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Solid Phase Peptide Synthesis employing Haloacylpolystyrene as a Polymer Support[1)]

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A modified solid phase method for peptide synthesis employing haloacyl polystyrene resins is described. For this purpose, several kinds of haloacylated copolystyrene-divinylbenzene were prepared, of which the bromoacetyl derivative was of choice. Protected amino acids could be attached to or released from this type of resin more smoothly than in the case of the benzyl type. Protected amino acids and their amide, hydrazide and ester derivatives were obtained by cleavage of the amino acid resins (III) by means of alkaline aqueous dioxane, sodium thiophenoxide in dimethylformamide, methanolic ammonia, hydrazine hydrate in methanol or methanol containing a catalyst under mild reaction conditions. This modification was successfully applied to syntheses of several small peptides.

Although the overall yields were slightly lower than in the original solid phase method, the peptides and their C-terminal amide derivatives could be prepared easily with preservation of all the protecting groups on them. Therefore, this method may also be useful for the preparation of peptide fragments in larger polypeptide synthesis.

Since Merrifield and his collaborators introduced in 1962 the ingenious method for peptide synthesis, known as solid phase peptide synthesis or Merrifield Synthesis,[3a–g)] rapid synthesis of various kinds of biologically active polypeptide such as insulin[3f)] and ribonuclease A[3g)] has been made feasible. This method consists of employing an insoluble polymer support, chloromethylated polystyrene beads, on which a peptide is synthesized stepweise by the dicyclohexylcarbodiimide (DCC) or active ester method, and of cleaving the completed peptide from the support with the aid of dry hydrogen bromide in trifluoroacetic acid or liquid hydrogen fluoride; all the isolation or purification steps except washing of the intermediates, and the neccessity of changing reaction vessels in the elongation steps are thus eliminated allowing a high yield of the product.

Several attempts have been made on modification of the polymer support in the last few years[4a,b)] for milder attachment of a protected amino acid to the resin, or more convenient cleavage of the finished peptide from the resin.

1) Preliminary communication, *Chem. Pharm. Bull.* (Tokyo), **17**, 411 (1969).

2) Location: *2–2–50, Kawagishi, Toda-shi, Saitama.*

3) *a*) R.B. Merrifield, *Federation Proc.*, **21**, 412 (1962); *b*) *Idem*, *Biochemistry*, **3**, 1385 (1964); *c*) *Idem*, *Science*, **150**, 178 (1965); *d*) V.A. Najjar and R.B. Merrifield, *Biochemistry*, **4**, 2394 (1965); *e*) G.R. Marshall and R.B. Merrifield, *ibid.*, **5**, 3765 (1966); *f*) A. Marglin and R.B. Merrifield, *J. Am. Chem. Soc.*, **88**, 5051 (1966); *g*) B. Gutte and R.B. Merrifield, *ibid.*, **91**, 501 (1969).

4) *a*) When the present work was almost finished, a brief communication of a modification similar to the present method appeared: *tert*-butyloxycarbonyl(BOC)-alanylalanine was attached to bromoacetyl copolystyrene-divinylbenzene and the peptide bond was elongated by the fragment condensation method employing N-hydroxysuccinimide esters of N-acyl peptides. The finished peptide resin was cleaved by treatment with sodium thiophenoxide. However, details or further development of this method has not yet been reported (F. Weygand, "Peptides 1968," ed. by E. Bricas, North Holland Publishing Co., Amsterdam, 1968, p. 183); *b*) Th. Wieland and Ch. Birr, *Chimia*, **21**, 581 (1967); M.M. Shemyakin, Yu A. Ovchinnikov, A.A. Kiryushkin and I.V. Kozhevnikova, *Tetrahedron Letters*, **1965**, 2323; M.A. Tilak and C.S. Hollinden, *ibid.*, **1968**, 1297; N. Inukai, K. Nakano and M. Murakami, *Bull. Chem. Soc. Japan*, **41**, 182 (1968); R. Camble, R. Garner and G.T. Young, *Nature*, **217**, 247 (1968); B. Green and L.R. Garson, *J. Chem. Soc.* (*C*), **1969**, 401; G.L. Southard, G.S. Brooke and J.M. Pettee, *Tetrahedron Letters*, **1969**, 3505; G. Losse, C. Madlung and P. Lorenz, *Chem. Ber.*, **101**, 1257 (1968); Yu A. Ovchinnikov, A.A. Kiryushkin and I.V. Kozhevnikova, *J. Gen. Chem. USSR* (Eng. Transl.), **38**, 2546 (1968).

This paper deals with a new type of polymer support, haloacylpolystyrene, and a preliminary evaluation thereof for peptide synthesis. Application of this type of resin may have advantage for esterification, deprotection and cleavage steps during peptide synthesis and would also provide a new method for preservation of protecting groups on a peptide released from the resin as acid, amide, hydrazide, or in some cases, ester derivative. The phenacyl ester group as used for protection of the carboxyl function in peptide chemistry has appeared in some reports,[5] in which it is noted that this group is more stable than the benzyl ester group to hydrogen bromide and more labile to strong nucleophilic reagents such as thiophenoxide ion.

Several kinds of haloacyl copolystyrene–divinylbenzene were prepared by direct haloacylation of polystyrene beads by the Friedel–Crafts reaction or by acetylation followed by bromination of the resin. Of these two routes examined, the former was considered to be more suitable for the preparation because of the simplicity of the reaction procedure and a better agreement in halogen content of the product with that calculated from weight increase. The best result was obtained when copolystyrene-2%-divinylbenzene (I), swelled in nitrobenzene, was treated with 0.4 equivalent amount of bromoacetyl bromide and aluminum chloride at room temperature. Dichloromethane could also been used as solvent, but with a slightly less extent of introduction of the haloacyl group to the resin. The 2-bromopropionyl resin (II: X=Br, R=CH_3) was prepared in this solvent from polystyrene and bromopropionyl bromide. The results obtained are summarized in Table I.

Esterification of protected amino acids with these resins was variously attempted using *tert*-butyloxycarbonyl (BOC)[6]-, *tert*-amyloxycarbonyl (AOC)[7]-, or *o*-nitrophenylsulphenyl (NPS)[8]-amino acids as shown in Table II. Ethyl acetate or dimethylformamide was successfully used as solvent, in which the reaction proceeded smoothly at room temperature in the presence of an equivalent amount of triethylamine. High reaction temperature was not effective in this esterification. Reactivity of the chloroacetyl group was less sufficient than that of the bromoacetyl type for the purpose of peptide synthesis. Concerning the effect of side chains of amino acids on the reactivity, the glycine derivatives seemed to be slightly more reactive than other amino acid derivatives. These results suggest that the bromoacetyl polystyrene may be superior to the chloromethyl resin which requires 48 hour reflux in ethanol for the esterification step.

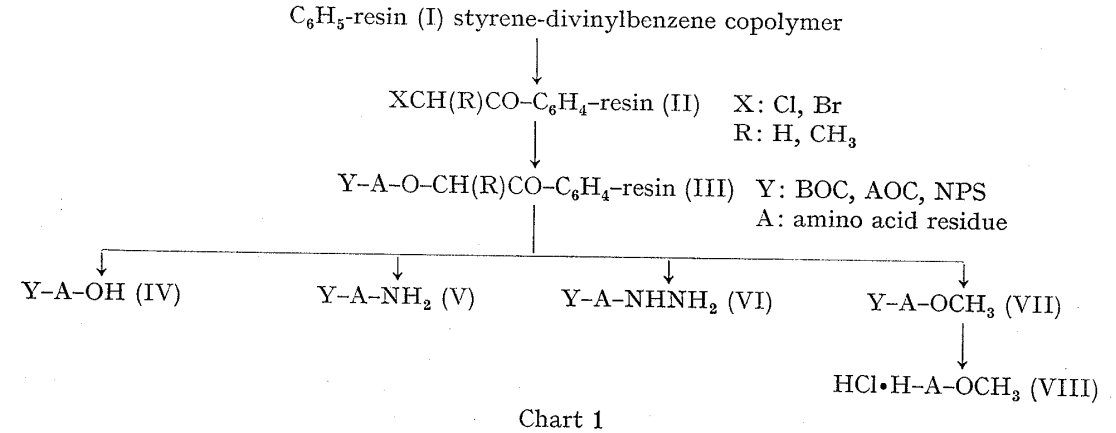


Chart 1

5) *a*) J.C. Sheehan, G.D. Dave and Jr., *J. Org. Chem.*, **29**, 2006 (1964); *b*) G.C. Stelakatos, A. Paganou and L. Zervas, *J. Chem. Soc.* (*C*), **1966**, 1191.
6) G.W. Anderson and A.C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).
7) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inoue and N. Inukai, *Bull. Chem. Soc. Japan*, **38**, 1522 (1965).
8) L. Zervas, D. Borovas and E. Gazis, *J. Am. Chem. Soc.*, **85**, 3660 (1963).

Release of peptides from the phenacyl type polystyrene was then examined taking several BOC-, AOC- or NPS-amino acid resins (III) as model compounds (Table III). BOC-Glycine, BOC-leucine and BOC-O-benzylserine were recovered in good yield by treatment of the corresponding protected amino acid resins (III) with alkali in aqueous dioxane or sodium thiophenoxide in dimethylformamide,[5a] whereas a poor yield was obtained in the alkaline hydrolysis of the NPS-O-benzylseryl resin (III: Y-A=NPS-O-benzyl-Ser, R=H). The latter result is consistent with the observation of Zervas, *et al.*[5b] that the rates of alkaline hydrolyses of NPS-amino acid alkyl esters are considerably low with the exception of the glycine derivative.

Hydrazinolysis also took place smoothly when the amino acid resins (III) were treated with an excess of hydrazine hydrate in methanol for several hours at room temperature. Experiments on AOC-L- and AOC-D-phenylalanine hydrazides showed that these reaction conditions did not affect the optical purity of the products.

An ester exchange reaction of the BOC-leucyloxyacetyl resin (III: Y-A=BOC-Leu, R=H) in methanol containing triethylamine as catalyst was successfully carried out affording BOC-leucine methyl ester (VII: Y-A=BOC-Leu) in 63% yield. VII gave optically pure leucine methyl ester hydrochloride (VIII: A=Leu) on treatment with methanolic dry hydrogen chloride. The BOC-glycyloxyacetyl resin (III: Y-A=BOC-Gly, R=H) also gave BOC-glycine methyl ester (VII: Y-A=BOC-Gly) in a good yield. These results are comparable with the observation of Stewart, *et al.* in their experiment with the benzyl ester type of polystyrene.[9] Imidazole was also found to be effective as catalyst in this transesterification.

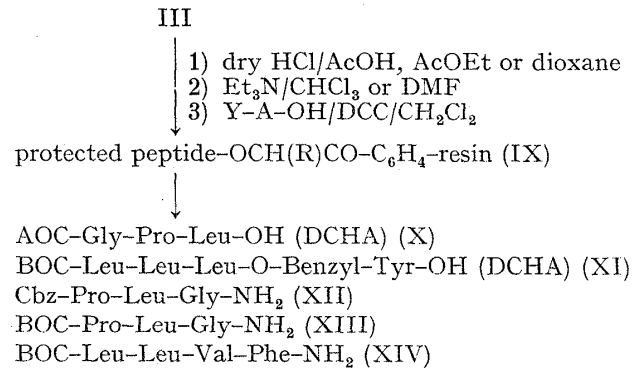


Chart 2

An application of the bromoacetyl and bromopropionyl resins for peptide synthesis was then attempted in syntheses of several small peptide derivatives.

Removal of the BOC or AOC group from the protected amino acid resin (III) was performed generally in the same manner as described by Merrifield, but in some cases, dry hydrogen chloride in ethyl acetate (concentration: *ca.* 2N) instead of acetic acid originally reported was employed for the deprotection, because the phenacyl ester group was found to be slightly more stable to this reagent in ethyl acetate than in acetic acid in a model experiment with BOC-glycine phenacyl ester.

A threefold excess of each protected amino acid and DCC in dichloromethane were used in the coupling steps according to the method of Merrifield and the completed peptide resins were cleaved in various ways as examined in the model experiments described above. The peptide derivatives thus obtained are X, XI, XII, XIII and XIV listed in Chart 2.

BOC-Leucylleucylleucyl-O-benzyltyrosine (XI) was obtained from the corresponding protected peptide-oxyacetyl (IX: R=H) and -oxypropionyl (IX: R=CH_3) resins in overall 36 and 14% yield, respectively and their dicyclohexylamine (DCHA) salts were practically identical. The methyl ester (XV), derived from XI in a good yield by treatment with diazomethane, was deprotected by hydrogenation in methanolic hydrogen chloride affording hydrochloride of leucylleucylleucyltyrosine methyl ester (XVI), whose melting point, specific

9) J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and Co., San Francisco, 1969, p. 12.

optical rotation and behavior on thinlayer chromatography and electrophoresis were satisfactorily identical with those of a specimen prepared by a conventional method.[1,10]

$$\underset{\text{XV}}{\text{BOC-Leu-Leu-Leu-O-benzyl-Tyr-OCH}_3} \longrightarrow \underset{\text{XVI}}{\text{HCl•H-Leu-Leu-Leu-Tyr-OCH}_3}$$

$$\text{XIV} \longrightarrow \underset{\text{XVII}}{\text{HCl•H-Leu-Leu-Val-Phe-NH}_2}$$

BOC-Prolylleucylglycine amide (XIII) was obtained by treatment of the corresponding peptide resin (IX: R=H) with methanolic ammonia overnight at room temperature (61% from III). Dimethylformamide was also useful as solvent for this ammonolysis. Ammonolysis of the BOC-leucylleucylvalylphenylalanyloxyacetyl resin (IX: R=H) gave the corresponding protected peptide amide (XIV) in an overall yield of 43% (calculated from the starting BOC-phenylalanyloxyacetyl resin (III: Y-A=BOC-Phe, R=H)). The product was deprotected to furnish leucylleucylvalylphenylalanine amide hydrochloride (XVII), which was identified with an authentic sample.[10]

Although all the esterification, hydrolysis, ammonolysis, hydrazinolysis and transesterification reactions in this modified solid phase method proceeded under mild reaction conditions as expected, overall yields of the peptides were somewhat lower than in the original method. This is partly due to some losses of the final products in the purification steps involving recrystallizations and also presumably due to cleavage to some extent of intermediates from the polymer supports during the repeated deprotection steps using hydrogen chloride in acetic acid or ethyl acetate.

Table I. Synthesis of Haloacylcopolystyrene-divinylbenzene (II)

Resin (I) (200—300 mesh) DVB (%)	$AlCl_3$ (mole)[a]	X-CH(R)CO-X	(mole)[a]	Solvent	Method[b]	Time(hr)	Product (II) (X: meq/g)[c]
0.5	0.25	X:Cl R:H	0.20	$C_6H_5NO_2$	A	24	Cl: 1.54
2	0.40	X:Cl R:H	0.40	CH_2Cl_2	A	21	Cl: 1.60
2	0.11	X:Br R:H	0.05	$C_6H_5NO_2$	A	22	Br: 0.52
1	0.23	X:Br R:H	0.10	$C_6H_5NO_2$	A	23	Br: 0.77
1	0.68	X:Br R:H	0.30	$C_6H_5NO_2$	A	21	Br: 1.88
2	0.40	X:Br R:H	0.40	$C_6H_5NO_2$	A	21	Br: 2.24
2	0.40	X:Br R:H	0.40	CH_2Cl_2	A	21	Br: 1.29
2	0.40	X:Br R:H	0.40	CH_2Cl_2	B	3.5	Br: 1.02
2[d]	0.30	X:Br R:H	0.30	$C_6H_5NO_2$	B	22	Br: 1.64
1	0.22	X:Br R:CH_3	0.10	$C_6H_5NO_2$	A	24	Br: 0.83
2	0.40	X:Br R:CH_3	0.40	CH_2Cl_2	A	21	Br: 1.55

a) mole for 100 g of the resin
b) Prepared by a procedure similar to Method A or B for the bromoacetyl resin described in the experimental part.
c) Measured by the modified Volhard method.[12]
d) 100 mesh

10) Details of the synthesis will be reported in a separated paper.

Table II. Attachment of Protected Amino Acids to the Resin (II)

Resin (II, 200—300 mesh) DVB (%)		Halogen content (meq/g)	Amino acid derivs	Solvent	Time(hr)	Product (III) (mmole amino acid/g)[a]
2	X:Cl, R:H	1.60	AOC-Gly-OH	AcOEt	21	0.36
2	X:Cl, R:H	1.60	AOC-Gly-OH	CH_2Cl_2	21	0.16
1	X:Br, R:H	0.61[b]	BOC-Gly-OH	AcOEt	19	0.34
1	X:Br, R:H	0.61[b]	BOC-Gly-OH	AcOEt	30 (at 50°)	0.34
2	X:Br, R:H	1.02	BOC-Gly-OH	AcOEt	22	0.71 0.73[c]
1	X:Br, R:H	1.88	AOC-Gly-OH	DMF	23	0.96
1	X:Br, R:H	1.88	AOC-Gly-OH	CH_2Cl_2	20	0.33
1	X:Br, R:H	1.88	AOC-Gly-OH	EtOH	20	0.12
2	X:Br, R:H	2.24	AOC-Phe-OH	AcOEt	23	0.92
2	X:Br, R:H	2.24	AOC-D-Phe-OH	DMF	23	0.85
2	X:Br, R:H	2.24	AOC-Phe-OH	CH_2Cl_2	23	0.61
1	X:Br, R:H	1.88	BOC-Leu-OH	AcOEt	23	1.05
2	X:Br, R:H	2.24	BOC-O-Bzl[d]-Tyr-OH	AcOEt	23	1.01
2[e]	X:Br, R:H	1.64	BOC-O-Bzl[d]-Ser-OH	AcOEt	22	0.96
2[e]	X:Br, R:H	1.64	NPS-O-Bzl[d]-Ser-OH	AcOEt	22	0.76
2	X:Br, R:CH_3	1.55	BOC-O-Bzl[d]-Tyr-OH	AcOEt	23	0.41

a) Measured by the modified Volhard method.[12]
b) Prepared by acetylation followed by bromination of the resin.
c) Measured by the method of Moore and Stein[13] after the alkaline hydrolysis of III (Experimental part).
d) benzyl
e) 100 mesh

Table III. Cleavage of the Protected Amino Acid Resin (III)

Resin (III, R:H)[a] DVB (%)	Y-A-	mmole/g	Reagent	Time (hr)	Product (IV,V, VI,VII)	Yield (%)	mp (°C)	$[\alpha]_D^{20}$
2	BOC-Gly-	0.69	0.5N NaOH/ dioxane	6	BOC-Gly-OH	quant.	82—85	—
1	BOC-Leu-	1.05	0.5N NaOH/ dioxane	6	BOC-Leu-OH·H_2O	99	82—85	−24.9° (*c*=2.2, AcOH)
2[b]	BOC-O-Bzl-Ser-	0.96	0.5N NaOH/ dioxane	6	BOC-O-Bzl-Ser-OH (DCHA)	67	129—9.5	+24.0° (*c*=2.5, MeOH)[c]
2[b]	BOC-O-Bzl-Ser-	0.96	C_6H_5SNa/ DMF	5	BOC-O-Bzl-Ser-OH (DCHA)	73	128—130	+25.0° (*c*=1.0, MeOH)[d]
2[b]	NPS-O-Bzl-Ser-	0.76	0.5N NaOH/ dioxane	6	NPS-O-Bzl-Ser-OH (DCHA)	27	155	−12.8° (*c*=1.4, DMF)[c]
2	AOC-Phe-	0.92	NH_3/MeOH	overnight	AOC-Phe-NH_2	87	126—127	−3.4° (*c*=0.9, AcOH)
2	AOC-Phe-	0.92	$N_2H_4\cdot H_2O$/ MeOH	3	AOC-Phe-$NHNH_2$	88	109	−5.1° (*c*=1.2, AcOH)
2	AOC-D-Phe-	0.85	$N_2H_4\cdot H_2O$/ MeOH	3	AOC-D-Phe-$NHNH_2$	92	109	+5.1° (*c*=1.3, AcOH)
2	BOC-Gly-	0.71	Et_3N/MeOH	23	BOC-Gly-OCH_3	82	oil	—
1	BOC-Leu-	1.05	Et_3N/MeOH	23	BOC-Leu-OCH_3	63	oil	—
1	BOC-Leu-	1.05	imidazole/ MeOH	35	BOC-Leu-OCH_3	72	oil	—

a) 200—300 mesh *b*) 100 mesh *c*) 25° *d*) 26°

Experimental[11]

Bromoacetyl Resin (II: X=Br, R=H)——Method A: A solution of 26.7 g (0.20 mole) of aluminium chloride in 200 ml of nitrobenzene was mixed with a solution of 40.4 g (0.20 mole) of bromoacetyl bromide in 25 ml of the same solvent at 5° and stirred for 10 min.

To this mixture was added portionweise copolystyrene–divinylbenzene (I, 2% DVB, 200—300 mesh, 50 g) and the whole was stirred for 1 hr at room temperature, diluted with 75 ml of the additional solvent and stirred further for 20 hr. The mixture was filtered and the resin was washed throughly with 80% MeOH, water, DMF, $CHCl_3$ and MeOH and dried *in vacuo* at 50—60° to give 70.75 g of the product (II) as pale yellow beads. A portion (212.3 mg) of this product suspended in 2.0 ml of pyridine was heated for 1 hr at 100°. The solvent was removed *in vacuo* and the residue was analyzed by the modified Volhard method.[12] Br: 2.24 meq/g (2.16 meq/g as determined by elemental analysis and 2.43 meq/g by weight increase).

Method B: Copolystyrene–divinylbenzene (I, 2% DVB, 100 mesh, 50 g) was stirred in 200 ml of nitrobenzene for 30 min at room temperature and then cooled to 8°. To this was added slowly a solution of 30.3 g (0.15 mole) of bromoacetyl bromide in 50 ml of nitrobenzene and after the mixture had been stirred for 10 min, 20 g (0.15 mole) of aluminium chloride was added in a period of 10 min. After being diluted with 25 ml of the additional solvent, the whole mixture was stirred at room temperature for 22 hr and worked up as described in Method A giving 68.0 g of pale yellow beads. IR ν_{max}^{KBr} cm^{-1}: 1663 (bromoacetyl). Br: 1.64 meq/g (modified Volhard method).[12]

2-Bromopropionyl Resin (II: X=Br, R=CH_3)——A solution of 25.9 g (0.12 mole) of 2-bromopropionyl bromide in 40 ml of CH_2Cl_2 was mixed slowly with 16.0 g (0.12 mole) of aluminium chloride in 50 ml of the same solvent with stirring and cooling in an ice–water bath. After 15 min, copolystyrene-divinylbenzene (I: 2% DVB, 200—300 mesh, 30 g) was added portionweise to this solution and the whole mixture was diluted with the solvent (60 ml) and stirred for 21 hr at room temperature. The resin was collected and washed as for the bromoacetyl resin described above to give II (39.5 g). IR ν_{max}^{KBr} cm^{-1}: 1670 (bromopropionyl). Br: 1.55 meq/g (modified Volhard method),[12] 1.55 meq/g (elemental analysis).

Attachment of Protected Amino Acids to Resins (II)——BOC-Glycyloxyacetyl Resin (III: Y–A=BOC–Gly, R=H): The bromoacetyl resin (II: 2% DVB, X=Br, R=H, 200—300 mesh, 20.81 g) containing 1.02 meq Br/g was stirred in 120 ml of AcOEt for 30 min.

To this was added a solution of BOC-glycine (3.72 g, 21.23 mmoles) and Et_3N (2.15 g, 21.23 mmoles) in 60 ml of AcOEt, and the whole mixture was stirred for 22 hr at room temperature. The resin was filtered, washed throughly with AcOEt, $CHCl_3$, dioxane–water (3:1, v/v), water, dioxane and MeOH and dried overnight *in vacuo* at room temperature to give 21.9 g of the product (III).

Determination of BOC-glycyl Residue on Resin (III)——a) A portion (277 mg) of the foregoing BOC-glycyloxyacetyl resin was stirred in 2.5N dry HCl/AcOEt for 40 min and filtered. The resin was washed throughly with AcOEt, dioxane, aqueous dioxane, water and MeOH. The resulting glycyloxyacetyl resin hydrochloride was then suspended in DMF, treated with 10 ml of DMF containing 1 ml of Et_3N, filtered and washed with DMF. The combined filtrate and washings were evaporated to dryness *in vacuo* and the residue, triethylamine hydrochloride, was analyzed by the modified Volhard method.[12] Gly=Cl:0.71 mmole/g.

b) A mixture of the BOC-glycyloxyacetyl resin (144 mg), 0.5 ml of 1N NaOH and 1.5 ml of dioxane was refluxed for 6 hr. The filtered solution was neutralized with 0.5 ml of 1N HCl and evaporated *in vacuo*. Dry HCl/AcOH (1.4N, 2 ml) was added to the residue and after being kept to stand for 30 min, the mixture was again evaporated *in vacuo*. The residue was dissolved in 0.2 ml of 1N NaOH, concentrated *in vacuo* and analyzed by the method of Moore and Stein.[13] Gly: 0.73 mmole/g.

Attachment of several other amino acid derivatives to the resins was attempted in a similar manner as for the BOC-glycyl resin described above. Experimental data thus obtained were summarized in Table II.

Cleavage of Protected Amino Acid Resins (III)——BOC-Leucine: A mixture of the BOC-leucyloxyacetyl resin (III: Y–A=BOC–Leu, R=H, Leu: 2.10 mmoles, 2 g) and a solution of 16.8 ml of dioxane and 8.4 ml of 0.5N NaOH was stirred for 3 hr at room temperature and filtered. The resin was washed several times with dioxane–water (2:1, v/v) and the combined filtrate and washings were neutralized with 1N HCl and evaporated *in vacuo*. The residue was dissolved in 10 ml of water, acidified with 1N HCl (pH 2) and extracted repeatedly with AcOEt. The combined organic extracts were washed with satd. NaCl solution, dried and evaporated to leave an oil, which was crystallized from water–EtOH to give 0.36 g of BOC-leucine as monohydrate, mp 82—85°, $[\alpha]_D^{20}$ −24.9° (c=2.2, AcOH) (lit.,[7] mp 67—72°, $[\alpha]_D^{25}$ −24° (c=2, AcOH)). This procedure was repeated on the residual resin and 0.16 g of second crop was obtained, mp 83—85°, $[\alpha]_D^{25}$ −24.7° (c=2.2, AcOH). Total yield, 0.52 g (99%).

11) Melting points were uncorrected.
12) R.B. Merrifield, personal communication; J.M. Stewart and J.D. Young, "Solid Phase Peptide Synthesis," W.H. Freeman and Co., San Francisco, 1969, pp. 6, 55—56.
13) S. Moore and W.H. Stein, *J. Biol. Chem.*, **211**, 907 (1954).

The product was identified with an authentic sample by mixed melting and IR analyses.

BOC-O-Benzylserine: a) BOC-O-Benzylseryloxyacetyl copolystyrene-divinylbenzene (III: Y-A=BOC-O-benzyl-Ser, R=H, 2% DVB, 100 mesh, Ser: 1.04 mmoles, 1.08 g) was saponified in a solution of dioxane (12 ml) and 0.5N NaOH (6 ml) for 6 hr at room temperature. The resin was filtered, washed with dioxane–water (2:1, v/v) and the combined filtrate and washings were neutralized and evaporated *in vacuo*.

The residue was dissolved in 10 ml of water, acidified with 1N HCl (pH 2) and extracted with AcOEt. The AcOEt extract was washed with satd. NaCl solution, dried and evaporated *in vacuo* to leave an oil, which was taken up in ether and precipitated by addition of DCHA (0.16 g) to give 0.33 g (67%) of BOC-O-benzylserine as DCHA salt, mp 129—129.5°, $[\alpha]_D^{25}$ +24.0° (c=2.5, MeOH). This product was identified with an authentic sample, mp 130.5—131°, $[\alpha]_D^{26}$ +25.5° (c=2.3, MeOH), by mixed melting test and IR analysis.

b) A mixture of the BOC-O-benzylseryloxyacetyl resin (1.01 g, Ser: 0.97 mmole), 10 ml of DMF and 0.53 g (4.01 mmoles) of Na thiophenoxide was stirred for 5 hr at room temperature. The resin was filtered off, washed with DMF and the combined filtrate and washings were evaporated *in vacuo*. The residue obtained was dissolved in 10 ml of water, acidified with 1N HCl (pH 2) and extracted three times with AcOEt. The combined extracts were re-extracted with dil. $NaHCO_3$ solution and the aqueous layer was acidified and extracted again with AcOEt. The extract was washed, dried and evaporated to give an oily residue, which was converted to DCHA salt as described in a).

Yield, 0.33 g (73%), mp 128—130°, $[\alpha]_D^{26}$ +25.0° (c=1.0, MeOH).

Hydrolysis of the BOC-glycyl and the NPS-O-benzylseryl resins was attempted in essentially the same manner as described in a). The data obtained were listed in Table III.

AOC-Phenylalanine Amide——To a suspension of AOC-phenylalanyloxyacetyl copolystyrene-divinylbenzene (III: Y-A=AOC-Phe, R=H, 2% DVB, 200—300 mesh, Phe: 2.76 mmoles, 3.0 g) in 30 ml of MeOH was introduced gaseous NH_3 for 3.5 hr with cooling in an ice-water bath and the mixture was stirred gently for 20 hr at room temperature in a stoppered bottle. The resulting mixture was then evaporated to leave a crystalline residue, which was washed with water, dried and recrystallized from AcOEt–iso-Pr_2O affording 0.67 g (87%) of colorless needles, mp 126—127°, $[\alpha]_D^{20}$ −3.4° (c=0.9, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1670 (AOC), 1634, 1622 (amide). *Anal.* Calcd. for $C_{15}H_{22}O_3N_2$: C, 64.72; H, 7.97; N, 10.07. Found: C, 64.84; H, 8.23; N, 9.75.

AOC-Phenylalanine Hydrazide——A mixture of 20 g of AOC-phenylalanyloxyacetyl copolystyrene-divinylbenzene (III: Y-A=AOC-Phe, R=H, 2% DVB, 200—300 mesh, Phe: 18.40 mmoles), 400 ml of MeOH and 10 g of 100% hydrazine hydrate was stirred for 3 hr at room temperature and filtered. The resin was washed well with MeOH and the combined filtrate and washings were evaporated *in vacuo* to give a crystalline residue, which was washed with water, dried and recrystallized from EtOH–iso-Pr_2O to give colorless needles. Yield, 4.74 g (88%), mp 109°, $[\alpha]_D^{20}$ −5.1° (c=1.2, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1670 (AOC), 1646, 1600 (hydrazide). *Anal.* Calcd. for $C_{15}H_{23}O_3N_3$: C, 61.41; H, 7.90; N, 14.33. Found: C, 61.29; H, 7.97; N, 14.39.

AOC-D-Phenylalanine Hydrazide——This was obtained by treatment of 2.5 g of AOC-D-phenylalanyloxyacetyl resin (III: Y-A=AOC-D-Phe, 2% DVB, 200—300 mesh, D-Phe: 2.12 mmoles) in 50 ml of MeOH with 1.25 g of hydrazine hydrate, and the work-up as for the L-isomer described above. Yield, 0.58 g (92%), mp 109°, $[\alpha]_D^{20}$ +5.1° (c=1.3, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1670 (AOC), 1646, 1600 (hydrazide). The IR spectrum was completely superimposable on that of the L-isomer.

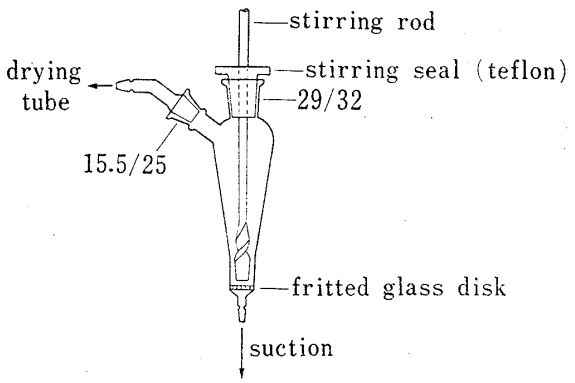
BOC-Leucine Methyl Ester——a) A suspension of 2.95 g of BOC-leucyloxyacetyl copolystyrene-divinylbenzene (III: Y-A=BOC-Leu, R=H, 2% DVB, 200—300 mesh, Leu: 3.10 mmoles) in 60 ml of MeOH containing 9.4 g of Et_3N was stirred for 23 hr at room temperature. The resin was filtered, washed with MeOH and the combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was dissolved in AcOEt, washed, dried and evaporated. Complete removal of the solvent from the residue under highly reduced pressure gave 0.48 g (63%) of BOC-leucine methyl ester as an oil. IR ν_{max}^{Liquid} cm^{-1}: 1735 (ester), 1705 (BOC). This product was identified with a specimen prepared by esterification of BOC-leucine monohydrate in MeOH with diazomethane, comparing their IR and NMR spectra. A portion (0.45 g) of this product was converted to leucine methyl ester hydrochloride by treatment with 10 ml of 20% dry HCl/MeOH for 30 min at room temperature. The solvent was removed and the residue was washed with acetone to give 0.23 g (87%) of the product, mp 149—150°, $[\alpha]_D^{16}$ +14.0° (c=1.3, water). Lit.[14] mp 146—148°, $[\alpha]_D^{20}$ +13.2° (c=4.9, water).

b) The above BOC-leucyloxyacetyl resin (Leu: 3.34 mmoles, 3.18 g) was transesterified in MeOH (64 ml) containing 6.8 g of imidazole as catalyst for 35 hr and worked up as described in a) to give 0.59 g (72%) of BOC-leucine methyl ester, IR ν_{max}^{Liquid} cm^{-1}: 1735 (ester), 1705 (BOC). A part of this product was converted to leucine methyl ester hydrochloride, mp 148—149°, which was identified with the authentic sample by mixed melting test and IR and NMR analyses.

AOC-Glycylprolylleucyloxyacetyl Resin——BOC-Leucyloxyacetyl copolystyrene-divinylbenzene (III Y-A=BOC-Leu, R=H, 1% DVB, 200—300 mesh, Leu: 15.75 mmoles, 15.0 g) suspended in 150 ml of 1.47N

14) J.R. Rachele, *J. Org. Chem.*, **28**, 2898 (1963).

dry HCl/AcOH was stirred at room temperature for 40 min in a reaction vessel designed as shown in Fig, and then filtered through the fritted glass disk at the bottom of the apparatus. The resin was washed with AcOH, dioxane, 50% aqueous dioxane, water, EtOH and $CHCl_3$ (three times each). The resultant leucyloxyacetyl resin hydrochloride was converted to the free base by stirring for 10 min in 150 ml of $CHCl_3$ containing 15 ml of Et_3N, filtered and washed well with $CHCl_3$ and CH_2Cl_2. The resin was then mixed with a solution of 10.83 g (47.25 mmoles) of AOC-proline in CH_2Cl_2 and after a few min, 9.75 g (47.25 mmoles) of DCC in CH_2Cl_2 was added and the mixture was diluted to 150 ml with CH_2Cl_2 and stirred for 2 hr at room temperature. The resin was collected and washed with CH_2Cl_2 and AcOH (three times each). AOC-Glycine (3.94 g, 47.25 mmoles) was then coupled in exactly the same way and the AOC-glycylprolylleucyloxyacetyl resin thus obtained was washed throughly with CH_2Cl_2, MeOH and ether and dried *in vacuo* at room temperature. Yield, 16.4 g. Peptide content: 0.87 mmole/g (modified Volhard method).[12]



Fig

AOC-Glycylprolylleucine DCHA Salt (X)——To a suspension of the foregoing AOC-glycylprolylleucyloxyacetyl resin (5 g, 4.35 mmoles) in 52 ml of dioxane and 13 ml of water was added 13 ml of 1N NaOH and the mixture was stirred at room temperature for 5 hr. The resin was filtered and washed with dioxane–water (4:1, v/v). The combined filtrate and washings were neutralized with 1N HCl and concentrated *in vacuo*. A small volume of water was added to the residue and the mixture was acidified with 1N HCl to pH 2 and extracted with AcOEt.

The AcOEt extract was washed with satd. NaCl solution, dried and evaporated to leave an oil, which was dissolved in a mixture of AcOEt and hexane and treated with 0.86 g of DCHA in AcOEt–hexane to yield AOC-glycylprolylleucine DCHA salt, 1.19 g, mp 154—157°, and second crop, 0.41 g, mp 153—156° (total 58% from the BOC-leucyloxyacetyl resin). Two recrystallizations from AcOEt–iso-Pr_2O afforded colorless prisms, mp 156—158°, $[\alpha]_D^{29}$ −65.6° (c=1.1, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1663 (amide), 1710 (AOC), 1625 (COO^-). *Anal.* Calcd. for $C_{31}H_{56}O_6N_4$: C, 64.10; H, 9.72; N, 9.65. Found: C, 64.00; H, 9.96; N, 9.38.

BOC-Leucylleucylleucyl-O-benzyltyrosyloxyacetyl Resin——BOC-O-benzyltyrosyloxyacetyl copolystyrene-divinylbenzene (III: Y–A=BOC-O-benzyl-Tyr, R=H, 2% DVB, 200—300 mesh, O-benzyl-Tyr: 5.05 mmoles, 5 g) was placed in the reaction vessel described above and stirred for 40 min in 50 ml of 1.47N HCl/AcOH to remove the BOC group. The resin was washed well with AcOH, dioxane and DMF (three times each) and then stirred for 10 min in 50 ml of DMF containing 5 ml of Et_3N to yield the O-benzyltyrosyloxyacetyl resin. After being washed with DMF and CH_2Cl_2 (three times each), the resin was mixed with a solution of BOC-leucine monohydrate (3.78 g, 15.15 mmoles) in CH_2Cl_2 and after a few min, a solution of 3.13 g (15.15 mmoles) of DCC in CH_2Cl_2 (total 50 ml) was added and the mixture was stirred for 3 hr at room temperature.

The resin was then filtered and washed throughly with CH_2Cl_2 and AcOH (three times each). In exactly the same manner, the peptide resin was acylated twice with BOC-leucine monohydrate (3.78 g, 15.15 mmoles each). The finished peptide resin was washed throughly with CH_2Cl_2 and MeOH and dried *in vacuo*. Yield, 4.99 g. Peptide content: 0.68 mmole/g (modified Volhard method).[12]

BOC-Leucylleucylleucyl-O-benzyltyrosine DCHA Salt (XI)——To a suspension of the above BOC-leucylleucylleucyl-O-benzyltyrosyloxyacetyl resin (4.5 g, 3.06 mmoles) in 36.8 ml of dioxane and 9.2 ml of water was added 9.2 ml of 1N NaOH with stirring and the mixture was stirred for 6 hr at room temperature. The resin was filtered off and washed with aqueous dioxane. The combined filtrate and washings were neutralized with 1N HCl, concentrated *in vacuo* to reomve dioxane, acidified to pH 2 with 1N HCl, saturated with NaCl and extracted with AcOEt. The extract was washed with satd. NaCl solution and dried. Removal of the solvent gave an oil, which was dissolved in AcOEt–hexane and treated with 0.50 g of DCHA in AcOEt–hexane. The mixture was kept standing in a refrigerator to precipitate BOC-leucylleucylleucyl-O-benzyltyrosine DCHA salt (XI), 1.44 g (36% from the starting BOC-O-benzyltyrosyloxyacetyl resin), mp 153—156°. Three recrystallizations from AcOEt–acetone afforded colorless crystalline powder, mp 163—164°, $[\alpha]_D^{26}$ −15.1° (c=0.9, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1681 (BOC), 1632 (amide and COO^-). Amino acid ratio:[15] Leu, 3.18: Tyr, 1.00. *Anal.* Calcd. for $C_{51}H_{81}O_8N_5$: C, 68.65; H, 9.15; N, 7.85. Found: C, 68.71; H, 9.25; N, 7.88.

15) Determined gas-chromatographically. C.W. Gehrke, D. Roach, R.W. Zumwalt, D.L. Stalling and L.L. Wall, "Quantitative Gas–Liquid Chromatography of Amino Acids in Protein and Biological Substances," Analytical Bio–Chemistry Labs., Inc. 1968.

BOC-Leucylleucylleucyl-O-benzyltyrosyloxypropionyl-(2) Resin——BOC-O-Benzyltyrosyloxypropionyl-(2) copolystyrene-divinylbenzene (III: Y-A=BOC-O-benzyl-Tyr, R=CH_3, 2% DVB, 200—300 mesh, BOC-O-benzyl-Tyr: 1.97 mmoles, 4.8 g) was deprotected by treatment with dry HCl/AcOH and $Et_3N/CHCl_3$. To the resultant O-benzyltyrosyloxypropionyl-(2) resin was coupled three times BOC-leucine in a similar manner as in the experiment with BOC-O-benzyloxyacetyl resin described above to yield 4.47 g of the BOC-leucylleucylleucyl-O-benzyltyrosyloxypropionyl-(2) resin. Peptide content: 0.15 mmole/g (modified Volhard method).[12]

BOC-Leucylleucylleucyl-O-benzyltyrosine DCHA Salt (XI)——A mixture of the foregoing BOC-leucyl-leucylleucyl-O-benzyltyrosyloxypropionyl-(2) resin (4 g, 0.62 mmole), 12.4 ml of dioxane, 3.1 ml of water and 3.1 ml of 1N NaOH was stirred for 5 hr at room temperature and worked up similarly to the experiment with the BOC-leucylleucylleucyl-O-benzyltyrosyloxyacetyl resin described above to give 0.21 g of XI as DCHA salt (13% from the BOC-O-benzyltyrosyloxypropionyl-(2) resin), mp 152—155°. Recrystallizations from AcOEt–acetone afforded colorless crystalline powder, mp 161—163°, $[\alpha]_D^{26}$ −14.2° (c=1.2, MeOH).

This product was identified with the specimen prepared using the acetyl type of the resin by mixed melting test and comparison of their IR spectra.

BOC-Leucylleucylleucyl-O-benzyltyrosine Methyl Ester (XV)——A 0.8 g portion of DCHA salt of XI was treated with an excess of 0.5M citric acid solution and extracted three times with AcOEt. The combined extracts were washed, dried and evaporated to give an oil, which was dissolved in 20 ml of MeOH and treated with a large excess of diazomethane in ether for 1 hr with cooling in an ice bath. The resulting solution was evaporated to dryness *in vacuo* to leave an oil, which was triturated with a small volume of hexane to give 0.16 g (94%) of white solid, mp 168—170°. Recrystallization from AcOEt–hexane afforded colorless fine needles, mp 169—170°, $[\alpha]_D^{26}$ −43.5° (c=0.8, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1732 (ester), 1678 (BOC), 1658, 1631 (amide). *Anal.* Calcd. for $C_{40}H_{60}O_8N_4$: C, 66.28; H, 8.34; N, 7.73. Found: C, 66.37; H, 8.59; N, 7.80.

Leucylleucylleucyltyrosine Methyl Ester Hydrochloride (XVI)——The above BOC derivative (XV, 0.51 g) suspended in 3N dry HCl/MeOH was shaken until the mixture had become clear. To this was added Pd–C (10%, 100 mg) in MeOH (2 ml) and the whole was hydrogenated for 4 hr at room temperature. The catalyst was filtered off and washed with MeOH. The combined filtrates were evaporated to dryness *in vacuo* to give a syrup, which was triturated with ether affording 0.36 g (90%) of colorless crystalline product mp 229—230° (decomp.), and mp 234° (decomp.) after recrystallization from MeOH–acetone–ether, $[\alpha]_D^{25}$ −27.8° (c=0.9, MeOH). Authentic sample:[10] mp 234° (decomp.), $[\alpha]_D^{25}$ −25.6° (c=1.0, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1723 (ester), TLC (Silica Gel G, $CHCl_3$–MeOH (4:1, v/v) and CCl_4–AcOEt–MeOH (5:5:2, v/v)): single spot, electrophoresis (Toyo Roshi No. 51, 0.5M pyridine–AcOH buffer (pH 3.5), 1000 V, 70 min, ninhydrin): single band. The data of these analyses were in good agreement with those of the authentic sample. *Anal.* Calcd. for $C_{28}H_{47}O_6N_4Cl$: C, 58.88; H, 8.30; N, 9.82; Cl, 6.21. Found: C, 58.68; H, 8.32; N, 9.87; Cl, 6.51.

Carbobenzoxy-prolylleucylglycine Amide (XII)——A 4.0 g portion of AOC-glycyloxyacetyl copolystyrene–divinylbenzene (III: Y-A=AOC-Gly, R=H, 2% DVB, 200—300 mesh, AOC-Gly: 1.48 mmoles) was deprotected by treatment with 35 ml of 1N dry HCl/AcOH and converted to the glycyloxyacetyl resin as described above. This was then acylated successively with AOC-leucine monohydrate (1.17 g, 4.46 mmoles) and carbobenzoxyproline (1.10 g, 4.46 mmoles) in the same manner as for the AOC-glycylprolylleucyl resin described above, using DCC (0.92 g, 4.46 mmoles each) as coupling reagent, affording the carbobenzoxy–prolylleucyl-glycyloxyacetyl resin. This was suspended in 30 ml of 18% NH_3/MeOH and stirred for 4 hr at room temperature.

The resin was then filtered off and washed with MeOH and the combined filtrate and washings were evaporated *in vacuo* leaving a crystalline residue, which was washed with water, dried and recrystallized from AcOEt to give 178 mg of colorless needles, mp 162—163°, $[\alpha]_D^{24}$ −72.4° (c=2.9, EtOH). Lit.[16] mp 163—163.5°, $[\alpha]_D^{18.5}$ −73.3° (c=2, 95% EtOH); lit.[17] mp 163°, $[\alpha]_D^{20}$ −71.9° (c=2.5, EtOH); lit.[18] mp 162—163°. IR ν_{max}^{Nujol} cm^{-1}: 1690 (shoulder, carbobenzoxy), 1655, 1645, 1604 (amide). Amino acid ratio:[15] Pro, 1.05: Leu, 1.00; Gly, 1.05. Ammonolysis was repeated on the residual resin and a second crop (45 mg) was obtained, mp 161—162°, $[\alpha]_D^{23}$ −69.3° (c=2.4, EtOH).

BOC-Prolylleucylglycine Amide (XIII)——a) BOC-Glycyloxyacetyl copolystyrene-divinylbenzene (III: Y-A=BOC-Gly, R=H, 2% DVB, 200—300 mesh, BOC-Gly: 2.13 mmoles, 3.0 g) was deprotected by treatment with 25 ml of 2.5N dry HCl/AcOEt and then converted to the glycyloxyacetyl resin as usual. To this was coupled BOC-leucine monohydrate (1.65 g, 6.61 mmoles) and BOC-proline (1.42 g, 6.61 mmoles) using DCC (1.36 g, 6.61 mmoles, each) as coupling reagent to give 3.27 g of the BOC-prolylleucylglycyloxyacetyl resin. A 3.03 g portion of this product was suspended in 35 ml of MeOH and gaseous NH_3 was bubbled for 100 min with cooling and the mixture was stirred for 20 hr at room temperature. The resin was filtered off and washed with MeOH. The combined filtrate and washings were evaporated *in vacuo* to leave an oil, which

16) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3107 (1954).
17) R.A. Boissonnas, St. Guttmann, P.A. Jaquenoud and J.P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).
18) M. Zaoral and J. Rudinger, *Collection Czech. Chem. Commun.*, **20**, 1183 (1955).

was crystallized from a small volume of water to a white solid (0.47 g, 61% from the BOC-glycyloxyacetyl resin), mp 137—139°. Recrystallizations from water and water–EtOH afforded colorless needles, mp 137—139°, $[\alpha]_D^{25}$ −72.3° (c=1.8, MeOH). Amino acid ratio:[15] Pro, 0.96: Leu, 1.00; Gly, 0.99. IR ν_{max}^{Nujol} cm^{-1}: 1680 (shoulder, BOC), 1661, 1614 (amide). *Anal.* Calcd. for $C_{18}H_{32}O_5N_4 \cdot 1/2\ H_2O$; C, 54.87; H, 8.44; N, 14.22. Found: C, 54.70; H, 8.50; N, 14.23.

b) The BOC-prolylleucylglycyloxyacetyl resin (11.2 g) was obtained from BOC-glycyloxyacetyl copolystyrene-divinylbenzene (III: Y–A=BOC–Gly, R=H, 2% DVB, 100 mesh, BOC–Gly: 10.50 mmoles, 10 g) in the same manner as described in a). A 2.0 g portion of the resulting peptide resin was suspended in DMF (30 ml) and treated with gaseous NH_3 for 7.5 hr and the mixture was stirred for 16.5 hr at room temperature. The work-up as described in a) afforded 0.51 g (69% from the BOC-glycyloxyacetyl resin) of XIII, mp 137—140°, $[\alpha]_D^{22}$ −71.8° (c=1.5, MeOH). This product was identified with the sample prepared by the method a) by mixed melting test and IR analysis.

BOC-Leucylleucylvalylphenylalanyloxyacetyl Resin——AOC-Phenylalanyloxyacetyl copolystyrene-divinylbenzene (III: Y–A=AOC–Phe, R=H, 2% DVB, 200—300 mesh, AOC–Phe: 4.90 mmoles, 5.38 g), swelled in dioxane, was treated with 60 ml of 4.2N dry HCl/dioxane to remove the AOC group (40 min, at room temperature) and washed with dioxane and DMF (three times each). Hydrochloride of the phenylalanyl resin thus obtained was then converted to the free base by stirring for 10 min in 60 ml of DMF containing 6 ml of Et_3N. After being washed with DMF and CH_2Cl_2 (three times each), the resin was mixed with a solution of AOC-valine (prepared from 6.07 g (14.70 mmoles) of AOC-valine DCHA salt) in CH_2Cl_2 and stirred for a few min. A solution of 3.03 g (14.70 mmoles) of DCC in CH_2Cl_2 was added and the mixture was made to a volume of 60 ml with CH_2Cl_2. The mixture was stirred for 3 hr at room temperature, filtered and the resin was washed with CH_2Cl_2 and dioxane. In a similar manner, the peptide resin was then acylated successively with AOC-leucine monohydrate (3.78 g, 14.70 mmoles) and BOC-leucine monohydrate (3.67 g, 14.70 mmoles) using DCC (3.03 g, 14.70 mmoles) as reagent. Following the last coupling reaction, the resin was washed throughly with MeOH and dried *in vacuo* affording 5.25 g of the protected peptide resin. Peptide content: 0.58 mmole/g (modified Volhard method).[12]

BOC-Leucylleucylvalylphenylalanine Amide (XIV)——Into a suspension of the foregoing BOC-leucylleucylvalylphenylalanyl resin (5.0 g) in 50 ml of MeOH was bubbled gaseous NH_3 for 3.5 hr with cooling and the mixture was stirred for 16.5 hr at room temperature. The resin was filtered off, washed with MeOH and $CHCl_3$ and the combined filtrate and washings were evaporated *in vacuo* to give a white solid, which was washed with water to give 1.17 g (43% from the AOC-phenylalanyloxyacetyl resin) of the crude amide (XIV), mp 254—256° (decomp.).

This was purified by a column chromatography using silica gel (80 g, elution: $CHCl_3$–acetone (5:2, v/v)) and recrystallized from MeOH–water affording colorless fine needles, mp 261—262° (decomp.), $[\alpha]_D^{29}$ −49.1° (c=1.0, AcOH), IR ν_{max}^{Nujol} cm^{-1}: 1732 (ester), 1678 (BOC), 1658, 1631 (amide). Amino acid ratio:[15] Leu, 1.93; Val, 1.00; Phe, 0.98. *Anal.* Calcd. for $C_{31}H_{51}O_6N_5$: C, 63.13; H, 8.72; N, 11.94. Found: C, 63.19; H, 8.76; N, 11.69.

Leucylleucylvalylphenylalanine Amide Hydrochloride (XVII)——A mixture of 0.25 g of the protected peptide amide (XIV) and 5 ml of 1.47N dry HCl/AcOH was kept to stand for 40 min at room temperature. The solvent was removed *in vacuo* leaving a syrup, which was crystallized from ether to give a white solid, mp >280°. Yield, 0.23 g (quantitative).

Recrystallization from MeOH–ether afforded colorless fine needles, mp >280°, $[\alpha]_D^{29}$ −18.4° (c=0.5, AcOH). Authentic sample:[10] mp >280°, $[\alpha]_D^{22}$ −18.4° (c=0.7, AcOH). IR ν_{max}^{Nujol} cm^{-1}: 1675 (shoulder), 1665, 1621 (amide). *Anal.* Calcd. for $C_{26}H_{44}O_4N_5Cl \cdot 1/2\ CH_3OH$: C, 58.70; H, 8.54; N, 12.92; Cl, 6.54. Found: C, 58.39; H, 8.30; N, 13.07; Cl, 7.09. This product was identified with a specimen prepared by a conventional method[10] by mixed melting test and comparison of their IR spectra. These two samples were also indistinguishable on TLC (solvent system: $CHCl_3$–MeOH (6:1, v/v) and $CHCl_3$–AcOH–MeOH (95:3:10, v/v)).

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