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Dehydrooligopeptides. XI. Facile Syntheses of Various Kinds of Dehydrodi- and tripeptides, and Dehydroenkephalins Containing ⊿Tyr Residue by Using *N*-Carboxydehydrotyrosine Anhydride[1]

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Several kinds of *N*-free and protected *N*-carboxydehydrotyrosine anhydride (⊿Tyr·NCA) derivatives, derived by both the cyclization of the corresponding *N*-benzyloxycarbonyldehydrotyrosine derivatives and the protection of the resulting ⊿NCA, were employed for a one-pot dehydrooligopeptide (DHP) synthesis. As a result, various kinds of DHPs with a wide variety of combinations of sequences could be synthesized in fairly good yields. In addition, by utilizing the obtained information, two kinds of leucine-dehydroenkephalins containing a ⊿Tyr[1] and both ⊿Tyr[1] and ⊿Phe[4] residues were also synthesized.

In a previous paper,[2] we reported in detail the syntheses of various kinds of *N,O*-diprotected dehydrotyrosine (⊿Tyr) derivatives from mainly two routes. That is to say, the ⊿Tyr derivatives were derived by the condensation of *O*-protected *p*-hydroxybenzaldehyde with *N*-acyl-2-(diethoxyphosphinyl) glycine[3] and by the reaction of *N*-carboxydehydrotyrosine anhydride (⊿Tyr·NCA), derived by the cyclization of *N*-benzyloxycarbonyl (Cbz)-dehydrotyrosine with $SOCl_2$, with methanol. On the other hand, recently, various modes of coupling of *N*-carboxy-*α*-dehydroamino acid anhydride (⊿NCA) with an amino (*N*-), carboxy (*C*-) or both two components of AA were carried out to give a number of dehydrooligopeptides (DHP) in fairly good yields.[4,5] However, there has only been a few reports concerning a useful synthetic method for DHP containing a ⊿Tyr residue,[6] although the hydrogenated one, namely L-tyrosine (Tyr) residue, is often an important bioactive site in opioid peptides, such as cholecystokinin-8.[7]

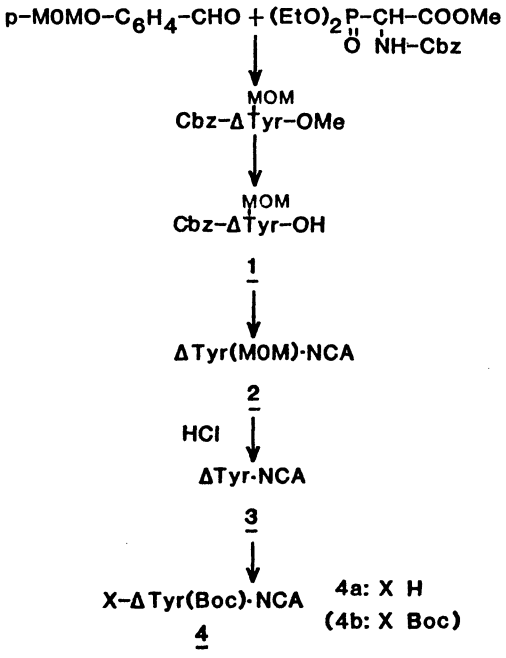
In this paper we wish to report on convenient syntheses of various kinds of DHP containing (*Z*)-configurational ⊿Tyr residues blocked with available *N*- and *O*-protecting groups, such as Cbz, *t*-butoxycarbonyl (Boc), and methoxymethyl (MOM), by the so-called ⊿NCA method.[4,8-10]

Moreover, in order to chemically modify naturally occurring peptides, leucine-enkephalin (Leu-Enk), as one of the numerous neuropeptides, was chosen and Tyr[1] or both Tyr[1] and phenylalanine (Phe[4]) residues were replaced by the corresponding ⊿Tyr and dehydrophenylalanine (⊿Phe) to obtain Δ^1 and $\Delta^{1,4}$-dehydroenkephalin derivatives (Δ^1Enk and $\Delta^{1,4}$Enk).[11]

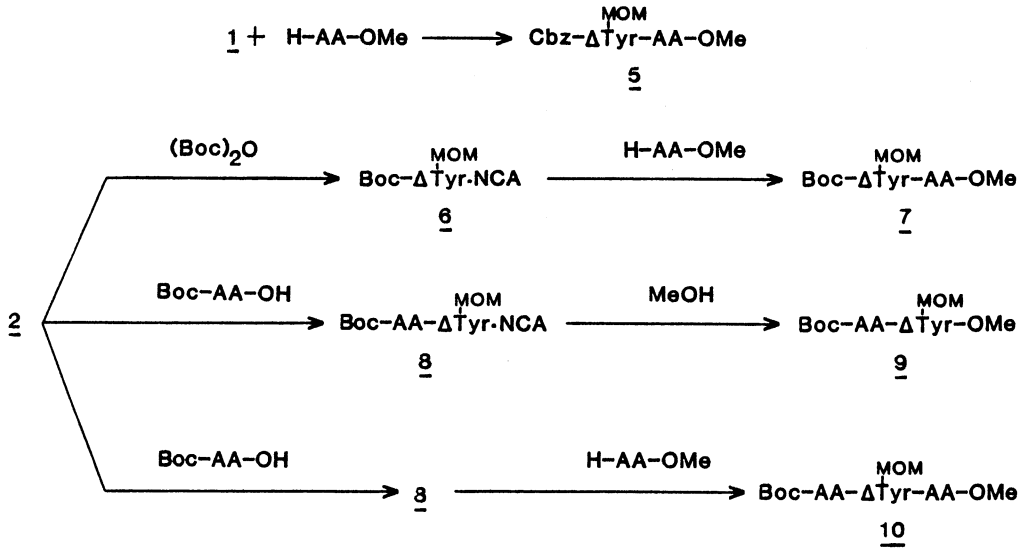
Results and Discussion

Syntheses of Dehydrodi- and tripeptides with ⊿Tyr Residue. We have already reported on the synthesis of (*Z*)-Cbz-(*O*-MOM)dehydrotyrosine [Cbz-⊿Tyr (MOM)-OH] (**1**), derived successively by the condensation of *p*-methoxymethoxy (MOMO) benzaldehyde with *N*-Cbz-2-(diethoxyphosphinyl)glycine methyl ester by the Schmidt method[3] and the ester hydrolysis of (*Z*)-Cbz-⊿Tyr(MOM)-OMe. Futhermore, the facile conversion of **1** with $SOCl_2$ to (*Z*)-*N*-carboxy-(*O*-MOM)dehydrotyrosine anhydride [⊿Tyr(MOM)·NCA] (**2**) and subsequent deprotection with HCl to ⊿Tyr·NCA (**3**) was also reported[2] (see Scheme 1).

In addition to the two compounds mentioned above (**2** and **3**), two more starting substrates were synthesized as follows. (*Z*)-*N*-Carboxy-(*O*-Boc)dehydrotyrosine anhydride [⊿Tyr(Boc)·NCA] (**4a**) was purely obtained in 70% yield by the selective acylation of a phenolic hydroxyl group alone of **3** with an equimolar di-*t*-butyl dicarbonate [$((CH_3)_3COCO)_2O$; $(Boc)_2O$] by the usual method.[2] Moreover, a similar treatment of **3** with more than two moles of $(Boc)_2O$ was carried out to give (*Z*)-*N*-carboxy-*N*-Boc-(*O*-Boc)dehydrotyrosine anhydride [Boc-⊿Tyr(Boc)·NCA] (**4b**) as an unstable



Scheme 1.



Scheme 2.

intermediate, although a pure isolation was unsuccessful. From the above results, in the case of the acylation of **3**, it can be seen that the side-chain phenolic hydroxyl group was more reactive than the ring imino group.

The four (*Z*)-*Δ*Tyr · NCA derivatives (**2**, **3**, **4a**, and **4b**) were subsequently subjected to the syntheses of numerous dehydrodi- and tripeptides by wide variety of combinations of appropriate AA and variously protected *Δ*Tyr residues, as illustrated in Schemes 2 and 3. Three more *N*-acyl-*Δ*Tyr · NCA intermediates were synthesized and then employed in situ to the synthesis of dehydrooligopeptides.

First, the stepwise elongation of **1** with L-*α*-amino acid methyl ester (H-AA-OMe) by using water-soluble [3-(dimethylamino)propyl]ethylcarbodiimide [C_2H_5-N=C=N-$(CH_2)_3$-N$(CH_3)_2$ · HCl; WSC] was readily carried out to give Cbz-*Δ*Tyr(MOM)-AA-OMe (**5**) in a 75% yield.

On the other hand, by taking advantage of two highly reactive sites of compound **2**, three sorts of *Δ*^{1},*Δ*^{2}-dehydrotripeptides (**7** and **9**) and *Δ*^{2}-dehydrotripeptides (**10**)[11] containing a *Δ*Tyr(MOM) residue at N, C-termini and at the center could be readily synthesized.[11] In the case of the synthesis of Boc-*Δ*Tyr(MOM)-AA-OMe (**7**), the acylation of **2** with $(Boc)_2O$ was carried out as the first step to form the corresponding intermediate, *N*-Boc-(*O*-MOM)-dehydrotyrosine anhydride [Boc-*Δ*Tyr(MOM) · NCA] (**6**), which was subsequently treated in situ with H-AA-OMe as an *N*-component to give **7** in 56% yield.

Similarly, a one-pot synthesis of *Δ*^{2}-dehydrodipeptides (**9**) containing C-terminal *Δ*Tyr(MOM) residue was also worked up by the following successive reactions. The coupling of **2** with Boc-AA-OH as a *C*-component in the presence of pyridine by the dicyclohexylcarbodiimide (DCC) method and a subsequent reaction of the resulting intermediate Boc-AA-*Δ* Tyr(MOM) · NCA (**8**) with methanol were carried out to give Boc-AA-*Δ*Tyr(MOM)-OMe (**9**) in 71% yield. Consequently, as can be seen by a comparison with the structures of two compounds, **7** and **9**, they are apparently related to each other as a regioisomer.

Furthermore, in an analogous manner (as mentioned above), *Δ*^{2}-dehydrotripeptides (**10**) containing *Δ*Tyr(MOM) residue at the center were also readily synthesized in one-pot by the condensation of **2** with, in turn, Boc-AA-OH and H-AA-OMe. In this case too,

Table 1. The Yields and Melting Points of **5**

Compound No. Cbz-*Δ*AA-AA-OMe	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/% C	H	N
5a -*Δ*Tyr(MOM)-Gly-	77	103—105	$C_{22}H_{24}N_2O_7$	61.63 (61.67	5.66 5.65	6.55 6.54)
5b -*Δ*Tyr(MOM)-Ala-	80	Syrup	$C_{23}H_{26}N_2O_7$	62.75 (62.43	6.15 5.92	6.03 6.33)
5c -*Δ*Tyr(MOM)-Leu-	77	60—61	$C_{26}H_{31}N_2O_7$	64.20 (64.45	6.75 6.66	5.91 5.78)
5d -*Δ*Tyr(MOM)-Phe-	66	58—60	$C_{29}H_{30}N_2O_7$	67.01 (67.17	5.89 5.83	5.45 5.40)

a) Colorless needles from a mixture of ethyl acetate and hexane.

Table 2. The Spectral and Optical Data of **5**

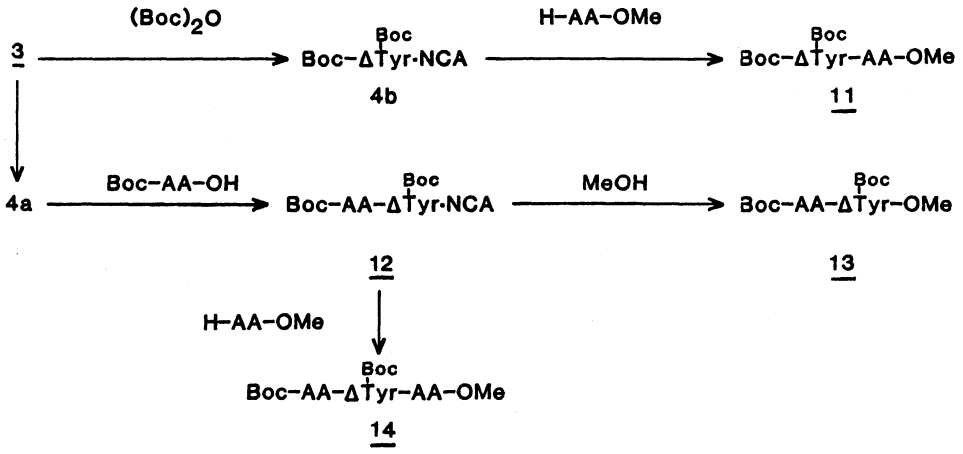
Compound No.	IR, $\tilde{\nu}$/cm^{-1} in KBr			^{1}H NMR (δ, $CDCl_3$)		$[\alpha]_D^{24}$/°
	-NHCO-		-C=C-	Ar-CH=	α-H[a] (*J*/Hz)	(*c* 1, in MeOH)
5a	1665	1520	1625	6.64s	4.10d (5.5)	—
5b	1665	1520	1630	7.18s	4.61dq (6.8, 6.8)	6.6
5c	1660	1515	1625	6.88—7.74[b]	4.74dt (6.5, 7.0)	−22.3
5d	1670		1630	6.82—7.54[b]	4.91dt (8.0, 7.0)	−21.2

a) Proton on α-carbon of L-α-amino acid residue. b) Overlapped on phenyl protons.

Table 3. The Yields and Melting Points of **7** and **9**

Compound No. Boc-⊿AA-AA-OMe (**7**) Boc-AA-⊿AA-OMe (**9**)	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/%		
				C	H	N
7a -⊿Tyr(MOM)-Ala-	45	Syrup	$C_{20}H_{28}N_2O_7$	58.75 (58.81	7.02 6.91	6.81 6.86)
7b -⊿Tyr(MOM)-Val-	85	Syrup	$C_{22}H_{32}N_2O_7$	60.27 (60.53	7.66 7.39	6.67 6.42)
7c -⊿Tyr(MOM)-Ile-	50	Syrup	$C_{23}H_{34}N_2O_7$	61.12 (61.34	7.90 7.61	6.19 6.22)
7d -⊿Tyr(MOM)-Phe-	43	Syrup	$C_{26}H_{32}N_2O_7$	64.21 (64.45	6.92 6.66	5.72 5.78)
9a -Ala-⊿Tyr(MOM)-	67	101—103	$C_{20}H_{28}N_2O_7$	58.92 (58.81	6.98 6.91	6.79 6.86)
9b -Val-⊿Tyr(MOM)-	73	117—118	$C_{22}H_{32}N_2O_7$	60.72 (60.53	7.45 7.39	6.40 6.42)
9c -Ile-⊿Tyr(MOM)-	70	109—110	$C_{23}H_{34}N_2O_7$	61.31 (61.34	7.76 7.61	6.31 6.22)
9d -Phe-⊿Tyr(MOM)-	74	114—116	$C_{26}H_{32}N_2O_7$	64.71 (64.45	6.70 6.66	5.71 5.78)

a) Colorless needles from a mixture of ethyl acetate and hexane.



Scheme 3.

after forming the intermediate **8** in the first place, *N*-component subsequently coupled with the resulting **8** to give the expected Boc-AA-⊿Tyr(MOM)-AA-OMe (**10**) in 76% yield. As summarized in Tables 3 and 5, the yields of **7**, **9**, and **10**, thus obtained, were found to attain to 68%.

From the above results, these three synthetic procedures were further ascertained to be appreciably efficient for the synthesis of the diversified and complicating peptides containing polyfunctional α-dehydroamino and/or amino acid residues.

On the other hand, in order to obtain similar dehy-

dropeptides blocked with the same protecting group to the amino and side-chain hydroxyl groups, *N*- and *O*-unsubstituted *N*-carboxydehydrotyrosine anhydride (**3**) was successively acylated with more than two moles of $(Boc)_2O$ and coupled with H-AA-OMe in one-pot to give the desired Δ^1-dehydrodipeptide [Boc-ΔTyr-(Boc)-AA-OMe] (**11**) in 58% yield. In this case too, it is almost certain to initially form Boc-ΔTyr(Boc)·NCA (**4b**) (as mentioned earlier) and then to couple with *N*-component.

In addition, for the purpose of the stepwise elongation of the N- and both N- and C-terminal ΔTyr(Boc) moiety, compound **4a** was subjected to the *N*-acylation with *C*-component, followed by the coupling with *N*-component α-amino acid. In a similar manner as in the case of **2**, the acylation of **4a** with an appropriate Boc-AA-OH took place to yield an intermediate regarded as *N*-carboxy-*N*-Boc-acyl-(*O*-Boc)dehydrotyrosine anhydride [Boc-AA-ΔTyr(Boc)·NCA] (**12**), which was immediately reacted with methanol to give Δ^2-dehydrodipeptide [Boc-AA-ΔTyr(Boc)-OMe] (**13**) in 57% yield. Similarly, according to Scheme 3, **12** was

Table 4. The Spectral and Optical Data of **7** and **9**

Compound No.	IR, $\tilde{\nu}$/cm^{-1} in KBr			^{1}H NMR (δ, $CDCl_3$)		$[\alpha]_D^{27}$/°
	-NHCO-		-C=C-	Ar-CH=	α-H[a] (J/Hz)	(c 1, in MeOH)
7a	1605	1515	1630	7.17s	4.72dq (6.8, 6.8)	15.3
7b	1610	1520	1630	7.14s	4.74dd (5.1, 8.6)	−21.8
7c	1615	1525	1620	7.24s	4.71dd (6.0, 9.0)	−8.4
7d	1610		1635	6.72—7.68[b]	5.20m	−12.0
9a	1690	1510	1640	7.36s	4.41dq (7.0, 7.0)	3.6
9b	1670	1525	1645	7.24s	3.99dd (7.5, 9.0)	4.3
9c	1665	1525	1640	7.28s	4.05dd (6.2, 9.1)	90.9
9d	1670		1635	7.04—7.60[b]	4.68dt (7.0, 8.0)	0.2

a) Proton on α-carbon of L-α-amino acid residue. b) Overlapped on phenyl protons.

Table 5. The Yields and Melting Points of **10**

Compound No. Boc-AA-ΔAA-AA-OMe	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/%		
				C	H	N
10a -Gly-ΔTyr(MOM)-Ala-	88	123—124	$C_{22}H_{31}N_3O_8$	57.07 (56.76	6.79 6.71	8.95 9.01)
10b -Gly-ΔTyr(MOM)-Ser-	86	49—51	$C_{22}H_{31}N_3O_9$	54.82 (54.88	6.55 6.49	8.59 8.73)
10c -Val-ΔTyr(MOM)-Ile-	62	161—163	$C_{28}H_{43}N_3O_8$	61.25 (61.18	7.95 7.89	7.55) 7.65)
10d -Val-ΔTyr(MOM)-Pro-	67	64—65	$C_{27}H_{39}N_3O_8$	60.34 (60.77	7.33 7.37	7.80 7.88)

a) Colorless needles from ethyl acetate.

Table 6. The Spectral and Optical Data of **10**

Compound No.	IR, $\tilde{\nu}$/cm^{-1} in KBr			^{1}H NMR (δ, $CDCl_3$)			$[\alpha]_D^{22}$/°
	-NHCO-		-C=C-	Ar-CH=	α-H[a] (J/Hz)	α-H[a]	(c 1, in MeOH)
10a	1695	1515	1630	7.18s	3.86d (4.5)	4.52dq (6.3, 6.3)	−5.6
10b	1705	1520	1635	7.12s	3.90m	4.64m	1.7
10c	1660	1525	1640	7.20s	4.18m	4.65dd (4.2, 8.0)	17.0
10d	1680	1510	1640	7.40s	3.98m	4.48m	125.2

a) Proton on α-carbon of two L-α-amino acid residues.

Table 7. The Yields and Melting Points of **11** and **13**

Compound No. Boc-⊿AA-AA-OMe (**11**) Boc-AA-⊿AA-OMe (**13**)	Yield %	Mp[a] θ_m/°C	Formula	Found (Calcd)/% C	H	N
11a -⊿Tyr(Boc)-Gly-	50	Syrup	$C_{22}H_{30}N_2O_8$	58.35 (58.65	6.48 6.71	6.13 6.22)
11b -⊿Tyr(Boc)-Ala-	70	47—48	$C_{23}H_{32}N_2O_8$	59.21 (59.46	7.05 6.94	5.92 6.03)
11c -⊿Tyr(Boc)-Leu-	47	72—73	$C_{26}H_{38}N_2O_8$	61.35 (61.64	7.67 7.56	5.61 5.53)
11d -⊿Tyr(Boc)-Phe-	69	36—38	$C_{29}H_{36}N_2O_8$	64.61 (64.43	6.80 6.71	5.01 5.18)
13a -Gly-⊿Tyr(Boc)-	36	Syrup	$C_{22}H_{30}N_2O_8$	58.39 (58.65	6.39 6.71	6.02 6.22)
13b -Ala-⊿Tyr(Boc)-	70	Syrup	$C_{23}H_{32}N_2O_8$	59.82 (59.46	7.12 6.94	5.85 6.03)
13c -Leu-⊿Tyr(Boc)-	58	Syrup	$C_{26}H_{38}N_2O_8$	61.29 (61.64	7.31 7.56	5.18 5.53)
13d -Phe-⊿Tyr(Boc)-	63	64—65	$C_{29}H_{36}N_2O_8$	64.51 (64.43	6.78 6.71	5.10 5.18)

a) Colorless needles from a mixture of ethyl acetate and hexane.

Table 8. The Spectral and Optical Data of **11** and **13**

Compound No.	IR, $\tilde{\nu}$/cm^{-1} in KBr -NHCO-		-C=C-	^{1}H NMR (δ, $CDCl_3$) Ar-CH=	α-H[a] (J/Hz)	$[\alpha]_D^{23}$/° (c 1, in MeOH)
11a	1665	1510	1630	7.02s	4.10d (5.5)	—
11b	1670	1515	1635	7.05s	4.62dq (7.0, 7.0)	13.3
11c	1665	1510	1640	7.12s	4.68dt (7.0, 8.0)	−33.0
11d	1670	1510	1640	7.00—7.60[b]	4.90dt (5.0, 7.0)	−9.3
13a	1690	1510	1650	7.32s	3.86d (6.0)	—
13b	1690	1515	1650	7.34s	4.34dq (6.5, 7.0)	50.0
13c	1675	1515	1650	7.36s	4.30m	32.1
13d	1690	1510	1650	7.34s	4.56dt (7.0, 7.0)	11.4

a) Proton on α-carbon of L-α-amino acid residue. b) Overlapped on phenyl protons.

Table 9. The Yields and Melting Points of **14**

Compound No. Boc-AA-⊿AA-AA-OMe	Yield %	Mp θ_m/°C	Formula	Found (Calcd)/% C	H	N
14a -Gly-⊿Tyr(Boc)-Ser-	39	69—71	$C_{25}H_{35}N_3O_{10}$	55.92 (55.86	6.58 6.56	7.81 7.82)
14b -Gly-⊿Tyr(Boc)-Val-	30	113—115	$C_{27}H_{39}N_3O_9$	59.13 (59.00	7.25 7.15	7.60 7.65
14c -Val-⊿Tyr(Boc)-Ser-	18	Syrup	$C_{28}H_{41}N_3O_{10}$	58.22 (58.02	7.06 7.13	7.10 7.25)
14d -Val-⊿Tyr(Boc)-Val-	32	116—118	$C_{30}H_{45}N_3O_9$	60.76 (60.89	7.63 7.67	7.22 7.10)

coupled with H-AA-OMe in one-pot to give the expected Δ^2-dehydrotripeptide [Boc-AA-⊿Tyr(Boc)-AA-OMe] (**14**), although the yield was lower than 30%.

The yields, melting points, and spectral data (IR and ^{1}H NMR) of **7**, **9**, **10**, **11**, **13**, and **14**, are summarized in Tables 3—9, and 10.

In the IR spectra of all the new DHPs, thus obtained, the characteristic absorption bands of second-

Table 10. The Spectral and Optical Data of **14**

Compound No.	IR, $\tilde{\nu}$/cm^{-1} in KBr			^{1}H NMR (δ, $CDCl_3$)			$[\alpha]_D^{25}$/°
	-NHCO-		-C=C-	Ar-CH=	α-H[a] (J/Hz)	α-H[a]	(c 1, in MeOH)
14a	1675	1510	1630	7.05s	3.86d (9.8)	4.66m	−1.2
14b	1670	1515	1635	7.02s	3.75d (5.7)	4.43dd (6.0, 9.1)	−11.5
14c	1700	1510	1635	7.08s	3.88m	4.58m	15.4
14d	1690	1525	1640	7.03s	3.96dd (6.0, 8.2)	4.48dd (5.1, 8.5)	23.3

a) Proton on α-carbon of L-α-amino acid residues.

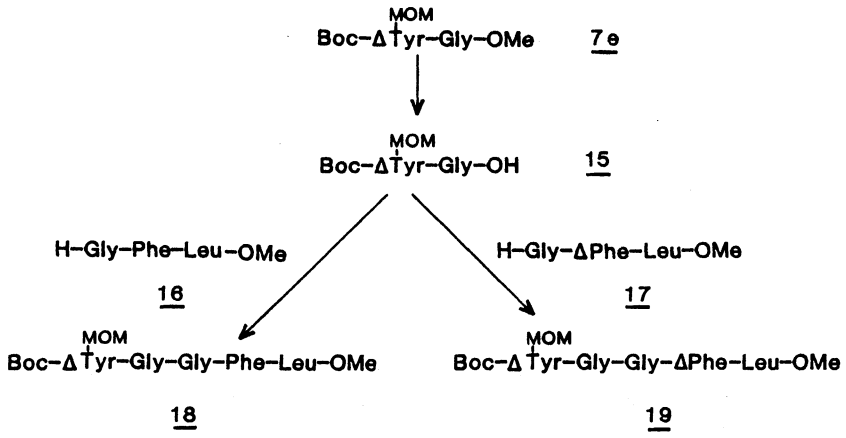
ary amide and carbon-carbon double bond functions appear at 1525—1510 and 1705—1605, and 1650—1620 cm^{-1} regions, respectively. Furthermore, in the ^{1}H NMR spectra, all of the olefin protons of the obtained DHPs shift at the δ 7.40—6.60 region as a singlet. Particularly, based on the chemical shifts of olefin protons, the configurations of the obtained Δ NCA and DHP could be tentatively confirmed to be (Z)-geometry, since the chemical shifts appeared at comparatively higher magnetic fields and the spectral patterns were quite similar to those of DHPs containing (Z)-α-dehydroamino acid (DHA) residues.[5]

From the above results and facts reported previously,[2,12] the selective protection of a hydroxyl group out of two functional groups (OH and NH) of ΔTyr·NCA was found to become feasible. Accordingly, in the case of obtaining various DHPs with a polyfunctional DHA residue as well as DHPs containing a ΔTyr residue protected by different protecting groups, the method developed here must be very effectively utilized. In addition, if α-amino acid and oligopeptide used as both the N- and C-components or segments are variously altered, even in the coupling of compound **2**, which is only one out of ΔTyr·NCAs, the further diversified types of DHP will be afforded. In fact, the various combinations of N- and C-components or moiety protected with the useful N- and O-protecting groups were already carried out to give the further wide variety of DHPs.[13]

Syntheses of Dehydroenkephalins. By utilizing the important N-terminal ΔTyr-Gly moiety, which was derived from Boc-ΔTyr(MOM)·NCA (**6**) and H-Gly-OMe by the ΔNCA method, the syntheses of two kinds of ΔEnk, e.g., Δ^1Enk and $\Delta^{1,4}$Enk, were tried, according to Scheme 4.

First of all, after an ester hydrolysis of the obtained Boc-ΔTyr (MOM)-Gly-OMe (**7e**) with 2 M LiOH, the resulting Boc-ΔTyr(MOM)-Gly-OH (**15**) was then coupled with H-Gly-L-Phe-L-Leu-OMe by the usual DCC method to give the expected Boc-ΔTyr (MOM)-Gly-Gly-Phe-Leu-OMe (**18**) in 45% yield. On the other hand, after preparation of Boc-Gly-(Z)-ΔPhe-L-Leu-OMe, derived by the one-pot reaction of N-carboxy dehydrophenylalanine anhydride (ΔPhe·NCA)[10] with in turn Boc-Gly-OH as an N-component and H-L-Leu-OMe as a C-component, and then conversion to H-Gly-(Z)-ΔPhe-L-Leu-OMe (**17**) by the N-deprotection with 4.5 M HCl, the fragment condensation of **15** with **17** was similarly worked up to give Boc-ΔTyr(MOM)-Gly-Gly-(Z)-ΔPhe-L-Leu-OMe (**19**) in 48% yield, according to Scheme 4.

In the ^{1}H NMR spectra of **18** and **19**, it was found that only the olefin protons of ΔTyr and ΔPhe residues could not be clearly assigned because of the overlap



Scheme 4.

with the signals of aromatic ring and NH protons in the δ 7.34—6.86 region. At least, however, the appearance of a carbon-carbon double bond at about 1630 cm^{-1} in the IR spectra and the satisfactory elemental analyses indicated the formation of **18** and **19**.

Subsequently, in order to deprotect the protecting groups of the N-terminal ⊿Tyr residue, treatment of **18** with 2 M HCl/acetic acid in THF was carried out at room temperature for 1 h. After concentrating the reaction solution under reduced pressure, the obtained crude crystalline residue was gradually changed into a syrup during purification by the usual procedure to give *p*-hydroxyphenylpyruvoylpeptide. Unfortunately, an attempt to isolate the protecting group-free ⊿[1]Enk was unsuccessful. An enthusiastic deprotection of **18** and **19** is now under way.

In conclusion, the one-pot reactions of ⊿Tyr·NCA protected with various combination of *N*- and *O*-protecting groups were performed very smoothly. More interestingly, due to the considerable stability and reactivity of ⊿NCA, it is firmly believed that not only stepwise elongation and fragment condensation but also ring-opening polymerization to poly(α-dehydroamino acid) would be possible and diversify further the field of DHP chemistry.

Experimental

General. Melting points were determined with a Yamato micro melting-point apparatus model (MP-21) and were uncorrected. The IR spectra were recorded with a Hitachi EPI-G2 grating spectrometer. The ^1H NMR spectra were measured with a JEOL JMN PS-100 spectrometer in a $CDCl_3$ solution with tetramethylsilane 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.).

⊿Tyr(Boc)·NCA (4a) and Boc-⊿Tyr(Boc)·NCA (4b). A solution of **3** (0.50 g, 2.43 mmol) and $(Boc)_2O$ (0.53 g, 2.43 mmol) in the presence of pyridine (0.2 ml) in THF (2 ml) was stirred at room temperature for 5 h. After removing the solvent under reduced pressure, the residual syrup was crystallized by adding a small amount of hexane. The crude crystals were collected and then recrystallized from benzene to give **4a** as pale-yellow needles. Yield 70%, mp 167—170 °C (decompd). IR ν_{max}^{KBr} (cm^{-1}) 3280 (NH), 1830, 1780, (CO-O-CO), 1665 (C=C); ^1H NMR ($CDCl_3$) δ=11.48 (bs, 1H, NH), 7.40 (dd, 4H, *J*=9.0, 46.0 Hz, C_6H_4), 6.64 (s, 1H, -CH=), 1.48 [s, 9H, $-C(CH_3)_3$]. Found: C, 59.29; H, 5.07; N, 4.64%. Calcd for $C_{15}H_{15}NO_6$: C, 59.01; H, 4.95; N, 4.59%.

In a similar manner, the treatment of **3** with two molar $(Boc)_2O$ was worked up to give **4b**, which was immediately utilized to the next reaction, without isolation.

Cbz-⊿Tyr(MOM)-AA-OMe (5). A solution of an appropriate H-AA-OH·HCl (2.0 mmol) in dry CH_2Cl_2 (10 ml) in the presence of triethylamine (TEA) (0.2 ml, 2.0 mmol) was stirred at −10 °C for 30 min. To the solution, thus prepared, was added **1** (0.6 g, 1.50 mmol) and WSC (0.3 g, 1.56 mmol), and then the resulting solution was further stirred at −10 °C for 1 h. After stirring continuously at room temperature for 8 h, the reaction solution was concentrated under reduced pressure. The residual substance was dissolved in ethyl acetate (30 ml) and the resultant solution was washed successively with water, 10% citric acid, water, saturated $NaHCO_3$ aqueous solution, and then water, and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crystalline residue, thus obtained, was recrystallized from a mixture of ethyl acetate and hexane to give **5** as colorless needles. See Tables 1 and 2.

Boc-⊿Tyr(MOM)-AA-OMe (7). A solution of **2** (1.25 g, 5.0 mmol) and $(Boc)_2O$ (1.31 g, 6.0 mmol) in dry THF (10 ml) in the presence of pyridine (50 μl) was stirred at room temperature for 8 h. With stirring, to the solution, thus prepared, was added a solution of an appropriate H-AA-OMe·HCl (7.0 mmol) and TEA (0.7 ml, 7.0 mmol) in dry THF (10 ml). To the resulting solution was added *N*-methylmorpholine (NMM) (0.76 ml, 7 mmol) and then stirred continuously at room temperature for 2 h. After removing the solvent under reduced pressure, the residual syrup was dissolved in ethyl acetate (30 ml); the resultant solution was washed successively with chilled 10% citric acid, water, saturated $NaHCO_3$ aqueous solution, and then water, and finally dried over anhydrous Na_2SO_4. The concentration of the solution under reduced pressure gave a residual crude syrup, which was purified on a silica-gel column using a mixture of benzene and acetone (15:1 v/v) as an eluent to give **7** as colorless syrup. See Tables 3 and 4.

Boc-AA-⊿Tyr(MOM)-OMe (9). A solution of an appropriate Boc-AA-OH (6.0 mmol) and DCC (1.24 g, 5.0 mmol) in dry THF (10 ml) was stirred at −10 °C for 20 min; to the solution prepared was added **2** (1.25 g, 5.0 mmol) and pyridine (0.4 g, 5.0 mmol). After stirring at −10 °C for 2 h, the resulting solution was returned slowly to room temperature and then stirred continuously for 12 h. Finally, after treating further with MeOH (15 ml) and stirring for 2 h, to the reaction solution was added NMM (1.1 ml, 10 mmol). After removing THF and MeOH under reduced pressure, the residue was dissolved in ethyl acetate (10 ml); the *N,N'*-dicyclohexylurea separated out was then filtered off. The filtrate was diluted in ethyl acetate (20 ml) and the resultant solution was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, water and then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude residue was purified on a silica-gel column using a mixture of benzene and acetone (15:1 v/v) as an eluent. The main fraction was concentrated under reduced pressure to give a viscous syrup, which was crystallized with petroleum ether. Recrystallization from a mixture of ethyl acetate and hexane gave **9** as colorless needles. See Tables 3 and 4.

Boc-AA-⊿Tyr(MOM)-AA-OMe (10). To a solution of an appropriate Boc-AA-OH (6.0 mmol) in dry THF (10 ml) was added DCC (1.24 g, 6.0 mmol) at −10 °C and, after stirring for 20 min, further added **2** (1.25 g, 5.0 mmol) and pyridine (0.4 g, 5.0 mmol). After stirring continuously for 2 h, the resultant solution was returned slowly to room temperature and then stirred for 12 h. To the reaction solution was added a solution of another appropriate H-AA-OMe·HCl (7.0 mmol) and TEA (0.7 ml, 7.0 mmol) in THF (10 ml); the resulting solution was then treated with NMM (0.76 ml, 7 mmol). After stirring for 2 h, and then removing the solvent under reduced pressure, the residue was dissolved in chilled ethyl acetate (10 ml) and the *N,N'*-dicyclohexylurea separated out was filtered off. The filtrate was similarly worked up to give a crude syrup, which was purified on a

silica-gel column using a mixture of $CHCl_3$ and acetone (15 : 1 v/v) to give a viscous syrup. After being crystallized with petroleum ether, the formed crystals were collected and then recrystallized from a mixture of ethyl acetate and hexane to give **10** as colorless needles. See Tables 5 and 6.

Boc-⊿Tyr(Boc)-AA-OMe (11). A solution of **3** (1.0 g, 5.0 mmol) and $(Boc)_2O$ (3.27 g, 15.0 mmol) in THF (10 ml) in the presence of pyridine (5.0 ml) was stirred at room temperature for 8 h. To the resulting solution was added a solution of an appropriate H-AA-OMe·HCl (10.0 mmol) and TEA (1.0 g, 10.0 mmol) in THF (10 ml). After stirring at room temperature for 4 h, as in the case of the synthesis of **7**, the reaction solution was similarly worked up to give **11** as a colorless syrup or crystals. See Tables 7 and 8.

Boc-AA-⊿Tyr(Boc)-OMe (13). A solution of **3** (1.0 g, 5.0 mmol) and $(Boc)_2O$ (1.1 g, 5.0 mmol) in THF (10 ml) in the presence of pyridine (5.0 ml) was stirred until the spot of **3** disappeared on thin-layer chromatography. To the reaction solution, which was chilled to −10 °C, was added successively Boc-AA-OH (5.0 mmol), DCC (5.0 mmol) and pyridine (0.4 g, 5.0 mmol) and then stirred continuously for 1 h. The resulting solution of the intermediate (**12**) was returned to room temperature and stirred continuously for 12 h. Finally, as in the case of the synthesis of **9**, the reaction solution was treated with MeOH (10 ml) and then similarly worked up to give **13** as a colorless syrup or crystals. See Tables 7 and 8.

Boc-AA-⊿Tyr(Boc)-AA-OMe (14). In a similar manner, a solution of the intermediate (**12**) in THF (10 ml) was prepared. Finally, as in the case of the synthesis of **10**, the resulting solution was further treated with H-AA-OMe; then the reaction solution was similarly worked up to give **14** as a colorless syrup or crystals. See Tables 9 and 10.

Boc-⊿Tyr(MOM)-Gly-OH (15). To a solution of **7e** (5.0 g, 12.7 mmol) in THF (20 ml) was added 1 M LiOH (17.8 ml) drop by drop, with stirring, under cooling. After stirring for 1 h, the resulting solution was returned to room temperature and stirred continuously for 2 h. Chilled water (40 ml) was added to the reaction solution; then the aqueous layer was washed twice with diethyl ether (20 ml). The aqueous layer was further chilled and neutralized with chilled 0.5 M HCl and then made acidic to pH 3 with 10% citric acid. The acidic solution was extracted three times with ethyl acetate (30 ml). The combined extracts were washed with a saturated NaCl aqueous solution and then dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave **15** as a colorless syrup. Yield 63%. IR ν_{max}^{KBr} (cm^{-1}) 1662, 1515 (CONH), 1630 (C=C). ^1H NMR (MeOH-d_4) δ=7.42 and 6.90 (d, 4H, J=9.5 Hz, C_6H_4-), 7.08 (s, 1H, -CH=), 5.09 (s, 2H, -O-CH_2-O-), 3.98 (s, 2H, -NH-CH_2-CO-), 3.38 (s, 3H, -CH_2OCH_3), 1.42 [s, 9H, -$C(CH_3)_3$]. Found: C, 56.64; H, 6.45; N, 7.19%. Calcd for $C_{18}H_{24}N_2O_7$: C, 56.83; H, 6.36; N, 7.37%.

HCl·H-Gly-⊿Phe-Leu-OMe (17). A solution of Boc-Gly-⊿Phe-Leu-OMe (0.3 g, 0.76 mmol) in 4.5 M HCl and ethyl acetate (20 ml) was stirred under cooling for 10 min. After removing the solvent under reduced pressure, the crude residue was dissolved in diethyl ether (20 ml) and the resulting solution was again concentrated. After adding a small amount of dry methanol and diethyl ether, the deposited crystals were collected. Yield 97%, mp 93—97 °C (decomp) IR ν_{max}^{KBr} (cm^{-1}) 3448 (NH_2), 1665, 1517 (CONH), 1635 (C=C). ^1H NMR (MeOH-d_4) δ=7.56—7.24 (m, 5H, C_6H_5), 7.09 (s, 1H, -CH=), 3.88 (s, 2H, -NH-CH_2-CO-), 3.72 (s, 3H, -CH_2OCH_3), 1.74 [m, 1H, -$CH(CH_3)_2$], 1.24 [m, 2H, -CH_2CH-$(CH_3)_2$], 0.98 [dd, 6H, J=7.3, 2.8 Hz, -$CH(CH_3)_2$]. $[\alpha]_D^{21}$ +39.3° (c 1.00, in MeOH). Found: C, 56.22; H, 6.90; N, 10.59%. Calcd for $C_{18}H_{26}N_3O_4Cl$: C, 56.32; H, 6.83; N, 10.95%.

Boc-⊿Tyr(MOM)-Gly-Gly-Phe-Leu-OMe (18). To a chilled solution of **15** (1.5 g, 4.0 mmol) in THF (20 ml) was added DCC (0.9 g, 4.4 mmol) and *N*-hydroxysuccinimide (0.5 g, 4.4 mmol). After stirring continuously for 30 min, a solution of **16** (1.7 g, 4.4 mmol) and TEA (0.5 ml, 4.9 mmol) in THF (10 ml) was added to the prepared solution; the combined solution was then further stirred under cooling for 2 h. The reaction solution was returned slowly to the room temperature for 24 h. The deposited *N,N'*-dicyclohexylurea were filtered off and the filtrate was concentrated under reduced pressure. The crude residue, thus obtained, was dissolved in ethyl acetate (70 ml) and the resulting solution was washed successively with 10% citric acid, saturated $NaHCO_3$ aqueous solution, and then saturated NaCl aqueous solution, and finally dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, crude crystalline substance, thus obtained, was recrystallized from a mixture of ethyl acetate and hexane to give **18** as a pale yellow amorphous. Yield 65%, mp 65.5—67.5 °C. IR ν_{max}^{KBr} (cm^{-1}) 1662, 1630 (NHCO), 1630 (C=C). ^1H NMR (DMSO-d_6) δ=7.75 (bs, 1H, NH), 7.32—6.86 (m, 13H, -CH=, C_6H_5, C_6H_4, NH), 6.56 (bs, 1H, NH), 5.13 (s, 2H, -OCH_2O-), 3.94 (d, 2H, -NH-CH_2-CO-), 3.60 (s, 3H, -$COOCH_3$), 3.45 (s, 3H, -OCH_3), 1.42 [s, 9H, -$C(CH_3)_3$)], 0.88 [dd, 6H, J=7.3, 2.8 Hz, -$CH(CH_3)_2$]. $[\alpha]_D^{24}$ −5.11° (c 1.25, in MeOH). Found: C, 60.52; H, 6.93; N, 9.83%. Calcd for $C_{36}H_{49}N_5O_{10}$: C, 60.74; H, 6.94; N, 9.84%.

Boc-⊿Tyr(MOM)-Gly-Gly-⊿Phe-Leu-OMe (19). In a similar manner, the coupling of **15** and **17** was worked up to give colorless syrup, which was purified on a silica-gel column using a mixture of chloroform and acetone (20 : 1 v/v) as the eluent to give **19** as a colorless syrup. Yield 50%. IR ν_{max}^{KBr} (cm^{-1}) 1665, 1525 (NHCO), 1632 (C=C). ^1H NMR (DMSO-d_6) δ=8.72 (bs, 1H, NH), 8.09 (bs, 1H, NH), 7.86 (bs, 1H, NH), 7.34—6.86 (m, 12H, -CH=, C_6H_4, C_6H_5, NH), 4.13 (d, 2H, J=8.0 Hz, -NH-CH_2-CO-). 4.02 (d, 2H, J=8.0 Hz, -NH-CH_2-CO-), 5.08 (s, 2H, -OCH_2O-), 3.57 (s, 3H, -OCH_3), 3.40 (s, 3H, CH_3O-), 1.28 [s, 9H, -$C(CH_3)_3$], 0.86 [dd, 6H, J=6.3, 2.8 Hz, -$CH(CH_3)_2$]. $[\alpha]_D^{24}$ −10.5° (c 0.9, in MeOH). Found: C, 60.88; H, 6.55; N, 9.86%. Calcd for $C_{36}H_{47}N_5O_{10}$: C, 60.91; H, 6.67; N, 9.87%.

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