Chem. Pharm. Bull. 17(8)1679—1686(1969)

UDC 547.964.4.07.04

Peptides. I. Selective Protection of α- or Side-chain Carboxyl Groups of Aspartic and Glutamic Acid. A Facile Synthesis of β-Aspartyl and γ-Glutamyl Peptides[1,2]

Masumi Itoh

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.[3]

(Received February 20, 1969)

(*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidineacetic acid (IIa) and (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidinepropionic acid (IIb) were utilized for the selective protection of side-chain carboxyl group of corresponding α-aminodicarboxylic acids and the synthesis of β-aspartyl and γ-glutamyl peptides as an α-carboxyl protected intermediates.

It was found that 5-oxazolidinone ring, the α-carboxyl protecting group, was easily removed under not only the condition of alkaline hydrolysis but also catalytic hydrogenolysis. Thus a facile synthesis of β-aspartyl and γ-glutamyl peptides was achieved by the coupling of IIa and IIb with benzyl ester of amino acids and peptides followed by hydrogenolytic removal of all the protecting groups.

The coupling reaction between an acylamino acid and an amino acid ester is nowadays a straightforward procedure for simple peptides. Most of biologically active peptides, however, possess side-chain substituted amino acids so that a number of investigations have been reported for selective protection of an amino, hydroxy, sulfhydryl or carboxyl group attached to the side-chain.[4]

As a most selective α-carboxyl protecting method for α-aminodicarboxylic acids it was reported that the reaction of paraformaldehyde with *p*-toluenesulfonylaminodicarboxylic acids gave 5-oxazolidinone derivatives[5,6] which were utilized for the peptide synthesis and preparation of homologous α-aminodicarboxylic acids. For example, γ-L-glutamylglycine,[5b] glutathione[7] and L-α-aminopimelic acid[6] were synthesized using 5-oxazolidinone derivatives. Preparation of 5-oxazolidinone compounds from benzyloxycarbonylamino acids except side-chain substituted amino acids were also reported without further application to peptide synthesis.[8]

In the present paper the synthesis of (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidineacetic acid (IIa)[9] and (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidinepropionic acid (IIb),[9] and these use for protection of a side-chain carboxyl group and peptide synthesis are described. IIa and IIb were synthesized by two procedures in good yield: (A) treatment of N-benzyloxycarbonyl-L-aspartic (Ia) or N-benzyloxycarbonyl-L-glutamic acid (Ib) with paraformaldehyde in a mixture of acetic anhydride, acetic acid and a trace of thionyl chloride[5]; (B) azeotropic distil-

1) Most part of this work was presented at the 6th Symposium on Peptide Chemistry, Kyushu Univ., Nov. 23—24, 1968.
2) Abbreviation used for protecting groups, amino acids and peptides are those recommended by IUPAC-IUB commission on biochemical nomenclature: *Biochemistry*, **5**, 2485 (1966).
3) Location: *1 Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press Inc., New York, 1966.
5) *a*) F. Micheel and S. Thomas, *Chem. Ber.*, **90**, 2906 (1957); *b*) F. Micheel and H. Haneke, *ibid.*, **92**, 309 (1959); *c*) F. Micheel and H. Haneke, *ibid.*, **95**, 1009 (1962).
6) H. Farkasova and J. Rudinger, *Collection Czech. Chem. Commun.*, **30**, 3117 (1965).
7) P. Baudet and I. Borecka, *Ann. Chim.* (Rome), **53**, 53 (1963) [*C.A.*, **59**, 2944 (1963)].
8) D. Ben-Ishai, *J. Am. Chem. Soc.*, **79**, 5736 (1957).
9) After this work was completed IIa and IIb were published in the two patents respectively; *a*) M. Murakami and N. Inukai, Japan. Patent 1968-2696 (Jan. 31, 1968); *b*) M. Murakami, I. Isaka, M. Takao, and M. Kochitani, Japan. Patent 1968-213 (Jan. 6, 1968).

lation of Ia or Ib with paraformaldehyde and *p*-toluenesulfonic acid in benzene.[8] The infrared spectra of IIa and IIb show the presence of 5-oxazolidinone ring at 1800 cm^{-1} and a free side-chain carboxyl group at 1710 cm^{-1}. The oxazolidinone ring is rather stable to acidic treatment than alkaline. IIb gave Ib or glutamic acid (IIIb) respectively by 1N sodium hydroxide treatment or catalytic reduction under the ring fission.

For the purpose of side-chain carboxyl protection, *tert*-butyl (*S*)-2-(3-benzyloxycarbonyl-5-oxo-4-oxazolidine-)acetate (IVa) and *tert*-butyl (*S*) 3-benzyloxycarbonyl-5-oxo-4-oxazolidine-propionate (IVb) were synthesized from IIa and IIb with isobutylene in the presence of concentrated sulfuric acid or *p*-toluenesulfonic acid, and were saponified with 1N sodium hydroxide to give *β-tert*-butyl N-benzyloxycarbonyl-L-aspartate (Va) and *γ-tert*-butyl N-benzyloxycarbonyl-L-glutamate (Vb). This procedure is convenient for preparation of Va and Vb than previously reported methods[10,11] because of its easier and selective α-carboxyl protection. *γ-tert*-Butyl L-glutamate (VIb)[12] was also prepared by the catalytic hydrogenation of IVb.

A oxazolidinone ring is a sort of so-called "active ester" so that ammonolysis of IIa and IIb gave N-benzyloxycarbonyl-L-isoasparagine (VIIa, R=H)[10,13] and N-benzyloxycarbonyl-L-isoglutamine (VIIb, R=H)[13,14], hydrolysis and hydrazinolysis of IIb gave also Ib and N-benzyloxycarbonyl-L-glutamic acid α-hydrazide (VIIc, $R=NH_2$)[14,15] respectively; furthermore aminolysis of IIb with ethyl glycinate gave ethyl N-benzyloxycarbonyl-α-L-glutamylglycinate (VIId, $R=CH_2CO_2Et$).[15] However, the rate of aminolysis of IIb with ethyl glycinate was so slow that the coupling reaction of IIb with ethyl glycinate by the mixed anhydride (MA) or dicyclohexylcarbodiimide (DCCI) method afforded ethyl (*S*)-3-(benzyloxycarbonyl-5-oxo-

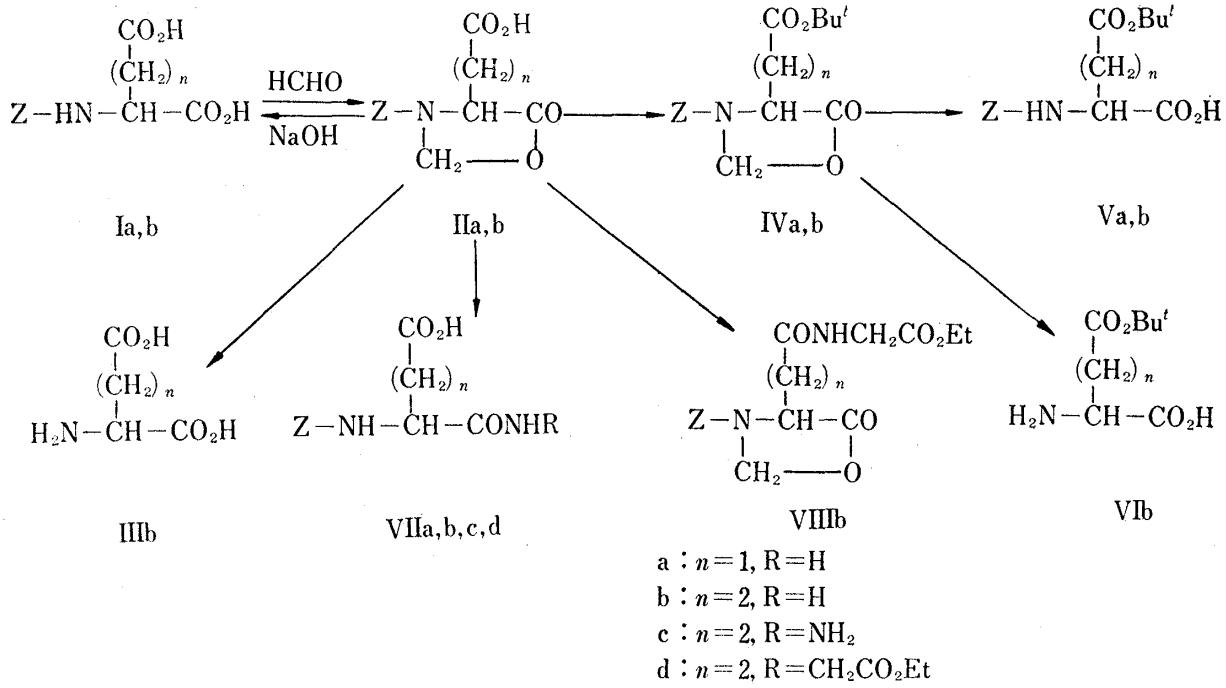
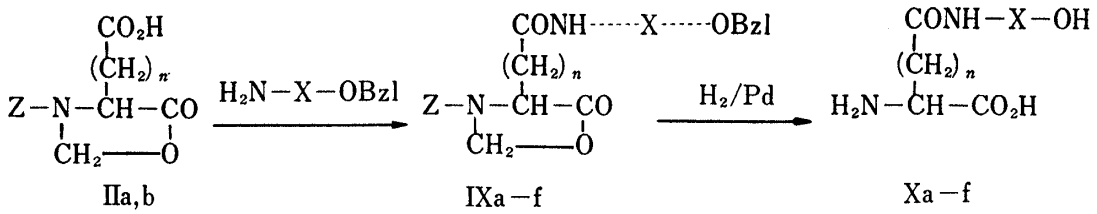


Chart 1

10) *a*) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 208 (1964); *b*) E. Wünsch and A. Zwick, *Chem. Ber.*, **99**, 105 (1966); *c*) B.O. Handford, T.A. Hyton, J. Preston, and B. Weinstein, *J. Org. Chem.*, **32** 1243 (1967).
11) E. Schröder and E. Klieger, *Ann. Chem.*, **673**, 196 (1964).
12) L. Zervas and C. Hamalidis, *J. Am. Chem. Soc.*, **87**, 99 (1965).
13) M. Bergman and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).
14) E. Klieger and H. Gibian, *Ann. Chem.*, **651**, 194 (1962).
15) W.J. LeQuesne and G.T. Young, *J. Chem. Soc.*, **1950**, 1959.

4-oxazolidine-)propionylglycinate (VIIIb) in good yield. Showing partial transpeptidation, saponification of both VIId and VIIIb gave the mixture of N-benzyloxycarbonyl-α- and γ-L-glutamylglycine. These facts suggest that the oxazolidinone ring is applicable to peptide synthesis as an α-carboxyl protecting group, but alkaline hydrolysis of ester groups is not suitable for their removal because of transpeptidation.



IIa,b IXa–f Xa–f

a : $n=1$, X=Gly– residue d : $n=2$, X=Ile– residue
b : $n=1$, X=Ile– residue e : $n=2$, X=Tyr– residue
c : $n=2$, X=Glu– residue f : $n=2$, X=Ala–Gly– residue

Chart 2

Table I. β-Aspartyl and γ-Glutamyl Peptides

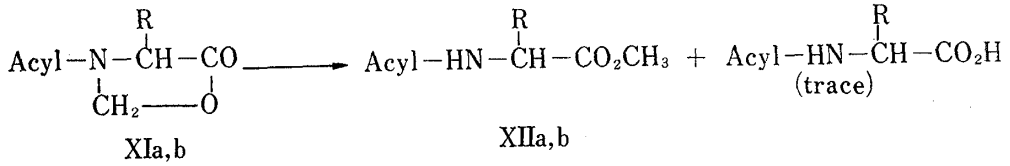
Peptides	Yield (%)	mp (°C) Found/Lit.	$[\alpha]_D^t$ (c, solvent) Found/Lit.	Analysis (%) (Calcd./Found) C	H	N
(Xa) β-Asp-Gly-OH	51	199 —200 decomp.	+18.6[20] (1.2, 1N HCl)	37.90	5.30	14.75
		163 decomp.[a]	+12.7[25] (2.0, H_2O+eq.HCl)[a]	37.51	5.35	15.05
(Xb) β-Asp-Ile-OH·1/4H_2O	27	196 —198	−4.1[23] (2.0, H_2O)[b]	47.89	7.43	11.17
		205[c]	−4.7[25] (2.0, H_2O)[c]	48.09	7.50	10.76
(Xc) γ-Glu-Glu(OH)$_2$	41	191 —192	+6.5[23] (1.0, 1N HCl)	43.48	5.84	10.14
		191.5—192[17]	+6.8[16] (1.0, 1N HCl)[17]	43.39	5.97	9.86
(Xd) γ-Glu-Ile-OH·1/2H_2O[d]	50	201 —203 decomp.	+17.2[20] (2.8, 1N HCl)[e]	49.06 49.17	7.86 7.80	10.40 9.94
(Xe) γ-Glu-Tyr-OH	38	219 —222 decomp.[f]	+25.3[23] (4.0, H_2O)[f]	54.19	5.85	9.03
		219 —221 decomp.[19]	+25.5[31] (4, H_2O)[19]	54.31	5.76	9.34
(Xf) γ-Glu-Ala-Gly-OH	72	193 —195 decomp.[g]	−28.3[23] (2.5, H_2O)[g]	43.63	6.23	15.27
		195 —197 decomp.[h]	−29.8[26] (2.4, H_2O)[h]	43.46	6.41	14.95

a) monohydrate. ref. 16)
b) +21.5[20] (1.9, 1N HCl)
c) anhydrate. ref. 16)
d) Isolated from Allium cepa. No physical data was given. ref. 18).
e) +3.2[23] (2.6, H_2O)
f) ppc: $Rf=0.57$ (solvent system C): Lit.[19] $Rf=0.57$
g) ppc: $Rf=0.12$ (solvent system A), $Rf=0.48$ (solvent system B): Lit.[20b] $Rf=0.54$ (solvent system B)
h) hemihydrate. ref. 20[a])

16) D.L. Buchanan, E.E. Haley, F.E. Dorer, and B.J. Corcoran, *Biochemistry*, **5**, 3240 (1966).
17) Y. Kakimoto, T. Nakajima, K. Kanazawa, M. Takesada, and I. Sano, *Biochim. Biophys. Acta*, **93**, 333 (1964).
18) A.I. Virtanen and E.J. Matikkala, *Suomen Kemistilehti*, **34B**, 53 (1961) [*C.A.*, **56**, 716 (1963)].
19) C.J. Morris and J.F. Thompson, *Biochemistry*, **1**, 706 (1962).
20) *a*) K. Ogata and S. Ishii, *Chem. Pharm. Bull.* (Tokyo), **15**, 707 (1967); *b*) G. Losse, H. Jeschkeit, and R. Hörn, *Ann. Chem.*, **676**, 222 (1964).

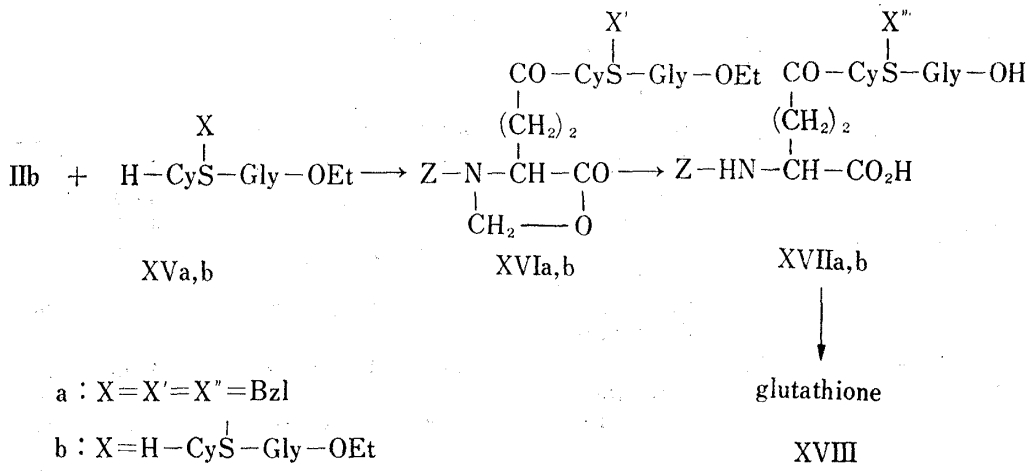
IIa and IIb were coupled with benzyl ester of amino acids and peptides by MA or DCCI method. The resulting product was hydrogenated to give the deblocked peptide. Thus prepared peptides were listed in the Table I.

It is well known that benzyloxycarbonyl (Z) and benzyl ester groups are easily removed by catalytic hydrogenolysis, but hydrogenolytic cleavage of the oxazolidinone ring has not been investigated. In fact the oxazolidinone ring is easily removed by catalytic hydrogenolysis, but it is doubtful whether its removal is hydrogenolytic. To clarify this point model compounds, 3-benzoyl-5-oxo-oxazolidine[8] and 3-*p*-toluenesulfonyl-5-oxo-4-oxazolidinepropionic acid[5b] were treated under the condition of hydrogenation. The major products were methyl benzoylglycinate (XIIa)[21] and α-methyl N-*p*-toluenesulfonyl-L-glutamate (XIIb)[22] respectively, showing the ring-fission at carbon–nitrogen bonding. As a further example, acidolytic cleavage of benzyl (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidineacetate (XIII) by hydrogen bromide in acetic acid was achieved to give β-benzyl L-aspartate (XIV)[12,23]. These facts suggest that the oxazolidinone ring becomes labile by means of the removal of Z group.



a : Acyl=C_6H_5CO-, R=H
b : Acyl=$p-CH_3-C_6H_4-SO_2-$, R=$-(CH_2)_2CO_2H$

Chart 3



a : X=X'=X''=Bzl
b : X=H–CyS–Gly–OEt

X'=Z–N–CH–CO (with $(CH_2)_2-CO-CyS-Gly-OEt$ and CH_2-O)

X''=Z–HN–CH–CO_2H (with $(CH_2)_2-CO-CyS-Gly-OH$)

Chart 4

21) H. Rinderknecht and C. Niemann, *J. Am. Chem. Soc.*, **70**, 2605 (1948).
22) G.H.L. Nefkens and R.J.F. Nivard, *Rec. Trav. Chim.*, **83**, 199 (1964).
23) L. Benoiton, *Can. J. Chem.*, **40**, 570 (1962).

IIb was also utilized for the synthesis of glutathione[24] as an α-carboxyl protected intermediate. IIb was coupled with ethyl S-benzyl-L-cysteinylglycinate (XVa) or diethyl L-cystinyldiglycinate (XVb) by DCCI or MA method using phosphorous oxychloride. When isobutyl chloroformate was used as a reagent for the coupling of IIb with XVa, ethyl N-isobutyloxycarbonyl-S-benzyl-L-cysteinylglycinate was obtained as a by-product. The fully protected peptides, ethyl (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine propionylglycinate (XVIa) and diethyl bis-[(*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl]cystinyldiglycinate (XVIb), were saponified with 1N sodium hydroxide, reduced with sodium in liquid ammonia and treated in the usual way to give pure glutathione.

Experimental

All melting points are uncorrected and taken on a Hoover "Uni-Melt" apparatus. Optical rotations were measured with a Yanagimoto photo-magnetic polarimeter, Model OR-10. All substances were pure as demonstrated by thin-layer chromatography on silica gel G in one of the following solvent systems either (A) *n*-butyl alcohol–acetic acid–water (4:1:1) or (B) chloroform–acetic acid–methanol (95:3:5). Spots were demonstrated by ninhydrin where the substance contained a free amino group and by the combination of hydrogen bromide and ninhydrin for N-benzyloxycarbonyl derivatives. Paper chromatography (ppc) was carried out on Toyo Filter Paper No. 51 by the ascending method. Solvent systems: (A) *n*-butyl alcohol–acetic acid–water (4:1:5, upper phase)[25]; (B) phenol–25% ammonia–water (79:1:20); (C) phenol–water (8:3).

(*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidineacetic Acid (IIa)——a): A mixture of N-benzyloxycarbonyl-L-aspartic acid[13] (Ia, 5.35 g), paraformaldehyde (1.8 g), acetic anhydride (4.0 g) and thionyl chloride (0.3 ml) in acetic acid (80 ml) was heated at 100° for 4 hr. Evaporation of acetic acid under reduced pressure gave an oily residue, which was dissolved in ethyl acetate and extracted with 5% $NaHCO_3$ solution. The aqueous layer was acidified with 6N HCl on ice-cooling and extracted with ethyl acetate. The extract was washed with water and dried over $MgSO_4$. Evaporation of the solvent gave 4.4 g (80%) of pale yellow syrup. A solution of the sample (1.0 g) in chloroform–ethyl acetate (3:1) was adsorbed on silica gel column (Wakogel Q-23, 20.0 g) which was eluted with same solvent system. Evaporation of the solvents gave a colorless syrup (80% recovery). *Anal.* Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.70; N, 5.02. Found: C, 55.37; H, 4.86; N, 4.82.

b): The product (89%) was prepared by the azeotropic distillation method as described below for IIb.

(*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidinepropionic Acid (IIb)——a): A mixture of N-benzyloxycarbonyl-L-glutamic acid[13,26] (Ib, 28.1 g), paraformaldehyde (5.0 g) and *p*-toluenesulfonic acid (1.0 g) in benzene (700 ml) was refluxed for 7 hr, the liberated water being removed azeotropically by means of a modified Dean–Stark distilling apparatus. The benzene solution was washed with water and extracted with 5% $NaHCO_3$ solution. The extract was treated in the same way as described for IIa. IIb was obtained as a syrup (28.6 g, 97.6% yield). For analysis a sample (1.0 g) was chromatographed on silica gel as described for IIa. *Anal.* Calcd. for $C_{14}H_{15}O_6N$: C, 57.33; H, 5.16; N, 4.78. Found: C, 57.56; H, 5.31; N, 4.98.

b): A syrupy product (90.6% yield) was obtained by the similar procedure as described for IIa. The product was identified by comparing its infrared spectrum with that of IIb prepared according to method *a*.

Saponification of IIb——A solution of IIb (1.5 g) in methanol (20 ml) was treated with 1N NaOH (10.0 ml) at room temperature for 4 hr. The reaction mixture was neutralized with 1N HCl, and the methanol was removed under reduced pressure. The product dissolved in ethyl acetate was extracted with 5% $NaHCO_3$ solution. The extract was washed once with fresh ethyl acetate, and acidified with 6N HCl. The acidified solution was extracted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. Evaporation of solvent gave an oily residue, which was solidified on standing. Recrystallization from water gave N-benzyloxycarbonyl-L-glutamic acid (Ib); 1.0 g, 71% yield, mp 120—121°, $[\alpha]_D^{20}$ −7.8° ($c=10$, acetic acid). (lit.[26] mp 120—121°, $[\alpha]_D$ −7.9° (acetic acid)).

Catalytic Reduction of IIb——A solution of IIb (2.2 g) in the mixture of methanol (25 ml), acetic acid (4 ml) and dioxane (20 ml) was hydrogenated in the presence of palladium black for 6 hr. The catalyst was removed by filtration, the filtrate was evaporated under reduced pressure and the residue was recrystal-

24) *a*) E. Schröder, and K. Lübke, "The Peptides," Vol. II, Academic Press Inc., New York, 1966, p. 260; *b*) M. Jeschkeit, G. Losse, and D. Knopf, *Pharmazie*, **18**, 658 (1963).
25) S.M. Partridge, *Biochem. J.*, **42**, 238 (1948).
26) S. Goldschmidt and C. Jutz, *Chem. Ber.*, **86**, 1116 (1953).

lized from water to give L-glutamic acid (IIIb); 0.8 g, 73% yield, mp 199—200° (decomp.), $[\alpha]_D^{23}$ +11.0° (c=0.7, H_2O), $[\alpha]_D^{23}$ +30.5° (c=1, 6N HCl), PPC Rf=0.20 (solvent system (A)). (lit.[27] mp 247—249° (decomp.), sublimes at 200°, $[\alpha]_D^{22.4}$ +31.4° (c=1.00, 6N HCl)).

***tert*-Butyl (*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidineacetate (IVa)**——IIa (4.4 g) was dissolved in dry dichloromethane (30 ml) containing concd. H_2SO_4 (0.2 ml) as a catalyst. Gaseous isobutene (about 15 g) was bubbled into the stirred solution at −10°. The solution was kept in a stoppered flask at room temperature for 48 hr. After addition of ethyl acetate (100 ml) the solution was washed with 5% $NaHCO_3$ solution and water, and then dried over $MgSO_4$. Solvents were removed under reduced pressure to give IVa as a pale yellow syrup; 4.9 g, 91.3% yield. The product was used for further reaction without any purification.

***tert*-Butyl (*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidinepropionate (IVb)**——As described above for IVa, IIb (4.0 g) was allowed to react with isobutene to give pale yellow syrup; 4.2 g, 90.0% yield. By use of *p*-toluenesulfonic acid in place of concd. H_2SO_4 IVb was obtained in 79.1% yield.

***β-tert*-Butyl N-Benzyloxycarbonyl-L-aspartate Dicyclohexylammonium Salt (Va)**——A solution of IVa (4.8 g) in ethanol (20 ml) was treated with 1N NaOH (7.1 ml) at room temperature for 4 hr. The syrupy product (2.8 g) was obtained in essentially same manner as described for saponification of IIb. Upon adding dicyclohexylamine (1.7 g) to a solution of above syrup in ether, 3.4 g (47.5% yield) of crystalline dicyclohexylammonium salt was obtained; mp 128—129° after recrystallization from water, $[\alpha]_D^{24}$ +6.5° (c=1.72, 90% acetic acid). (lit.[10a] mp 123—124°, $[\alpha]_D^{25}$ +7.7° (c=1, 95% acetic acid); lit.[28] mp 125—126.5°, $[\alpha]_D^{23}$ +5.5±1° (c=1.72, 90% acetic acid)). *Anal.* Calcd. for $C_{28}H_{44}O_6N_2$: C, 66.64; H, 8.79; N, 5.55. Found: C, 66.71; H, 9.07; N, 5.56.

***γ-tert*-Butyl N-Benzyloxycarbonyl-L-glutamate Dicyclohexylammonium Salt (Vb)**——A solution of IVb (4.7 g) in ethanol (40 ml) was treated with 1N NaOH (13.5 ml) at room temperature for 4 hr. The syrupy product (3.9 g) was obtained in same manner as described for saponification of IIb. Upon adding dicyclohexylamine (2.1 g) to a solution of above syrup in ethyl acetate, 3.2 g (45.8% yield) of dicyclohexylammonium salt was obtained; mp 138—140° after recrystallization from water, $[\alpha]_D^{25}$ +6.1° (c=3.9, methanol). (lit.[29] mp 138—140.5°, $[\alpha]_D^{20}$ +6.5° (c=2.0, methanol)). *Anal.* Calcd. for $C_{29}H_{46}O_6N_2$: C, 67.28; H, 8.76; N, 5.41. Found: C, 67.68; H, 8.91; N, 5.62.

***γ-tret*-Butyl L-Glutamate (VIb)**——A solution of IVb (2.0 g) in methanol (20 ml) was hydrogenated and treated as described above for the reduction of IIb. Crude product (940 mg, 80.8% yield) was reprecipitated from methanol–ether, mp 174—176°, 527 mg (45.3% yield), $[\alpha]_D^{22.5}$ +11.5° (c=2, H_2O), $[\alpha]_D^{23}$ +20.4° (c=1.9, 95% acetic acid). (lit.[12] mp 182°, $[\alpha]_D^{27}$ +9.83° (c=2, H_2O), lit.[11] mp 186—187°, $[\alpha]_D^{25}$ +10.1° (c=1, H_2O), $[\alpha]_D^{25}$ +18.1° (c=1, 95% acetic acid)). *Anal.* Calcd. for $C_9H_{17}O_4N$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.26; H, 8.65; N, 7.02.

N-Benzyloxycarbonyl-L-isoasparagine (VIIa)——Dried ammonia gas was bubbled into a solution of IIa (1.0 g) in absolute methanol (15 ml). The solution was allowed to stand at room temperature for 24 hr. After the evaporation 10% HCl was added to the residue, and crystalline product was filtered and recrystallized from hot water (20 ml) to give needles, mp 167—168°, 300 mg (30% yield), $[\alpha]_D^{23}$ −26.5° (c=1.02, Dimethylformamide). (lit.[10a] mp 164°, $[\alpha]_D^{25}$ −25.5° (c=1, Dimethylformamide)). *Anal.* Calcd. for $C_{12}H_{14}O_5N_2$: C, 54.13; H, 5.30; N, 10.52. Found: C, 54.06; H, 5.21; N, 10.63.

N-Benzyloxycarbonyl-L-isoglutamine (VIIb)——This substance was prepared in the same manner as described above for VIIa, mp 171.5—173°, 29% yield. (lit.[13] mp 175° (corr.)). *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.49; H, 5.66; N, 10.05.

N-Benzyloxycarbonyl-L-glutamic Acid α-Hydrazide (VIIc)——A solution of IIb (1.5 g) and 100% hydrazine hydrate (3 ml) in ethanol (3 ml) was allowed to react at room temperature for 43 hr. The mixture was added water (12 ml), acidified to pH 2 with concd. HCl and neutralized to pH 5 with sodium acetate powder. The solution was stored in refrigerator for 3 days. The crystalline product was filtered, washed with water and dried, 1.3 g (88% yield). Recrystallization from aqueous methanol gave a crystalline powder, mp 167—168°, 819 mg (55.5% yield), $[\alpha]_D^{23}$ −19.0° (c=2.9, 0.5N HCl). (lit.[14] mp 168—170°, $[\alpha]_D^{25}$ −20.5° (c=7.19, 0.5N HCl)). *Anal.* Calcd. for $C_{13}H_{17}O_5N_3$: C, 52.87; H, 5.80; N, 14.23. Found: C, 52.50; H, 5.59; N, 14.47.

Catalytic Reduction of 3-Benzoyl-5-oxazolidinone——A solution of 3-benzoyl-5-oxazolidinone (4.1 g) in the mixture of methanol (40 ml), acetic acid (5 ml) and water (5 ml) was hydrogenated in the presence of palladium black for 7 hr. Methyl benzoylglycinate (XIIa)[21] was obtained in 88% yield as major product with a trace of benzoylglycine.

***α*-Methyl N-*p*-Toluenesulfonyl-L-glutamate (XIIb)**——A solution of (*S*)-3-*p*-toluenesulfonyl-5-oxo-4-oxazolidinepropionic acid[5b] was hydrogenated and treated in essentially same manner as described for the

27) P.G. Stecher, M. Windholz, D.S. Leahy, D.M. Bolton, and L.G. Eaton, "Merck Index," 8th Ed., Merck and Co., Inc., Rahway, N.J., U.S.A., 1968, p. 497.
28) R. Schwyzer and H. Dietrich, *Helv. Chim. Acta*, **44**, 2003 (1961).
29) G. Losse and H. Weddige, *Ann. Chem.*, **678**, 148 (1964).

reduction of IIb to give syrupy product. Upon adding dicyclohexylamine to a solution of above syrup in ethanol, dicyclohexylammonium salt was obtained in 68% yield; mp 201—203° after recrystallization from water, $[\alpha]_D^{23}$ +67.6° (c=1.8, dimethylformamide). *Anal.* Calcd. for $C_{25}H_{40}O_6N_2S$: C, 60.45; H, 8.12; N, 5.64. Found: C, 59.82; H, 8.12; N, 5.76. Free acid XIIb was prepared in the usual manner, mp 158—160°, $[\alpha]_D^{23}$ −20.6° (c=1, dimethylformamide). (lit.[22] mp 158—161°, $[\alpha]_D^{23\text{-}5}$ −20.8° (c=1, dimethylformamide)).

General Procedures for the Coupling of IIa and IIb with Esters of Amino Acids and Peptides——a) DCCI Method[30]: A mixture of IIa or IIb (0.005 mole), hydrochloric or *p*-toluenesulfonic acid salt of amino acid ester or peptide ester (0.005 mole) and triethylamine (0.005 mole) in dry chloroform (25 ml) was added DCCI (0.005 mole) with stirring at 0°C for 4—6 hr. After the filtration, the product was extracted with ethyl acetate, washed with water, 1N HCl, 5% $NaHCO_3$ solution and water, wand dried over $MgSO_4$. Evaporation of the solvent gave the crude product. Benzyl (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine acetyl-L-isoleucinate (IXb) and benzyl (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine acetylglycinate (IXa) were prepared as a syrup in 84—90% yield, and used for next step without further purification.

b) MA Method Using Isobutyl Chloroformate[31]: A solution of isobutyl chloroformate (0.005 mole) in dry chloroform (5 ml) was added dropwise into a mixture of IIa or IIb (0.005 mole) and triethylamine (0.005 mole) in chloroform (20 ml) with stirring at −15° and stirring was continued for 15 min. Then, a solution of hydrobromic or *p*-toluenesulfonic acid salt of amino acid ester (0.005 mole) and triethylamine (0.005 mole) in chloroform (20 ml) was added to the reaction mixture; the whole mixture was allowed to react for 30 min at −10°C and then for 20 hr at room temperature. The reaction mixture was treated as described above. Benzyl (*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl-L-glutamate (IXc), benzyl (*S*)-3-3-benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl-L-isoleucinate (IXd), and benzyl (*S*)-3-benzyl-oxycarbonyl-5-oxo-4-oxazolidine propionyl-L-tyrosinate (IXe) were prepared as a syrup in 88—93% yield, and used for next step without purification.

c) MA Method Using $POCl_3$[32]: A solution of $POCl_3$ (0.01 mole) in chloroform (5 ml) was added dropwise into a stirred solution of IIb (0.01 mole), hydrochloric or hydrobromic acid salt of peptide ester (0.01 mole) and triethylamine (0.02 mole) in chloroform (40 ml) below 0°. The mixture was stirred for 1 hr at same temperature, for 1.5 hr at room temperature and then allowed to stand overnight. The reaction mixture was treated as described above.

Benzyl *tret*-Butyloxycarbonyl-L-alanylglycinate——*tert*-Butyloxycarbonyl-L-alanine was coupled with benzyl glycinate *p*-toluenesulfonate by MA method using isobutyl chloroformate in the similar manner as described above. Recrystallization from ethyl acetate–petroleum ether gave needles, mp 85—86°, 85.8% yield, $[\alpha]_D^{23}$ +57.3° (c=1.26, methanol). *Anal.* Calcd. for $C_{17}H_{24}O_5N_2$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.60; H, 7.15; N, 8.54.

Benzyl (*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl-L-alanylglycinate (IXf)——Benzyl *tert*-butyloxycarbonyl-L-alanylglycinate (1.7 g) was treated with 2.5N HCl in ethyl acetate (25 ml) for 2 hr at room temperature. Evaporation left oily benzyl L-alanylglycinate hydrochloride which was dried on NaOH pellet in a desiccator overnight and coupled with IIb by DCCI method and MA method using $POCl_3$ as described above in 87% and 76% yield respectively. A solution of the sample (1.0 g) in chloroform–methanol (2:1) was adsorbed on silica gel column which was eluted with same solvent system. Evaporation of the solvents gave colorless syrup (0.85 g) which was solidified on standing. Recrystallization from ethyl acetate–petroleum ether gave crystalline product, mp 107—109°, $[\alpha]_D^{23}$ +27.7° (c=1.68, ethyl acetate). *Anal.* Calcd. for $C_{26}H_{29}O_8N_3$: C, 61.05; H, 5.71; N, 8.22. Found: C, 61.13; H, 5.95; N, 7.91.

General Procedure for the Reduction of Oxazolidinyl Derivatives (IXa—f)——A solution of fully protected peptide (IXa—f, 0.004 mole) in the mixture of methanol (25 ml), acetic acid (5 ml) and water (3 ml) was hydrogenated in the presence of palladium black at room temperature for 4—6 hr. After the filtration,the solvent were removed under reduced pressure. The syrupy product was triturated with absolute ethanol or ethyl acetate, filtered and recrystallized from suitable solvent (Table I).

Ethyl(*S*)-3-Benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl-S-benzyl-L-cysteinylglycinate (XVIa)——Ethyl N-benzyloxycarbonyl-S-benzyl-L-cysteinylglycinate[32] (4.0 g) was dissolved in 25% hydrogen bromide in acetic acid (30 ml). After 1 hr at room temperature dry ether (400 ml) was added to precipitate hydrobromide of peptide ester. The supernatant liquid was decanted and oily residue was washed with ether and dried on NaOH pellet in a desiccator to give syrupy ethyl S-benzyl-L-cysteinylglycinate hydrobromide, 3.3 g (90%). IIb was coupled with above peptide ester by DCCI method and MA method using isobutyl chloroformate or $POCl_3$ as described above in 53, 30 and 64% yield respectively to give syrupy product which was used for the next step without purification. In the MA method using isobutyl chloroformate a by-product, ethyl N-isobutyloxycarbonyl-S-benzyl-L-cysteinylglycinate, was isolated by means of column chromatography (adsorbent: silica gel, solvent: chloroform–ethyl acetate (3:1)), mp 85—87° (recrystallized

30) J.C. Sheehan and G.P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).
31) J.R. Vaughan, Jr. and R.L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).
32) T. Wieland and B. Heinke, *Ann. Chem.*, **688**, 238 (1956).

from ethyl acetate-petroleum ether). *Anal.* Calcd. for $C_{19}H_{28}O_5N_2S$: C, 57.56; H, 7.12; N, 7.07. Found: C, 57.81; H, 7.28; N, 7.27.

Diethyl Bis-[(*S*)-3-benzyloxycarbonyl-5-oxo-4-oxazolidine propionyl]-L-cystinyldiglycinate (XVIb)——Diethyl N,N'-dibenzyloxycarbonyl-L-cystinyldiglycinate[33] was treated with 25% hydrogen bromide in acetic acid as described above for XVIa for 30 min. Dry ether was added to precipitate the product, which was filtered, washed with ether and dried on NaOH pellet to give hygroscopic powder in quantitative yield. IIb was coupled with above peptide ester by MA method using isobutyl chloroformate or $POCl_3$ in 76 and 82% yield respectively. Syrupy product was solidified and recrystallized from ethyl acetate–petroleum ether, mp 100—103°, $[\alpha]_D^{25}$ −54.7° (c=1.85, ethyl acetate). *Anal.* Calcd. for $C_{42}H_{52}O_{16}N_6S_2$: C, 52.49; H, H, 5.45; N, 8.74; S, 6.67. Found: C, 52.47; H, 5.52; N, 8.49; S, 6.46.

N-Benzyloxycarbonyl-γ-L-glutamyl-S-benzyl-L-cysteinylglycine (XVIIa)——A solution of XVIa in ethanol was treated with 1N NaOH in similar manner as described for saponification of IIb. Crude product was triturated with water, filtered and reprecipitated from ethanol–water, mp 93—106°, 75% yield, $[\alpha]_D^{23}$ −32.5° (c=2.1, absolute ethanol). (lit.[34a] mp 92—95°, $[\alpha]_D^{17}$ −31±1° (c=1.12, 96% ethanol); lit.[34b] mp 120° (sintered at 85°), $[\alpha]_D^{20}$ −23° (c=2, ethanol)). *Anal.* Calcd. for $C_{28}H_{34}O_8N_3S$: C, 56.49; H, 5.50; N, 7.91. Found: C, 56.63; H, 5.69; N, 8.21.

Bis-(N-Benzyloxycarbonyl-γ-L-glutamyl)-L-cystinyldiglycine Monohydrate (XVIIb)——A solution of XVIb in the mixture of ethanol and dioxane (1:1) was treated with 1N NaOH as described above. The product was extracted with ethyl acetate containing dioxane and reprecipitated from ethyl acetate–petroleum ether, amorphous powder mp 90—95° (decomp.), 97% yield. (lit.[35] mp 108—120° (decomp.)). *Anal.* Calcd. for $C_{36}H_{44}O_{16}N_6S_2 \cdot H_2O$: C, 48.10; H, 5.16; N, 9 35. Found: C, 47.94; H, 5.20; N, 9.13.

Glutathione (XVIII)———a) XVIIa (2.0 g) was dissolved in dry liquid NH_3 (100 ml) with stirring. Sodium (*ca.* 0.5 g) was introduced in small portions, untill a permanent blue color was obtained. $(NH_4)_2CO_3$ was added to discharge blue color, the liquid NH_3 was evaporated and the residue was placed in a desiccator over conc. H_2SO_4 overnight. A solution of the crude product in 0.1N HCl (130 ml) was washed with ether, added a solution of $CdSO_4$–XH_2O (2.5 g) in water (30 ml) and adjusted to pH *ca.* 6[36] Formed precipitate was collected and washed at the centrifuge with water. H_2S gas was bubbled into a suspension of the Cd salt in water (20 ml), formed sulphide was centrifuged and the supernatant liquid was treated with IRA-402 (H^+ form, 7 ml) which was washed 5% acetic acid. The combined aqueous eluate was lyophilized in a conventional apparatus. Glutathione was obtained as a bulky amorphous solid, 522 mg (45.4% yield), PPC Rf=0.17 (solvent system A) which was identical with that of authentic sample. Recrystallization from aqueous ethanol gave pure glutathione, mp 189—191° (decomp.), 322 mg (28% yield), $[\alpha]_D^{23}$ −21.4° (c=2.0, water). (lit.[37] mp 186—190° (decomp.), $[\alpha]_D^{27}$ −21° (c=2.74, water)).

b) Crude product obtained from XVIIa was purified through the Hg salt[38] and IRA-402 treatment. Amorphous product was obtained in 19.4% yield.

c) XVIIb (2.0 g) was reduced with sodium in liquid NH_3. A solution of the crude product in water was acidified with acetic acid, washed with ether and added a solution of lead acetate (4.4 g) in water (20 ml). The precipitate was collected and washed at centrifuge with water. H_2S gas was bubbled into a suspension of the Pb salt in water (20 ml), formed sulphide was centrifuged and the supernatant liquid was lyophilized; amorphous solid 572 mg (41% yield).

d) Crude product obtained from XVIIb was purified through the Cu salt and subsequent H_2S treatment.[38] Amorphous product was obtained in 15.5% yield.

Acknowledgement The author express his deep gratitude to Dr. S. Sakakibara of Institute for Protein Research, Osaka University, and Dr. H. Nakano of this laboratory for their kind advices and encouragements. Thanks are also to Mr. D. Morino for his skillful technical assistance.

33) E. Schnabel, *Ann. Chem.*, **688**, 238 (1965).
34) *a*) J. Rudinger and F. Šorm, *Collection Czech. Chem. Commun.*, **16**, 214 (1951); *b*) W. Ried and G. Franz, *Ann. Chem.*, **644**, 141 (1961).
35) M. Murakami, I. Isaka, N. Inukai, and K. Kubo, Japan. Patent 16529 (1965) [*C.A.*, **63**, 16466 (1965)].
36) F. Kögl and A. M. Akkerman, *Rec. Trav. Chim.* **65**, 216 (1946)[*C.A.*, **40** 5017 (1946)].
37) S. Goldschmidt, W. Lautenschlager, B. Kolb, and G. Zumach, *Chem. Ber.*, **97**, 2434 (1964).
38) M. Murakami, I. Isaka, and M. Kochitani, Japan. Patent 17727-1967[*C.A.*, **68**, 87596(1968)].