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Dehydrooligopeptides. IV.[1] Synthesis of Various Types of Dehydrodi- and tripeptides by Fragment Condensation and Base-Catalyzed β-Elimination

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The synthesis of dehydrotripeptides, composed of an α-amino acid and two α-dehydroamino acid (DHA) residues, by the direct coupling of *N*-protected Δ^1-dehydrodipeptide with DHA ester and by the β-elimination of Δ^1-dehydrotripeptide containing a threonine residue is described. Moreover, the configurational determination of all the new products is discussed.

Keywords——α-dehydroamino acid; fragment condensation; β-elimination; dehydrooligopeptide; geometric configuration

In connection with the synthesis of dehydrooligopeptides, we have already reported the synthesis of two kinds of α-dehydroamino acids (DHA; ΔAA) as amine and carboxyl components.[2-5] In addition, not only the direct synthesis of a dehydrooligopeptide by the coupling of the two DHAs, but also an indirect synthesis by the β-elimination reaction of a dipeptide having a suitable leaving group, such as a hydroxyl or chlorine group, was reported.[1,6,7]

Although there are a few reports on the synthesis of dehydrooligopeptides by the use of unsaturated azlactones[8-11] and by another method,[12] no report on the synthesis of dehydrotripeptides by fragment condensation has yet appeared in the literature.

In this paper, we wish to report the synthesis of dehydrodi- and tripeptides containing one or two DHA residues by both the fragment condensation and the β-elimination methods.

After the hydrolysis of *N*-acetyl-Δ^1-(*Z*,L)-dehydrodipeptide ethyl ester[13] with 10% NaOH aqueous solution in dioxane, the resulting dehydrodipeptide (**1**) was coupled with DHA ester. The condensation of **1** with α-dehydrovaline (ΔVal) ethyl ester (**2**) as an amine component in the presence of dicyclohexylcarbodiimide (DCC) by the usual procedure gave the expected *N*-acetyl-$\Delta^{1,3}$-(*Z*,L,*Z*)-dehydrotripeptide ethyl ester (**4**),[13] even though the yield was low (*ca.* 25%). Unfortunately, it was found that similar coupling of **1** with other DHA esters scarcely proceeded except in the case of α-dehydrovaline ester. The difference between the above two coupling reactions is considered to be a result of the weak nucleophilicity of DHA ester as compared with that of ΔVal ester.

On the other hand, the fragment condensation of benzyloxycarbonyl (Cbz)-(*Z*)-DHA (**3**) as a carboxyl component with an appropriate (L,L)-dipeptide ester by the DCC method mentioned above was achieved to give Cbz-Δ^1-(*Z*,L,L)-dehydrotripeptide ester (**5**) in *ca.* a 65% yield.

The above results suggest that the use of DHA ester as an amine component in the fragment condensation as well as the stepwise elongation may be disadvantageous. Therefore, in order to synthesize further unsaturated peptides, the synthesis of dehydrooligopeptides having one or more leaving groups by condensation followed by base-catalyzed β-elimination of the resulting peptide might be more effective. In fact, the fragment condensation of **3** with dipeptide methyl ester containing a DL-threonine or DL-erythro-2-amino-3-chlorobutanoic

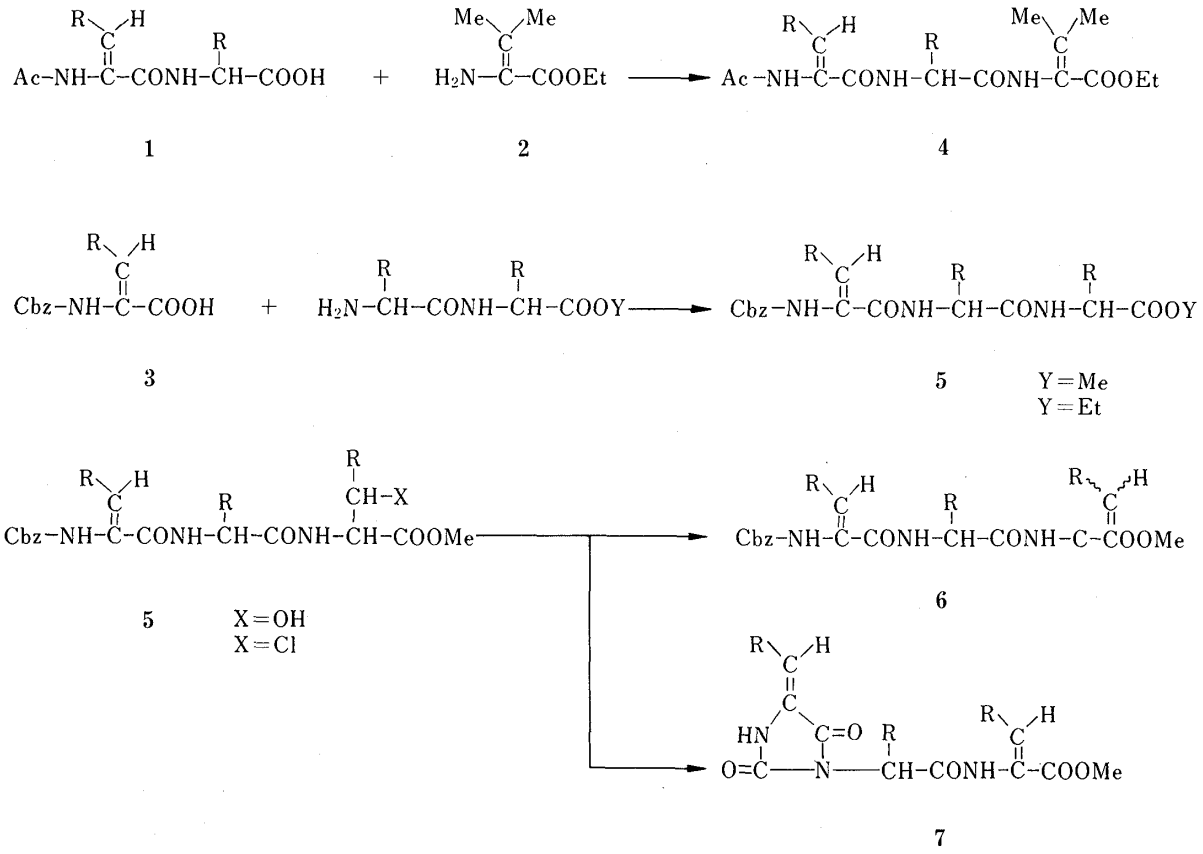


Chart 1

acid[14] residue provided Cbz-Δ^1-(*Z*,L,DL)-dehydrotripeptide (**5**: β-OH and β-Cl respectively) in good yield. As shown in Table 1, the ratio of the diastereomeric isomers of **5** thus obtained was evaluated from the intensity of the olefinic proton signals in the nuclear magnetic resonance (NMR) spectrum. Furthermore, the mesylation of **5** (β-OH) with methanesulfonyl chloride and subsequent elimination with three moles of triethylamine below 0 °C gave the desired Cbz-$\Delta^{1,3}$-dehydrotripeptide ester (**6**) in a good yield. In this case, the corresponding mesyloxy intermediate as well as the unsaturated hydantoin derivative,[1] resulting from the cyclization of the final reaction product (**6**), were not obtained. Moreover, similar elimination reaction of **5** (β-Cl) with triethylamine gave the corresponding $\Delta^{1,3}$-dehydrotripeptide ester (**6**) in *ca.* 77% yield.

On the other hand, it was found that the elimination of **5** (β-Cl) and the subsequent cyclization of the resulting product by using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in place of triethylamine proceeded to give the expected unsaturated hydantoin derivative (**7**) in 67% yield. Accordingly, if a strong base such as DBU is used, it can be expected that **5** (β-Cl) will be converted into the corresponding hydantoin derivative.

The yields, physical constants, and spectral data of **5** and **6** are summarized in Tables I and II.

In the NMR spectra of **5** and **6**, the signal of the olefinic proton of **5** due to the (*Z*)-DHA residue appeared in the δ 7.10—6.03 region, and the signals of the olefinic proton of **6** newly formed by the elimination of the mesyl derivative of **5** (β-OH) and by that of **5** (β-Cl) appeared in the δ 6.70—6.63 and δ 6.75—6.60 regions, respectively. Comparison of the chemical shifts of the olefinic proton of **6** derived from **5** (β-OH) and **5** (β-Cl) indicated that the differences between (*Z*)- and (*E*)-DHA residues of C-terminus in **6** could not be clearly distinguished. However, by comparing the signal of the γ-methyl protons of the DHA residue of the C-

terminus in **6** with that of the Cbz-$\Delta^{1,3}$-(*Z*,L,*E*)-dehydrotripeptide ester prepared previously,[1] the configurational structure of **6** obtained from **5** (β-OH and β-Cl) could be readily determined to be Cbz-$\Delta^{1,3}$-(*Z*,L,*Z*)-dehydrotripeptide ester and Cbz-$\Delta^{1,3}$-(*Z*,L,*E*)-dehydrotripeptide ester, respectively. As was reported in the preceding paper,[1] the γ-methyl protons of the (*E*)-DHA residue resonate at lower magnetic field ($\Delta\delta$ *ca.* 0.33 ppm) in the δ 2.10—2.04 region as a doublet, as shown in Table II.

Moreover, in the NMR spectrum of **7** obtained from **5** (β-Cl), the signals of the γ-methyl protons at δ 1.75 and two olefinic protons at δ 6.82 and 5.86 were shifted to higher field. Accordingly, the configuration of **7** derived from **5** (β-Cl and -OH) could be determined to be (*Z*, *Z*) [(*Z*)-5-alkylidene-hydantoin-3-yl]-Δ^2-(*Z*,L)-dehydrodipeptide methyl ester.

Thus, in the synthesis of **7**, the elimination occurred smoothly and then the resulting product (**6**) presumably isomerized immediately to give the (*Z*, *Z*)-isomer, because of the presence of a strong base.

Experimental

All the melting points are uncorrected. The infrared (IR) spectra were recorded with a Hitachi EPI-G3 spectrometer. The NMR spectra were measured with a JNM-PS-100 spectrometer (Japan Electron Laboratory Co., Ltd.), using tetramethylsilane as the internal standard.

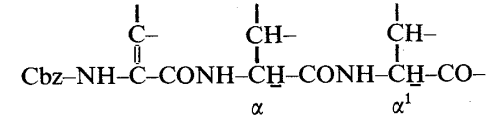
Ac-Δ^1-(*Z*)-dehydrodipeptide (1). By the Hydrolysis of the Dipeptide Ester——A 10% solution of NaOH (30 ml), was added to a solution of Ac-Δ^1-dehydrodipeptide methyl ester (20 mmol) in dioxane (15 ml) with stirring, at room temperature. Water (30 ml) was added to the resulting solution, and the aqueous layer was acidified with 1 M HCl to pH 2.0. The resultant solution was extracted well with ethyl acetate. The combined extract was washed with saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave a residual syrup, which gradually crystallized. Recrystallization from ethyl acetate gave colorless needles. Ac–ΔPhe–Phe–OH, yield 87%, mp 213—215 °C.[9] Ac–ΔPhe–Leu–OH, yield 80%, mp 218—219 °C.[9]

From the Coupling of α-Amino Acid with Azlactone——According to the method reported by Bergmann *et al.*,[8] A 10% NaOH (100 ml) was added to a mixture of α-amino acid (20 mmol) in acetone (100 ml) with stirring and, after a few minutes, 2-methyl-4-benzylidene-5-oxazolone (20 mmol) was added at room temperature. After cooling of the solution at 0 °C for 1 h, precipitated crystals were collected and dissolved in K_2CO_3 aqueous solution. When 6 M HCl was added to the resulting solution, colorless crystals separated out. Ac–ΔPhe–Phe–OH, yield 80%. Ac–ΔPhe–Leu–OH, yield 85%.

Ac-$\Delta^{1,3}$-(*Z*, L)-dehydrotripeptide Ester (4)——DCC (10 mmol) was added to a solution of **1** (10 mmol) in dry dimethylformamide (DMF, 12 ml) below −12 °C. The mixture was stirred for 30 min, then α-dehydrovaline ethyl ester (**2**; 12 mmol) was added, and the whole was kept at the same temperature for 6 h, then at room temperature for 48 h with stirring. Ethyl acetate (100 ml) was added, *N*,*N*′-dicyclohexylurea deposited was filtered off, and the filtrate was washed successively with 1 M HCl, water, saturated $NaHCO_3$ aqueous solution, and water and then finally dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified on a silica-gel column using a mixture of benzene and acetone (5 : 1, v/v) as the eluent to give colorless crystals. Recrystallization from chloroform gave **4** as colorless prisms. Ac–ΔPhe–Phe–ΔVal–OEt, yield 25%, mp 219—221 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.76 (s, –CH=), 4.62 (m, –CHCO–), 1.99 and 1.74 [6H, s, $(CH_3)_2C$=]. *Anal.* Calcd for $C_{27}H_{31}N_3O_5$: C, 67.90; H, 6.54; N, 8.80. Found: C, 67.85; H, 6.51; N, 8.89. Ac–ΔPhe–Leu–ΔVal–OEt, yield 25%, mp 213—215 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.02 (s, –CH=), 4.28 (m, –CHCO–), 1.99 and 1.74 [6H, s, $(CH_3)_2C$=]. *Anal.* Calcd for $C_{24}H_{33}N_3O_5$: C, 64.99; H, 7.50; N, 9.47. Found: C, 65.05; H, 7.45; N, 9.40.

Cbz-Δ^1-(*Z*,L,L)-dehydrotripeptide Ester (5)——Boc-(L,L)-dipeptide methyl ester (11 mmol) was added to methanol (30 ml) saturated with hydrogen chloride under cooling. The mixture was left to stand for 1 h and evaporated to dryness, then methanol (30 ml) was once more added to the resulting residue. The above procedure was repeated till the excess hydrogen chloride had been removed completely. The residue obtained was dissolved in dry CH_2Cl_2 (10 ml). Triethylamine (11 mmol) was added to the above solution under cooling, and after 20 min, Cbz-α-dehydroamino acid (**3**; 10 mmol), 1-hydroxybenzotriazole (HOBT, 11 mmol), and DCC (11 mmol) were added at −10 °C; this temperature was held for 1 h. The mixture was then stirred at room temperature for 48 h and evaporated to dryness. Ethyl acetate (100 ml) was added to the residue and insoluble material was filtered off. The filtrate was washed successively with 1 M HCl, water, saturated $NaHCO_3$ aqueous solution, and water and then finally dried over anhydrous Na_2SO_4. After removal of the solvent, the residual syrup was purified on a silica-gel column using a mixture of benzene and ethyl acetate (2 : 1, v/v) as the eluent. Concentration of the eluate under reduced pressure gave colorless crystals, which were recrystallized from a mixture of chloroform and cyclohexane (1 : 10, v/v) to give **5** as colorless needles.

TABLE I. Cbz-Δ^1-dehydrotripeptides (**5**)

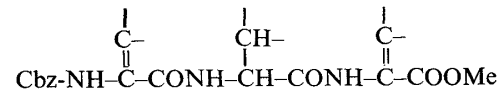


Compound	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd (Found)			^{1}H-NMR (δ, $CDCl_3$)			Diastereomer ratio
				C	H	N	R–CH= (J, Hz)	α^1	–CH– (J, Hz)	
⊿But–Gly–L-Phe–OEt	75	136—138	$C_{25}H_{29}N_3O_6$	64.22 (64.34	6.25 6.43	8.99 8.78)	6.50 q (7.0)	4.76 dt (7.0, 7.0)	3.87 dd (6.0, 7.8)	
⊿But–L-Ala–L-Val–OEt	50	Syrup	$C_{22}H_{31}N_3O_6$	60.95 (61.07	7.21 7.42	9.69 9.48)	6.55 q (7.0)	4.50 m	4.70 m	
⊿But–L-Phe–L-Val–OEt	65	71—72	$C_{28}H_{35}N_3O_6$	65.99 (66.07	6.92 6.97	8.25 8.21)	6.22 q (7.0)	4.35 dd (5.5, 8.0)	4.82 dt (7.0, 7.0)	
⊿Phe–L-Ala–L-Leu–OEt	60	115—117	$C_{20}H_{25}N_3O_6$	65.44 (65.45	6.71 6.65	8.48 8.41)	7.10 s	4.56 m	(2H)	
⊿But–L-Val-DL-But–OMe (Cl)	63	119—121	$C_{22}H_{30}ClN_3O_6$	56.46 (56.38	6.46 6.80	8.98 8.90)	6.50 6.45 q (7.0)	4.28 m	4.70 m	1 : 1
⊿Leu–L-Ala-DL-Thr–OMe	76	137—139	$C_{22}H_{31}N_3O_7$	58.78 (58.88	6.95 6.74	9.35 9.56)	6.03 d (10.0)	4.40 m	(2H)[b]	1 : 1
⊿But–-L-Leu-DL-Thr–OMe	45	54—55	$C_{23}H_{33}N_3O_7$	59.59 (59.71	7.18 7.19	9.07 9.02)	6.26 6.44 q (7.0)	4.56 m	(2H)	1 : 1

⊿But–L-Val–DL-Thr–OMe	40	39—41	$C_{22}H_{31}N_3O_7$	58.83 (58.83	6.95 6.90	9.35 9.32)	6.30 6.44 q (7.0)	4.50 m	(2H) m	1 : 1
⊿But–L-Phe–DL-Thr–OMe	60	44—46	$C_{26}H_{31}N_3O_7$	62.76 (62.77	6.28 6.32	8.45 8.44)	6.50 6.45 q (7.0)	4.60 m	4.80 m	1 : 1
⊿But–L-Phe–DL-But–OMe (Cl)	65	105—107	$C_{26}H_{30}ClN_3O_6$	60.52 (60.60	5.86 5.89	8.14 8.21)	6.50[c] q (7.0)	4.66 dd (3.5, 9.0)	4.80 dt (7.0, 7.0)	3 : 1
⊿But–L-Ala–DL-Thr–OMe	70	155—157	$C_{20}H_{27}N_3O_7$	57.00 (57.18	6.46 6.54	9.97 9.96)	6.24 6.38 q (7.0)	4.53 m	(2H)	1 : 1
⊿Leu–L-Val–DL-But–OMe (Cl)	75	Syrup	$C_{24}H_{34}ClN_3O_6$	58.11 (58.09	7.39 7.66	8.47 8.49)	6.21 6.28 d (10.0)	4.30 m	4.52 dd (5.5, 8.0)	1 : 1
⊿Leu–L-Val–DL-Thr–OMe	68	58—60	$C_{24}H_{35}N_3O_7$	60.36 (60.30	7.39 7.50	8.80 8.75)	6.26 6.06 d (10.0)	4.40 m	(2H)	3 : 1
⊿But–L-Ala–DL-But–OMe (Cl)	64	136—137	$C_{20}H_{26}ClN_3O_6$	54.61 (54.55	5.96 6.03	9.55 9.50)	6.40 6.47 q (7.0)	4.70 m	4.30 m	4 : 3

a) Colorless needles from a mixture of cyclohexane and chloroform (10 : 1, v/v).
b) Measured in DMSO-d_6.
c) Not split.

TABLE II. Cbz-$\Delta^{1,3}$-dehydrotripeptides (**6**)



Compound Z, L, Z (Z, L, E)	Yield (%)	mp[a] (°C)	Formula	Analysis (%) Calcd (Found)			^{1}H-NMR (δ, $CDCl_3$)			
				C	H	N	R–CH=	CH_3–CH=	N–CH–CO (J, Hz)	CH_3–CH=
ΔBut–L-Phe–ΔBut	90	111—113	$C_{27}H_{31}N_3O_6$	65.70 (65.77	6.33 6.30	8.51 8.57)	6.39 q (7.0)	6.70 q (7.0)	4.93 m	1.62 d
ΔBut–L-Leu–ΔBut	92	116—118	$C_{23}H_{31}N_3O_6$	62.00 (61.89	7.01 7.28	9.43 9.38)	6.22 q (7.0)	6.64 q (7.0)	4.65 m	1.70 d
ΔBut–L-Val–ΔBut	90	88—89	$C_{22}H_{29}N_3O_6$	61.24 (60.95	6.77 6.77	9.74 9.54)	6.40 q (7.0)	6.63 q (7.0)	4.50 dd (7.0, 8.5)	1.68 d
ΔBut–L-Ala–ΔBut	95	174—175[b]	$C_{20}H_{25}N_3O_6$	59.54 (59.11	6.25 6.25	10.42 10.33)	6.44 q (7.0)	6.73 q (7.0)	4.67 dq (7.0, 7.0)	1.70 d
ΔLeu–L-Val–ΔBut	86	112—114	$C_{23}H_{31}N_3O_6$	62.72 (62.57	7.24 7.29	9.14 9.08)	6.25 d (10.0)	6.70 q (7.0)	4.58 dd (7.0, 8.5)	1.68 d
(ΔBut–L-Ala–ΔBut)	75	146—148[b]	$C_{20}H_{25}N_3O_6$	59.54 (59.46	6.25 6.30	10.42 10.32)	6.50 q (7.0)	6.75 q (7.3)	4.59 dq (7.0, 7.0)	2.01 d
(ΔLeu–L-Val–ΔBut)	78	128—129	$C_{23}H_{31}N_3O_6$	62.72 (62.95	7.24 7.36	9.14 9.14)	6.27 d (10.0)	6.60 q (7.3)	4.46 dd (7.0, 8.5)	2.00 d
(ΔBut–L-Val–ΔBut)	93	90—92	$C_{22}H_{29}N_3O_6$	61.24 (61.19	6.77 6.75	9.74 9.77)	6.44 q (7.0)	6.64 q (7.3)	4.39 dd (7.0, 8.5)	2.00 d

a) Colorless needles from a mixture of cyclohexane and chloroform (10:1, v/v).
b) Colorless prisms from a mixture of cyclohexane and chloroform (10:1, v/v).

Cbz-$\Delta^{1,3}$-(*Z*,L,*Z*)-dehydrotripeptide Ester (6)——Methanesulfonyl chloride (3 mmol) and then triethylamine (6 mmol) were added dropwise to a solution of **5** (2 mmol) containing a Thr residue in CH_2Cl_2 (5 ml) below 0 °C. The mixture was stirred at room temperature for 24 h, CH_2Cl_2 (20 ml) was further added, and the resulting solution was washed with 1 M HCl and water, then dried over anhydrous $MgSO_4$. Removal of the solvent gave colorless crystals, which were recrystallized from a mixture of chloroform and cyclohexane (1 : 10, v/v) to give **6** as colorless needles.

Cbz-$\Delta^{1,3}$-(*Z*,L,*E*)-dehydrotripeptide Ester (6)——Similar treatment of **5** (2 mmol) containing a 2-amino-3-chlorobutanoic acid residue in CH_2Cl_2 (5 ml) in the presence of triethylamine (6 mmol), but for 12 h, gave colorless crystals, which were recrystallized from a mixture of chloroform and cyclohexane (1 : 10, v/v) to give colorless needles (**6**).

Hydantoin Derivative (7)——Similarly, treatment of **5** (X=Cl) (4.5 mmol) in CH_2Cl_2 (20 ml) in the presence of DBU (5 mmol), but for 5 h, gave crude crystals, which were purified on a silica-gel column using a mixture of benzene and ethyl acetate (5 : 1, v/v) as the eluent. Concentration of the eluate under reduced pressure gave colorless crystals, which were recrystallized from isopropyl alcohol to give **7** as colorless needles. [(*Z*)-5-ethylidene-hydantoin-3-yl]-(L,*Z*)-Phe-ΔBut-OMe. Yield 67%, mp 143—145 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.82 (1H, q, J=7.5 Hz, –CH=), 5.09 (1H, t, J=8.0 Hz, –NH–CH–CO–). *Anal.* Calcd for $C_{20}H_{22}N_4O_6$: C, 57.96; H, 5.35; N, 13.52. Found: C, 58.11; H, 5.45; N, 13.42.

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