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Amino Acids and Peptides. I. Novel Peptide Bond Formation Catalyzed by Metal Ions. I.[1] Formation of Glycine Peptide Esters

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Treatment of glycine ester with Cu (II) ion and other metal ions in a non-aqueous solution at room temperature resulted in the simultaneous formation of di-, tri-, and tetraglycine peptide esters. Each peptide ester was separated by column chromatography after carbobenzoxylation. Diketopiperazine was not formed in this reaction. Various reaction conditions were examined in detail.

There are many reports on the formation of peptide bonds by chemical reactions, but few are concerned with peptide bond formation using metal complexes. Buckingham[3] and Collman[4] independently found peptide bond formation in the coordination sphere of a cobalt (III) complex with the reaction of a cobalt (III) glycine ester complex with glycine ester. Nakahara[5] has also recognized a different type of peptide bond formation in the reaction of a Cu (II) complex of the Schiff base derived from salicylaldehyde and glycinamide with glycine ester. However, no isolation of peptides formed from these metal complexes has been reported in any of the papers cited above.[3,4,5]

It is apparent that (1) the Cu (II) atom[6] protects the amino group of amino acids in the reaction of the Cu (II) complex of glycine with acetaldehyde for the synthesis of Akabori's threonine synthesis[7] and in the ω-carbobenzoxylation of lysine,[8] and that (2) the rate of hydrolysis of α-amino ester is extremely accelerated in aqueous solution in the presence of Cu (II) ion[9] and other metal ions[9a,c,e,10] by the activation of ester carbonyl for nucleophilic attack. Considering (1) and (2), metal complexes of α-amino acid esters whose α-amino group is protected and whose ester carbonyl group is activated by metal ions might be expected to react with other amino acid esters to form dipeptide esters in non-aqueous solution. We previously communicated[1] that peptide bond formation easily occurred in the reaction of Cu (II) ion with amino acid esters, and that the peptides formed could be isolated with high optical purity after Cu (II) ion was removed by treatment with hydrogen sulfide, and that in the case of

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3) *a*) D.A. Buckingham, L.G. Marzilli, and A.M. Sargeson, *J. Am. Chem. Soc.*, **89**, 2772 (1967); *b*) *Idem, ibid.*, **89**, 4539 (1967).
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5) T. Higashiyama, H. Ueda, and A. Nakahara, *Bull. Chem. Soc. Japan*, **41**, 3028 (1968).
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7) M. Sato, K. Okawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **30**, 937 (1957).
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9) *a*) H. Kroll, *J. Am. Chem. Soc.*, **74**, 2036 (1952); *b*) M.L. Bender and B.M. Turnquest, *ibid.*, **79**, 1889 (1957); *c*) R. Mathur and N.C. Li, *ibid.*, **86**, 1289 (1964); *d*) W.A. Conner, M.M. Jones, and D.L. Tuleen, *Inorg. Chem.*, **4**, 1129 (1965); *e*) R.J. Angelici and B.E. Leach, *J. Am. Chem. Soc.*, **89**, 4605 (1967).
10) *a*) M.D. Alexander and D.H. Busch, *J. Am. Chem. Soc.*, **88**, 1130 (1966); *b*) R.W. Hay and L.J. Porter, *J. Chem. Soc.*, **1969**, 127.
11) Abbreviations in this paper are: H-Gly-OEt, H-Gly_2-OEt, H-Gly_3-OEt, H-Gly_4-OEt, Z-Gly_2-OEt: glycine ethyl ester, diglycine ethyl ester, triglycine ethyl ester, tetraglycine ethyl ester and N-carbobenzoxy diglycine ethyl ester and so on.

H-Gly-OEt,[11] peptide esters, H-Gly$_2$-OEt, H-Gly$_3$-OEt, and H-Gly$_4$-OEt were formed at the same time.

We now report a fundamental study of this novel peptide bond formation employing glycine esters, Cu(II) ion and other metal ions in various conditions.

1. Peptide Bond Formation with Dichlorobis-(ethyl glycinate)-copper (II); Cu(H-Gly-OEt)$_2$Cl$_2$

There are few papers written on the preparation of copper amino acid ester complexes.[12,13] Copper glycine ester complex was prepared according to methods in the literature.[12,13] The $\nu_{C=O}$ in the IR spectrum of the solid state (KBr) of this complex is split into two peaks, 1747 and 1695 cm^{-1}, as previously noted by Springer[12] and Hay.[13] They concluded that this complex was formed by a copper-nitrogen bond only, although the possibility of weak interaction between Cu (II) ion and the carbonyl group could not be excluded. Similar splitting was observed at 1745 and 1715 cm^{-1} with nearly equal intensity in the IR spectrum of this complex in diluted acetonitrile solution. This complex is thought to exist in solution as it does in the solid state. This complex should have characteristics by which the α-amino group of the ester is protected by Cu (II) ion and the carbonyl group is activated for nucleophilic attack. Reaction of this complex with H-Gly-OEt as nucleophile was attempted first.

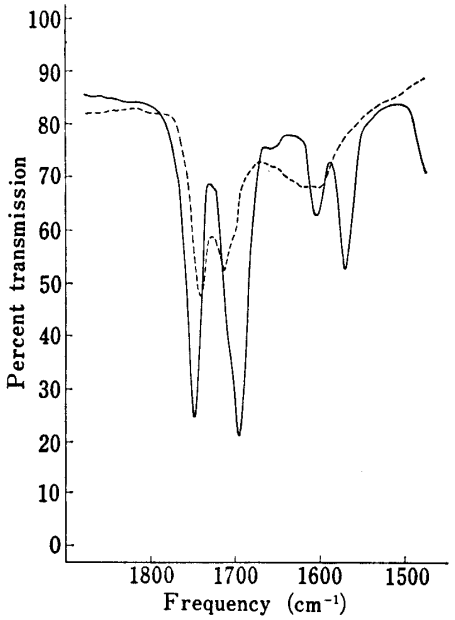

Fig. 1. Infrared Spectrum of Cu(H-Gly-OEt)$_2$Cl$_2$

——— in KBr disk
------- in CH_3CN solution

$$\mathrm{Cu(H\text{-}Gly\text{-}OEt)_2Cl_2} \xrightarrow[\text{2) } \mathrm{Cl\cdot COOCH_2C_6H_5,\ Et_3N \text{ in } CHCl_3}]{\text{1) H-Gly-OEt in Anhydrous EtOH}} \begin{cases} \mathrm{Z\text{-}Gly_2\text{-}OEt} \\ + \\ \mathrm{Z\text{-}Gly_3\text{-}OEt} \\ + \\ \mathrm{Z\text{-}Gly_4\text{-}OEt} \end{cases}$$

Chart 1

When an anhydrous ethanolic solution containing a large excess of H-Gly-OEt·HCl and an equimolar amount of Et_3N was added to a suspension of Cu(H-Gly-OEt)$_2$Cl$_2$ in anhydrous ethanol, the solution immediately became blue and clear, and peptide bond formation occurred at room temperature. After the reaction was complete, the copper complex was decomposed by adding ethanol solution saturated with hydrogen chloride. Cu (II) ion was removed as cupric sulfide by treatment with hydrogen sulfide. Not only H-Gly$_2$-OEt but H-Gly$_3$-OEt and H-Gly$_4$-OEt as well were synthesized in the reaction mixture in a single step reaction. For the easy and quantitative isolation of each glycine peptide ester formed, the reaction mixture was carbobenzoxylated after changing the solvent from ethanol to chloroform. Using silica gel column chromatography, carbobenzoxylated glycine peptide esters were isolated. These were identified by comparison of their melting points and infrared (IR) spectra with those of authentic samples, or values from the literature. Results are summarized in Table I.

When the molar ratio of H-Gly-OEt/Cu(H-Gly-OEt)$_2$Cl$_2$ is small, dipeptide ester is obtained as the main product, tri- and tetrapeptide esters increase as the molar ratio increases. With

12) M.P. Springer and C. Curran, *Inorg. Chem.*, **2**, 1270 (1963).
13) R.W. Hay and L.J. Porter, *Australian J. Chem.*, **20**, 675 (1967).

Table I. Reaction of $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$ with H-Gly-OEt in Anhydrous EtOH

Run	Starting material Molar ratio		Reaction time (hr)	Yields of peptide esters formed[a] (%)		
	$Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$	H-Gly-OEt		Z-Gly_2-OEt	Z-Gly_3-OEt	Z-Gly_4-OEt
1	1[b]	1	4	24.5 (18.3)[c]	Trace	—
2	1	2	3	32.3 (32.3)[c]	Trace	—
3	1	10	3	26.7 (81.0)[c]	22.2 (45.5)[c]	2.7 (4.0)[c]

a) Yields based on added H-Gly-OEt.
b) The starting material $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$ was recovered in 52.9% yield.
c) Figures in parenthesis are yields of peptides based on $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$ assuming that two moles of peptide ester are formed from one mole of $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$.

a large excess of H-Gly-OEt (Run 3), the total yields of glycine peptide esters (di-, tri-, and tetrapeptide esters) are about 130%, based on $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$, assuming that two moles of peptide esters are formed from one mole of $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$. As the yield exceeds 100%, this peptide formation is a catalytic, not a stoichiometric reaction by $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$. The detailed reaction mechanism will be reported in a subsequent paper. This result suggests that added H-Gly-OEt coordinates the $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$ and/or copper peptide complex to drive peptide esters formed in the copper complex into solution by ligand exchange reactions.

II. Peptide Bond Formation with $CuCl_2$

The copper glycine ester complex, $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$ is prepared from $CuCl_2$ and H-Gly-OEt in ethanol.[12,13] Hence, instead of $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$, the direct reaction of $CuCl_2$ with excess H-Gly-OEt in anhydrous solvent is successful for this peptide bond formation.

$$\text{H-Gly-OEt} \xrightarrow[\text{2) } Cl\cdot COOCH_2C_6H_5,\ Et_3N \text{ in } CHCl_3]{\text{1) } CuCl_2 \text{ in Anhydrous EtOH}} \left\{ \begin{array}{l} \text{Z-Gly}_2\text{-OEt} \\ + \\ \text{Z-Gly}_3\text{-OEt} \\ + \\ \text{Z-Gly}_4\text{-OEt} \end{array} \right.$$

Chart 2

Table II. Reaction of $CuCl_2$ with H-Gly-OEt in Anhydrous EtOH

Run	Starting material Molar ratio		Reaction time (hr)	Yields of peptide esters formed[a] (%)		
	$CuCl_2$	H-Gly-OEt		Z-Gly_2-OEt	Z-Gly_3-OEt	H-Gly_4-OEt·HCl
1	1	6	3	41.3 (62.0)[b]	14.3 (24.3)[b]	Trance
2	1	12	3	28.1 (84.5)[b]	22.7 (45.4)[b]	2.7 (4.0)[b]
3	1	16	66	17.9 (71.5)[b]	16.7 (44.4)[b]	11.2 (22.5)[b]

a) Yields based on added H-Gly-OEt.
b) Figures in parentheses are yields of peptides calculated from $CuCl_2$ used. The calculation is based on the assumption that two moles of peptide are produced from one mole of $CuCl_2$.

Peptide bond formation occurred by stirring a mixture of anhydrous $CuCl_2$, H-Gly-OEt·HCl, and Et_3N in anhydrous ethanol at room temperature, followed by work-up above. Results of the reaction of $CuCl_2$ and H-Gly-OEt with various molar ratios are listed in Table II. Comparing Run 3 in Table I with Run 2 in Table II, the molar ratio of the starting materials, Cu (II) ion and H-Gly-OEt, is the same (1: 12) and yields and the product ratio of di-, tri-, and tetra peptides are very similar. The two reactions, therefore, seem to proceed *via* the same reaction path through $Cu(H\text{-}Gly\text{-}OEt)_2Cl_2$. Yields of total peptide ester (Run 2 and 3 in Table II) exceed 100%, based on $CuCl_2$; assuming as above, that two moles of

peptide are formed from one mole of $CuCl_2$. This reaction is also explained by the catalytic peptide bond formation by $CuCl_2$. The reaction of H-Gly_2-OEt with $CuCl_2$ in anhydrous ethanol did not form any H-Gly_4-OEt. Thus, H-Gly_4-OEt is formed by stepwise elongation of the peptide bond from H-Gly_2-OEt. No formation of H-Gly_5-OEt was observed. Isolation of H-Gly_4-OEt, in this case, was achieved as the hydrochloride without carbobenzoxylation because of its insolubility in ethanol under ice-cooling. When an ethanolic solution of H-Gly-OEt was stirred at room temperature for 240 hrs without $CuCl_2$, the total yield of H-Gly_2-OEt was less than 4% and no formation of diketopiperazine was observed.

This reaction presents a convenient method for the preparation of di-, tri- and tetraglycine peptide esters by the reaction of H-Gly-OEt with $CuCl_2$ in anhydrous ethanol at room temperature. Detailed reaction conditions were examined as follows.

Solvent Effects——In an aqueous solution, amino acid esters are rapidly hydrolyzed in the presence of Cu (II) ion,[9] aqueous solvents are not used for this peptide bond formation. Various solvents, *i.e.* anhydrous ethanol, DMF, chloroform, DMSO, tetrahydrofuran, and benzene were investigated for this peptide bond formation. Results are summarized in Table III.

Table III. Solvent Effects on Peptide Bond Formation[a]

Run	Solvent	Yields of peptide esters formed (%)[b]		
		Z-Gly_2-OEt	Z-Gly_3-OEt	Z-Gly_4-OEt
1	EtOH	41.3	14.3	trace
2	DMF	35.0	12.0	trace
3	$CHCl_3$	28.0	8.0	trace
4	DMSO	22.6	6.8	—
5	THF	15.9	4.7	—
6	C_6H_6	2.0	—	—

a) Reactions were performed at room temperature with 3 hr of stirring under a 6:6:1 molar ratio of H-Gly-OEt, Et_3N, and $CuCl_2$ in each solvent.
b) Yields based on added H-Gly-OEt.

$CuCl_2$ is easily soluble in ethanol and the reaction proceeds smoothly. However in solvents other than ethanol, $CuCl_2$ is sparingly soluble and reactions do not proceed as smoothly as in ethanol. After decomposition of the complex by the addition of hydrochloric acid, hydrogen sulfide was passed through the reaction mixture and no precipitates of cupric sulfide appeared in the solvents, except for chloroform and ethanol. In these instances, solvents were removed and ethanol was added to the reaction mixture, after which hydrogen sulfide was passed throguh it to precipitate cupric sulfide.

The yield of peptide ester has a strong correlation with the solubility of $CuCl_2$ or the complex in each solvent. Solvents with high polarity generally gave good results; reactivity for peptide bond formation is of the following order; EtOH>DMF>$CHCl_3$>DMSO>THF>C_6H_6.

Effects of Ester Groups——To investigate the effect of ester groups on peptide bond formation, methyl, ethyl, benzyl, and isopropyl esters of glycine were used for peptide formation. Results are given in Table IV.

The reaction was carried out in chloroform at room temperature with stirring for 3 hr under 6:1 molar ratio of glycine ester and $CuCl_2$, which was followed by the above work-up. Methy and ethyl esters of glycine showed nearly the same reactivity for peptide bond formation. Benzyl ester gave only the dipeptide ester under the same reaction conditions, but with bulky isopropyl ester no peptide bond formation was observed, probably because of its steric hindrance.

Effects of Cu (II) salts——To examine the effect of anions on cupric compounds, $CuSO_4$, $Cu(OAc)_2$, and CuO besides $CuCl_2$ were used in peptide bond formation.

Table IV. Effects of Ester Groups on Peptide Bond Formation in $CHCl_3$

Run	H-Gly-OR -R	Yields of peptide esters formed (%)[a]		
		Z-Gly_2-OR	Z-Gly_3-OR	Z-Gly_4-OR
1	Me	30.0	8.7	trace
2[b]	Et	28.0	8.0	trace
3	Bzl	20.0	—	—
4	iso-Pr	—	—	—

a) Yields based on added H-Gly-OEt.
b) cited from Run 3 in Table III

Reactions were performed in anhydrous ethanol at room temperature with stirring for 3 hr under a 6: 6: 1 molar ratio of H-Gly-OEt·HCl, Et_3N, and cupric salt. $CuSO_4$ and $Cu(OAc)_2$ are insoluble in ethanol. However, when H-Gly-OEt·HCl and Et_3N are added to a suspension of $CuSO_4$ or $Cu(OAc)_2$ in ethanol, a clear blue solution is gradually formed and yields of peptides are fairly comparable with that of $CuCl_2$. As CuO is insoluble in ethanol, 90% of the CuO recovered from the reaction mixture and only the dipeptide ester was obtained in low yield. Results are shown in Table V.

Table V. Effects of Cu(II) salts on Peptide Bond Formation in EtOH

Run	Cu Salts	Yields of peptide esters formed (%)[a]		
		Z-Gly_2-OEt	Z-Gly_3-OEt	Z-Gly_4-OEt
1[b]	$CuCl_2$	41.3	14.3	trace
2	$CuSO_4$	37.1	16.0	trace
3	$Cu(OAc)_2$	37.1	10.3	trace
4	CuO	13.6	trace	—

a) Yields based on added H-Gly-OEt.
b) cited from Run 1 in Table II

III. Formation of Peptide Bonds with α-, β-, and γ- Amino Acid Esters

As shown above, glycine ester easily formed peptide esters with Cu (II) ion. Whether or not β- and γ-amino acid esters, β-alanine ester and γ-amino butyric acid ester form the corresponding peptide ester was investigated.

For comparison, all reactions were carried out in methanol or ethanol with stirring for 3 hr under a 12: 12: 1 molar ratio of amino acid ester hydrochloride, Et_3N, and $CuCl_2$ followed by the usual work-up. After carbobenzoxylation, each peptide ester was isolated by column chromatography. Results are given in Table VI.

Table VI. Formation of Peptide Bonds with α-, β-, and γ-Amino Acid Esters

Run	α-, β-, and γ-Amino acid esters H_2N-$(CH_2)_n$-COOR		Solvent	Peptide esters formed and Yields (%)[a]	
	n	-R			
1[b]	1	Et	EtOH	Z-Gly_2-OEt	28.1
				Z-Gly_3-OEt	22.7
				Z-Gly_4-OEt	2.7
2	2	Me	MeOH	Z-β-Ala-β-Ala-OMe	12.1
3	3	Et	EtOH	——	

a) Yields based on the H_2N-$(CH_2)_n$-COOR used.
b) cited from Run 2 in Table II

β-Alanyl-β-alanine methyl ester from β-alanine methyl ester was obtained in a 12.1% yield, which is below half the total yields of di- and triglycine esters, and no formation of β-alanine tripeptide ester was observed in the reaction mixture. γ-Amino-butyric acid ethyl ester gave neither peptide nor 2-pyrrolidone and the starting ester was recovered in good yield. Ease of peptide bond formation among the α-, β-, and γ-amino acid esters has the following order, $\alpha>\beta>\gamma$. The stability of the copper chelate complex of α-, β-, and γ-amino acids is reported to be of the same order, since α-amino acid copper chelate complex forms the most stable five membered chelate ring.[14] Stability of a six and seven membered copper chelate ring is greatly decreased. We have postulated a mechanism for peptide formation which invloves a five membered metal chelate complex shown as A or B.

$n=1, 2, 3$

IV. Effects of Metal Ions other than Cu (II) Ion on Peptide Bond Formation

We examined this peptide bond formation further using H-Gly-OEt and several metal chlorides to study whether or not this reaction would occur with metal ions other than Cu(II) ion.

Table VII. Peptide Formation with H-Gly-OEt Using Metal Chloride in Anhydrous EtOH

Run	Metal chloride MCl_n	Reaction time (hr)	Isolation procedure[a]	Yields of peptide esters formed (%)[b]	
				Z-Gly_2-OEt	Z-Gly_3-OEt
1	$MgCl_2$	3	B	16.8	6.8
2	$AlCl_3$	3	B	15.0	6.3
3	$CrCl_3$	240	C	9.1	9.7
4	$MnCl_2$	3	B	8.6	2.3
5	$FeCl_2$	240	C	11.3	12.0
6	$FeCl_3$	3	B	10.9	10.8
7	$CoCl_2$	3	A	9.5	2.8
8	$NiCl_2$	24	A	14.5	0.9
9	CuCl	3	A	35.8	10.8
10	$CuCl_2$	3	A	43.3	12.5
11	$ZnCl_2$	48	D	—	—
12	$PdCl_2$	18	D	—	—
13	$SnCl_4$	4	A	11.8	12.0
14	$HAuCl_4$	3	E	—	—
15	HgCl	120	E	—	—
16	$HgCl_2$	24	E	—	—
17	$PbCl_2$	48	C	21.7	13.1

a) Procedure A: After completion of the reaction, metal ion was removed as metal sulfide by treatment with H_2S. Filtration and evaporation gave a residue which was submitted to N-carbobenzoxylation.
Procedure B: After completion of the reaction, the reaction mixture was evaporated without treatment with H_2S, to give a residue which was submitted to N-carbobenzoxylation.
Procedure C: After completion of the reaction insoluble metal chloride was filtered. The filtrate was treated as was reaction mixture in Procedure B.
Procedure D: When the reaction was completed, a precipitate of metal chloride complexes with glycine ester, $M(H\text{-}Gly\text{-}OEt)_2Cl_2$ was obtained only by filtration. No formation of peptide ester from the filtrate was observed.
Procedure E: After completion of the reaction, gold and mercury, which were reduced, were filtered off. No formation of peptide ester from the filtrate was observed.
b) Yields are based on H-Gly-OEt used. Wherever peptide bond formation reaction was observed, formation of a trace amount of H-Gly_4-OEt was also seen.

14) A. Nakahara, J. Hidaka, and R. Tsuchida, *Bull. Chem. Soc. Japan*, **29**, 925 (1956).

The reaction was carried out in ethanol at room temperature under a 6:6:1 molar ratio of H-Gly-OEt·HCl: Et_3N: metal chloride.

Isolation procedures for the peptides formed are arranged in Table VII according to properties of the metal ions used. Results summarized in Table VII clearly demonstrate that the series of metal ions examined, other than Cu (II) ion, are less effective in this peptide bond formation than is Cu (II) ion, and that the difference in efficiency for peptide formation between Cu (II) ion and Co (II) and Ni (II) ions is slightly greater than that for metal-promoted hydrolyses of α-amino acid esters[9a] and amides.[15] It is also evident that not only transition metal ions such as Cu (II), and Ni (II) ions, but also non-transition metal ions show peptide bond formation. Interestingly, Zn (II) and Pd(II) ions disclosed no peptide bond formation, Zn(H-Gly-OEt)$_2$Cl$_2$ and Pd(H-Gly-OEt)$_2$Cl$_2$ were, instead, obtained in high yields. Moreover, with Au (III), Hg (I), and Hg (II), the reduced metal (gold or mercury) precipitated and no formation of peptide ester was observed.

Professor Akabori proposed poly-glycine as a prebiotic peptide, and formation of poly-glycine was thought to proceed from formaldehyde α-amino nitrile.[16] In our reaction, glycine tetrapeptide ester is formed at room temperature by metal salt catalysts through step-wise elongation of peptide bonds. Further elongation of peptide bonds from tetraglycine ester and glycine ester might result in poly-glycine. Glycine ester is thought to be the methyl ester which is most reactive in this peptide bond formation, and would be derived from methane and formaldehyde. This process suggests a reasonable pathway for the prebiotic formation of poly-glycine proposed by Akabori.

Experimental[17]

Materials——Commercially available metal salts were employed. These were dried over P_2O_5 *in vacuo* at about 120° before use. All amino acid ester hydrochlorides were prepared according tio general methods in the literature.[18] H-Gly-OMe·HCl mp 175°, H-Gly-OEt·HCl mp 144°, H-Gly-OPri·HCl mp 86—87° (Lit.[19] mp 84—86°), H-Gly-OBzl·HCl mp 139°, methyl β-alaninate·HCl mp 90—92° (Lit.[20] mp 86°), ethyl γ-aminobutyrate·HCl mp 71—72° (Lit.[21] mp 70—72°).

Dichlorobis-(ethyl glycinate)-copper (II); Cu(H-Gly-OEt)$_2$ Cl$_2$——Cu(H-Gly-OEt)$_2$Cl$_2$ was prepared essentially as described by Springer and Curran.[12] mp 105—106° (decomp.) IR ν_{max}^{KBr} cm^{-1}: 3300, 3270, 3240, 3180, 3140, 1747, 1695, 1605, 1570, 710. (Lit.[12] 3300, 3263, 3236, 3170, 3125, 1748, 1700, 1607, 1570, 708). IR $\nu_{max}^{CH_3CN}$ cm^{-1}: 1747, 1715.

Reaction of Cu(H-Gly-OEt)$_2$Cl$_2$ with H-Gly-OEt (Table I)——Run 3: A mixture of H-Gly-OEt·HCl (3.50 g, 25 mmoles) and Et_3N (2.53 g, 25 mmoles) in anhyd. EtOH (30 ml) was added at once to a suspension of Cu(H-Gly-OEt)$_2$Cl$_2$ (850 mg, 2.5 mmoles) in anhyd. EtOH (15 ml) with stirring. The clear blue solution formed was stirred at room temperature for 3 hr, then it was cooled to 0—5° in an ice–bath. The Cu (II) complex in the solution was decomposed by adding ethanol saturated with hydrogen chloride. After Cu (II) ion was removed as CuS by treatment with H_2S, ethanol was removed under reduced pressure to obtain a mixture of glycine peptide ester hydrochlorides. The residue was dissolved in $CHCl_3$ (50 ml) in an ice–bath. A solution of carbobenzoxy chloride (6.2 g, 36 mmoles) in $CHCl_3$ (10 ml) and a $CHCl_3$ solution (10 ml) of Et_3N (7.1 g, 70 mmoles) were alternatingly added dropwise to the $CHCl_3$ solution with stirring to keep the solution basic.

The solution was stirred for another 30 min at 0—5°, then for an hour at room temperature. A $CHCl_3$-insoluble white powder, Z-Gly$_4$-OEt was obtained by filtration (80 mg, 2.7% based on H-Gly-OEt, mp 198—200° (decomp.), (Lit.[22] mp 205°). IR ν_{max}^{KBr} cm^{-1}: 3300, 1745, 1695, 1650, 1550, 1284, 1245, 1215. *Anal.*

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16) S. Akabori, *Kagaku (Tokyo)*, **25**, 54 (1955).
17) All melting points are uncorrected. IR spectra measurements were performed with a Spectrophotometer, Model 402, Japan Spectroscopic Co., Ltd.
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20) A.S. Dutha and N. Anand, *Indian. J. Chem.*, 3, 323 (1965).
21) T. Jakobiec, *Acta. Polon. Pharm.*, 23, 111 (1966).
22) Reference 18, p. 1128.

Calcd. for $C_{18}H_{21}O_7N_4$ (Z-Gly$_4$-OEt): C, 52.93; H, 5.92; N, 13.72. Found: C, 52.58; H, 5.92; N, 14.11. The $CHCl_3$ layer was washed with 10% HCl, satd. $NaHCO_3$ and satd. NaCl, and dried over $MgSO_4$. After evaporation of $CHCl_3$, half the residue was purified by column chromatography using silica gel (solvent, $CHCl_3$: EtOH=97:3) giving Z-Gly$_2$-OEt (590 mg, 26.7% based on H-Gly-OEt) and successively Z-Gly$_3$-OEt (390 mg, 22.2% based on H-Gly-OEt). Recrystallization of crude Z-Gly$_2$-OEt from $CHCl_3$–ether gave pure Z-Gly$_2$-OEt (mp 80—81°). Pure H-Gly$_3$-OEt (mp 165—167°) was obtained by recrystallization from $CHCl_3$–EtOH. These peptide esters were identified by comparison of their IR spectra with those of authentic samples. Their mixed melting points compared with authentic samples showed no depression.

Run 1 and 2 were treated in a manner similar to that for Run 3.

Reaction of H-Gly-OEt with $CuCl_2$ (Table II)——Run 3: An ethanolic solution (55 ml) of H-Gly-OEt·HCl (5.59 g, 40 mmoles) and Et_3N (4.05 g, 40 mmoles) was added to a solution of anhyd. $CuCl_2$ (340 mg, 2.5 mmoles) in EtOH (5 ml). The blue solution obtained was stirred for 66 hr at room temperature. The Cu (II) complex was decomposed by adding ethanolic hydrogen chloride solution, after which a white precipitate appeared. This crude H-Gly$_4$-OEt·HCl (350 mg, 11.2% based on H-Gly-OEt) was obtained by filtration, mp 188—192°. An analytical sample was prepared by recrystallization from aq. EtOH, mp 212—213° (decomp.) (Lit.[23] mp 213—214° (decomp.)). IR ν_{max}^{KBr} cm^{-1}: 3300, 1744, 1637, 1590, 1210. Mass Spectrum, M^+ 274. *Anal.* Calcd. for $C_{10}H_{19}O_5N_4Cl$ (H-Gly$_4$-OEt·HCl): C, 38.65; H, 6.16; N, 18.03. Found: C, 38.81; H, 5.89; N, 18.19.

Z-Gly$_2$-OEt (1.05 g, 17.9% based on H-Gly-OEt), and Z-Gly$_3$-OEt (0.78 g, 16.7% based on H-Gly-OEt) were obtained from an ethanolic soluton by treatment similar to that above.

Runs 1 and 2 were performed in a manner similar to that for Run 3.

Reaction of H-Gly-OEt with $CuCl_2$ in Various Solvents (Table III)——A mixture of H-Gly-OEt·HCl (15 mmoles), Et_3N (15 mmoles), and anhyd. $CuCl_2$ (2.5 mmoles) in dry solvent (20 ml) was stirred at room temperature for 3 hr. The copper complex was decomposed by treatment with HCl gas and the solvent was removed on a steam bath under reduced pressure. The residue was dissolved in EtOH (30 ml). A subsequent work-up as above gave results shown in Table III. The Z-peptide ethyl esters obtained were isolated by silica gel chromatography (solvent: $CHCl_3$: EtOH=97:3).

Reaction of Various Glycine Esters with $CuCl_2$ (Table IV)——A mixture of H-Gly-OR·HCl (R=-Me, -Et, -Bzl, or -Pr^i) (15 mmoles), Et_3N (15 mmoles), and anhyd. $CuCl_2$ (2.5 mmoles) in dry $CHCl_3$ was stirred at room temperature for 3 hr. After the copper complex was decomposed by treatment with HCl gas, $CHCl_3$ was removed and the residue was dissolved in MeOH (Run 1), EtOH (Run 2) or iso-PrOH (Run 3 and 4). A subsequent work-up as above gave the results shown in Table IV. All peptide esters obtained were isolated by silica gel chromatography after carbobenzoxylation.

Run 1: Solvent of chromatography: $CHCl_3$:MeOH=97:3. Z-Gly$_2$-OMe, mp 62—64°, yield 30% based on H-Gly-OMe. Recrystallization from $CHCl_3$–ether gave colorless prisms, mp 63—64° (Lit.[22] mp 63—65°). IR ν_{max}^{KBr} cm^{-1}: 3330, 1750, 1695, 1665, 1545, 1295. *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.47; H, 5.60; N, 10.01. Z-Gly$_3$-OMe, mp 151—154°, yield 8.7% based on H-Gly-OMe. Colorless powders were obtained by recrystallization from MeOH–$CHCl_3$, mp 153—155° (Lit.[22] mp 151—155°). IR ν_{max}^{KBr} cm^{-1}: 3300, 1755, 1695, 1660, 1650, 1550, 1290. *Anal.* Calcd. for $C_{15}H_{19}O_6N_3$: C, 53.40; H, 5.68; N, 12.46. Found: C, 53.28; H, 5.63; N, 12.48.

Run 2: See Run 3 in Table III.

Run 3: Solvent of chromatography: $CHCl_3$: iso-PrOH=98:2. Z-Gly$_2$-OBzl, mp 107—109°, yield 20% based on H-Gly-OBzl. Recrystallization from $CHCl_3$–ether gave colorless needles, mp 109—110° (Lit.[22] mp 110°). IR ν_{max}^{KBr} cm^{-1}: 3350, 1740, 1710, 1650, 1540, 1200, 700. *Anal.* Calcd. for $C_{19}H_{20}O_5N_2$: C, 64.03; H, 5.66; N, 7.86. Found: C, 64.01; H, 5.71; N, 7.82.

Run 4 Solvent of chromatography: $CHCl_3$: iso-PrOH=98:2. No Z-peptide ester was obtained.

Reaction of H-Gly-OEt with Various Cu (II) Salts (Table V)——A mixture of H-Gly-OEt·HCl (15 mmoles), Et_3N (15 mmoles) and anhyd. Cu (II) salt (2.5 mmoles) in anhyd. EtOH (20 ml) was stirred at room temperature for 3 hr. Z-peptide esters were obtained by the work-up as above.

Reaction of Methyl β-Alaninate or Ethyl γ-Amino Butyrate with $CuCl_2$ (Table VI)——A mixture of amino acid ester hydrochloride (15 mmoles), Et_3N (15 mmoles), and anhyd. $CuCl_2$ (1.25 mmoles) in anhyd. EtOH (20 ml, Runs 1 and 3) or MeOH (20 ml, Run 2) was stirred at room temperature for 3 hr. Z-peptide esters were obtained by the work-up as above.

Run 1: See Run 2 in Table II.

Run 2: Using column chromatography on silica gel (solvent: CH_2Cl_2:MeOH=98:2), Z-β-alanyl-β-alanine methyl ester was obtained in a 12.1% yield based on β-alanine methyl ester, mp 108—109°. Recrystallization from benzene gave a pure sample, mp 110—111°. IR ν_{max}^{KBr} cm^{-1}: 3320, 3300, 1740, 1680, 1637, 1550. IR $\nu_{max}^{CHCl_3}$: 3460, 1735, 1670, 1510. *Anal.* Calcd. for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.58; H, 6.48; N, 9.27.

Run 3: This reaction gave neither peptide nor Z-pyrrolidone.

23) Reference 18, p. 1192.

Table VIII

Run	Metal chloride	Solubility of Metal chloride in EtOH	Color of reaction
1	$MgCl_2$	soluble	colorless
2	$AlCl_3$	partially soluble	colorless
3	$CrCl_3$	partially soluble	dark blue
4	$MnCl_2$	soluble	pale orange
5	$FeCl_2$	partially soluble	light brown
6	$FeCl_3$	soluble	brown
7	$CoCl_2$	soluble	violet
8	$NiCl_2$	soluble	sky blue
9	CuCl	soluble	blue
10	$CuCl_2$	soluble	blue
11	$ZnCl_2$	soluble	colorless precipitate[a]
12	$PdCl_2$	soluble	yellow precipitate[b]
13	$SnCl_4$	soluble	colorless
14	$HAuCl_4$	soluble	pale green[c]
15	HgCl	partially soluble	red[d]
16	$HgCl_2$	soluble	red[e]
17	$PbCl_2$	partially soluble	colorless

a) Dichlorobis-(ethyl glycinate)-zinc (II), $Zn(H\text{-}Gly\text{-}OEt)Cl_2$[13] was obtained in 97% yield based on $ZnCl_2$ used. Recrystallization from $CHCl_3$–EtOH gave colorless prisms, mp 165—169°. IR ν_{max}^{KBr} cm^{-1}: 3295, 3255, 3165, 1728, 1600. (Lit.[13] 3295, 3257, 3165, 1730, 1600). *Anal.* Calcd. for $(C_4H_9NO_2)_2ZnCl_2$: C, 28.06, H, 5.29; N, 8.18. Found: C, 27.99; H, 5.26; N, 8.27.

b) Dichlorobis-(ethyl glycinate)-palladium (II), $Pd(H\text{-}Gly\text{-}OEt)_2Cl_2$[13] was obtained in 76% yield based on $PdCl_2$ used. Recrystallization from $CHCl_3$–EtOH gave yellow needles, mp 184—185°. IR ν_{max}^{KBr} cm^{-1}: 3280, 3225, 1750, 1585, 706. (Lit.[13] 3279, 3226, 1751, 1585, 704). *Anal.* Calcd. for $(C_4H_9NO_2)_2PdCl_2$: C, 25.05; H, 4.73; N, 7.30. Found: C, 25.05; H, 4.68; N, 7.40.

c) Metal Au was obtained in 79% yield based on $HAuCl_4$.

d) Metal Hg was obtained in 98% yield based on HgCl.

e) Metal Hg was obtained in 52% yield based on $HgCl_2$.

Reaction of H-Gly-OEt with Various Metal Chlorides (Table VII)——Anhyd. metal chloride (2.5 mmoles) was added to an ethanolic solution (40 ml) of H-Gly-OEt·HCl (15 mmoles) and Et_3N (15 mmoles). The reaction mixture was stirred at room temperature. Reaction time and isolation procedures are shown in Table VII. The appearances of the reaction are briefly summarized in the above table.

Synthesis of Authentic Samples——Z-Gly_2-OEt or Z-Gly_3-OEt for authentic samples was synthesized from H-Gly-OEt and Z-Gly-OH in $CHCl_3$ or H-Gly_2-OEt and Z-Gly-OH in acetonitrile by the DCCD method.

Z-Gly_2-OEt: mp 80—81° (Lit.[22] mp 80—81°). IR ν_{max}^{KBr} cm^{-1}: 3330, 3290, 1745, 1690, 1660, 1550, 1310, 1295, 695.

Z-Gly_3-OEt: mp 164—166° (Lit.[22] mp 166—167°). IR ν_{max}^{KBr} cm^{-1}: 3340, 3270, 1748, 1694, 1664, 1545, 1285, 1245, 1198.