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The γ-Phenacyl and γ-*p*-Nitrobenzyl Esters to Minimize Side Reactions during Treatment of Glutamyl Peptides with Hydrogen Fluoride-Anisole Mixture[1,2]

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For the synthesis of peptide containing glutamic acid residue(s) by solid phase peptide synthesis, γ-phenacyl or γ-nitrobenzyl ester group, which is stable to anhydrous hydrogen fluoride (HF), was used as γ-carboxyl protecting group of glutamic acid to minimize the side reactions. The protected peptide resins were treated with HF-anisole mixture followed by deprotection of the γ-carboxyl protecting group under mild conditions. This strategy gave a satisfactory result without detectable side reactions in the preparations of two model dipeptides, H–Ala–Glu–OH and H–Asn–Glu–OH, by solid phase peptide synthesis.

Keywords——Boc–Asn–Glu(ONb)–resin; H–Asn–Glu(ONb)–OH; catalytic hydrogenolysis; H–Asn–Glu–OH; Boc–Asn–Glu(OPac)–resin; H–Asn–Glu(OPac)–OH; zinc in 90% AcOH

Side reactions of peptide derivatives containing γ-benzyl glutamyl residue (s) have been found by Sano[4] and Feinberg[5] during anhydrous hydrogen fluoride (HF)-anisole mixture treatment which has been widely used in the peptide synthesis.[6] γ-*p*-Methoxybenzoyl-α-aminobutylic acid residue was detected in the by-product.[5,7] In addition, pyrrolidone-containing peptide was also detected as a by-product.[8] The rate of the side reactions was shown to be dependent on both the amino acid sequence[7] and the temperature of HF-anisole treatment.[5,9]

This paper describes a strategy for the minimization of the side reactions. The strategy was designed in which the protecting groups stable to HF, such as γ-phenacyl or γ-*p*-nitrobenzyl ester, were used and the protected peptides were treated with HF-anisole mixture to remove protecting groups other than γ-phenacyl or γ-*p*-nitrobenzyl ester group. The γ-carboxyl protecting group was removed with the other procedures. As model di-peptides for this synthetic strategy, several derivatives of H-Ala-Glu-OH and H-Asn-Glu-OH were

1) A part of this work was presented at the 13th Symposium on Peptide Chemistry, Tokyo, 1974; K. Suzuki, N, Endo, and Y. Sasaki, "Proceedings of the 13th Symposium on Peptide Chemistry," ed. by S. Yamada, Protein Research Foundation, Minoh, Osaka, 1975, p. 77.
2) Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: DMF=dimethylformamide, DCC=dicyclohexylcarbodiimide. The -resin represents ester bond derived from N-protected amino acid or peptide with chloromethylated polystyrene 2% divinylbenzene.
3) Location: *Komatsushima, Sendai, 983, Japan.*
4) S. Sano and S. Kawanishi, *Biochem. Biophys. Res. Commun.*, **51**, 46 (1973).
5) R.S. Feinberg and R.B. Merrifield, *J. Am. Chem. Soc.*, **97**, 3485 (1975).
6) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967); See for review article, S. Sakakibara, Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 1, ed. by B. Weinstein, Marcel Dekker, Inc., New York, 1971, p. 51.
7) *a*) S. Sano and S. Kawanishi, *J. Am. Chem. Soc.*, **79**, 3480 (1975); *b*) *Idem*, "Proceedings of the 12th Symposium on Peptide Chemistry," ed. by H. Yajima, Protein Research Foundation, Minoh, Osaka, 1975, p. 28.
8) C.C. Yang and R.B. Merrifield, *J. Org. Chem.*, **41**, 1032 (1976).
9) Y. Ogata, K. Igano, K. Inoue, and S. Sakakibara, "Proceedings of the 12th Symposium on Peptide Chemistry," ed. by H. Yajima, Protein Research Foundation, Minoh, Osaka, 1975, p. 35.

chosen because it was reported that the resin derivatives, Boc-Ala-Glu(OBzl)-resin and Boc-Asn-Glu(OBzl)-resin, were derived to by-products in 70 to 80% yields.[7b]

H-Glu(ONb)-OH (I), which was one of the important starting materials, was prepared by a modification of Ledger and Stewart's method[10] in a 44% yield based on *p*-nitrobenzylbromide used. H-Glu(OPac)-OH (II) was similarly prepared in a 46% yield. Crystalline Z(OMe)-Glu(ONb)-OH (III) was obtained in a 78% yield in the usual manner. Similarly, Boc-Glu(OPac)-OH (IV) was obtained in a 41% yield. As model dipeptide resin derivatives, Boc-Ala-Glu(ONb)-resin (V), Boc-Asn-Glu(ONb)-resin (VI), Boc-Ala-Glu(OPac)-resin (VII) and Boc-Asn-Glu(OPac)-resin (VIII), were prepared by a standard solid phase method. The model dipeptide resins were treated with HF-anisole mixture by the usual method. Amino acid analyses of the acid hydrolysates of the resulting crude dipeptide derivatives, H-Ala-Glu(ONb)-OH (Va), H-Asn-Glu(ONb)-OH (VIa), H-Ala-Glu(OPac)-OH (VIIa) and H-Asn-Glu(OPac)-OH (VIIIa), gave correct ratios as shown in Table I. This implies that formation of γ-*p*-methoxybenzoyl-α-aminobutylic acid does not occur during HF-anisole mixture treatment.

Table I. Amino Acid Ratios of Acid Hydrolysates of Crude Dipeptide Derivatives

Compound	Amino acid ratios			
	Glu	Ala	Asp	NH_3
H-Ala-Glu(ONb)-OH (Va)	1.00	1.01	—	—
H-Ala-Glu(OPac)-OH (VIIa)	1.00	0.91	—	—
H-Asn-Glu(ONb)-OH (VIa)	1.00	—	1.05	0.92
H-Asn-Glu(OPac)-OH (VIIIa)	1.00	—	1.00	1.02

Acid hydrolysis was carried out with constant-boiling HCl at 110° for 22 hr.

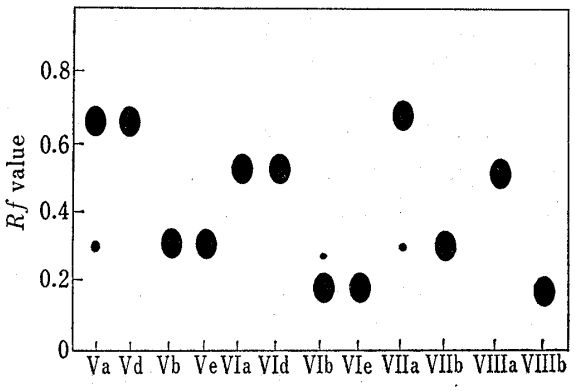

Fig. 1. Paper Chromatograms of Dipeptide Derivatives treated with HF, Dipeptides treated Further with the Other Reagents and Authentic Dipeptides

Solvent system: *n*-BuOH–AcOH–pyridine–H_2O (15 : 3 : 10 : 12).

The crude dipeptide derivatives described above were compared on paper chromatograms with authentic dipeptide derivatives, H-Ala-Glu(ONb)-OH (Vd) and H-Asn-Glu(ONb)-OH (VId) derived from Boc-Ala-Glu(ONb)-OH·DCHA (Vc) and Boc-Asn-Glu(ONb)-OH (VIc), which were prepared by the conventional solution method. Concurrently, *p*-nitrobenzyl ester group in Va and VIa was deblocked by catalytic hydrogenolysis to give free dipeptides, H-Ala-Glu-OH (Vb) and H-Asn-Glu-OH (VIb), which were compared on paper chromatograms with authentic dipeptides, H-Ala-Glu-OH (Ve) and H-Asn-Glu-OH (VIe). Phenacyl ester group in VIIa and VIIIa was deblocked with zinc powder in 90% acetic acid to give free dipeptides, H-Ala-Glu-OH (VIIb) and H-Asn-Glu-OH (VIIIb). VIIb and VIIIb were compared on paper chromatograms with Ve and VIe. These results are shown in Fig. 1. As is shown in Table I and Fig. 1, no by-product was detected under such a strategy. The strategy could be applied to the conventional peptide synthesis as well.

10) R. Ledger and F.H.C. Stewart, *Aust. J. Chem.*, **18**, 1477 (1965).

Experimental

All melting points are uncorrected. Unless otherwise mentioned, Boc- and Z(OMe)-group of the protected peptides were deblocked with 4 N HCl in dioxane 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);[11] *Rf*(B), BuOH–AcOH–pyridine–H_2O (15: 3: 10: 12).[12] Amino acid analysis was carried out on a Hitachi Model KLA-3B amino acid analyzer according to the directions given by Moore, *et al.*[13]

H-Glu(ONb)-OH (I)——This compound was synthesized in a similar method to that described previously for the preparation of H-Asp(ONb)–OH by Suzuki, *et al.*[14] This was a modification of Ledger and Stewart's method;[10] yield 44%, needles; mp 166—167° (lit. 165—166°,[5] 171—172°,[10] 158—159°,[15] 171°[16]); $[\alpha]_D^{19}$ +19.3° (c=1.4, 1 N HCl) (lit. +19.2°,[10] +19.52°[16]); *Rf*(A) 0.60, *Rf*(B) 0.65, single spot positive to ninhydrin.

H-Glu(OPac)-OH (II)——This compound was synthesized in a similar manner for the preparation of I. The product was recrystallized from H_2O; yield 46%, plates; mp 163—165° (dec.); $[\alpha]_D^{19}$ +10.3° (c=1.8, AcOH); *Rf*(A) 0.50, *Rf*(B) 0.70, single spot positive to ninhydrin. *Anal.* Calcd. for $C_{13}H_{15}NO_5$: C, 58.86; H, 5.70; N, 5.28. Found: C, 59.11; H, 5.86; N, 5.29.

Z(OMe)-Glu(ONb)-OH (III)——This compound was prepared from I (2.82 g) and *p*-methoxybenzyl S-4,6-dimethylpyrimidyl-2-thiocarbonate (3.55 g) in dimethylsulfoxide (40 ml) containing Et_3N (2.1 ml) according to the procedure given by Nagasawa, *et al.*[17] The product was reprecipitated from EtOAc and petroleum ether; amorphous powder, yield 3.5 g (78%); mp 77—79°; $[\alpha]_D^{19}$ −11.7° (c=0.9, DMF). *Anal.* Calcd. for $C_{21}H_{22}N_2O_9$: C, 56.50; H, 4.94; N, 6.28. Found: C, 56.23; H, 4.96; N, 6.21.

Boc-Glu(OPac)-OH (IV)——A solution of II (5.3 g), Boc–N_3 (3.15 g) and Et_3N (11.2 ml) in DMF (40 ml) was stirred at room temperature for 2 days. The solution was evaporated to small volume in vacuum. The residue was diluted with 1 N $NaHCO_3$ (150 ml) and washed with EtOAc (40 ml×2) rapidly. The aqueous layer was acidified with solid citric acid under cooling and extracted with EtOAc (60 ml×2). The combined extracts were washed with 1 N citric acid and H_2O and dried over $MgSO_4$. Evaporation of EtOAc gave oily residue which was crystallized in a refrigerator. The product was reprecipitated from EtOAc and *n*-hexane; amorphous powder, yield 3.0 g (41%); mp 101—104°; $[\alpha]_D^{19}$ −19.5° (c=0.8, DMF). *Anal.* Calcd. for $C_{18}H_{23}NO_7$: C, 59.18; H, 6.33; N, 3.83. Found: 59.37; H, 6.36; N, 3.92.

Preparations of V, VI, VII and VIII[18]——Z(OMe)–Glu(ONb)–resin and Boc–Glu–(OPac)–resin were prepared by the procedure of Gisin.[19] The contents of glutamic acid in the amino acyl resin were 0.258 and 0.181 mM/g respectively from the results of the amino acid analysis in the acid hydrolysate of the dry resin.[20] V was prepared from Boc–Ala–OH and Z(OMe)–Glu(ONb)–resin according to simplified solid phase procedure described by Suzuki, *et al.*[21] Similarly, VII was prepared from Boc–Ala–OH and Boc–Glu(OPac)–resin. VI was prepared from Boc–Asn–ONp and Z(OMe)–Glu(ONb)–resin according to the active ester method.[22] Similarly, VIII was prepared from Boc–Asn–ONp and Boc–Glu(OPac)–resin.

HF-anisole Treatment of Protected Dipeptidyl Resins——The general procedure was as follows: For example, VII (240 mg, 36 μmol of peptide) was treated with HF (10 ml) in the presence of anisole (0.6 ml, 150 eq.). After stirred for 60 min at 0°, excess HF was rapidly evaporated in vacuum. The residue was suspended in 10% AcOH (10 ml), and the suspension was stirred for 20 min and then filtered on a glass filter. The extraction was repeated twice. The combined aqueous layers were washed several times with EtOAc and lyophilized to yield VIIa (23 mg).

Preparations of Vb and VIb——These compounds were prepared as follows: Va in H_2O was hydrogenated over 5% Pd-C for 6 hr to give Vb. Similarly, VIa was treated to give VIb. The products were applied on paper chromatograms (see Fig. 1).

11) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
12) S.G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).
13) S. Moore, D.H. Spackman, and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).
14) K. Suzuki, K. Nitta, and Y. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 3025 (1976).
15) R.L. Prestidge, D.R.K. Harding, J.E. Battersby, and W.S. Hancock, *J. Org. Chem.*, **40**, 3287 (1975); R.L. Prestidge, D.R.K. Harding and W.S. Hancock, *ibid.*, **41**, 2579 (1976).
16) M. Goodman, A.M. Felix, C.M. Deber, A.R. Bause, and G. Schwartz, *Biopolymers*, **1**, 371 (1963).
17) T. Nagasawa, K. Kuroiwa, K. Narita, and Y. Isowa, *Bull. Chem. Soc. Jpn.*, **46**, 1269 (1973).
18) In the case of VIII, the recovery of aspartic acid was a half of that of glutamic acid as analyzed the acid hydrolysate with conc. HCl-propionic acid (130°, 5 hr). The reasons are not clear. However, when the hydrolysate with conc. HCl-dioxane (110°, 22 hr)[22] was rehydrolyzed with conc. HCl-propionic acid (130°, 5 hr) according to the procedure given by Stewart, *et al.*,[22] the ratio of the recovered aspartic acid and glutamic acid was 1: 1.
19) B.F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
20) J. Scotchler, R. Lozier, and A.B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970).
21) K. Suzuki, K. Nitta, and N. Endo, *Chem. Pharm. Bull.* (Tokyo), **23**, 222 (1975).
22) J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and Co., San Francisco, 1969, p. 51.

Preparations of VIIb and VIIIb——These compounds were prepared as follows: VIIa in 90% AcOH was treated with zinc powder (5 eq.) in three portions at 5 min interval under cooling and stirred for 1 hr at room temperature to give VIIb. Similarly VIIIa was treated to give VIIIb. The products were applied on paper chromatograms (see Fig. 1).

Boc-Ala-Glu(ONb)-OH·DCHA (Vc)——To a solution of I (0.70 g), and $NaHCO_3$ (0.42 g) in H_2O (8 ml) was added a solution of Boc–Ala–ONSu[23] (0.71 g) in EtOH (8 ml) and the mixture was stirred at room temperature overnight. After removal of EtOH in vacuum, the residual solution was washed with EtOAc, acidified with solid citric acid under cooling and extracted with EtOAc. The combined extracts were washed with H_2O, dried over $MgSO_4$ and evaporated in vacuum. The resulting oil was dissolved in MeOH (2 ml) and dry ether (50 ml). To this solution DCHA (0.5 ml) was added. After stirred for 30 min, the precipitate was collected and washed with dry ether. Recrystallization from MeOH and dry ether gave a fine needles, yield 1.47 g (92%); mp 143—145°; $[\alpha]_D^{19}$ −7.7° (c=1.2, MeOH). *Anal.* Calcd. for $C_{20}H_{27}N_3O_9 \cdot C_{12}H_{23}N$: C, 60.55; H, 7.94; N, 8.83. Found: C, 60.25; H, 8.11; N, 8.54.

Boc-Asn-Glu(ONb)-OH (VIc)——To a solution of Boc–Asn–OH (232 mg) and HONSu (115 mg) in a mixture of dioxane (10 ml) and EtOAc (10 ml) was added DCC (208 mg) at 0° and the solution was stirred at 5° for 15 hr. The precipitate was filtered off and washed with EtOAc. The combined filtrates were concentrated in vacuum to yield crude Boc–Asn–ONSu, yield 350 mg. The crude product (250 mg) was dissolved in DMF (6 ml) and the solution was added to a suspension of I (163 mg) and $NaHCO_3$ (97 mg) in H_2O (6 ml). After stirred for 20 hr at room temperature, a small amount of insoluble material was filtered off and the filtrate was diluted with H_2O (30 ml) and washed with EtOAc. The aqueous layer was acidified with solid citric acid under cooling and extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated in vacuum. The product was recrystallized from EtOAc; amorphous powder, yield 230 mg (81%); mp 174—176°; $[\alpha]_D^{19}$ −15.6° (c=0.5, DMF). *Anal.* Calcd. for $C_{21}H_{28}N_4O_{10}$: C, 50.80; H, 5.68; N, 11.29. Found: C, 50.66; H, 5.67, N, 10.88.

H-Ala-Glu(ONb)-OH (Vd)——Vc (200 mg) was treated with 1 N citric acid in the usual manner. The oily product was treated with 4 N HCl in dioxane (4 ml) for 30 min at room temperature. The reaction mixture was diluted with dry ether. The precipitate was collected by centrifugation, washed with dry ether and dried over KOH pellets in vacuum. The dried product was reprecipitated from MeOH and dry ether; amorphous powder, yield 90 mg (67%); mp 68—80°; $[\alpha]_D^{25}$ −3.1° (c=1.3, H_2O); *Rf*(A) 0.62, *Rf*(B) 0.65, single spot positive to ninhydrin. *Anal.* Calcd. for $C_{15}H_{19}N_3O_7 \cdot HCl \cdot 2H_2O$: C, 42.30; H, 5.68; N, 9.87. Found: C, 42.57; H, 5.90; N, 9.62.

H-Ala-Glu-OH (Ve)——Vd (60 mg) was hydrogenated in H_2O (10 ml) over 5% Pd–C for 6 hr. The catalyst was removed by filtration with the aid of celite. The solution was evaporated to dryness and the residue was dried over P_2O_5 in vacuum. The solution of the crude product in H_2O (6 ml) was added to a Dowex 1×2 (acetate form) column (1.7×18 cm) which was eluted with 1 N AcOH. Fractions of 5 ml each were collected. The eluates in tubes No. 37 to 43, which were positive to Cl-*o*-tolidine reagent, were pooled, evaporated to dryness in vacuum and lyophilized; amorphous powder, yield 26 mg (52% based on I); $[\alpha]_D^{22}$ +3.6° (c=1.1, H_2O); *Rf*(A) 0.24, *Rf*(B) 0.29, single spot positive to ninhydrin; amino acid ratios in the AP–M digest: Ala 1.05, Glu 0.95 (average recovery 86%).

H-Asn-Glu(ONb)-OH (VId)——VIc (200 mg) was treated with 4 N HCl in dioxane (4 ml) for 30 min at room temperature. The reaction mixture was worked up in a similar manner as described for the preparation of Vd. The dried product was reprecipitated from MeOH and dry ether; amorphous powder, yield 120 mg (68%); mp 90—105°; $[\alpha]_D^{25}$ 0° (c=1.0, H_2O); *Rf*(A) 0.47, *Rf*(B) 0.59, single spot positive to ninhydrin. *Anal.* Calcd. for $C_{16}H_{20}N_4O_8 \cdot HCl$: C, 44.31; H, 4.89; N, 12.95. Found: C, 44.21; H, 5.19; N, 12.88.

H-Asn-Glu-OH (VIe)——VId (80 mg) in H_2O (10 ml) was hydrogenated in the usual manner for 10 hr. The hydrogenated product was submitted to column chromatography in the same manner as described for the preparation of Ve. The eluates in tubes No. 25 to 35 containing the desired peptide were pooled, evaporated to dryness in vacuum and lyophilized; amorphous powder, yield 34 mg (44% based on I); $[\alpha]_D^{22}$ +2.5° (c=1.6, H_2O); *Rf*(A) 0.16, *Rf*(B) 0.18, single spot positive to ninhydrin; amino acid ratios in the AP–M digest: Asn 0.96, Glu 1.04 (average recovery 94%).

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