Dehydrooligopeptides. XV. Useful Syntheses of Dehydrodipeptides by the Enzymatic Coupling of α-Dehydroglutamate with Various α-Amino Acid Amides Using Proteases[1)]

Chung-gi SHIN,* Masashi SEKI, Takeshi KAKUSHO, and Nobuyuki TAKAHASHI
Laboratory of Organic Chemistry, Faculty of Technology, Kanagawa University, Rokkakubashi, Kanagawa-ku, Yokohama 221

(Received December 22, 1992)

Novel enzymatic syntheses of various dehydrodipeptides by the coupling of α,γ-dimethyl *N*-benzyloxycarbonyl(*Z*)-α-dehydroglutamate [Cbz–ΔGlu(OMe)–OMe; **1**] with α-amino acid amides (H–AA–NHR) using proteases, such as papain and α-chymotrypsin A (CT), are described. It was found that there was conspicuous difference between the catalytic actions of papain and CT toward **1**. In the case of papain, the selective coupling of α-ester of **1** with H–AA–NHR took place to give Cbz–ΔGlu(OMe)–AA–NHR, while in the case of CT the coupling of γ-ester of **1** proceeded predominantly to give Cbz–ΔGlu(AA–NHR)–OMe. The present study suggests that both papain and CT may become a versatile tool for the organic synthesis by the coupling of not only ΔGlu derivatives but also the other α-dehydroamino acids with α-amino acids or peptides.

In recent years, it has been widely recognized that, whenever the enzymatic hydrolysis of L-α-amino acid esters (H–AA–OR) and their peptides proceed by the catalytic action of such proteases as papain and α-chymotrypsin A (CT) and so on, the reverse peptide bond formation of AA or peptide almost takes place.[2—5)] We have been really surprised to find that, even in the case of unusual α-amino acid ester such as α-dehydroamino acid (ΔAA), both the enzymatic hydrolysis and coupling take place smoothly.[6—10)] In fact, in the preceding papers, we have briefly reported that the very selective enzymatic hydrolyses of α- and γ-methyl esters of α,γ-dimethyl *N*-benzyloxycarbonyl(*Z*)-α-dehydroglutamate [Cbz–ΔGlu(OMe)–OMe; **1**] with papain (EC 3.4.22.2)[6,9)] and CT (EC 3.4.21.1)[8)] proceeded to give Cbz–ΔGlu(OMe)–OH and Cbz–ΔGlu–OMe, respectively, as shown in Scheme 1. Furthermore, according to our expectation, the selective enzymatic synthesis of dehydrodipeptides by the coupling of a carboxyl (C–) component **1** with an amine (N–) component L-leucinamides (H–Leu–NHR) has been successful and has also been briefly reported.[7)]

In this paper, we wish to report on the detailed study of the peptide synthesis by the enzymatic couplings of **1** with various kinds of H–AA–NHR, in which the two types of the products, Cbz–ΔGlu(OMe)–AA–NHR (**3** and **5**) and Cbz–ΔGlu(AA–NHR)–OMe (**6** and **7**), were obtained by using papain and CT, respectively. Besides, in order to look for the optimum reaction conditions, the coupling of **1** with H–Leu–NHC_6H_5 (**2d**) was also studied in detail.

Experimental

General. The melting points were determined with a Yamato Mp-21 micro melting-point apparatus, and were uncorrected. The IR spectra were recorded with a Hitachi EPI-G2 spectrometer. The ^1H NMR spectra were measured with a JEOL LMN-PS-100 spectrometer in a $CDCl_3$ solution with tetramethylsilane used as the internal standard. The specific rotations were measured in a 0.5 dm tube using a JASCO DIP-4 polarimeter (Japan Spectroscopic Co., Ltd.).

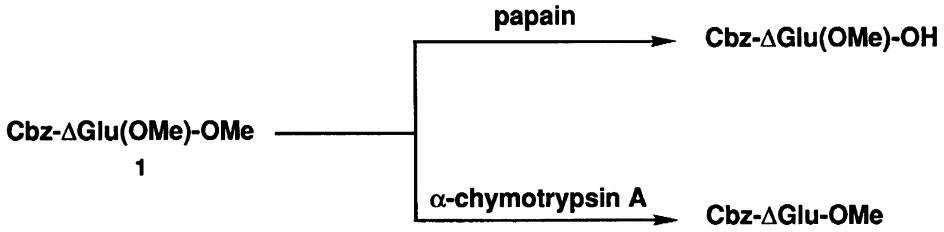
Enzymes. Papain (2.8 units mg^{-1}, crude powder, P3375) and α-chymotrypsin A (CT) (39 units mg^{-1}, Type II, C4129), purchased from Sigma Chemical Co., U. S. A., were used without further purification.

Cbz–ΔGlu(OMe)–OMe (1) as the C-Component. Substrate **1** was prepared by the direct condensation of dimethyl 2-oxoglutarate with benzyl carbamate, according to the method reported previously.[11)]

α-Amino Acid Amides as the N-Component. Various kinds of H–Leu–NHR (**2**) and H–AA–NHPh (**4**) used here as the N-component were prepared by the usual methods or purchased from Tokyo Kasei Co., Ltd. and Kanto Kagaku Co., Ltd.

General Enzymatic Coupling of 1 with 2 or 4 Using Papain. A suspension (10 ml) of **1** [1 mM (1 M=1 mol dm^{-3})], an appropriate HCl·H–AA–NHR (5 mM), and papain (15 g dm^{-3}) in the presence of 2-mercaptoethanol (0.1 ml) in McIlvaine buffer was incubated, with shaking, at pH 8.0 and at 35 °C for 24 h. The reaction solution was acidified with 10% aqueous citric acid solution and further diluted with water (50 ml) and then extracted twice with ethyl acetate (20 ml). The combined extracts were washed with water (50 ml) and brine (60 ml) and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crude syrup or crystals, which were purified by HPLC using a mixture of MeOH–water (60→20% in 70 min) as the eluent. The eluate was concentrated in vacuo to give crystals, which were recrystallized from a mixture of hexane and ethyl acetate to give colorless powder (**3** and **5**). See Tables 1, 2, and 3.

General Enzymatic Coupling of 1 with 2 or 4 Using α-Chymotrypsin A (CT). A suspension (20 ml) of **1** (0.5 mM), an appropriate HCl·H–AA–NHR (1.5 mM), and CT (3.5 g dm^{-3}) in the presence of $CaCl_2$ (40 mg) in McIlvaine buffer was incubated, with shaking, at pH 8.0 and 35 °C for 24 h. The reaction solution was acidified with 10% aqueous citric acid solution and extracted three times with ethyl acetate (60 ml). The combined extracts were washed with brine (80 ml) and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crude crystals, which were recrystallized from a mixture of hexane and ethyl acetate to



Scheme 1.

Table 1. The Yields and Physical Constants of Cbz–ΔGlu(OMe)–Leu–NHR (**3**)

Compd	Yield	Mp[a]	Formula	Found (Calcd)/%			IR, ν/cm^{-1}[b]		^{1}H NMR, δ[c]		$[\alpha]_D^{20}$/°
							–NH–	–CONH–	–CONH–	–CH=	
No.	%	θ_m/°C		C	H	N	(–C=C–)		(J/Hz)		(c 1, in MeOH)
3a	18	Syrup	$C_{20}H_{27}N_3O_6$	59.30	6.39	10.08	3310	1675	7.22bs	6.43t	−21.93
				(59.24	6.71	10.37)	(1635)	1530		(7.0)	
3b	33	53—54	$C_{21}H_{29}N_3O_6$	60.14	7.02	10.09	3310	1655	7.18d	6.36t	−29.42
				(60.13	6.97	10.02)	(1655)	1530	(7.0)	(7.0)	
3c	34	82—83	$C_{22}H_{31}N_3O_6$	60.98	7.34	9.99	3315	1650	7.66—[d]	6.36t	−34.75
				(60.95	7.21	9.69)	(1645)	1535	7.00m	(7.0)	
3d	77	78—79	$C_{26}H_{31}N_3O_6$	64.56	6.36	8.59	3310	1675	6.97t	6.32t	−39.59
				(64.85	6.49	8.73)	(1640)	1535	(7.0)	(7.0)	
3e	11	71—72	$C_{24}H_{35}N_3O_6$	62.45	7.99	9.00	3310	1650	6.82t	6.35t	−31.67
				(62.45	7.64	9.11)	(1650)	1540	(7.0)	(7.0)	
3f	13	63—64	$C_{26}H_{32}N_4O_6$	62.80	6.53	11.22	3285	1680	6.80d	6.34t	−22.44
				(62.89	6.50	11.28)	(1660)	1525	(7.0)	(7.0)	

a) Colorless powder from a mixture of hexane and ethyl acetate. b) Recorded in KBr. c) Measured in $CDCl_3$. d) Overlapped with proton of Ph.

Table 2. Synthesis of Cbz–ΔGlu(OMe)–AA–NHPh (**5**)

Reaction AA (Yield/%)				No reaction AA		
Gly	(58)	Ser	(53)	Asp(OBzl)	Asp	Asn
Ala	(53)	Thr	(68)	Glu(OBzl)	Glu	Gln
Val	(52)	Phe	(54)	Orn(Cbz)	Lys(Cbz)	
Leu	(77)	Tyr	(34)	His(Tos)	Pro	Trp
Ile	(38)	Nle	(39)[a]	Tle[c]	Sar	Gle[d]
Cys(Bzl)	(27)	H	(55)[b]	Met		

a) Norleucine. b) Aniline. c) *t*-Leucine (3-Methylvaline). d) 4-Methylleucine.

give pale yellow powder. In the present stage the ratio of the structural isomers (**3**, **5**, **6**, and **7**) were determined and then separated by HPLC using a mixture of MeOH–water (60→20% in 70 min) as the eluate. The each fraction was concentrated in vacuo to give crystals, which were recrystallized from a mixture of hexane and ethyl acetate to give colorless powder (**6** and **7**). See Tables 4, 5, 6, and 7.

Results and Discussion

Enzymatic Coupling of 1 with AA Amides Using Papain. Quite similarly as in the case of the hydrolysis and coupling of **1** reported briefly in the preceding papers,[6,7,9] the following enzymatic reactions using papain were worked up. First, the coupling of **1** with six kinds of H–Leu–NHR (**2**: **a**; R=H, **b**; R=Me, **c**; R=Et, **d**; R=Ph, **e**; R=Bu^t, **f**; R=NHPh) was fully

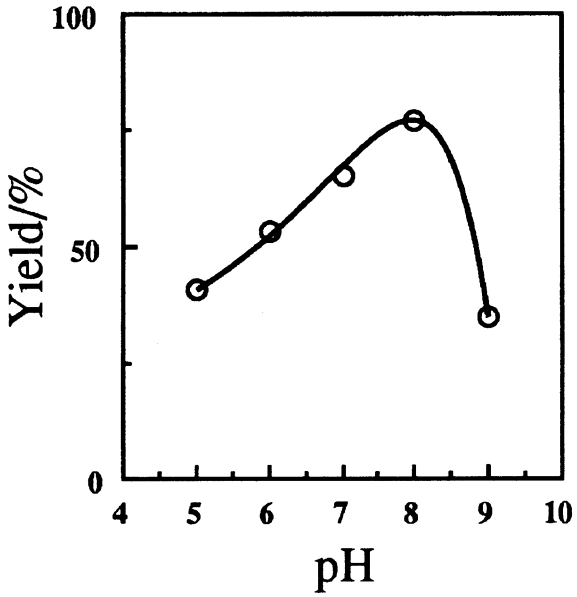


Fig. 1. Effect of pH on the yields of **3d**. The reaction mixture (5 ml), containing 100 mM **1** and 500 mM **2d** and 15 g dm^{-3} papain (2.8 units mg^{-1}) in McIlvaine buffer, was shaken at various pH's at 35 °C for 24 h.

carried out to give Cbz–ΔGlu(OMe)–Leu–NHR (**3a**—**f**), in which the yield of Cbz–ΔGlu(OMe)–Leu–NHPh (**3d**) was found to be the highest to reach 77%, as summarized in Table 1. Accordingly, various anilides (H–AA–

Table 3. The Physical Constants of Cbz–ΔGlu(OMe)–AA–NHPh (**5**)

Compd No.	Mp[a] θ_m/°C	Formula	Found (Calcd)/%			IR, ν/cm^{-1}[b]		^{1}H NMR, δ[c]		$[\alpha]_D^{20}$/°
			C	H	N	–NH– (–C=C–)	–NHCO–	–CONH–	–CH=	(*c* 1, in MeOH)
								(*J*/Hz)		
5a	149—150	$C_{22}H_{23}N_3O_6$	62.40	5.48	9.75	3365	1685	7.04d	6.23t	—
			(62.16	5.45	9.88)	(1640)	1530	(7.0)	(7.0)	
5b	107—108	$C_{23}H_{25}N_3O_6$	62.98	5.45	9.49	3330	1675	7.02d	6.30t	−18.64
			(62.86	5.73	9.56)	(1665)	1535	(7.0)	(7.0)	
5c	129—130	$C_{23}H_{25}N_3O_7$	60.44	5.47	9.09	3285	1660	7.04d	6.34t	−15.69
			(60.65	5.53	9.23)	(1640)	1530	(7.0)	(7.0)	
5d	86—87	$C_{24}H_{27}N_3O_7$	61.66	5.77	8.88	3315	1670	6.94d	6.26t	−36.01
			(61.40	5.80	8.95)	(1645)	1520	(7.0)	(8.0)	
5e	Syrup	$C_{25}H_{29}N_3O_6$	64.41	6.28	8.69	3315	1670	7.00bs	6.29t	−20.41
			(64.22	6.25	8.99)	(1640)	1535		(7.0)	
5f	58—59	$C_{29}H_{29}N_3O_6$	67.27	5.54	8.23	3290	1675	6.84d	6.16t	−38.61
			(67.56	5.67	8.15)	(1640)	1535	(7.0)	(7.0)	
5g	63—64.5	$C_{29}H_{30}N_3O_7$	65.39	5.19	7.92	3310	1680	6.36d	6.36t	−9.90
			(65.40	5.18	7.89)	(1655)	1535	(7.0)	(7.0)	
5h	159—160.5	$C_{26}H_{31}N_3O_6$	64.77	6.48	8.88	3310	1670	7.11d	6.38t	−16.17
			(64.85	6.49	8.73)	(1635)	1535	(7.0)	(7.0)	
5i	117—118	$C_{26}H_{31}N_3O_6$	64.91	6.60	8.74	3315	1665	7.06d	6.40t	−26.97
			(64.85	6.49	8.73)	(1640)	1530	(7.0)	(7.2)	
5j	92—93	$C_{30}H_{31}N_3O_6S$	63.89	5.51	7.41	3320	1670	6.84—[d]	6.28t	−42.84
			(64.16	5.56	7.48)	(1635)	1525	7.84m	(7.0)	
5k	107—108	$C_{20}H_{20}N_2O_5$	65.26	5.52	7.61	3300	1670	7.10d	6.32t	
			(65.21	5.47	7.61)	(1650)	1555	(8.0)	(7.0)	

a) Colorless powder from a mixture of hexane and ethyl acetate. b) Recorded in KBr. c) Measured in $CDCl_3$. d) Overlapped with proton of Ph.

Table 4. Coupling of **1** with H–Leu–NHR (**2**) by using CT

N-Component **2**		Yield/%	
No.	R	**6**	**3**
2a	H	10	3
2d	Ph	64	7
2f	NHPh	10	3
2g	Bzl	22	6

NHPh) were chosen and used as the N-components, as shown in Table 2. As the results, in the cases using the neutral aliphatic and aromatic AA anilides [**4**: AA; **a**; Gly, **b**; Ala, **c**; Ser, **d**; Thr, **e**; Val, **f**; Phe, **g**; Tyr, **h**; Ile, **i**; Nle (norleucine), **j**; Cys(Bzl) (Bzl=benzyl)] as well as Leu (**2d**) and aniline (**4k**), the expected coupling of **1** proceeded successfully to give Cbz–ΔGlu(OMe)–AA–NHPh (**3d** and **5a**—**j**) and Cbz–ΔGlu(OMe)–NHPh (**5k**) in ca. 51% yield, as summarized in Table 3. Unfortunately, however, in the case using α-amino acid ester (H–AA–OR) as the N-component, at present, the coupling was found to scarcely proceed.

In order to obtain the optimal conditions for the peptide synthesis, the similar enzymatic coupling of **1** with H–Leu–NHPh (**2d**) was thoroughly reexamined, because the preliminary experiment was already achieved to give **3d** in good yield.[7] That is, besides the detailed examination on the effect of pH,[7] the effect of the reaction time and those of the concentrations of enzyme and

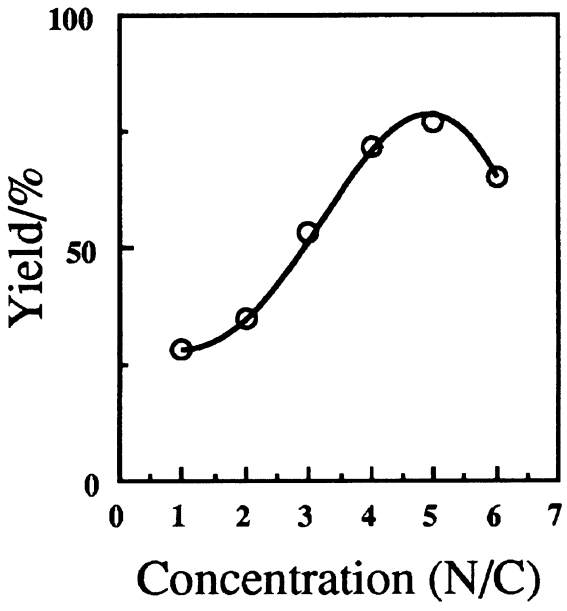


Fig. 2. Effect of N-component concentration on the yields of **3d**. The reaction mixture (5 ml), containing 100 mM **1** and various concentrations of **2d** and 15 g dm^{-3} papain (2.8 units mg^{-1}) in McIlvaine buffer, was shaken at pH 8 at 35 °C for 24 h.

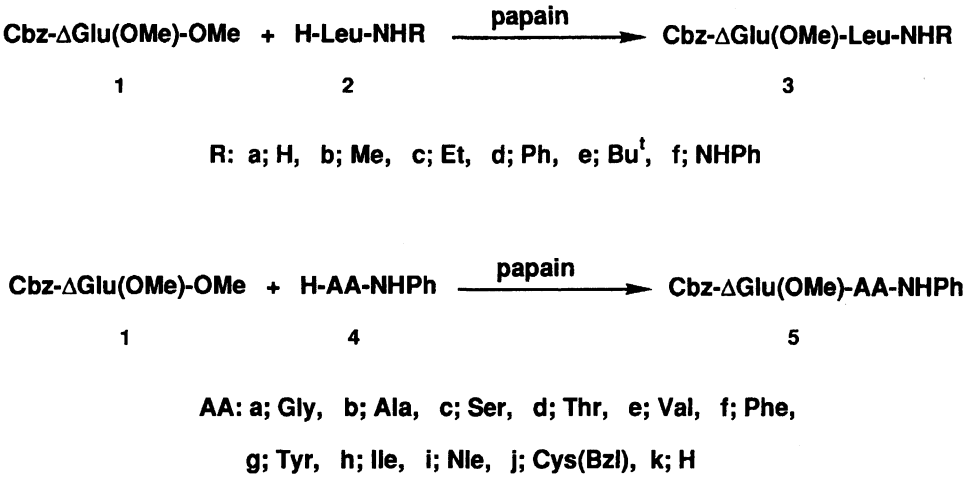
two substrates C- and N-components, were also studied in detail, as shown in Figs. 1, 2, 3, 4, and 5.

Generally, in the case using papain in McIlvaine buffer, it is well-known that the optimal pH value of the

Table 5. The Physical Constants of Cbz–ΔGlu(Leu–NHPh)–OMe (**6**)

Compd No.	Mp[a] θ_m/°C	Formula	Found (Calcd)/%			IR, ν/cm^{-1}[b]		^{1}H NMR, δ[c]		$[\alpha]_D^{25}$/°
			C	H	N	–NH– (–C=C–)	–CONH–	–CONH–	–CH=	(c 1, in MeOH)
								(J/Hz)		
6a	161—162	$C_{20}H_{27}N_3O_6$	59.34	6.53	10.45	3320	1695	6.94d	6.74t	−2.56
			(59.24	6.71	10.37)	(1675)	1545	(7.0)	(8.0)	
6d	169—171	$C_{26}H_{31}N_3O_6$	64.91	6.48	8.88	3285	1695	7.10d	6.65t	−11.51
			(64.85	6.49	8.73)	(1670)	1545	(7.0)	(7.0)	
6f	177—179	$C_{26}H_{32}N_4O_6$	62.54	6.39	11.01	3335	1695	7.15d	6.82t	−9.51
			(62.89	6.50	11.28)	(1660)	1540	(7.0)	(7.0)	
6g	135—136	$C_{27}H_{33}N_3O_6$	65.45	6.54	8.73	3280	1695	7.00—[d]	6.70t	−5.78
			(65.44	6.71	8.48)	(1670)	1555	7.20m	(8.0)	

a) Colorless powder from a mixture of hexane and ethyl acetate. b) Recorded in KBr. c) Measured in $CDCl_3$. d) Overlapped with proton of Ph.



Scheme 2.

Table 6. Coupling of **1** with H–AA–NHPh (**4**) by Using CT

N-Component **4**		Yield/%	
No.	AA	**7**	**5**
4a	Gly	23.9	1.6
4b	Ala	38.7	1.1
4e	Val	40.0	4.5
4f	Phe	48.0	9.0

usual coupling is around 7. In the present study, however, the pH value was variously changed to examine the coupling ability of the uncommon AA (**1**). As a result, in the case using an equimolar **1** and N-component (**2d**), the yield of **3d** was found to increase gradually in the range of the pH from 6 to 9 and come up to the highest at pH 8.0, even though the yield was considerably low. Consequently, upon increasing the amount of the N-component from 1 to 6 mmoles to **1** in the presence of papain (15 g dm^{-3}) at pH 8.0 and at 35 °C, the yield was found to increase exceedingly and steeply to reach 77% at the component ratio (N/C) 5.0, as Figs. 1 and 2 show. From the result, it is not too much to say that the formation of **3d** from **1** and **2d** is extremely dependent upon the pH value and the substrate concentrations. Namely, quite similarly as in the case of the hydrolysis of **1**,[7] Fig. 1 indicates that a rather alkaline pH may be favorable to such dehydropeptide formation reaction. Subsequently, the time course of the peptide synthesis from the similar C- and N-component ratio at pH 8.0 and at 35 °C was also examined. As Fig. 3 shows, the peptide formation was comparatively slow and completed to reach the utmost yield over 24 h.

Furthermore, regarding the amount of papain, the enzyme concentration at around 15 g dm^{-3} provided the highest yield. Figure 4 indicates that a considerable large amount of enzyme is required for the coupling. In addition, Fig. 5 indicates that the C-component concentration ranging from 50 to 150 mM affords high yield more than 60%.

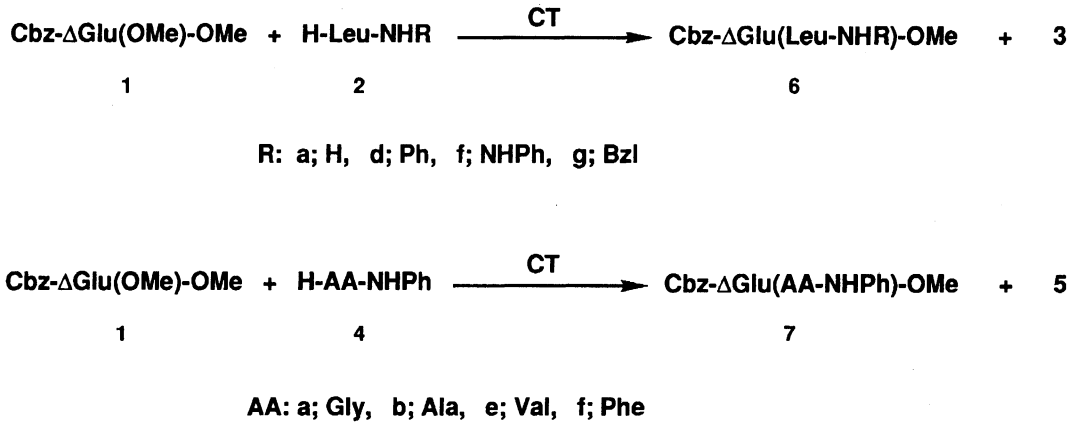
Consequently, the substrates (C-) concentration of 100 mM was found to be the most effective.

As can be seen from Figs. 1, 2, 3, 4, and 5, the results show that the enzymatic coupling of **1** with **2d** require the considerably prolonged reaction time and higher concentration of enzyme, but closely depends upon the comparatively lower concentration of C- and N-components under slightly alkaline pH.

Table 7. The Physical Constants of Cbz–ΔGlu(AA–NHPh)–OMe (**7**)

Compd No.	Mp[a] θ_m/°C	Formula	Found (Calcd)/%			IR, ν/cm^{-1}[b]		^{1}H NMR, δ[c]		$[\alpha]_D^{25}$/°
						–NH–	–CONH–	–CONH–	–CH=	
			C	H	N	(–C=C–)		(J/Hz)		(c 1, in MeOH)
7a	156—157	$C_{22}H_{23}N_3O_6$	62.34	5.15	10.03	3295	1695	7.12d	6.78t	—
			(62.16	5.45	9.75)	(1675)	1555	(7.0)	(7.0)	
7b	143—145	$C_{23}H_{25}N_3O_6$	62.74	5.49	9.79	3335	1695	7.11d	6.70t	−18.45
			(62.86	5.73	9.56)	(1660)	1540	(8.0)	(7.0)	
7e	183—185	$C_{25}H_{29}N_3O_6$	63.95	6.39	9.00	3290	1695	7.09d	6.76t	−8.50
			(64.22	6.25	8.99)	(1660)	1540	(7.0)	(7.0)	
7f	115—116	$C_{29}H_{29}N_3O_6$	67.70	5.72	8.26	3305	1695	7.10d	6.72t	−8.87
			(67.56	5.67	8.15)	(1675)	1550	(7.0)	(7.0)	

a) Colorless powder from a mixture of hexane and ethyl acetate. b) Recorded in KBr. c) Measured in $CDCl_3$.



Scheme 3.

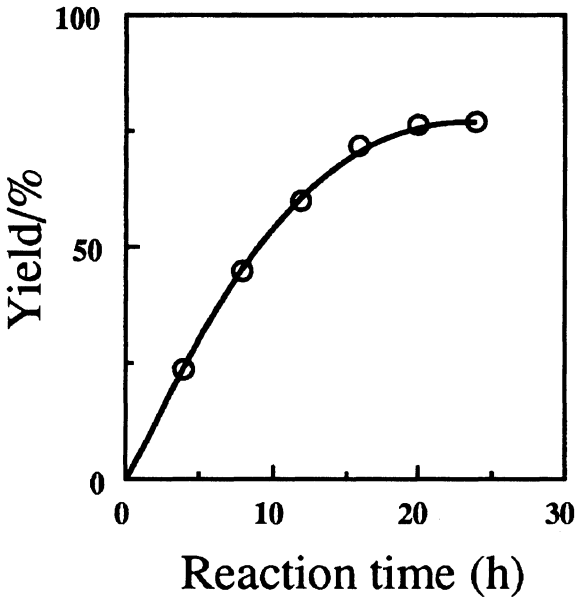


Fig. 3. Effect of reaction time on the yields of **3d**. The reaction mixture (5 ml), containing 100 mM **1** and 500 mM **2d** and 15 g dm^{-3} papain (2.8 units mg^{-1}) in McIlvaine buffer, was shaken at pH 8 at 35 °C for various hours.

Thus, the optimum reaction conditions were found to be papain (15 g dm^{-3}), substrate concentration ratio (N/C=5.0) under C-component concentration (100 mM) in McIlvaine buffer at pH 8.0 and 35 °C for 24 h. The optimum conditions were applied to all the similar peptide formation reactions of **1** with **2**, according to Scheme 2.

From the above results and the facts that many kinds of the other α-dehydroamino acid and Δ^1-dehydrodipeptide esters[12] as the C-component also coupled enzymatically with the N-component Δ^2-dehydrodipeptide esters,[12,13] our present study indicates that papain is a potent catalyst and widely applicable for the various dehydropeptide syntheses. Particularly, with regard to the structural characteristics of the N-component substrates, as can be seen from Table 2, irrespective of the small or large side chain, the neutral aliphatic and aromatic AA anilides are indispensable to the coupling with the C-component **1**.

Moreover, in these cases, it has been found that Ser, Thr, and Tyr without the O-protecting group also couple with Cbz–ΔGlu–OMe as well as **1**.[13]

The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **3** and **5** are summarized in Tables 1, 2, and 3

Enzymatic Coupling of 1 with AA Amides Us-

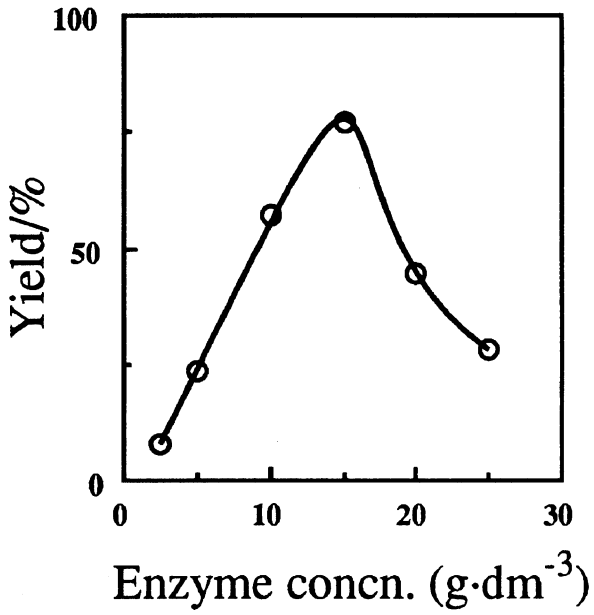


Fig. 4. Effect of enzyme concentration on the yields of **3d**. The reaction mixture (5 ml), containing 100 mM **1** and 500 mM **2d** and various concentrations of papain in McIlvaine buffer, was shaken at pH 8 at 35 °C for 24 h.

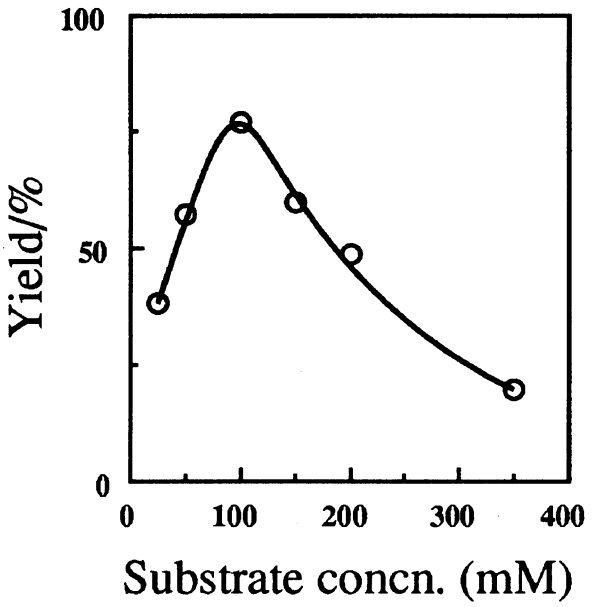


Fig. 5. Effect of substrate concentration on the yields of **3d**. The reaction mixture (5 ml), containing various concentration of **1** and 5 equivalent of **2d** and 15 g dm^{-3} papain (2.8 units mg^{-1}) in McIlvaine buffer, was shaken at pH 8 at 35 °C for 24 h.

ing CT. Similarly as in the case using papain, the C-component **1** (0.5 mM) was subjected to the coupling with H–AA–NHR (**2**; 1.5 mM) by using CT (3.5 g dm^{-3}) under the optimal conditions mentioned in the case using papain. As a result, contrary to the expectation, the coupling of γ-ester of **1** with **2** took place predominantly to give Cbz–ΔGlu(Leu–NHR)–OMe (**6**: **a**; R=H, **d**; R=Ph, **f**; R=NHPh, **g**; R=Bzl), as summarized in Tables 4 and 5. In these cases, regarding of the amount of the total yields, it is accompanying with a small amount of **3** by all means. Among the above examples, the yield of **6d** from **1** and **2d** was found to be the highest reaching 64%, along with **3d** in ca. 8% yield.

Accordingly, the anilides of the hydrophobic AA–NHPh (AA: **a**; Gly, **b**; Ala, **e**; Val, **f**; Phe) were selected as the N-component and submitted to the similar coupling with **1**, according to Scheme 3. The expected coupling took place to give a mixture of Cbz–ΔGlu(AA–NHPh)–OMe (**7**) and **5** in 44 and 5% yields, respectively, in a ratio 9:1, although the total yields were relatively low. The obtained mixture could be readily separated by the HPLC method, as summarized in Tables 6 and 7.

The yields, melting points, and physical constants (IR, ^{1}H NMR, and specific rotation) of **6** and **7** are summarized in Tables 4, 5, 6, and 7.

In conclusion, it is worth noting that the enzymatic coupling of uncommon α-amino acid ester such as α-dehydroglutamate with α-amino acid amides is first successful, indicating the further many and wonderful possibilities of the various couplings for the organic syntheses. In particular, it is interesting that the coupling of γ-ester of Cbz–ΔGlu(OMe)–OMe in the reaction using CT took place predominantly.

References

1) Part XIV: C. Shin, S. Honda, K. Morooka, and Y. Yonezawa, *Bull. Chem. Soc. Jpn.*, **66**, 1844 (1993).
2) Y. Isowa, M. Ohmori, M. Sato, and K. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2766 (1977).
3) K. Morihara and T. Oka, *Biochem. J.*, **163**, 531 (1977).
4) Y. Isowa, M. Ohmori, T. Ichikawa, and K. Mori, *Tetrahedron Lett.*, **1979**, 2611.
5) K. Morihara and T. Oka, *J. Biochem.*, **89**, 385 (1981).
6) C. Shin, N. Takahashi, and Y. Yonezawa, *Chem. Lett.*, **1988**, 2001.
7) C. Shin and N. Takahashi, *Chem. Lett.*, **1989**, 747.
8) C. Shin, M. Seki, and N. Takahashi, *Chem. Lett.*, **1990**, 2089.
9) C. Shin, N. Takahashi, and M. Seki, *Bull. Chem. Soc. Jpn.*, **64**, 3575 (1991).
10) C. Shin and M. Seki, *Chem. Lett.*, **1991**, 887.
11) C. Shin, Y. Yonezawa, and E. Watanabe, *Tetrahedron Lett.*, **26**, 85 (1985).
12) In this paper, the symbols Δ^1 and Δ^2 indicate the position number of double bond of ΔAA residue from the N-terminus in sequence.
13) C. Shin, M. Seki, and H. Tano, "61st National Meeting of the Chemical Society of Japan," Yokohama, April 1991, Abstr., No. 3D104; C, Shin, T. Kakusho, and M. Seki, "64th National Meeting of the Chemical Society of Japan," Niigata, October 1992, Abstr., No. 1C301.