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AMINO ACIDS AND PEPTIDES—IV[1]

NOVEL PEPTIDE BOND FORMATION CATALYSED BY METAL IONS—II[2]
FORMATION OF OPTICALLY ACTIVE PEPTIDE ESTERS[3]

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Abstract—The novel peptide bond formation previously reported in the reaction of glycine ester with Cu(II) ion in an anhydrous solvent, was examined using several kinds of optically active amino acid esters. Various reaction conditions were studied in detail for Phe-OEt. From Phe-OEt, Ala-OMe, Leu-OMe, Trp-OMe, Ser-OMe, and Met-OMe, the expected dipeptide esters with the same amino acid components were obtained without racemization. Asp(OEt)-OEt, and Glu(OMe)-OMe gave only optically active α-dipeptide esters No formation of dipeptide esters was observed with Ile-OMe, Cys-OMe, His-OMe, and Pro-OEt. However, Lys-OMe, and Orn-OMe afforded optically active lactam derivatives. Some explanations of these abnormal observations have been given.

Attempts to prepare di- and tri-peptide esters carrying different kinds of amino acid units were also studied.

Previously,[2] we reported that the simultaneous formation of di-, tri-, and tetra-glycine esters occurred when a glycine ester was treated with several kinds of metal ions in a completely anhydrous solvent. This novel reaction was originally understood to be the result of the same activation of the ester CO group by metal ions as that observed in the well-known acceleration of hydrolysis rate of amino acid ester in aqueous solution.[4,5] In peptide bond formation, Cu(II) ion was the most powerful among metal ions examined.[2] Moreover, the peptide esters formed were easily isolated from the reaction mixture by decomposing the Cu(II) complex with alcoholic hydrogen chloride, followed by the removal of liberated Cu(II) ion with hydrogen sulfide.[2] These facts clearly demonstrate that this novel reaction has a completely different character from the reactions independently developed by Buckingham[6] and Collman[7] and that many applications to the synthetic peptide chemistry can be foreseen for this Cu(II)-catalysed reaction.

We report an application of this novel reaction in the preparation of optically active peptide esters from optically active amino acid esters.*

*All amino acids, except glycine and DL-2,3-diaminopropionic acid, have 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; EtOH = Ethanol; MeOH = Methanol; DMF = Dimethyl formamide; EtOAc = Ethyl acetate; THF = Tetrahydrofuran; $CHCl_3$ = Chloroform, CH_2Cl_2 = Dichloromethane.

I. *Reaction of ethyl* L-*phenylalaninate* (Phe-OEt) *with cupric chloride* ($CuCl_2$).

The reaction of Phe-OEt with $CuCl_2$ was first examined under various conditions in order to discover the optimum conditions for this peptide formation using optically active amino acid esters.

$$\text{Phe-OEt} \xrightarrow[\text{(2) ClCOOCH}_2\text{C}_6\text{H}_5,\ \text{Et}_3\text{N in CHCl}_3]{\text{(1) CuCl}_2\text{ in anhyd EtOH}} \text{Z-Phe-Phe-OEt}$$

CHART 1

A clear blue solution obtained by adding anhydrous $CuCl_2$ (1·0 eq) to a mixture of Phe-OEt-hydrochloride (HCl) (12·0 eq) and triethylamine (12·0 eq) in anhydrous ethanol was stirred under the conditions indicated in Table 1. The Cu(II) complex which formed was decomposed by adding ethanolic hydrogen chloride. The liberated Cu(II) ion was removed from the mixture as cupric sulfide by passing hydrogen sulfide through the acidic ethanolic mixture. The entire product was carbobenzoxylated as usual,[2] and the N-carbobenzoxypeptide ester was isolated using silica gel column chromatography. The above procedure was almost the same as that used in the reaction of glycine ester with $CuCl_2$.[2]

Being completely different from the reaction of glycine ester,[2] dipeptide ester, ethyl N-carbobenzoxy-L-phenylalanyl-L-phenylalaninate (Z-Phe-Phe-OEt), was obtained as the sole product, no formation of tri-, and tetra-peptide esters was observed. Comparison of the optical rotation of the isolated N-protected dipeptide ester with that of an authentic sample independently prepared,[8] clearly

Table 1. Reaction of Phe-OEt with $CuCl_2$

Run	Starting materials (Molar ratio) Phe-OEt-HCl	Et_3N	$CuCl_2$	Reaction conditions Time (hr)	Temp (°C)	Z-Phe-Phe-OEt Isolated[a] Yield (%) based on Phe-OEt	M.P.[b] (°C)	$[\alpha]_D^{20}$ (c, EtOH)[b]
1	12	12	1	3	0–5	11·4	134–135 [138	−16·9° (2·0) −17·4° (2·0)][c]
2	12	12	1	3	Room Temp	18·2	130–134 [137–138	−16·7° (1·1) −17·4° (1·1)][c]
3	12	12	1	18	Room Temp	32·3[d]	130–134	−16·7° (0·5)
4	12	12	1	3	Reflux	4·2	125–130	−1·0° (0·5)
5	12[e]	0	1	3	Room Temp	23·5	136–138	−16·8° (0·6)
6	12[e]	12	1	3	Room Temp	20·2	134–136	−16·7° (0·6)

[a]Measurements of m.p. and optical rotation were carried out using a sample purified by silica gel column chromatography.

[b]Authentic Z-Phe-Phe-OEt showed m.p. 138° and $[\alpha]_D^{20}$ −17·5° (c = 1·0, EtOH) and $[\alpha]_D^{20}$ +10° (c = 0·6, EtOAc). Reported values[8] were m.p. 136–138° and $[\alpha]_D^{23}$ +11·0° (c = 0·91, EtOAc).

[c]Values in parentheses were measured with a sample obtained by one recrystallization from EtOAc-light petroleum.

[d]In addition to Z-Phe-Phe-OEt, Z-Phe-NH_2, m.p. 160–164° and $[\alpha]_D^{20}$ +11·0° ($CHCl_3$) was obtained in 1·0% yield based on the Phe-OEt used. This was identified with an authentic sample[8,9] m.p. 166° and $[\alpha]_D^{20}$ +11·9° ($CHCl_3$), prepared independently.

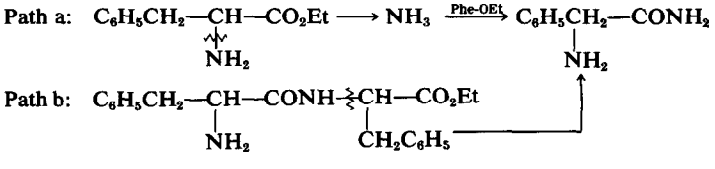
[e]Oily Phe-OEt was used instead of its crystalline hydrochloride.

disclosed that the Z-Phe-Phe-OEt prepared had a high optical purity. Experimental results obtained by changing reaction conditions are summarized in Table 1.

Changes in the reaction temperature influenced the yield of dipeptide ester, and seriously effected the optical purity of the isolated product. That is, when peptide bond formation was carried out at room temperature, the Z-Phe-Phe-OEt obtained in 18·2% yield, showed a high optical purity. However, the reaction performed at 0–5° afforded the dipeptide ester of a similar high optical purity in a lower yield. Reflux of the ethanolic reaction mixture drastically lowered the yield of Z-Phe-Phe-OEt, and there was almost complete racemization of the isolated peptide ester (Table 1, runs 1, 2, and 4).

To improve the yield of Z-Phe-Phe-OEt, the reaction period was extended from 3·0 hr to 18·0 hr, to give the desired dipeptide ester in 32·3% yield. Although extension of the reaction period again afforded no tripeptide ester, the N-carbobenzoxy-L-phenylalanine amide (Z-Phe-NH_2) could be isolated from the reaction mixture in 1·0% yield in addition to the normal product (Table 1, run 3). The amide obtained was identified with the independently prepared authentic sample[8,9] by mixed m.p.m. and by comparison of their IR spectra.

Two possible reaction paths were anticipated for the formation of Phe-NH_2. One is the formation of ammonia by cleavage of the C—N bond of Phe-OEt, followed by the capture of ammonia by the other molecule of Phe-OEt (Scheme 1, path a). The other reaction path is the direct formation of Phe-NH_2 from ethyl L-phenylalanyl-L-phenylalaninate (Phe-Phe-OEt), by cleavage of its C—N bond (Scheme 1, path b). However, path a is tentatively considered responsible for the abnormal formation



SCHEME 1.

of Phe-NH_2 because treatment of Phe-Phe-OEt with anhydrous $CuCl_2$ in ethanol for 65·0 hr affords no trace amount of Phe-NH_2 and because ammonia gas has been reported to be evolved when amino acid is irradiated with UV light in the presence of metal oxide.[10]

The effect of the presence of triethylamine on peptide formation was studied using an oily free base of Phe-OEt. As shown in Table 1, no clear difference between the presence and the absence of triethylamine in the reaction was observed in the yield of the formed dipeptide ester and its optical purity (Table 1, runs 5, and 6).

II. *Reaction of optically active amino acid ester other than* Phe-OEt *with* $CuCl_2$.

Based on the results with Phe-OEt, the reaction of optically active amino acid esters, other than Phe-OEt, with $CuCl_2$ was examined. Usually, anhydrous $CuCl_2$ (1·0 eq) was added to a mixture of amino acid ester hydrochloride (12·0 eq) and triethylamine (12·0 eq) in anhydrous methanol (or ethanol), then the reaction mixture was stirred at room temperature for 3·0 hr. After the reaction was over, the whole mixture was worked up as in the case of Phe-OEt. The N-protected peptide esters obtained were respectively identified with authentic samples independently prepared from N-carbobenzoxy-L-amino acid and L-amino acid ester by the DCCD (dicyclohexyl carbodiimide) method,[11] using mixed m.p.m. and a comparison of physical constants. Results are summarized in Table 2.

When methyl L-alaninate (Ala-OMe) was submitted to the reaction described, methyl N-carbobenzoxy-L-alanyl-L-alaninate (Z-Ala-Ala-OMe) was obtained in 34·5% yield, based on the Ala-OMe used. One recrystallization from ethyl acetate—light petroleum afforded the pure dipeptide ester showing m.p. 104–105°, and $[\alpha]_D^6$ −53·5° (MeOH). A trace amount of tripeptide ester, methyl N-carbobenzoxy-L-alanyl-L-alanyl-L-alaninate (Z-Ala-Ala-Ala-OMe) was also isolated by column chromatography, and was identified with the authentic sample.[12] However, when methyl L-leucinate (Leu-OMe), which should have a larger steric hindrance than Ala-OMe, was treated in a manner similar to Ala-OMe, methyl N-carbobenzoxy-L-leucyl-L-leucinate (Z-Leu-Leu-OMe) was obtained in 27·1% yield based on Leu-OMe. Formation of the tripeptide ester could not be observed in the case of Leu-OMe. Changes in the molar ratio of Leu-OMe and $CuCl_2$ from 12:1 to 6:1 and in the reaction time from 3·0 hr to 18·0 hr, produced a slight improvement in the yield of Z-Leu-Leu-OMe (Table 2, runs 2, and 3). The same peptide bond formation attempted using methyl L-isoleucinate (Ile-OMe), which carries a side chain with much greater steric hindrance than Leu-OMe, simply produced the starting ester instead of the desired methyl N-carbobenzoxy-L-isoleucyl-L-isoleucinate (Z-Ile-Ile-OMe).

The decrease in the reactivity of amino acid esters observed in the order of Ala-OMe > Leu-OMe > Ile-OMe is undoubtedly due to the change in steric bulkiness of their side chains; i.e. the methyl, 2-methylpropyl, and 1-methylpropyl groups.

Methyl L-tryptophanate (Trp-OMe) carrying an aromatic ring in its side chain similar to that of Phe-OEt, also afforded the corresponding dipeptide ester, methyl N-carbobenzoxy-L-tryptophanyl-L-tryptophanate (Z-Trp-Trp-OMe), showing high optical purity, in 12·5% yield.

Reaction of methyl L-serinate (Ser-OMe), methyl L-methioninate (Met-OMe), and methyl L-cysteinate (Cys-OMe) were considered of interest since these amino acid esters carry one more ligand, e.g. hydroxyl, methylthio, and thiol groups, in their side chains, which should coordinate with Cu(II) ion, in addition to the normal amino and ester groups. Although treatments of Ser-OMe and Met-OMe with $CuCl_2$ afforded the expected dipeptide esters, the mixture prepared with Cys-OMe and $CuCl_2$ in methanol immediately precipitated bis-(methyl L-cysteinato)-copper(II) (Cu(Cys-$OMe)_2$), and gave no trace amount of the desired peptide derivative.

Hay, *et al.*[13] reported the formation of bis-(methyl-L-cysteinato)-metal(II) by the reaction of Cys-OMe with Hg^{++}, Ni^{++}, Rd^{++}, and Cd^{++} ions. They postulated a chelating structure for these metal complexes, in which the amino and thiolate functions were coordinated to central divalent metal ions. We feel it is reasonable to assign the same kind of structure, shown in Chart 2, to the Cu(II) complex of Cys-OMe.

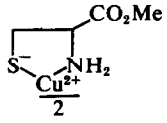


CHART 2

When esters of acidic amino acids, such as diethyl L-aspartate (Asp(OEt)-OEt) and dimethyl L-glutamate(Glu(OMe)-OMe), were submitted to peptide bond formation using $CuCl_2$, only α-dipeptide esters were obtained, and no formation of β- and γ-dipeptide esters was observed.

Peptide bond formation was also undertaken with several kinds of basic amino acid esters. With methyl L-histidinate(His-OMe), no peptide formation occurred and simple recovery of the starting His-OMe was observed. This result may be due to the chelated ring formation postulated in Chart 3 by coordination of the α-amino group and the imidazole ring of His-OMe to Cu(II) ion, which conceivably prohibits normal peptide bond formation.

Table 2. Reaction of optically active amino acid esters, other than Phe-OEt, with $CuCl_2$[a]

Run	Amino acid esters used	Solv.	Formed N-carbobenzoxy-peptide esters[b]	Yield (%) Based on amino acid esters	M.P. (°C)	Optical rotations	Authentic N-carbobenzoxy-peptide esters[d] M.P. (°C)	Optical rotations
1	Ala-OMe	MeOH	Z-Ala-Ala-OMe[c]	34·5	98–101	$[\alpha]_D^{16}-51·5°$ ($c = 0·8$, MeOH)	106	$[\alpha]_D^{16}-53·5°$ ($c = 0·9$, MeOH)[f]
					[104–105]	[$[\alpha]_D^{16}-53·5°$ ($c = 0·7$, MeOH)][e]		
2	Leu-OMe	MeOH	Z-Leu-Leu-OMe	27·1	94–96	$[\alpha]_D^{25}-34·2°$ ($c = 0·8$, EtOH)	97–98	$[\alpha]_D^{24}-35·5°$ ($c = 1·0$, EtOH)[g]
					[97–98	[$[\alpha]_D^{24}-35·8°$ ($c = 0·4$, EtOH)][e]		
3[h]	Leu-OMe	MeOH	Z-Leu-Leu-OMe	34·9	95–97	$[\alpha]_D^{24}-34·1°$ ($c = 0·6$, EtOH)		
4	Ile-OMe	MeOH	Z-Ile-Ile-OMe	0				
5	Trp-OMe	MeOH	Z-Trp-Trp-OMe	12·5	182–185	$[\alpha]_D^{20}-9·9°$ ($c = 1·7$, MeOH)	185–186	$[\alpha]_D^{20}-12·0°$ ($c = 1·5$, MeOH)[j]
					[184–185	[$[\alpha]_D^{20}-11·7°$ ($c = 1·5$, MeOH)][e]		
6	Ser-OMe	MeOH	Z-Ser-Ser-OMe	21·5	138–141	$[\alpha]_D^{22}-3·0°$ ($c = 1·5$, MeOH)	143–144[k]	$[\alpha]_D^{22}-3·5°$ ($c = 1·2$, MeOH)
					[142–143	[$[\alpha]_D^{24}-3·3°$ ($c = 0·9$, MeOH)][i]		
7	Met-OMe	MeOH	Z-Met-Met-OMe	21·7	98–100	$[\alpha]_D^{19}-25·6°$ ($c = 0·7$, MeOH)	104–105	$[\alpha]_D^{19}-28·0°$ ($c = 1·0$, MeOH)[l]
					[103–104	[$[\alpha]_D^{18}-27·8°$ ($c = 0·8$, MeOH)][e]		
8	Cys-OMe	MeOH	Z-Cys-Cys-OMe	0				
9	Asp(OEt)-OEt	EtOH	Z-Asp(OEt)-Asp-OEt	10·8	81–85	$[\alpha]_D^{24}-12·2°$ ($c = 1·4$, EtOH)	89–90	$[\alpha]_D^{18}-12·3°$ ($c = 1·2$, EtOH)
					[89–90	[$[\alpha]_D^{18}-12·3$ ($c = 0·9$, EtOH)][m]		
10	Glu(OMe)-OMe	MeOH	Z-Glu(OMe)-Glu-OMe	7·4	105–109	$[\alpha]_D^{13}-20·7°$ ($c = 1·0$, MeOH)	109–110	$[\alpha]_D^{13}-21·0°$ ($c = 0·8$, MeOH)[n]
					[108–109	[$[\alpha]_D^{13}-21·1°$ ($c = 1·0$, MeOH)][m]		
11	His-OMe	MeOH	Z-His-His-OMe	0				
12	Lys-OMe	MeOH	Z-Lys-Lys-OMe	0[o]				
13	Orn-OMe	MeOH	Z-Orn-Orn-OMe	0[p]				
14	Pro-OEt	EtOH	Z-Pro-Pro-OEt	0				

[a]The molar ratio of amino acid ester and $CuCl_2$ was 12:1. All reactions were carried out at room temperature for 3·0 hrs.

[b]Measurements of melting point and optical rotation were made with a sample purified by silica gel column chromatography.

[c]In addition to Z-Ala-Ala-OMe, trace amount of Z-Ala-Ala-Ala-OMe, m.p. 180–184°, was isolated by silica gel column chromatography. This was identified with an authentic sample[12] by mixed m.p.m. and by spectral data.

[d]Independently prepared using the DCCD method from N-carbobenzoxy-L-amino acid and L-amino acid ester.[11]

[e]Values in parentheses were obtained from a sample recrystallized once from EtOAc-light petroleum.

[f]Reported values are m.p. 105·5° and $[\alpha]_D^{21}-40·2°$ (MeOH). I. Imita, J. Oohashi, T. Tokuda, and M. Nakajima, *Nippon Nogei Kagaku Kaishi, Japan*, **39**, 378 (1965).

[g]Reported values are m.p. 97–98·5° and $[\alpha]_D^{24}-35·3°$ (EtOH). Reference 11, p. 1133.

[h]The molar ratio of Leu-OMe and $CuCl_2$ was 6:1. The reaction was carried out for 18·0 hr.

[i]Values in parentheses were obtained from a sample recrystallized once from EtOAc.

[j]Reported values are m.p. 196° and $[\alpha]_D^{25}-13°$ (MeOH). M. Wilchek and A. Patchornik, *J. Org. Chem.*, **28**, 1874 (1963).

[k]The reported value is m.p. 143–145°. J. S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).

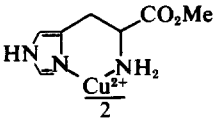


CHART 3.

When methyl L-lysinate(Lys-OMe) carrying a ω-amino group in its side chain was similarly treated, L-3-amino-caprolactam was obtained in 61·0% yield instead of the expected peptide ester. L-3-Amino-caprolactam purified as its hydrochloride was confirmed by spectral data and elemental analysis, and by comparison of its physical constants with reported values.[14] No lactam formation was achieved without $CuCl_2$ under the same conditions as employed before. The α-amino group of Lys-OMe had an important role in this abnormal reaction since treatment of methyl 6-aminocaproate under the same reaction conditions as those used with Lys-OMe afforded no trace amount of the expected caprolactam.

While methyl L-ornitinate(Orn-OMe) also afforded L-3-amino-valerolactam in 75·7% yield under the same conditions as those used with Lys-OMe, and afforded it in a very low yield without $CuCl_2$, a clear acceleration of lactam formation due to the presence of $CuCl_2$ was achieved. However the reaction of methyl DL-2,3-diamino-propionate with $CuCl_2$ produced no β-lactam. This is probably due to formation of the chelation structure, shown in Chart 4, from the ester and $CuCl_2$.[15] Elucidation of the lactam formation mechanism from Lys-OMe

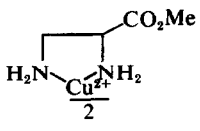


CHART 4

and Orn-OMe will be discussed in an accompanying paper.[5] The lack of peptide formation with ethyl L-prolinate(Pro-OEt) is probably the result of its intrinsically large steric hindrance, which overcomes the stronger basicity of the secondary amino group.

III. *Reactions of dichloro-bis(ethyl glycinato)-copper*(II) ($Cu(Gly\text{-}OEt)_2Cl_2$) *and dichloro-bis-(ethyl* L*-alaninato)-copper*(II) *($Cu(Ala\text{-}OEt)_2Cl_2$) with amino acid esters.*

In the preceding section, peptide bond formation affording peptide esters of the same amino acid sequence was studied using several kinds of optically active amino acid esters. We anticipated the formation of peptide esters containing different kinds of amino acid components if two different kinds of amino acid esters are employed.

When a mixture of $Cu(Gly\text{-}OEt)_2Cl_2$ (1·0 eq),[2] Phe-OEt-HCl (4·0 eq), and triethylamine (4·0 eq) in anhydrous ethanol was stirred at room temperature for 3·0 hr, then worked up as usual, a mixture of four different kinds of peptide esters was obtained, as shown in Chart 5, due to the ligand ex-

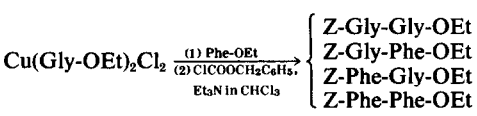


CHART 5

change. Then, the yields of formed peptide esters summarized in Table 3 were calculated, based on the amount of $CuCl_2$ in the reaction mixture. Purification using silica gel column chromatography afforded Z-Phe-Phe-OEt, ethyl N-carbobenzoxyglycylglycinate (Z-Gly-Gly-OEt), and a mixture of dipeptide esters consisting of glycine and L-phenylalanine units. The first two dipeptide esters were identified with authentic samples prepared previously,[2] and the latter mixture was further purified with column chromatography using silica gel. Pure ethyl N-carbobenzoxy-L-phenylalanylglycinate (Z-Phe-Gly-OEt) which was isolated in a low yield, was identified with the authentic sample prepared using the DCCD method.[16] Unfortunately, isolation of pure ethyl N-carbobenzoxyglycyl-L-phenylalaninate(Z-Gly-Phe-OEt) was unsuccessful because of its insufficient separation from Z-Phe-Gly-OEt. However, formation of Z-Gly-Phe-OEt by this reaction was definitely confirmed by a comparison of the NMR spectrum measured in a mixture of Z-Phe-Gly-OEt and Z-Gly-Phe-OEt with those of respective authentic samples.

Table 2. (Contd.)

[l]M. Brenner and R. W. Pfister, *Helv. Chim. Acta* **34**, 2085 (1951)

[m]These values were observed with a sample obtained by one recrystallization with benzene–light petroleum.

[n]Reported values were m.p. 108–109° and $[\alpha]_D^{20} - 19{\cdot}8°$ (MeOH). I. Tomita, T. Tokuda, J. Oohashi and M. Nakajima, *Nippon Nogei Kagaku Kaishi Japan*, **39**, 385 (1965)

[o]L-3-Amino-caprolactam hydrochloride was obtained in 61·0% yield based on Lys-OMe-2HCl. It had a m.p. > 270° and $[\alpha]_D^{24} - 26{\cdot}4°$ ($c = 1{\cdot}3$, N-HCl) after recrystallization from MeOH. The reported rotation[14] is $[\alpha]_D^{22} - 24{\cdot}5 \pm 1{\cdot}2°$ (N-HCl).

[p]L-3-Amino-valerolactam hydrochloride was obtained in 75·7% yield based on Orn-OMe-2HCl. It had a m.p. 218–220° (dec) and $[\alpha]_D^{20} + 3°$ ($c = 0{\cdot}6$, N-HCl) after recrystallization from MeOH-isopropanol.

Table 3. Reactions of $Cu(Gly\text{-}OEt)_2Cl_2$ and $Cu(Ala\text{-}OEt)_2Cl_2$ with amino acid esters[a]

Run	Cu(II) complexes (Molar ratio)	Amino acid esters	Formed N-carbobenzoxy-peptide esters	Yield (%)[b]
1	$Cu(Gly\text{-}OEt)_2Cl_2$ (1:4)	Phe-OEt	Z-Phe-Phe-OEt	10·4
			Z-Phe-Gly-OEt }	82·2
			Z-Gly-Phe-OEt }	
			Z-Gly-Gly-OEt	13·6
2	$Cu(Ala\text{-}OEt)_2Cl_2$ (1:4)	Gly-OEt	Z-Gly-Gly-OEt	52·6[c]
			Z-Gly-Ala-OEt }	66·6[c]
			Z-Ala-Gly-OEt }	
			Z-Ala-Ala-OEt	trace

[a]All reactions were carried out at room temperature for 3·0 hr using EtOH as the solvent. For physical constants of each authentic sample, see the experimental section.

[b]Based on $CuCl_2$.

[c]The molar ratio of Z-Gly-Gly-OEt and a mixture of Z-Gly-Ala-OEt and Z-Ala-Gly-OEt was determined to be 43:57 according to the NMR spectrum measured with a mixture containing three different kinds of peptide esters (Experimental).

The reaction of $Cu(Ala\text{-}OEt)_2Cl_2$, prepared according to the procedure reported by Springer, *et al.*,[17] with Gly-OEt again afforded a mixture of four possible dipeptide esters. This time too, only Z-Ala-Ala-OEt could be isolated in a pure state by silica gel column chromatography. Structures of the other three dipeptide esters were confirmed by comparing IR and NMR spectra for the mixture with those of the three authentic samples independently prepared by the DCCD method.[11]

An attempt to prepare a single dipeptide ester using the N-protected amino acid ester and ordinary one, such as ethyl N-carbobenzoxy-glycinate-(Z-Gly-OEt), and Ala-OEt, in the presence of $CuCl_2$ was completely unsuccessful in affording objective ethyl N-carbobenzoxy-glycyl-L-alaninate(Z-Gly-Ala-OEt).

Obviously this novel peptide bond formation can not be applied to the preparation of dipeptide esters containing two different kinds of amino acid components since it usually gives a mixture of dipeptide esters whose separation, even by column chromatography, is practically impossible. However, the results obtained here played an important role in elucidating the mechanism, which is discussed in detail in the accompanying paper.[5]

IV. *Formation of tripeptide esters using ethyl glycyl-glycinate* (Gly-Gly-OEt) *and* $CuCl_2$

Although tripeptide ester formation was easily achieved when Gly-OEt was treated with $CuCl_2$ in anhydrous ethanol,[2] the products obtained in the reaction of amino acid esters other than Gly-OEt with $CuCl_2$ mainly consist of dipeptide ester, and preparation of the tripeptide ester using the same experiment was virtually unsuccessful.* As the absence of tripeptide ester formation is thought to be due to steric hindrance caused by the side chains of amino acid esters and/or dipeptide esters, we investigated whether the reaction of Gly-Gly-OEt, which was expected to have the smallest steric hindrance among the dipeptide esters, with an ordinary amino acid ester in the presence of $CuCl_2$, would afford the desired tripeptide ester.

A blue solution obtained by the addition of a mixture of amino acid ester hydrochloride (10·0 eq) and triethylamine (10·0 eq) in an anhydrous alcohol to an anhydrous ethanolic solution of Gly-Gly-OEt (2·0 eq) and $CuCl_2$ (1·0 eq) was stirred at room temperature, then it was worked up as usual. The product was isolated using column chromatography with silica gel after carbobenzoxylation. Results are summarized in Table 4.

In run 1, ethyl N-carbobenzoxy-L-alanyl-glycyl-glycinate(Z-Ala-Gly-Gly-OEt) was obtained in 31·9% yield based on the Gly-Gly-OEt used, and no formation of ethyl N-carbobenzoxy-glycyl-glycyl-L-alaninate (Z-Gly-Gly-Ala-OEt) containing N-terminal glycine unit was achieved. In this reaction, the formation of di-, and tri-peptide esters prepared simply from Ala-OEt, also occurred since a large excess of Ala-OEt was employed. However, no formation of glycine tetra-peptide ester by Gly-Gly-OEt and $CuCl_2$ was observed. The reaction of Ser-OMe with Gly-Gly-OEt similarly afforded ethyl N-carbobenzoxy-L-seryl-glycyl-glycinate (Z-Ser-Gly-Gly-OEt) as the sole tripeptide ester product (Table 4, run 2). On the other hand, no tripeptide ester was formed in the reaction of Phe-OEt with Gly-Gly-OEt. This seems to be due to a steric hindrance by the side chain of Phe-OEt larger than those by Ala-OEt and Ser-OMe.

The mechanism of this novel reaction will be studied in an accompanying paper,[5] but the remark-

*With Ala-OEt, a trace amount of tripeptide ester was isolated from the reaction mixture when this amino acid ester was treated with $CuCl_2$.

Table 4. Formation of tripeptide esters using Gly-Gly-OEt and $CuCl_2$[a]

Run	Amino acid esters used	Solv.	N-Carbobenzoxy-tripeptide esters isolated[b]	Yield[c] (%)	M.P.(°C)	Optical rotations	Authentic samples[d] M.P.(°C)	Optical rotations
1	Ala-OEt	EtOH	Z-Ala-Gly-Gly-OEt	31·9	—	$[\alpha]_D^{17}+2°$ ($c=1·2$, EtOH)	134	$[\alpha]_D^{17}+2°$ ($c=0·8$, EtOH)[f]
					[133–134	$[\alpha]_D^{17}+2°$ ($c=1·3$, EtOH)][e]		
2	Ser-OMe	EtOH	Z-Ser-Gly-Gly-OEt	25·0	136–138	$[\alpha]_D^{23}+6·3°$ ($c=1·0$, THF)	139–140	$[\alpha]_D^{23}+6·9°$ ($c=0·9$, THF)
					[139–140	$[\alpha]_D^{23}+6·8°$ ($c=1·0$, THF)][e]		
3	Phe-OEt	EtOH	Z-Phe-Gly-Gly-OEt	0				

[a]The molar ratio of amino acid ester, Gly-Gly-OEt and $CuCl_2$ is 10:2:1. All reactions were carried out at room temp for 20·0 hr.

[b]Measurements of melting point and optical rotation were made with a sample purified by column chromatography.

[c]Based on Gly-Gly-OEt.

[d]Independently prepared using the DCCD method from N-carbobenzoxy amino acid and dipeptide ester.[11]

[e]Values in parentheses were obtained from a sample recrystallized from EtOAc–light petroleum.

[f]Reported values are m.p. 135·5–136·5° and $[\alpha]_D^{27}+3·7°$ (DMF). H. D. Jakubke and A. Voigl, *Chem. Ber.* **99**, 2944 (1966).

able results obtained here clearly demonstrate that tripeptide ester formation occurs at the N-terminus of the dipeptide ester.

EXPERIMENTAL

All m.ps are uncorrected. IR spectra measurements were performed with a spectrometer, Model 402, Japan Spectroscopic Co, Ltd. NMR spectra were measured with a spectrometer, Model 3H-60, Japan Electron Optics Lab. and data are reported in parts per million downfield from internal TMS. Optical activities were determined with a Yanagimoto Photo Direct Reading Polarimeter, Model OR-20. Mass spectra measurements were performed with a Hitachi RMU-6D instrument using a direct inlet system.

Materials. Commercially available $CuCl_2$ was used throughout this work after dried *in vacuo* at *ca* 120° over P_2O_5. All amino acid ester hydrochlorides were prepared according to the established procedure reported in the literature.[18] Completely anhyd MeOH or EtOH was used as the reaction solvent.

Reaction of Phe-OEt *with* $CuCl_2$ (Table 1)

Run 3. A clear blue soln obtained by adding anhyd $CuCl_2$ (170 mg, 1·25 mmole) to a mixture of Phe-OEt-HCl (3·45 g, 15·0 mmole) and triethylamine (1·51 g, 15·0 mmole) in EtOH (18·0 ml), was stirred at room temp for 18·0 hr. After the mixture was cooled to 0–5° in an ice-water bath, the Cu(II) complex formed was decomposed by adding ethanolic HCl. Cu(II) ion was removed as cupric sulfide by passing H_2S gas through the acidic mixture. The clear ethanolic soln obtained by the filtration of CuS, was concentrated *in vacuo*, to give crude dipeptide ester hydrochloride.

A chloroform soln (50·0 ml) of carbobenzoxy chloride (3·30 g, 19·0 mmole) and that (5·0 ml) of triethylamine (3·20 g, 32·0 mmole) were alternately added to the chloroform soln (25 ml) of the crude peptide ester hydrochloride with stirring at 0°. The whole was stirred at 0–5° for 30 min, then at room temp for 1·0 hr. The mixture was successively washed with 10% HCl, sat $NaHCO_3$ aq, and sat NaCl aq, then it was dried over anhyd $MgSO_4$. Filtration and evaporation *in vacuo* afforded the crude product, which was purified with column chromatography using silica gel (solvent: $CHCl_3$:EtOH 97:2), to give crude Z-Phe-Phe-OEt (1·15 g, 32·3% based on Phe-OEt), m.p. 130–134°, $[\alpha]_D^{20}-16·7°$ ($c=1·1$, EtOH), and Z-Phe-NH_2 (50 mg, 1·0% based on Phe-OEt), m.p. 160–164°, $[\alpha]_D^{20}+11·0°$ ($c=0·5$, $CHCl_3$). Recrystallization of the crude dipeptide ester from EtOAc-light petroleum afforded a pure sample, m.p. 137–138°, $[\alpha]_D^{20}-17·4°$ ($c=1·1$, EtOH). Pure Z-Phe-NH_2, m.p. 167°, $[\alpha]_D^{20}-12°$ ($c=0·2$, $CHCl_3$), was also obtained by the recrystallization from EtOAc-light petroleum. The peptide ester and amide were identified with authentic sample independently prepared,[8,9] by a comparison of their IR spectra and by mixed m.ps.

Runs 1, 2 *and* 4. These reactions were carried out in a manner similar to that described for run 3.

Run 5. The free base of Phe-OEt was prepared according to the usual procedure.[19] Triethylamine (1·51 g, 15·0 mmole) was added to an ethanolic soln (6·0 ml) of the ester hydrochloride (3·45 g, 15·0 mmole) with stirring. After anhyd ether (150 ml) was added to the ethanolic mixture, the whole was effectively shaken and allowed to stand at −30–−20° for 30 min. The triethylamine hydrochloride which precipitated was filtered off, and the clear filtrate was evaporated *in vacuo* to give a slightly colored oil which was directly used in the next reaction.

A blue ethanolic soln (20 ml) containing Phe-OEt (2·90 g, 15·0 mmole) and anhyd $CuCl_2$ (170 mg, 1·25 mmole) was stirred at room temp for 3·0 hr. The mixture was treated as described, to afford Z-Phe-Phe-OEt (840 mg, 23·5% based on Phe-OEt), m.p. 136–138°, $[\alpha]_D^{20}-16·8°$ ($c=0·6$, EtOH).

Reaction of optically active amino acid ester other than Phe-OEt with $CuCl_2$ (Table 2)

General procedure. Anhyd $CuCl_2$ (1·25 mmole) was added with stirring to a methanolic (or ethanolic) soln

(18·0 ml) of amino acid methyl (or ethyl) ester hydrochloride (15·0 mmole) and triethylamine (15·0 mmole). A blue soln (except in run 8) was stirred at room temp for 3·0 hr. The Cu(II) complex formed in the mixture was decomposed by the addition of methanolic (or ethanolic) HCl. After Cu(II) ion was removed as CuS by the usual treatment with H_2S, evaporation of the clear alcoholic filtrate, followed by the work-up described in the reaction of Phe-OEt, gave the dipeptide esters listed in Table 2.

Solvent system of chromatographic separation. Z-Ala-Ala-OMe ($CHCl_3$·MeOH 98·2), Z-Leu-Leu-OMe ($CHCl_3$:MeOH 98:2), Z-Trp-Trp-OMe (CH_2Cl_2:MeOH 98:2), Z-Ser-Ser-OMe (EtOAc·MeOH 98·2), Z-Met-Met-OMe ($CHCl_3$·MeOH 98.2), Z-Asp (OEt)-Asp-(OEt)-OEt ($CHCl_3$:EtOH 98:2), and Z-Glu(OMe)-Glu(OMe)-OMe($CHCl_3$.MeOH 98.2).

Run 8. Bis(methyl L-cysteinato)copper(II) in a quantitative yield was obtained as a gray powder, m.p. 205–210° (dec) (a color change gradually occurred *ca* 180°); and had the following IR spectrum, IR ν_{max}^{KBr} cm^{-1}: 3340, 3300, 1740, 1580, 1470, 1450, 1390, 1300, 1240, 1194, 1175. (Found; C, 29·09; H, 4·90; N, 8·02. Calcd. for $C_8H_{16}N_2O_4S_2Cu$· C 28·93; H, 4·87; N, 8·44%).

Run 11. A clear blue methanolic soln (25 ml) containing His-OMe-2HCl (3·64 g, 15·0 mmole) and anhyd $CuCl_2$ (170 mg, 1·25 mmole) was stirred at room temp for 3·0 hr. The formed Cu(II) complex was decomposed by the addition of methanolic HCl, and liberated Cu(II) ion was removed as CuS as usual. Filtration and evaporation *in vacuo* afforded a residue, from which triethylamine hydrochloride was extracted with $CHCl_3$, to afford the chloroform insoluble His-OMe-2HCl (3·55 g, 97·2% recovery). The His-OMe-2HCl recovered here was identified with the starting material by comparison of their spectral data.

Run 12. A methanolic soln (25·0 ml) of Lys-OMe-2HCl (3·51 g, 15·0 mmole), triethylamine (3·03 g, 30·0 mmole) and anhyd $CuCl_2$ (170 mg, 1·25 mmole) was stirred at room temp for 3·0 hr. The Cu(II) complex formed was decomposed with methanolic HCl. After Cu(II) ion was removed with H_2S, the clear methanolic soln was evaporated *in vacuo*. One half of the evaporation residue was diluted with $CHCl_3$, to extract the triethylamine hydrochloride. A sat $NaHCO_3$ aq was added to chloroform insoluble residue, and the aqueous mixture was evaporated to dryness *in vacuo*. The evaporation residue was again washed with ether, after which it was extracted with MeOH. Evaporation of the methanolic extract after addition of an excess of methanolic HCl, afforded crude L-3-amino-caprolactam hydrochloride (750 mg, 61·0% based on Lys-OMe-2HCl) as a pale yellow powder. This was recrystallized from MeOH to give a pure sample as colorless needles, m.p. > 270°, $[\alpha]_D^{24} - 26·4°$ ($c = 1·3$, N-HCl). IR ν_{max}^{KBr} cm^{-1}: 3190, 3100, 2960, 1665, 1567, 1490, 1310, 1130, 750. Mass spectrum: M^+ *m/e* 128. (Found: C, 43·76; H, 8·06; N, 17·17. Calcd. for $C_6H_{12}ON_2$-HCl: C, 43·78; H, 7·96; N, 17·02%).

The remaining evaporation residue directly obtained by the removal of Cu(II) ion as CuS, gave no peptide derivative even when isolation of the peptide ester was attempted after carbobenzoxylation.

Run 13. The same treatment as described in run 12 gave L-3-amino-valerolactam hydrochloride in 75·7% yield based on Orn-OMe-2HCl. Recrystallization from MeOH-isopropanol afforded colorless needles, m.p 218–220° (dec) and $[\alpha]_D^{20} + 3·0°$ ($c = 0·6$, N-HCl); IR ν_{max}^{KBr} cm^{-1}; 3150, 3080, 2920, 2850, 1680. Mass spectrum; M^+ *m/e* 114. (Found C, 39·50; H, 7·23; N, 18·61 Calcd. for $C_5H_{10}ON_2$-HCl: C, 39·87; H, 7·36; N, 18·60%).

Reaction of $Cu(Gly\text{-}OEt)_2Cl_2$ *and* $Cu(Ala\text{-}OEt)_2Cl_2$ *with amino acid esters* (Table 3)

Run 1. The addition of a mixture of Phe-OEt-HCl (2·30 g, 10·0 mmole), triethylamine (1·01 g, 10·0 mmole) in EtOH (15·0 ml) to a suspension of $Cu(Gly\text{-}OEt)_2Cl_2$[2] (850 mg, 2·50 mmole) in EtOH (10·0 ml), afforded a clear blue soln, which was stirred at room temp for 3·0 hr, then it was worked up as usual. Chromatographic separation of the products using silica gel gave Z-Phe-Phe-OEt (100 mg, 10·4% based on $CuCl_2$) from the $CHCl_3$-eluted fraction, and a mixture of Z-Gly-Phe-OEt and Z-Phe-Gly-OEt (790 mg, 82·2% based on $CuCl_2$) as a yellow oil from the $CHCl_3$-MeOH (97·3)-eluted fraction The presence of Z-Gly-Phe-OEt and Z-Phe-Gly-OEt in the latter mixture was confirmed by comparing the NMR spectrum of the mixture with spectra obtained from the respective pure authentic samples. NMR (in $CDCl_3$). 1·20 (tri, $COOCH_2C\underline{H}_3$ of Z-Gly-Phe-OEt, $J = 15$ c/s), 1·25 (tri, $COOCH_2C\underline{H}_3$ of Z-Phe-Gly-OEt, $J = 15$ c/s), 5·05 (s, $NHCOOC\underline{H}_2C_6H_5$ of Z-Gly-Phe-OEt and Z-Phe-Gly-OEt). The latter mixture was further purified by silica gel column chromatography using the same solvent system as used before, to give crude Z-Phe Gly-OEt (100 mg, 10·4% based on $CuCl_2$) as a pale yellow powder m.p. 100–105°, $[\alpha]_D^{30} - 17·1°$ ($c = 0·92$, EtOH) Recrystallization from EtOAc-light petroleum gave a pure product, m.p. 109–110°, $[\alpha]_D^{30} - 17·0°$ ($c = 0·52$, EtOH), as colorless needles. This sample showed no depression on mixed m.p.m. with the authentic sample. The IR spectrum of these needles was also identical with that of the authentic sample.

Further elution of the original silica gel column using $CHCl_3$-EtOH (97:3) afforded Z-Gly-Gly-OEt (100 mg, 13·6% based on $CuCl_2$), m.p. 77–79°, which was also identified with an authentic sample[2] in the usual manner.

Run 2. $Cu(Ala\text{-}OEt)_2Cl_2$ was prepared according to the following procedure [17] Addition of an ethanolic soln (15·0 ml) containing Ala-OEt (2·34 g, 20·0 mmole) to anhyd $CuCl_2$ (1·34 g, 10·0 mmole) dissolved in EtOH (10·0 ml) at 5–10° precipitated $Cu(Ala\text{-}OEt)_2Cl_2$ as blue leaflets. These were collected by filtration and washed with EtOH. They weighed 3·0 g (81·0%), and showed m.p. 134° (dec); IR ν_{max}^{KBr} cm^{-1}: 3440, 3340, 3240, 1745, 1715, 1580, 1205, 1125, 1025. (Found: C, 32·58; H, 5·92; N, 7·63 Calcd. for $C_{10}H_{22}O_4N_2Cl_2Cu$: C, 32·58; H, 6·09; N, 7·59%)

A mixture of Gly-OEt-HCl (1·40 g, 10·0 mmole) and triethylamine (1·01 g, 10·0 mmole) in EtOH (15 ml) was gradually added at room temp to an ethanolic soln (5·0 ml) of $Cu(Ala\text{-}OEt)_2Cl_2$ (920 mg, 2·50 mmole) The whole mixture was stirred at the same temp for 3·0 hr, then worked up as usual. Separation using silica gel column chromatography (solvent: $CHCl_3$ EtOH 98 2) afforded a trace amount of Z-Ala-Ala-OEt, and a mixture of three kinds of dipeptide ester, Z-Ala-Gly-OEt, Z Gly-Ala-OEt, and Z-Gly-Gly-OEt (900 mg) as a pale yellow semisolid. Structures of the latter three kinds of peptide esters were confirmed by comparison of IR and NMR spectra measured with the pale yellow semisolid with those of respective authentic samples; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1150 (due to Z-Gly-Ala-OEt), 1120 (due to Z-Gly-Gly-OEt and Z-Ala-Gly-OEt), 1070 (due to Z-Ala-Gly-OEt); NMR (in $CDCl_3$)· 1·15 (9H, tr, $COOCH_2C\underline{H}_3$ of Z-Ala-Gly-OEt,

Z-Gly-Ala-OEt and Z-Gly-Gly-OEt, $J = 15$ c/s), 1·27 (d, CH_3CH of Z-Ala-Gly-OEt and Z-Gly-Ala-OEt, $J = 12$ c/s), 5·05 (6H, s, $CH_2C_6H_5$ of Z-Ala-Gly-OEt, Z-Gly-Ala-OEt and Z-Gly-Gly-OEt). According to the above-mentioned NMR spectrum, the molar ratio of Z-Gly-Gly-OEt and a mixture of Z-Ala-Gly-OEt and Z-Gly-Ala-OEt was calculated to be 43:57. Then the yields of Z-Gly-Gly-OEt and a mixture of Z-Ala-Gly-OEt and Z-Gly-Ala-OEt were determined to be 52·6 and 66·6% based on $CuCl_2$.

Formation of tripeptide esters using Gly-Gly-OEt *and* $CuCl_2$ (Table 4)

Run 1. An ethanolic soln (15·0 ml) of Ala-OEt-HCl (1·84 g, 12·0 mmole) and triethylamine (1·20 g, 12·0 mmole) was added with stirring at room temp to a mixture of Gly-Gly-OEt-HCl (470 mg, 2·40 mmole), triethylamine (240 mg, 2·40 mmole) and anhyd $CuCl_2$ (150 mg, 1·20 mmole) in EtOH (5·0 ml) after the latter soln had first been stirred for 10·0 min. The mixture obtained was stirred at room temp for 20·0 hr, then worked up as usual after carbobenzoxylation.

Column chromatography using silica gel and $CHCl_3$ EtOH (97.3) as the eluent successively gave a mixture of Z-Ala-Ala-OEt and Z-Gly-Gly-OEt (in a ratio of 2 3, based on its NMR spectrum), Z-Ala-Ala-Ala-OEt[12] (trace amount) and Z-Ala-Gly-Gly-OEt (280 mg, 31·9% based on Gly-Gly-OEt), m.p. 130–132° and $[\alpha]_D^{17} + 2°$ ($c = 1·2$, EtOH). Recrystallization of the latter product from EtOAc-light petroleum gave a pure sample as colorless crystals, m.p. 133–134°, $[\alpha]_D^{17} + 2·2°$ ($c = 1·3$, EtOH), which was identified with an authentic sample independently prepared, by mixed m.p.m. and by comparison of its IR spectrum with the authentic one.

Run 2. A mixture of Ser-OMe-HCl (1·73 g, 11·0 mmole) and triethylamine (1·10 g, 11·0 mmole) in EtOH (15·0 ml) was added with stirring to an ethanolic soln (5·0 ml) of Gly-Gly-OEt-HCl (460 mg, 2·30 mmole), triethylamine (230 mg, 2·30 mmole), and anhyd $CuCl_2$ (150 mg, 1·10 mmole). The mixture was stirred at room temp for 20·0 hr, then was worked up as usual. Separation using silica gel column chromatography (solvent: CH_2Cl_2: EtOAc 1:1) afforded Z-Ser-Ser-OMe (170 mg, 10·0% based on Ser-OMe), m.p. 141–142°, then gave Z-Ser-Gly-Gly-OEt (220 mg, 25·0% based on Gly-Gly-OEt), m.p. 136–138° and $[\alpha]_D^{23} + 6·3°$ ($c = 0·95$, THF). The latter tripeptide ester was further purified by recrystallization from EtOAc-light petroleum, giving a pure sample, m.p. 139–140° and $[\alpha]_D^{23} + 6·8°$ ($c = 0·95$, THF) This was identified with an authentic sample prepared independently, by comparing its physical data with data for the authentic sample.

Syntheses of authentic peptides

All the authentic peptides, except those described below, were prepared from Z-amino acids and amino acid ester or peptide ester hydrochlorides using triethylamine and dicyclohexyl carbodiimide (DCCD), according to the established procedure.[11] Physical constants of these authentic samples are shown in Tables 1, 2, and 4.

Authentic samples necessary for use in Section III, were also synthesized as cited above. Their physical constants are as follows.

Z-Phe-Gly-OEt: Colorless needles, m p. 109–110°, $[\alpha]_D^{26} - 16·7°$ ($c = 2·13$, EtOH) (Lit.,[18] m.p. 110–111°, $[\alpha]_D^{25} - 16·9°$ ($c = 5$, EtOH).)

Z-Gly-Phe-OEt: Pale yellow oil (Lit.,[20] oil)

Z-Gly-Ala-OEt: m.p. 56–59°, $[\alpha]_D^{31} - 24·4°$ ($c = 1·04$, EtOH) (Lit.,[21] m.p. 54–56°)

Z-Ala-Gly-OEt· m.p. 99–100°, $[\alpha]_D^{22} - 24·4°$ ($c = 1·05$, EtOH) (Lit.,[22] m.p. 97·5–98°, $[\alpha]_D^{20} - 24·4°$ ($c = 1$, EtOH))

Z-Asp(OEt)-Asp(OEt)-OEt. Carbobenzoxy chloride (8·5 g, 50 mmole) was added with stirring at 0–5° to an aqueous soln (60 ml) of $NaHCO_3$ (8·4 g, 100 mmole) and Asp(OEt)-OH-HCl[23] (6·0 g, 30 mmole). The whole was stirred at 0–5° for 3·0 hr, then at room temp for 2·0 hr. Unreacted carbobenzoxy chloride was recovered from the mixture by extraction with ether. The residual aqueous phase was acidified with conc HCl, then extracted with EtOAc. A washing with sat NaCl soln, then drying over $MgSO_4$, followed by evaporation *in vacuo*, gave crude Z-Asp(OEt)-OH as a thick oil (7·7 g, 85·5%).

An anhyd THF soln (5·0 ml) of triethylamine (0·50 g, 5·0 mmole) was added with stirring at 0–5° to a mixture of Z-Asp(OEt)-OH (1·50 g, 5·0 mmole), DCCD (1·03 g, 5·0 mmole) and Asp(OEt)-OEt-HCl (1·13 g, 5·0 m mole) in anhyd THF (25·0 ml). The whole was stirred at 0–5° for 4·0 hr, then at room temp for an additional 4·0 hr Removal of dicyclohexyl urea by filtration was followed by evaporation of the clear filtrate. The evaporation residue, thus obtained, was dissolved in EtOAc, which was successively washed with dil HCl, sat $NaHCO_3$, and sat NaCl solns. Final evaporation of the organic phase afforded crude Z-Asp(OEt)-Asp(OEt)-OEt as a pale yellow powder. Recrystallization from benzene-light petroleum gave a pure sample (1·30 g, 55·7%) as colorless needles, m.p. 89–90°, $[\alpha]_D^{18} - 12·3°$ ($c = 1·2$, EtOH); IR ν_{max}^{KBr} cm^{-1} 3310, 3300, 1745, 1733, 1697, 1650, 1535. (Found C, 56·90; H, 6·33; N, 6·14. Calcd for $C_{22}H_{30}O_9N_2$ C, 56·64; H, 6·48; N, 6·01%).

Z-Ser Gly-Gly-OEt. Crude Z-Ser-Gly-Gly-OEt (230 mg, 50·0%) was prepared from Z-Ser-OH (290 mg, 1·2 mmole) and Gly-Gly-OEt-HCl (240 mg, 1·2 mmole) using the procedure described for Z-Asp(OEt)-Asp(OEt)-OEt. Recrystallization of the crude product from EtOAc-light petroleum gave a pure sample as colorless needles, m.p. 139–140°, $[\alpha]_D^{23} + 6·9°$ ($c = 0·87$, THF), IR ν_{max}^{KBr} cm^{-1} 3280, 1745, 1687, 1655, 1560, 1513. (Found· C, 53·54, H, 6·05; N, 11·00. Calcd. for $C_{17}H_{23}O_7N_3$: C, 53·53; H, 6·08; N, 11·02%).

REFERENCES

[1]Part III: H. Seki, K. Koga and S. Yamada, *Chem. Pharm. Bull. Tokyo* **20**, 361 (1972)

[2]Part I: S. Yamada, M. Wagatsuma, Y. Takeuchi and S. Terashima, *Ibid.* **19**, 2380 (1971)

[3]Part of this report was communicated, S. Yamada, S. Terashima and M. Wagatsuma, *Tetrahedron Letters* 1501 (1970)

[4a]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, N. 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)

[5]For detailed discussion on the peptide bond formation mechanism, see M. Wagatsuma, S. Terashima and S. Yamada, *Tetrahedron* **29**, 1499 (1973)

[6a]D. A. Buckingham, L. G. Marzilli and A. M. Sargeson,

J. Am. Chem. Soc. **89**, 2772 (1967); [b]*Ibid.* **89**, 4539 (1967)
[7]J. P. Collman and E. Kimura, *Ibid.*, **89**, 6096 (1967)
[8]A. Barth, *Liebigs Ann.* **683**, 216 (1965)
[9]D. Ben-Ishai, *J. Am. Chem. Soc.* **79**, 5736 (1957)
[10]M. A. Khenokh and N. P. Bogdanova, *Dokl. Akad. Nauk. USSR.* **182**, 715 (1968); *Chem. Abstr.* **70**, 38068m (1969)
[11]G. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids* pp. 1016–1024. Wiley, New York, London (1961)
[12]M. Wagatsuma, S. Terashima and S. Yamada, *Chem. Pharm. Bull., Tokyo* **21**, 422 (1973)
[13]R W. Hay and L. J. Proter, *Australian J. Chem.* **20**, 675 (1967)
[14]H. Brenner and H. R. Rickenbacker, *Helv. Chim. Acta* **41**, 181 (1958)
[15]W. Sehaeg and F. Schenider, *Z. Physiol. Chem.* **326**, 40 (1961)
[16]Ref. 11, p. 1135
[17]M. P. Springer and C. Curran, *Inorg. Chem.* **2**, 1270 (1963)
[18]Ref. 11, pp. 925–932
[19]J. R. Vangham and J. A. Eichler, *J. Am. Chem. Soc.* **75**, 5556 (1953)
[20]J. Gante, *Angew Chem.* **78**, 602 (1966)
[21]Idem , *Ibid.* **78**, 334 (1966)
[22]Ref 11, p. 1114
[23]R. Koch and H. Hanson, *Z. Physiol. Chem.* **292**, 180 (1953)