gave colourless needles, mp 116.5—117.5°, which was identified by direct comparison of mp and IR with those of an authentic sample of **4a.**

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An Improved Attachment of N-Protected Amino Acid and Peptide to Chloromethylated Polystyrene-divinylbenzene Resin[1)]

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N-Protected amino acyl- or peptidyl-resin was prepared in good yield without racemization, when about a half equivalent amount of 1,5-diaza-bicyclo[4,3,0]nonene-5 (DBN) or 1,8-diaza-bicyclo[5,4,0]undecene-7 (DBU) salt of N-protected amino acid or peptide was allowed to react with chloromethylated polystyrene–2%-divinylbenzene resin (chlorine content 0.66 millimole per gram) at 50° for 28 hours. The unchanged chloromethyl group was acetylated quantitatively by the reaction with large excess of DBN salt of acetic acid.

Keywords——ester bond formation; 1,5-diaza-bicyclo[4,3,0]nonene-5; 1,8-diaza-bicyclo[5,4,0]undecene-7; N-protected amino acids; N-protected peptides; chloromethylated polystyrene–2%-divinylbenzene; racemization test

In solid phase peptide synthesis[3)] the most widely used starting material is a Boc-amino acid bound *via* a benzyl ester linkage to an insoluble copolymer of styrene and divinylbenzene (Boc-amino acyl-resin). Among many procedures for the preparation of Boc-amino acyl-resin investigated so far, it has been reported that the reaction of chloromethylated polystyrene-co-1%-divinylbenzene resin with the cesium salts of Boc-amino acid give Boc-amino acyl-resin in good yield.[4)] However, the procedure did not always give satisfactory result.[5)] On the other hand, for the synthetic strategy of fragment condensation on the solid support, it is required the attachment of large peptide to the solid support in good yield without racemization,[6)] although this has not been extensively tested.

The present communication deals with an improved attachment of N-protected amino acid and peptide to the solid support. As a basic catalyst, 1,5-diaza-bicyclo[4,3,0]nonene-5 (DBN)[7)] or 1,8-diaza-bicyclo[5,4,0]undecene-7 (DBU)[8)] was used in the reaction of chloromethylated polystyrene–2%-divinylbenzene resin (chlorine content 0.66 millimole or 1.10 millimole per gram) with Boc-amino acid or peptide in dimethylformamide (DMF). When

1) Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: HOBt=1-hydroxybenztriazole, DCC=dicyclohexylcarbodiimide. The -resin represents ester bond derived from N-protected amino acid or peptide with chloromethylated polystyrene 2% divinylbenzene.
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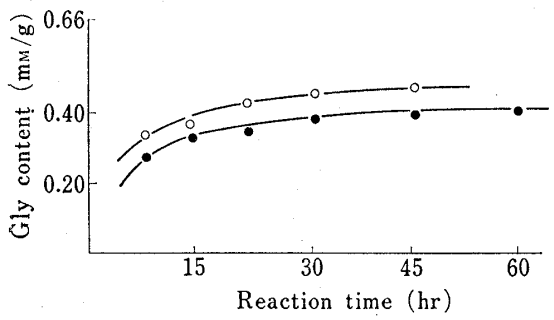

Fig. 1. Relationship between Reaction Time and Incorporation of Amino Acid or Peptide onto the Solid Support

Experimental conditions are described in text.
—○—: Boc-Gly-OH; —●—: Boc-Gly-Ile-OH

DBU salt of Boc-Gly-OH and the solid support (chlorine content 0.66 millimole per gram) in equimolar in 6 milliliter of DMF per gram of the solid support were allowed to react at 50° for 45 hours, the incorporation of glycine onto the solid support was reached maximum (0.50 millimole Gly per gram). Under the same conditions, Boc-Gly-Ile-OH, which has a side chain of rather steric hindrance, was incorporated by 0.40 millimole per gram.[9] Relationship between reaction time and incorporation of amino acid or peptide onto the solid support is shown in Fig. 1. Thus, quantitative incorporation of amino acid or peptide was not observed. However, in practical solid phase peptide synthesis 0.1 to 0.5 millimole of amino acid content per gram of the solid support is preferable.[10] A half equivalent amount

Table I. Esterification of DBN or DBU Salts of N-Acyl Amino Acids or Peptides with the Chloromethylated Solid Support in DMF (6 ml/g of resin) at 25 to 28 hr

Compound	Base	Base and compound eq. to Cl-content	Cl-content (mM/g of resin)	Amino acid incorporated onto resin (mM/g)[a]	Approximate yield (%)[b]
Boc–Gly–OH	DBN	0.50	0.66	0.29	88
	DBU	0.50	0.66	0.28	86
	DBN	0.50	1.10	0.33	61
	DBN	0.33	1.10	0.26	71
Boc–Ala–OH	DBN	0.50	0.66	0.32	98
	DBU	0.50	0.66	0.31	95
Boc–Val–OH	DBN	0.50	0.66	0.26	80
	DBU	0.50	0.66	0.24	72
Boc–Ile–OH	DBN	0.50	0.66	0.28	85
	DBU	0.50	0.66	0.31	93
Boc–Tyr(Bzl)–OH	DBN	0.50	0.66	0.27	80
Z(OMe)–Asp(ONb)–OH[c]	DBN	0.50	0.66	0.26[d]	79
Boc–Arg(Tos)–OH	DBN	0.50	0.66	0.20[d]	61
Boc–Glu(OPac)–OH[e]	DBN	0.50	0.66	0.19[d]	58
Z(OMe)–Glu(ONb)–OH[e]	DBN	0.50	0.66	0.23[d]	72
Boc–Gly–Ile–OH	DBN	0.50	0.66	0.33 (Gly)[f]	100
	DBU	0.50	0.66	0.28 (Gly)[f]	83
	DBN	0.50	1.10	0.25 (Gly)[f]	59
	DBN	0.33	1.10	0.33 (Gly)[f]	69
Boc–Trp–Gly–OH	DBN	0.50	0.66	0.24 (Gly)[f]	72
Z–Leu–Ala–OH	DBN	0.50	0.66	0.30 (Ala)[f]	90
Z–Val–Phe–Gly–OH	DBN	0.50	0.66	0.32 (Gly)[f]	98
	DBU	0.50	0.66	0.33 (Gly)[f]	100

a) determined by amino acid analysis of the acid hydrolysate of the resin

b) calculated as follows: $\% = \frac{\text{mM of incorporated amino acid or peptide/g}}{\text{mM of N-acyl amino acid or peptide used/g}} \times 100$

c) K. Suzuki, K. Nitta and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 3025 (1976)

d) N-Protected amino acyl-resin was acetylated as described in experimental parts.

e) "in preparation"

f) Amino acid in parentheses was determined as the indication of incorporation rate of the peptide.

9) DBU was not used under the conditions, because in a preliminary experiment at room temperature the incorporation of glycine with the use of DBU was much lower than that of DBN.

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of DBN salt of amino acids or peptides (0.33 millimole) to the chlorine in the solid support (0.66 millimole) was allowed to react under the same conditions as described above. DBU salt was also tested in the same manner. The incorporation rate of amino acids or peptides were shown in Table I. Thus, Boc-amino acids or peptides were incorporated onto the solid support in good yield with the use of either DBN or DBU. When the incorporation rates were tested on the solid support containing much more higher chlorine (1.10 millimole per gram) under the same conditions, the results observed were shown in Table I. From the data, it seems that the incorporation rates does not increase proportionally to the increase of chlorine content of the solid support. Since the presence of unchanged chloromethyl group is not preferable,[4] the group was acetylated with 4 equivalent amount of DBN salt of acetic acid to the chlorine in the solid support under the same conditions for the incorporation of Boc-amino acids. Determination of chlorine content[10] of Boc-amino acyl-resin thus obtained showed that acetylation reaction was almost quantitative. Determination of amino acid content before and after of the acetylation showed that transacylation did not occur during the acetylation reaction. Racemization rate of isoleucine residue in Boc-Gly-Ile-resin was determined according to the directions given by Bodanszky, *et al.*[11] Z-Leu-Ala-resin was treated with anhydrous hydrogen fluoride in the usual manner to liberate the dipeptide. Racemization rate of alanine residue in the dipeptide was determined according to the directions given by Manning, *et al.*[12] The results of these tests for the racemization showed that this procedure for the incorporation of N-protected peptide onto the solid support did not cause racemization. N-Protected peptides used in this study were synthesized by the conventional solution method as described in Experimental.

This procedure provides an easy way to prepare N-protected amino acyl-resin and even N-protected peptidyl-resin in good yield without racemization.

Experimental

All melting points are uncorrected. Unless otherwise mentioned, Boc- and Z-groups of the protected peptides were deblocked with 4 N HCl in dioxane and 4.6 N HBr in AcOH respectively, and paper chromatography was performed on Toyo Roshi No. 51 with the following solvent systems: *Rf* (A), BuOH–AcOH–H_2O (4:1:5, upper layer);[13] *Rf* (B), BuOH–AcOH–pyridine–H_2O (15:3:10:12).[14] Amino acid analysis was carried out on a Hitachi Model KLA-3B amino acid analyzer according to the directions given by Moore, *et al.*[15]

Syntheses of Peptide Derivatives

Boc-Gly-Ile-OH (I)——To a suspension of isoleucine (394 mg) and $NaHCO_3$ (504 mg) in H_2O (7 ml), a solution of Boc–Gly–ONSu[16] (817 mg) in dioxane (7 ml) was added and stirred at room temperature for 5 hr. The reaction mixture was diluted with H_2O (30 ml), washed with EtOAc (15 ml × 3), acidified with solid citric acid under cooling with ice, and extracted with EtOAc. The extract was washed with 1 N citric acid and saturated NaCl successively, and dried over $MgSO_4$. The solution was evaporated in vacuum and the residue was recrystallized from EtOAc and petr. ether; plates, yield 700 mg (81%); mp 101—107°; $[\alpha]_D^{13}$ −22.7° (c=1.1, DMF); de-Boc peptide HCl salt, *Rf* (A) 0.50, *Rf* (B) 0.58, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{13}H_{24}O_5N_2$: C, 54.15; H, 8.39; N, 9.72. Found: C, 53.72; H, 8.58; N, 9.74.

Boc-Trp-Gly-OBzl (II)——H–Gly–OBzl·Tos[17] (3.0 g) was dissolved in DMF (25 ml). To the solution, Boc-Trp-OH (2.5 g) and HOBt (1.1 g) were added. After chilled this mixture, Et_3N (1.3 ml) and DCC (1.9 g) were added, stirred at 5° overnight and kept at room temperature for 1 hr. DCC-urea thereby formed was removed by filtration and filtrate was diluted with EtOAc. The solution was washed with 1 N citric acid, saturated NaCl, 1 N $NaHCO_3$ and saturated NaCl respectively. The EtOAc solution was dried over $MgSO_4$, decolored with activated charcoal and concentrated to a small volume. The residue was recrystallized

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from EtOAc and petr. ether; needles, yield 3.6 g (94%); mp 111—112°; $[\alpha]_D^{13}$ −12.7° (c=1.1, DMF); de-Boc peptide ester HCl salt, *Rf* (A) 0.71, *Rf* (B) 0.90, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{25}H_{29}O_5N_3$: C, 66.50; H, 6.47; N, 9.31. Found: C, 66.53; H, 6.64; N, 9.11.

Boc-Trp-Gly-OH (III)——To a solution of II (3.0 g) in MeOH (25 ml), 1 N NaOH (7.3 ml) was added and stirred at room temperature for 90 min. The reaction mixture was evaporated in vacuum until it became *ca.* 15 ml. After addition of H_2O (50 ml), the solution was washed with EtOAc (20 ml×3), acidified with solid citric acid under cooling, and extracted with EtOAc. The extract was worked up in the usual manner. The product was recrystallized from EtOAc and petr. ether; plates, yield 2.3 g (96%); mp 101—105°; $[\alpha]_D^{13}$ +16.0° (c=1.0, DMF); de-Boc peptide HCl salt, *Rf* (A) 0.41, *Rf* (B) 0.44, single spot positive to ninhydrin and Ehrlich reagents. *Anal.* Calcd. for $C_{18}H_{23}O_5N_3 \cdot H_2O$: C, 56.98; H, 6.64; N, 11.08. Found: C, 57.10; H, 6.87; N, 10.93.

Z-Val-Phe-Gly-OEt (IV)——Z–Phe–Gly–OEt[18] (1.92 g) was hydrogenated in MeOH (15 ml) and AcOH (2 ml) over 5% Pd-C for 5 hr. The catalyst was removed by the aid of Cellite. To a solution of this product in DMF (8 ml), Z–Val–ONp[19] (1.97 g) was added, followed by Et_3N to keep the solution slightly alkaline. After stirred at room temperature for 20 hr, the reaction mixture was diluted with 1N NH_4OH (1.5 ml) and stirred for 1 hr. The mixture was poured into cold 1N NH_4OH (100 ml) with stirring. To the suspenstion, 50% NH_4OAc was added dropwise with stirring to form precipitate. The precipitate was collected and washed successively with 1N NH_4OH, H_2O, 1N HCl and H_2O. The product dried over P_2O_5 was recrystallized from EtOAc; amorphous powder, yield 1.6 g (68%); mp 170—184°; $[\alpha]_D^{13}$ −19.0° (c=1.0, DMF); de-Z peptide ester AcOH salt, *Rf*(A) 0.83, *Rf*(B) 0.93, single spot positive to ninhydrin reagent; *Anal.* Calcd. for $C_{26}H_{33}O_6N_3$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.54; H, 6.99; N, 8.70.

Z-Val-Phe-Gly-OH (V)——To the solution was evaporated to dryness and dried over KOH pellets in vacuum. To a solution of IV (339 mg) in dioxane (6 ml), 1 N NaOH (0.8 ml) was added and stirred at room temperature for 90 min. The reaction mixture was worked up in the usual manner. The product was reprecipitated from EtOAc and petr. ether; amorphous powder, yield 250 mg (79%); mp 201—204°; $[\alpha]_D^{13}$ −10.9° (c=1.1, DMF); de-Z peptide HBr salt, *Rf*(A) 0.59, *Rf*(B) 0.67, single spot positive to ninhydrin reagent. *Anal.* Calcd. for $C_{24}H_{29}O_6N_3$: C, 63.28; H, 6.42; N, 9.23. Found: C, 63.40; H, 6.48; N, 9.29.

General Procedure for the Preparation of N-Protected Amino Acyl- or Peptidyl-resin——To a suspension of chloromethylated resin (400 mg, 0.66 mM of Cl/g) in DMF (2.5 ml), a half eq. amount of DBN or DBU salt of N-protected amino acid or peptide (0.132 mM) were added and the mixture was stirred at 50° for 25 hr. The resin was then washed with the following solvents (10 ml each) respectively; DMF, EtOH, H_2O and EtOH, on filter and dried to constant weight in vacuum.

Acetylation of Unchanged Chloromethyl Group in the Solid Support——After the incorporation of Boc-amino acids, 4 fold excess of DBN salt of acetic acid were added into the reaction mixture and stirring was continued at 50° for 20 hr. The resin thereby obtained was washed with the following solvents respectively; DMF, EtOH, H_2O and EtOH, on filter and dried to constant weight in vacuum. The acetylated resin was confirmed by the disappearance of Cl by the Vorhard method.[10]

Racemization Test——(A) Boc–Gly–Ile–resin, which was prepared by the procedure used DBN or DBU salt in the same manner as described above, was hydrolized with the mixture of conc. HCl–propionic acid, according to the procedure of Robinson, *et al.*[19] Samples taken for analysis were about 0.75 μmole as content of isoleucine, since in the range of sample about 1% racemate, D-alloisoleucine, could be detectable and determined on the amino acid analyzer.

(B) Z–Leu–Ala–OH, which was prepared by the procedure of Anderson, *et al*,[16] was allowed to react with the resin in the same manner as described above. The dried resin thereby obtained was treated with anhydrous hydrogen fluoride as usual manner. After lyophylization, the sample was analized and determined racemate, H–Leu–D–Ala–OH, according to the procedure of Manning, *et al.*[12]

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