ml) was added DEPC (0.34 g, 2.1 mM), followed by the addition of TEA (0.445 g, 4.4 mM) at 0°. The mixture was stirred at 0° for 4 hr and then at room temperature overnight. It was then diluted with AcOEt–benzene (3: 1, 200 ml), washed successively with 10% aq. citric acid (×2), H_2O (×1), sat. aq. NaCl (×1), sat. aq. $NaHCO_3$ (×2), H_2O (×2), and sat. aq. NaCl (×2), and then dried over anhydrous Na_2SO_4 (this washing and drying procedure being described as "treated as usual"). Removal of the solvent gave a solid, which was recrystallized from AcOEt–hexane to afford pure Boc–Leu–Gly–OMe as crystals, (0.52 g, 86%), mp 136—137° (lit.[20] mp 131°), $[\alpha]_D^{20}$ −20.9° (c=2, DMF) (lit.[20] $[\alpha]_D^{25}$ = −20.7° (c=2, DMF)). *Anal.* Calcd for $C_{14}H_{26}N_2O_5$: C, 55.61; H, 8.67; N, 9.27. Found: C, 55.39; H, 8.82; N, 9.13.

Boc–Pro–Leu–Gly–OMe: Boc–Leu–Gly–OMe (0.43 g, 1.42 mM) was treated with 2.5 N MeOH–HCl (6 ml) at room temperature for 1 hr. The solvent was removed *in vacuo*. After repeated evaporation with MeOH, Leu–Gly–OMe·HCl obtained was dried over KOH pellets *in vacuo* and dissolved in DMF (6 ml). Boc–Pro (0.26 g, 1.28 mM) and DEPC (0.21 g, 1.28 mM) were added to the above solution with ice-cooling, followed by the addition of TEA (0.265 g, 2.63 mM). The mixture was stirred at 0° for 4 hr, then at room temperature overnight. It was then diluted with AcOEt–benzene (3: 1, 200 ml), and treated as usual. After removal of the solvent, the residual solid was recrystallized from AcOEt–pet. ether to give pure Boc–Pro–Leu–Gly–OMe as crystals (0.415 g, 86%), mp 107—110° (lit.[20] mp 97—98°), $[\alpha]_D^{20}$ −58° (c=2.1, DMF) (lit.[20] $[\alpha]_D^{25}$ −49.2° (c=2, DMF)). *Anal.* Calcd for $C_{19}H_{33}N_3O_6$: C, 57.12; H, 8.33; N, 10.52. Found: C, 56.81; H, 8.32; N, 10.17.

Boc–Pro–Leu–Gly–NH_2: Boc–Pro–Leu–Gly–OMe (0.12 g, 0.3 mM) was dissolved in MeOH (30 ml) and gaseous NH_3 was bubbled through the solution for 100 min with cooling, then the mixture was stirred at room temperature overnight. After removal of MeOH by evaporation, the residual oil was crystallized by the addition of a small volume of water. Recrystallization from H_2O–EtOH gave pure Boc–Pro–Leu–Gly–NH_2 as colorless needles (0.11 g, 94%), mp 136—138°, $[\alpha]_D^{25}$ −71.8° (c=1.64, MeOH).

Racemization Test during Fragment Condensation (Izumiya Test). Boc-Leu-resin——Boc–Leu–resin (Boc–Leu: 0.42 mM/g) was prepared from the cesium salt of Boc–Leu (0.45 g, 1.81 mM) and chloromethyl resin[19] (3.5 g, Cl: 1.7 mM/g) in DMF (24 ml) at 50° for 24 hr.

Boc–Gly–Ala–OBzl: To a stirred mixture of Boc–Gly (1.23 g, 7 mM) and Ala–OBzl·*p*-TsOH [2.7 g (7.7 mM), prepared according to the reported procedure,[21] mp 112°, $[\alpha]_D^{20}$ −6.7° (c=2, H_2O), lit.[21] mp 114°, $[\alpha]_D^{20}$ −6.8° (c=2, H_2O)] in DMF (15 ml) was added DPPA (1.93 g, 7 mM), followed by the addition of TEA (1.49 g, 14.7 mM) at 0°. The mixture was then stirred at 0° for 4 hr, and at room temperature overnight. It was diluted with AcOEt–benzene (4: 1, 500 ml) and treated as usual. After removal of the solvent, the residual oil was purified by column chromatography (silica gel, ether–hexane=3: 1) to give pure Boc–Gly–Ala–OBzl as an oil (2.0 g, 85%), $[\alpha]_D^{20}$ −29° (c=1.3, MeOH). NMR (in $CDCl_3$), 1.35 (3H, d, CH_3, J=8 Hz), 1.45 (9H, s, $(CH_3)_3$), 3.9 (2H, d, CH_2), 4.35 (1H, CH), 5.1 (2H, s, $C\underline{H}_2C_6H_5$), 5.75 (1H, NH), 6.85 (1H, CONH), 7.3 (5H, s, C_6H_5). IR ν_{max}^{film} cm^{-1}: 1720, 1670.

Boc–Gly–Ala–OH: A stirred solution of Boc–Gly–Ala–OBzl (1.9 g, 5.65 mM) in MeOH (50 ml) was hydrogenated at room temperature for 5 hr using 5% palladium on charcoal (0.5 g). The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give an oil, which was crystallized from $CHCl_3$–AcOEt–hexane to afford pure Boc–Gly–Ala–OH as crystals (1.1 g, 79%), mp 127—128° (lit.[17a] mp 124—129°), $[\alpha]_D^{20}$ −14° (c=1, DMF). *Anal.* Calcd for $C_{10}H_{18}N_2O_5$: C, 48.77; H, 7.37; N, 11.36. Found:

20) A.S. Dutta and J.S. Morley, *J. Chem. Soc.* (*C*)., **1971**, 2896.
21) N. Izumiya and S. Makisumi, *Nippon Kagaku Zasshi*, **78**, 662 (1957).

C, 48.49; H, 7.48; N, 11.26.

Boc–Gly-L-Ala-L-Leu–OBu^t: To a stirred mixture of Z-L-Ala (0.335 g, 1.5 mM) and Leu–OBu^t [0.31 g (1.65 mM), $[\alpha]_D^{25}$ +21.5° (c=2.3, EtOH), prepared according to the published procedure,[22] lit.[22] $[\alpha]_D^{25}$ +21.6° (c=2.5, EtOH)] in DMF (8 ml) was added DEPC (0.245 g, 1.5 mM) at 0°, followed by the addition of TEA (0.152 g, 1.5 mM). The mixture was stirred at 0° for several hours, then at room temperature overnight, diluted with AcOEt–benzene (4: 1, 100 ml), and treated as usual. Removal of the solvent gave crude Z–Ala–Leu–OBu^t as an oil (0.27 g, quantitative), NMR (in $CDCl_3$): 0.9 (6H, d, $(CH_3)_2$, J=6 Hz), 1.3—1.7 (15H, $(CH_3)_3$, CH_3, $C\underline{H}_2C\underline{H}(CH_3)_2$), 4.3 (2H, 2×CH), 5.1 (2H, s, $C\underline{H}_2C_6H_5$), 5.6 (1H, NH), 6.6 (1H, CONH), 6.3 (5H, s, C_6H_5). This oil was dissolved in MeOH (15 ml) and hydrogenated for 10 hr in the presence of 5% palladium on charcoal (0.2 g). Removal of the catalyst followed by concentration gave an oil, which was dissolved in DMF (8 ml). To this solution were added Boc–Gly (0.263 g, 1.5 mM) and DEPC (0.245 g, 1.5 mM) at 0°, followed by the addition of TEA (0.152 g, 1.5 mM). The mixture was stirred at 0° for several hours, then at room temperature overnight. It was diluted with AcOEt–benzene (4: 1, 100 ml) and treated as usual. Removal of the solvent gave an oily solid, which was purified by column chromatography (silica gel, ether–hexane=5: 1) to give pure Boc–Gly-L-Ala-L-Leu–OBu^t as a solid (0.36 g, 58% based on Leu–OBu^t), mp 80—85°, $[\alpha]_D^{20}$ −47.4° (c=0.5, MeOH). *Anal.* Calcd for $C_{20}H_{37}N_3O_6$: C, 57.81; H, 8.98; N, 10.11. Found: C, 57.79; H, 8.98, N, 10.12.

Boc–Gly-D-Ala-L-Leu–OBu^t: The title compound was synthesized from Leu–OBu^t (0.14 g, 0.74 mM), Z-D-Ala [0.15 g (0.67 mM), prepared according to the published procedure,[23] mp 82—84°, $[\alpha]_D^{20}$ +14° (c=1.6, AcOH), lit.[23] mp 84—85°, $[\alpha]_D^{24}$ +13.5° (c=8.5, AcOH)], and Boc–Gly (0.105 g, 0.6 mM) using DEPC by the procedure described for the synthesis of the L-isomer. Yield 0.18 g (64%). mp 90—93° (AcOEt–hexane), $[\alpha]_D^{20}$ +2° (c=0.5, MeOH). *Anal.* Calcd for $C_{20}H_{37}N_3O_6$: C, 57.81; H, 8.98; N, 10.11. Found: C, 57.60; H, 8.98; N, 9.70.

Coupling of Boc-Gly-Ala with Leu-resin——Boc–Leu-resin (0.3 g, Boc–Leu: 0.126 mM) was deprotected by treatment with TFA–CH_2Cl_2 (1: 1, 2 ml, 30 min) and converted to the leucyl resin with TEA–DMF (0.13 g/2 ml, 10 min) according to the general procedure. A solution of Boc–Gly–L–Ala (93 mg, 0.38 mM) in DMF (1 ml) was added, followed by the addition of DPPA (104 mg, 0.38 mM) or DEPC (62 mg, 0.38 mM) in DMF (0.5 ml). Next, a solution of TEA (37 mg, 0.38×0.95 mM) in DMF (0.5 ml) was added and the mixture was stirred at 0° or at room temperature for 2 hr. After the coupling, the resin was washed with DMF, EtOH, and CH_2Cl_2, and dried *in vacuo*. A portion (about 120 mg) of the resin was suspended in TFA (3 ml) and gaseous HBr was bubbled through the suspension at room temperature for 40 min. The resin was filtered off and washed with TFA. Combined TFA was evaporated *in vacuo* to give a residue, which was dissolved in distilled water (50 ml) and analyzed with an amino acid analyzer.

Each diastereomer of Boc–Gly-L-Ala-L-Leu–OBu^t and Boc–Gly-D-Ala-L-Leu–OBu^t (about 10 mg) was treated with TFA (2 ml) at room temperature for 1 hr. After removal of the TFA, the residue was dissolved in distilled water (25 ml) and analyzed with an amino acid analyzer. Each diastereomer was separated completely using a JEOL amino acid analyzer (JLC-5H) with LC-R-1 JEOL resin in a 0.8×50 cm column under the following conditions: flow rate 0.83 ml/min, jacket temperature 55°, 0.2 M standard citrate buffer at pH 4.25 as a solvent (L-L was eluted at 104 ml and D-L at 126 ml).

The results of racemization tests are summarized in Table III.

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