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Amino Acids and Peptides. VI.[1] Novel Peptide Bond Formation catalyzed by Metal Ions. IV.[1] Formation of optically Active Amino Acid Amides and Peptide Amides[2]

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The Cu(II)-catalyzed peptide bond formation previously reported by us, was applied to the synthesis of optically active amino acid amides and peptide amides.

Treatment of an optically active amino acid ester with a primary amine in the presence of anhydrous $CuCl_2$ afforded the desired amino acid secondary amide, without racemization, in 60—70% yield. However, the same reaction with a secondary amine with larger steric hindrance than primary amine gave an optically active tertiary amide in a very low yield as expected from the proposed mechanism. Almost all the amino acid amides could be isolated as hydrochlorides or as free bases.

Some peptide amides, *i.e.* Z-Gly-Gly-NH-Bzl, and Z-Ala-Gly-NH-Bzl, were also prepared.

In the preparation of optically active amino acid amides, the usual synthetic methods, *i.e.* the N-carboxyanhydride method and mixed anhydride method, which are frequently used in peptide synthesis,[4] are employed. Only one report by Hougton, *et al.*[5] is concerned with the formation of racemic amino acid amides using a metal complex.

In privious communications,[1,6,7] we reported that treatment of amino acid esters with anhydrous cupric chloride ($CuCl_2$) in an anhydrous solvent afforded dipeptide ester without racemization, and in some instances was accompanied with tri-, and tetra-peptide esters, by way of a reaction mechanism[1] in which the Cu(II)-co-ordinating amino group attacked the non-activated ester carbonyl group.

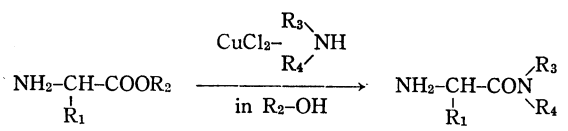

Chart 1

We have now applied this Cu(II)-catalyzed peptide bond formation to the syntheses of optically active amino acid amides and peptide amides,[8] and have found that the desired amides are obtainable in fairly good yields. Formed amino acid amides were isolated from reaction mixtures as the hydrochlorides or by direct distillation of their free bases, which

1) Part V and Part III: M. Wagatsuma, S. Terashima, and S. Yamada, *Tetrahedron*, in press.
2) Presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan. Fukuoka, April, 1971.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John-Wiley & Sons, Inc., New York, London, 1961, p. 763.
5) K. Blazevic, R.P. Hougton, and C.S. Williams, *J. Chem. Soc.* (*C*), **1968**, 1704.
6) S. Yamada, M. Wagatsuma, Y. Takeuchi, and S. Terashima, *Chem. Pharm. Bull.* (Tokyo), **19**, 2380 (1971).
7) S. Terashima, M. Wagatsuma, and S. Yamada, *Tetrahedron*, in press.
8) The amino acids, except glycine, had L-configuration. Abbreviations used for amino acid and peptide derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, **5**, 2485 (1966). The following abbreviations are used: *Z*=carbobenzoxy; *n*-Bu=*n*-butyl; *t*-Bu=*t*-butyl; *n*-Pr=*n*-propyl; Bzl=benzyl; MeOH=methanol; EtOH=ethanol; MeCN=acetonitrile.

differs from the synthesis of peptide esters[6,7] where products were purified by column chromatography after carbobenzoxylation.

I. Formation of Amino Acid Amides

1. Reaction of Amino Acid Esters with Ammonia in the Presence of $CuCl_2$——That treatment of an amino acid ester (methyl or ethyl ester) with ammonia in alcohol at room temperature, usually affords the desired amino acid primary amide has been well established. However, when the reaction of ethyl L-phenylalaninate (Phe-OEt) (2.0 eq.) with ammonia (2.0 eq.) in the presence of anhydrous $CuCl_2$ (1.0 eq.) in anhydrous ethanol was attempted, in order to study the effect of the Cu(II) ion on primary amide formation, the Cu(II)-ammonia complex simply precipitated just after preparation of the reaction mixture. Absolutely no formation of L-phenylalanine amide (Phe-NH_2) was observed.[9] This clearly shows that Cu(II) ion has no effect on the formation of the amino acid primary amide with an amino acid ester and ammonia.

2. Reaction of Amino Acid Esters with Primary Amines in the Presence of $CuCl_2$——An anhydrous blue ethanolic mixture of Phe-OEt (2.0 eq.), *n*-butylamine (2.0 eq.) and anhydrous $CuCl_2$ (1.0 eq.) was stirred at room temperature for 16.0 hr. The whole was worked up in a manner similar to that reported for peptide ester formation,[6,7] giving L-phenylalanine *n*-butylamide (Phe-NH-*n*-Bu) in 30% yield (see Table I, run 1). The amide produced was isolated as its hydrochloride and identified with an authentic sample independently prepared by the mixed-anhydride method,[4] using mixed melting point measurement and a comparison of its infrared (IR) spectrum with that of an authentic sample.

To improve the yield of Phe-NH-*n*-Bu, another experimental procedure, the addition of *n*-butylamine (3.0 eq.) to a heterogeneous green solution of dichloro-mono(ethyl L-phenylalaninato)-copper(II) (Cu(Phe-OEt)Cl_2)[10] prepared with Phe-OEt (1.0 eq.) and anhydrous $CuCl_2$(1.0 eq.) in anhydrous ethanol, was attempted. The clear blue solution obtained was stirred at room temperature for 5.0 hr, then was worked up as usual to give Phe-NH-*n*-Bu-hydrochloride(HCl) in 66% yield (see Table I, run 2). In place of Phe-OEt, the hydrochloride (Phe-OEt-HCl) could be used for this amide formation in the presence of an equivalent amount of triethylamine (see Table I, run 3).

Further examples of secondary amide formation using Phe-OEt, ethyl L-leucinate (Leu-OEt), methyl L-alaninate (Ala-OMe), ethyl glycinate (Gly-OEt), and methyl L-glutaminate (Gln-OMe) are given in Table I.

As shown in the Table, almost all the amino acid primary amides can be obtained in moderate yields without racemization. With Leu-OEt and Gly-OEt (see Table I, runs 5, and 7) the amides formed could be isolated by distillation instead of by preparation of the hydrochlorides. The absence of the desired amide formation, when *t*-butylamine was used as an amine counterpart (see Table I, run 8), seems to be due to steric hindrance produced by the bulky *t*-butyl group, which is easily understood by considering the proposed formation mechanism.[1]

It is of interest that reaction of Gln-OMe with ethyl γ-aminobutyrate afforded the desired amide, ethyl N-L-glutaminyl-γ-aminobutyrate, as the sole product even though in a low yield, since Gln-OMe usually affords the cyano derivative when submitted to a dehydrative condition such as treatment with dicyclohexyl carbodiimide.

3. Reaction of Amino Acid Esters with Secondary Amines in the Presence of $CuCl_2$——The same reactions examined with primary amines were carried out using secondary amines. Yields of formed amino acid tertiary amides were very much lower than those observed on

9) Preferential formation of the Cu(II)-ammonia complex seems to be due to its larger stability, as compared to that of the Cu(II)-amino acid ester complex, because of steric hindrance.

10) After being collected by filtration and dried *in vacuo*, this Cu(II) complex showed mp 172° (decomp.). *Anal.* Calcd. for $C_{11}H_{15}O_2NCl_2Cu$: C, 40.32; H, 4.61; N, 4.27. Found: C, 40.70; H, 4.73; N, 4.54.

TABLE I. Reaction of Amino Acid Esters with Primary and Secondary Amines in the Presence of $CuCl_2$[a]

Run	Amino acid esters used[b]	Amines used	Solv.	Reac. time (hr)	Formed amino acid amides[c]				Authentic sample[e]	
						Yield[d] (%)	mp (bp) (°C)	Optical rotation $[\alpha]_D$ (t, c, solv.)	mp (bp) (°C)	Optical rotation $[\alpha]_D$ (t, c, solv.)
1	Phe-OEt[f]	*n*-$BuNH_2$	EtOH	16	Phe-NH-*n*-Bu-HCl	30	141—143	+56.8° (20, 1.0, MeOH)	145—146	+59.3° (23, 0.62, MeOH)
							[145—146	+59.0° (20, 1.02, MeOH)][g]		
2	Phe-OEt	*n*-$BuNH_2$	EtOH	5	Phe-NH-*n*-Bu-HCl	66	140—143	+57.1° (23, 1.02, MeOH)		
							[145—146	+59.0° (24, 1.04, MeOH)][g]		
3	Phe-OEt[h]	*n*-$BuNH_2$	EtOH	5	Phe-NH-*n*-Bu-HCl	60	139—141			
4	Phe-OEt	*n*-$PrNH_2$	EtOH	5	Phe-NH-*n*-Pr-HCl	62	150—153	+62.0° (24, 0.80, MeOH)		
							[155—155.5	+62.9° (24, 0.62, MeOH)][i]		
5	Leu-OEt	*n*-$BuNH_2$	EtOH	3	Leu-NH-*n*-Bu[j]	58	(124—125 (0.3 mmHg))	+8.7° (23, 2.11, EtOH)	(124—126 (0.3 mmHg))	+8.8° (25, 2.46, EtOH)
6	Ala-OMe	*n*-$BuNH_2$	MeOH	3	Ala-NH-*n*-Bu-TosOH[k]	68	118—121	+4.6° (20, 2.00, MeOH)		
							[122.5—123.5	+5.3° (20, 1.00, MeOH)][i]		
7	Gly-OEt[f]	$C_6H_5CH_2NH_2$	EtOH	3	Gly-NH-Bzl[f]	59	(180—185 (0.5 mmHg))		(155—160[m] (0.01 mmHg))	
8	Phe-OEt	*t*-$Bu-NH_2$	EtOH	5	Phe-NH-*t*-Bu	0				
9	Gln-OMe[f]	$NH_2(CH_2)_3$-COOEt	EtOH	3	Z-Gln-NH$(CH_2)_3$-COOEt[n]	10	160—164	−2.0° (24, 1.02, EtOH)		
							[165—167	−2.0° (24, 1.31, EtOH)][o]		
10	Leu-OEt	C_4H_8NH	EtOH-MeCN	5	Leu-NC_4H_8-HCl	6	[186—187	−21.0° (25, 0.30, MeOH)][g]	189	+21.0° (25, 0.61, MeOH)
11	Phe-OEt	Me_2NH	EtOH-MeCN	18	Phe-NMe_2[f]	7	(145—147 (5 mmHg))	+103.9° (25, 0.5, EtOH)	(148—150 (4 mmHg))	+105.3° (13, 0.97, EtOH)[p]
12	Phe-OEt	Et_2NH	EtOH-MeCN	18	Phe-NEt_2	0				

a) The molar ratio of amino acid ester, $CuCl_2$ and amine was 1:1:3. All reactions were carried out at room temperature.
b) Free bases of amino acid esters were used.
c) Measurements of melting points and optical rotations were made with a crude sample.
d) Calculated based on an amino acid esters.
e) Independently prepared according to the authorized procedure (see Experimental).
f) The molar ratio of amino acid ester, $CuCl_2$, and amine was 2:2:1.
g) Values in parentheses were obtained with a sample recrystallized from EtOH-ether.
h) Equivalent amounts of Phe-OEt-HCl and triethylamine were used instead of Phe-OEt.
i) Values in parentheses were obtained with a sample recrystallized from EtOH-ether. Colorless needles, IR ν_{max}^{KBr} cm^{-1}: 1655 ($\nu_{C=O}$). *Anal.* Calcd. for $C_{15}H_{14}ON_4 \cdot HCl$: C, 59.39; H, 7.89; N, 11.49. Found: C, 59.21; H, 7.93; N, 11.30.
j) Isolated as the free base.
k) *p*-toluenesulfonate
l) Values in parentheses were obtained with a sample recrystallized from ethyl acetate. Colorless needles, IR ν_{max}^{KBr} cm^{-1}: 1680 ($\nu_{C=O}$). *Anal.* Calcd. for $C_{14}H_{18}O_4N_2S$: C, 53.14; H, 7.65; N, 8.86. Found: C, 53.23; H, 7.32; N, 8.69
m) bp 170—190° (0.5 mmHg) was reported. J. Brunker and G. Back, *Chem. Ber.*, **89**, 1363 (1956).
n) Purified by column chromatography using silica gel after carbobenzoxylation.
o) Values in parentheses were obtained with a sample recrystallized from EtOH.
p) bp 148—150° (3 mmHg), and $[\alpha]_D^{25}$ +104.1° (*c*=0.97, EtOH) were reported. I. Saito, Y. Kikugawa, and Shun-ichi Yamada, *Chem. Pharm. Bull.* (Tokyo), **18**, 1731 (1970)

secondary amide formation. That is, treatment of Leu-OEt with pyrrolidine in a mixture of ethanol and acetonitrile gave L-leucine pyrrolydilamide (Leu-NC_4H_8) in only 6% yield (see Table I, run 10), and L-phenylalanine dimethylamide (Phe-NMe_2) was obtained in 7% yield (see Table I, run 11). Diethylamine, whose steric hindrance is clearly larger than that of dimethylamine, did not afford the desired amide (see Table I, run 12).

The decrease in the reactivity of secondary amine was also observed in the metal-promoted amide formation reported by Hougton.[5]

Using the proposed mechanism for Cu(II)-catalyzed peptide bond formation previously reported,[1] these low yield of amino acid tertiary amides can be understood to be the result of increased steric hindrance of the secondary amine, as compared with the primary amine.

II. Formaion of Peptide Amides

The applicability of this amino acid amide formation to the preparation of peptide amides was studied, using Glycine benzylamide (Gly-NH-Bzl) as the amine component. Formed peptide amides were isolated according to the established procedures[6,7] after carbobenzoxylation. Results are summarized in Table II.

The reaction of Gly-NH-Bzl with Gly-OEt afforded N-carbobenzoxy-glycyl-glycine benzylamide (Z-Gly-Gly-NH-Bzl) in 30% yield with the concomitant formation of di-, tri-, and

TABLE II. Reaction of Amino Acid Esters with Gly-NH-Bzl in the Presence of $CuCl_2$[a]

Run	Amino acid esters used	Solv.	Formed N-carbobenzoxy-peptide benzylamide[b]	Yield (%)	mp (°C)	Optical rotation $[\alpha]_D$ (t, c, solv.)
1	Gly-OEt	EtOH	Z-Gly-Gly-NH-Bzl	30	157—159	
2	Ala-OMe	MeOH	Z-Ala-Gly-NH-Bzl	48	128	+9.6° (16, 1.80, MeOH)
3	Phe-OEt	EtOH	Z-Phe-Gly-NH-Bzl	0		

a) The molar ratio of Gly-NH-Bzl: $CuCl_2$: Amino Acid Ester was 2:1:10. All reactions were carried out at room temperature for 20.0 hr. Amino acid esters were used as the hydrochlorides in the presence of an equivalent amount of triethylamine.
b) Purified by column chromatography using silica gel.

tetra-glycine esters. The Z-Gly-Gly-NH-Bzl formed was freed from the accompanying peptide esters by treating all the N-carbobenzoxy peptide products with aqueous alkaline before chromatographic purification. Identification with the authentic sample was made in the usual manner.

A similar reaction using Ala-OMe gave N-carbobenzoxy-L-alanyl-glycine benzylamide (Z-Ala-Gly-NH-Bzl) in 48% yield. Its structure was confirmed by elemental analysis and by spectral data.

But, when Phe-OEt was treated in a similar manner, absolutely no formation of the desired peptide amide was observed. Using the formation mechanism previously established for this Cu(II)-catalyzed peptide bond formation,[1] steric hindrance inherently present in Phe-OEt and Gly-NH-Bzl probably caused the experimental result obtained here.

Experimental[11]

Materials——Commercially available $CuCl_2$ was used after drying *in vacuo* at *ca.* 120° over P_2O_5. All amino acid ester hydrochlorides were prepared according to the usual procedure.[12] Their free bases were obtained by the treatment reported in the literature.[13] Reaction solvents, such as MeOH and EtOH, were used in a completely anhydrous condition throughout this work.

Formation of Amino Acid Amides (Table I)——Run 1: A clear blue solution obtained by the addition of *n*-$BuNH_2$ (730 mg, 10.0 mmole) in EtOH (5.0 ml) to an ethanolic solution (10.0 ml) of Phe-OEt (1.93 g, 10.0 mmole) and anhydrous $CuCl_2$ (670 mg, 5.0 mmole), was stirred at room temperature for 16.0 hr. After the reaction was over, the Cu(II) complex formed was decomposed by the addition of ethanolic hydrogen chloride, then the Cu(II) ion was removed as cupric sulfide by passing hydrogen sulfide gas through the acidic ethanolic solution. The residue obtained by successive filtration and evaporation *in vacuo*, was diluted with an excess amount of diluted aqueous sodium bicarbonate solution and the alkaline solution was extracted with chloroform. Chloroform extracts were washed with satd. sodium chloride solution, and dried over anhyd. $MgSO_4$. Filtration and evaporation *in vacuo* gave a residue which was separated by column chromatography using neutral alumina (44 g). After unreacted Phe-OEt was eluted with benzene, elution with chloroform afforded the desired Phe-NH-*n*-Bu, which was dissolved in ether and isolated as its hydrochloride by passing dry hydrogen chloride gas through the ethereal solution. The Phe-NH-*n*-Bu-HCl obtained as a pale yellow powder, weighed 780 mg (30% based on Phe-OEt), and showed mp 141—143°, and $[\alpha]_D^{20}$ +56.8° (c=1.01, MeOH). Recrystallization from EtOH–ether afforded a pure sample, mp 145—146°, $[\alpha]_D^{20}$ +59.0° (c=1.02, MeOH) as colorless needles. This showed no depression on mixed melting point measurement with an authentic sample independently prepared. The IR spectrum of this product was also identical with that of an authentic sample in the same state.

Run 3: *n*-$BuNH_2$ (1.10 g, 15.0 mmole) in EtOH (5.0 ml) was added with stirring to a heterogeneous solution of Cu(II) complex prepared by the addition of anhydrous $CuCl_2$ (670 mg, 5.0 mmole) to an ethanolic solution (15.0 ml) of Phe-OEt (970 mg, 5.0 mmole), to afford a clear blue solution. After the reaction mixture was stirred at room temperature for 5.0 hr, the Cu(II) complex was decomposed, and liberated Cu(II)

11) All melting points are uncorrected. IR spectra measurements were performed using a spectrometer, Model 402, Japan Spectroscopic Co., Ltd. Optical activities were determined with a Yanagimoto Photo Direct Reading Polarimeter, Model OR-20.
12) Reference 4, pp. 925—932.
13) J.R. Vaughan and J.A. Eichler, *J. Am. Chem. Soc.*, **75**, 5556 (1953).

ion was removed in a manner similar to that used in run 1. The residue obtained by evaporation of the ethanolic solution was made alkaline with diluted aqueous sodium bicarbonate solution, then it was extracted with chloroform. Organic layers were washed with satd. sodium chloride solution, then dried over anhyd. $MgSO_4$. After chloroform was removed *in vacuo*, the evaporation residue was dissolved in ether. The Phe-NH-*n*-Bu isolated as the hydrochloride, showed the physical constants given in Table I.

Run 3—Run 8: All reactions were carried out in a manner similar to the case of run 2. Reaction products were isolated as the hydrochlorides or as free bases.

Run 9: An ethanolic mixture (10.0 ml) of ethyl γ-amino-butyrate hydrochloride (1.50 g, 9.0 mmole) and triethylamine (910 mg, 9.0 mmole) was added to a mixture of Gln-OMe-HCl (590 mg, 3.0 mmole), triethylamine (300 mg, 3.0 mmole), and anhydrous $CuCl_2$ (410 mg, 3.0 mmole) in EtOH (10.0 ml). The clear blue solution was stirred at room temperature for 3.0 hr, then Cu(II) ion was removed from the reaction mixture by the usual procedure. The residue obtained by evaporation of the acidic ethanolic solution was dissolved in dimethyl formamide (10.0 ml), to which carbobenzoxy chloride (2.60 g, 15.0 mmole) in chloroform (10.0 ml) and triethylamine (3.03 g, 30.0 mmole) in chloroform (10.0 ml) were alternately added at 0—5°. The whole was stirred at 0—5° for 1.0 hr, then at room temperature for 2.0 hr. The reaction mixture was poured into ice-water, then extracted with chloroform. The aqueous phase was further extracted with ethyl acetate. Two lots of the organic extracts were separately washed with diluted hydrochloric acid, satd. sodium bicarbonate solution, and finally satd. sodium chloride solution. After being dried over anhyd. $MgSO_4$, the combined organic layers were evaporated *in vacuo* producing a residue. This was submitted to silica gel column chromatography (100 g, solvent: methylene chloride: ethyl acetate 1:1), and gave Z-Gln-NH$(CH_2)_3COOC_2H_5$ (115 mg, 10% based on Gln-OMe) as a pale yellow powder, mp 160—164° (decomp.), $[\alpha]_D^{24}$ −2° (c=1.02, EtOH). Recrystallization from EtOH afforded a pure sample as colorless crystals, mp 165—167° (decomp.), $[\alpha]_D^{24}$ −2° (c=1.31, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3450, 3320, 1730, 1685, 1655, 1645, 1535, 1260, 1245, 1190, 695. *Anal.* Calcd. for $C_{19}H_{27}O_6N_3$: C, 58.00; H, 6.92; N, 10.68. Found: C, 58.15; H, 6.99; N, 10.50.

Run 10: An acetonitrile solution (10.0 ml) of pyrrolidine (1.06 g, 15.0 mmole) was added with stirring at room temperature to a mixture of Leu-OEt (800 mg, 5.0 mmole) and anhydrous $CuCl_2$ (670 mg, 5.0 mmole) in EtOH (5.0 ml) and MeCN (5.0 ml). After stirred for 5.0 hr, the reaction mixture was worked up as was run 1. The crude reaction product was purified by column chromatography using neutral alumina (50 g). Elution of the column with methylene chloride afforded Leu-NC_4H_8, which was isolated as the hydrochloride. Recrystallization with EtOH gave a pure sample as hygroscopic colorless needles (70 mg, 6% based on Leu-OEt), mp 186—187°, $[\alpha]_D^{25}$ −21.0° (c=0.30, MeOH). This sample was identified with an authentic sample prepared independently by comparison of their IR spectra and by mixed melting point measurement.

Run 11: An acetonitrile solution (10.0 ml) of dimethylamine (680 mg, 15.0 mmole) was added with stirring to a mixture of Phe-OEt (970 mg, 5.0 mmole) and anhydrous $CuCl_2$ (670 mg, 5.0 mmole) in EtOH (5.0 ml). The whole was stirred at room temperature for 18.0 hr, then worked up as usual. The crude product was distilled and gave Phe-NMe_2 (70 mg, 7% based on Phe-OEt), bp 145—147° (5 mmHg), $[\alpha]_D^{25}$ +103.9° (c=0.5, EtOH), as a pale yellow oil. The IR spectrum of this sample was identical with that of an authentic sample in the same state.

Formation of Peptide Amides (Table II)——Run 1: The clear blue solution, obtained by the addition of anhydrous $CuCl_2$ (200 mg, 1.5 mmole) to an ethanolic solution (10.0 ml) of Gly-NH-Bzl-HCl (600 mg, 3.0 mmole), and triethylamine (300 mg, 3.0 mmole), was stirred at room temperature for 10 min, then a mixture of Gly-OEt-HCl (2.10 g, 15.0 mmole) and triethylamine (1.51 g, 15.0 mmole) in EtOH (20 ml) was added. After the whole was stirred at room temperature for 20.0 hr, Cu(II) ion was removed from the reaction mixture as usual. A residue obtained by evaporation of the acidic ethanolic solution was submitted to carbobenzoxylation using carbobenzoxy chloride (4.1 g, 24.0 mmole) and triethylamine (4.0 g, 40.0 mmole) in chloroform as in run 11 in Table I. After the reaction was over, water (15 ml) was added to the residue obtained by evaporating the chloroform. The water-insoluble product was collected and washed with ether. A pale yellow powder obtained was treated with MeOH (15 ml) and 1.0N sodium hydroxide solution at room temperature for 4.0 hr. Evaporation of MeOH and water *in vacuo* gave a residue, which was washed with water. After being dried *in vacuo*, the water-insoluble product was purified with silica gel column chromatography. Elution of the column with tetrahydrofuran afforded the desired Z-Gly-Gly-NH-Bzl as a colorless powder weighing 320 mg (30% based on Gly-NH-Bzl) and showing mp 157—159°. This sample showed no depression on mixed melting point measurement with an authentic sample. Its IR spectrum was also identical with that of the authentic sample in the same state.

Run 2: A methanolic solution (20 ml) of Ala-OMe-HCl (2.10 g, 15.0 mmole) and triethylamine (1.51 g, 15.0 mmole) was added to a mixture of Gly-NH-Bzl-HCl (600 mg, 3.0 mmole), triethylamine (300 mg, 3.0 mmole), and anhydrous $CuCl_2$ (200 mg, 1.5 mmole) in MeOH (10 ml). After stirred at room temperature for 20.0 hr, the whole was worked up as in run 1. Then it was purified with column chromatography using silica gel. A fraction eluted with chloroform afforded Z-Ala-Ala-OMe (420 mg, 18% based on Ala-OMe) mp 101—103°. This sample was identified with an authentic one[7] using mixed melting point measurement. A second fraction eluted with chloroform–ethyl acetate (1:3) gave Z-Ala-Ala-Ala-OMe (80 mg,

4% based on Ala-OMe). Recrystallization with ethyl acetate–petr. ether produced a pure sample as colorless needles, mp 187—188° (decomp.), $[\alpha]_D^{18}$ −72.0° (c=0.848, MeOH). *Anal.* Calcd. for $C_{18}H_{25}O_6N_3$: C, 56.98; H, 6.64; H, 11.08. Found: C, 56.45; H, 6.73; N, 10.85. Elution of the column with chloroform–ethyl acetate (1:6) gave Z-Ala-Gly-NH-Bzl (520 mg, 48% based on Gly-NH-Bzl), mp 128° and $[\alpha]_D^{18}$ +9.6° (c=1.80, MeOH). Recrystallization from ethyl acetate–petr. ether gave a pure product as colorless needles, mp 130°, $[\alpha]_D^{22}$ +10° (c=1.10, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3280, 1712, 1655, 1545, 1255. *Anal.* Calcd. for $C_{20}H_{23}O_4N_3$: C, 65.02; H, 6.28; N, 11.28. Found: C, 65.17; H, 6.39; N, 11.55.

Syntheses of Authentic Samples——Phe-NH-*n*-Bu-HCl: Ethyl chloroformate (510 mg, 5.0 mmole) in anhydrous tetrahydrofuran (10 ml) was added at −5° to a mixture of Z-Phe-OH[14] (1.50 g, 5.0 mmole) and triethylamine (500 mg, 5.0 mmole) in anhydrous tetrahydrofuran (10 ml). After 13.0 min, *n*-$BuNH_2$ (360 mg, 5.0 mmole) in anhydrous tetrahydrofuran (5.0 ml) was added to the mixture at the same temperature. The mixture was stirred at the same temperature for 2.0 hr, then at room temperature for 2.0 hr. Filtration of triethylamine hydrochloride and evaporation of tetrahydrofuran afforded a residue, which was dissolved in ethyl acetate. The ethyl acetate solution was successively washed with 5% hydrochloric acid, satd. sodium bicarbonate, and satd. sodium chloride solutions, and finally dried over anhyd. $MgSO_4$. Filtration and evaporation *in vacuo*, followed by recrystallization of the evaporation residue, gave Z-Phe-NH-*n*-Bu (1.20 g, 68%) as colorless needles, mp 144—145°. IR ν_{max}^{KBr} cm^{-1}: 3300, 1690, 1645, 1530, 1280, 1240, 745, 700.

A mixture of Z-Phe-NH-*n*-Bu (750 mg, 2.0 mmole), hydrogen chloride satd. EtOH (2.0 ml), and 5% palladium on charcoal (200 mg) in EtOH (100 ml) was hydrogenated at room temperature under atmospheric pressure for 4.0 hr. Filtration and evaporation of the reaction mixture, followed by recrystallization with EtOH–ether, gave Phe-NH-*n*-Bu-HCl (390 mg, 72%) as colorless needles, mp 145—146°, $[\alpha]_D^{23}$ +59.3° (c=0.62, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3330, 2960, 2870, 1655, 1565, 1485, 1260, 745, 705. *Anal.* Calcd. for $C_{13}H_{20}ON_2$-HCl: C, 60.81; H, 8.24; N, 10.95. Found: C, 60.59; H, 8.25; N, 10.74.

Leu-NH-*n*-Bu: A similar treatment of Z-Leu-OH[15] (2.70 g, 10.0 mmole) to that of Z-Phe-OH afforded Leu-NH-*n*-Bu (1.18 g, 64%) as a colorless oil, bp 125—126° (0.3 mmHg), $[\alpha]_D^{25}$ +8.8° (c=2.46, MeOH). IR $\nu_{max}^{Cap.}$ cm^{-1}: 3300, 2960, 2880, 1645, 1530, 1470. *Anal.* Calcd. for $C_{10}H_{22}ON_2$: C, 64.47; H, 11.90. N, 15.04. Found: C, 64.09; H, 11.74; N, 15.03.

Gly-NH-Bzl: This amide was prepared according to the reported procedure (Table I, footnote m). Bp 155—160° (0.01 mmHg).

Leu-NC_4H_8-HCl: This amide was prepared using Z-Leu-OH (2.70 g, 10.0 mmole), triethylamine (1.01 g, 10.0 mmole), ethyl chloroformate (1.10 g, 10.0 mmole), and pyrrolidine (710 mg, 10.0 mmole). The preparation procedure was exactly the same as that described for Phe-NH-*n*-Bu. Leu-NC_4H_8, isolated as the hydrochloride, was recrystallized from EtOH–ether. The colorless needles (1.00 g, 45%) obtained, showed mp 189° and $[\alpha]_D^{25}$ −21.0° (c=0.61, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 1685, 1560, 1475, 1450. *Anal.* Calcd. for $C_{10}H_{20}ON_2$-HCl: C, 54.41; H, 9.59; N, 12.60. Found: C, 54.19; H, 9.67; N, 12.60.

Z-Gly-Gly-NH-Bzl: A mixture of Gly-NH-Bzl-HCl (1.00 g, 5.0 mmole) and triethylamine (500 mg, 5.0 mmole) in anhydrous dimethyl formamide (15 ml) was added at 0—5° to an anhydrous dimethyl formamide solution prepared by the addition of dicyclohexyl carbodiimide (1.14 g, 5.0 mmole) to Z-Gly-Gly-OH[16] (1.05 g, 5.0 mmole) in anhydrous dimethyl formamide (10.0 ml). The whole was stirred at the same temperature for 3.0 hr, then at room temperature for 3.0 hr. After insoluble material had been removed by filtration and dimethyl formamide had been evaporated *in vacuo*, the residue was recrystallized from tetrahydrofuran, to give Z-Gly-Gly-NH-Bzl (300 mg, 18%) as colorless needles, mp 163°. IR ν_{max}^{KBr} cm^{-1}: 3300, 1690, 1650, 1565, 1285, 1240. *Anal.* Calcd. for $C_{19}H_{21}O_4N_3$: C, 64.21; H, 5.96; H, 11.83. Found: C, 64.02; H, 5.79; N, 12.03.

14) Reference 4, p. 893.
15) M. Bergmann, L. Zervas, and J.S. Fruton, *J. Biol. Chem.*, **115**, 593 (1936)
16) Reference 4, p. 892.