October, 1980] *Bull. Chem. Soc. Jpn.*, *53*, *2905—2909 (1980)* 2905

Dehydrooligopeptides. I. The Facile Coupling of α-Amino Acids with α-Dehydroamino Acids to Dehydrodipeptides

Yasuchika YONEZAWA, Chung-gi SHIN,* Yuji ONO, and Juji YOSHIMURA[†]
Laboratory of Organic Chemistry, Faculty of Technology, Kanagawa University, Rokkakubashi, Kanagawa-ku, Yokohama 221
[†]*Laboratory of Chemistry for Natural Products, Faculty of Science, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227*

(Received December 14, 1979)

N-Cbz- or *N*-Tos-α-dehydroamino acid (DHA) and its ester were prepared by the condensation of 2-oxo carboxylic acid or ester with benzyl carbamate or *p*-toluenesulfonamide. Subsequently, the N-protected DHA and its N-free ester thus obtained were coupled with several L-α-amino acid esters and N-protected (Boc or Cbz)-L-α-amino acid respectively by the usual peptide synthetic methods to give a number of different types of dehydrodipeptide. The configurations of all the new DHA and dehydrodipeptides obtained were shown to have (*Z*)-geometry.

Recently, much interest has been directed to the synthesis and bioactivity of dehydropeptide (DHP), containing α-dehydroamino acid (DHA) residues, which are very important constituents in an increasing number of antibiotic and phytotoxic peptides.[1—3] The previous methods of synthesizing the simple DHP has been almost exclusively indirect, *i.e.*, methods using the reaction of unsaturated azlactone with α-amino acid[4,5] and the *β*-elimination of peptide, which has a leaving group.[6—8] Concerning the direct condensation, no report has been published on the coupling of DHA with α-amino acid, except for two examples[9,10] involving earlier work by one of the present authors (C. S.).

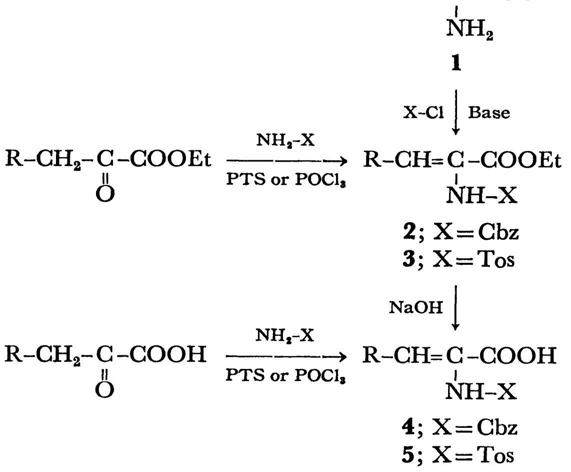
In a previous paper, we briefly reported the direct coupling of (*Z*)-2-(benzyloxycarbonylamino)-2-alkenoic acid (**4**) as a carbonyl component with the L-leucine ethyl ester by the usual synthetic method.[11]

In the present paper, we wish to report in detail on the general preparation of the DHA N-protected with the benzyloxycarbonyl (Cbz) group (**2** and **4**) or the *p*-tolylsulfonyl (Tos) group (**3** and **5**), and on the facile synthesis of the two kinds of N-protected DHP from the N-protected DHA (ΔAA)[12] and several L-α-amino acid (AA) esters, and from N-protected AA and ethyl (*Z*)-2-amino-2-alkenoate (**1**)[13,14] by the mixed anhydride, DCC, or azide method.

Results and Discussion

N-Protected (Z)-α-Dehydroamino Acids. In order to prepare the DHA with a useful protecting group as a carboxyl component, according to the method reported previously by us,[15] the condensation reaction of α-oxoalkanoic acids with amides under various acidic conditions have been extensively reinvestigated. As a result, the optimum experimental conditions for the preparation of 2-Cbz- or 2-Tos-amino-2-alkenoic acids (**4** and **5**) and ethyl 2-Cbz- or 2-Tos-amino-2-alkenoates (**2** and **3**) were established as follows. The condensation reaction of equimolar 2-oxoalkanoic acid with benzyl carbamate or *p*-toluenesulfonamide in benzene in the presence of *p*-toluenesulfonic acid (PTS) as the catalyst under reflux for *ca.* 4 h gives **4** and **5** in yields *ca.* 75 and *ca.* 51% respectively. On the other hand, in the case of the reaction of ethyl 2-oxoalkanoate with benzyl carbamate or *p*-toluenesulfonamide, it was found that a similar condensation readily occurred in the presence of phosphoryl chloride rather than PTS to give **2** and **3** in *ca.* 69 and 72% yields respectively. However, the attempt to effect a similar direct condensation of 2-oxoalkanoic acid or its ester with *t*-butyl carbamate failed because of the unstability of the *t*-butoxycarbonyl (Boc) group under acidic conditions.

The α-dehydroamino acid structure of **2**—**5** was supported by the characteristic signals of olefinic (*δ* 5.63—6.94), vinyl (*δ* 5.68—6.22), and *γ*-protons (*δ* 1.68—3.19) in the NMR spectra and the characteristic absorption bands of NH (3220—3310 cm^{-1}), ester (1720—1740 cm^{-1}), carbonyl (1705—1710 cm^{-1}), and carbon-carbon double bond (1640—1665 cm^{-1}) functions in the IR spectra. Although the configuration of **2**—**5** could not be determined from the above spectroscopic data, the geometric structures of the esters were unambiguously determined to have (*Z*)-geometry by comparison with **2** and **3** prepared independently by the acylation of (*Z*)-**1**[14] with benzyloxycarbonyl chloride (Cbz-Cl) or tosyl chloride (Tos-Cl) respectively in the usual way. Moreover, the configurational structures of the free acid prepared by the hydrolysis



a; R=H, **b**; R=CH_3, **c**; R=C_2H_5, **d**; R=*n*-C_3H_7, **e**; R=*i*-C_3H_7, f; R=C_6H_5

Scheme 1.

TABLE 1. ETHYL (*Z*)-2-(CBZ-AMINO)- AND (*Z*)-2-(TOS-AMINO)-2-ALKENOATES (**2** and **3**)

Compd No.	Yield(%)		Mp/°C	Formula	Found (Calcd), %			NMR spectrum, δ in $CDCl_3$		
	A[a]	B[b]			C	H	N	Olefinic proton (J_{Hz})	γ-Proton (J_{Hz})	NH
2a	61		34—35[c]	$C_{13}H_{15}NO_4$	62.51 (62.64	6.16 6.07	5.48 5.62)	6.22s, 5.80d		
2b	69	96	syrup	$C_{14}H_{17}NO_4$	63.76 (63.86	6.55 6.51	5.12 5.23)	6.67q (7.2),	1.78d (7.2),	6.32s
2c	69	85	syrup	$C_{15}H_{19}NO_4$	65.02 (64.96	6.77 6.91	4.98 5.05)	6.58t (7.5),	2.10p (7.5),	6.64s
2d	72	88	syrup	$C_{16}H_{21}NO_4$	66.01 (65.95	7.23 7.27	4.99 4.81)	6.64t (7.8),	2.26q (7.5),	6.28s
2e	70	81	syrup	$C_{16}H_{21}NO_4$	66.07 (65.95	7.34 7.27	4.69 4.81)	6.45d (9.8),	2.70m,[e]	6.28s
2f	71	22	60—62[c]	$C_{19}H_{19}NO_4$	70.22 (70.14	5.65 5.89	4.27 4.31)	7.14—7.60m,		6.44s
3a	51		76—77[d]	$C_{12}H_{15}NO_4S$	54.00 (53.53	5.51 5.62	5.31 5.20)	5.69s,		7.40bs
3b	76	66	93—95[d]	$C_{13}H_{17}NO_4S$	55.31 (55.12	6.12 6.05	4.90 4.95)	6.94q (7.5),	2.09d (7.5),	6.21s
3c	77	63	45—46[d]	$C_{14}H_{19}NO_4S$	56.50 (56.56	6.41 6.44	4.68 4.71)	6.82t (7.5),	2.55p (7.8),	6.11s
3d	75	62	59—60[d]	$C_{15}H_{21}NO_4S$	57.80 (57.86	6.88 6.80	4.42 4.50)	6.82t (8.0),	2.49q (7.8),	6.22s
3e	71	64	79—80[d]	$C_{15}H_{21}NO_4S$	57.81 (57.86	6.71 6.80	4.45 4.50)	6.58d (11.0),	3.19m,	6.14s
3f	80	80	89—91[d]	$C_{18}H_{19}NO_4S$	62.71 (62.60	5.08 5.55	4.12 4.06)	7.12—7.90m,[e]		6.37s

a) Yield from ethyl 2-oxoalkanoate and benzyl carbamate or *p*-toluenesulfonamide. b) Yield from ethyl 2-amino-2-alkenoate and Cbz-Cl or Tos-Cl. c) Colorless needles from hexane–ethyl acetate (5: 2 v/v). d) Colorless needles from diisopropyl ether. e) Overlapped with phenyl protons.

TABLE 2. (*Z*)-2-(CBZ-AMINO)- AND (*Z*)-2-(TOS-AMINO)-2-ALKENOIC ACIDS (**4** and **5**)

Compd No.	Yield(%)		Mp/°C	Formula	Found (Calcd), %			NMR spectrum, δ in $CDCl_3$ (**4**) or DMSO-d_6 (**5**)		
	A[a]	B[b]			C	H	N	Olefinic proton (J_{Hz})	γ-Proton (J_{Hz})	NH
4a	42		100—101[c]	$C_{11}H_{11}NO_4$	59.65 (59.72	5.19 5.01	6.25 6.33)	5.92s, 5.68s,		8.62bs
4b	73	84	152—153[d]	$C_{12}H_{13}NO_4$	61.29 (61.27	5.63 5.57	5.68 5.96)	6.50q (7.0),	1.68d (7.0),	8.54s
4c	80	92	100—101[d]	$C_{13}H_{17}NO_4$	62.44 (62.64	5.60 6.07	6.02 5.62)	6.40t (7.0),	2.19p (7.0),	8.55s
4d	82	91	126—127[d]	$C_{14}H_{17}NO_4$	63.81 (63.86	6.67 6.51	5.08 5.32)	6.41t (7.0),	2.10q (7.0),	8.54s
4e	79	93	105—106[d]	$C_{14}H_{17}NO_4$	63.62 (63.86	6.46 6.51	5.30 5.32)	6.27d (10.0),	2.62m,	8.50s
4f	92	92	170—171[d]	$C_{17}H_{15}NO_4$	68.60 (68.67	5.21 5.08	4.68 4.71)	7.35—7.70m,[h]		8.94s
5b	41	18	138—140[e]	$C_{11}H_{13}NO_4S$	51.71 (51.76	5.22 5.13	5.44 5.49)	6.93q (7.2),	1.68d (7.2),	9.11s
5c	45	17	128—129[f]	$C_{12}H_{15}NO_4S$	53.48 (53.53	5.70 5.62	5.26 5.20)	6.77t (7.8),	2.14p (7.8),	9.13s
5d	50	16	149—150[f]	$C_{13}H_{17}NO_4S$	55.25 (55.12	6.10 6.05	4.90 4.95)	6.63t (7.8),	2.05q (7.8),	8.90s
5e	47	21	140—141[f]	$C_{13}H_{17}NO_4S$	55.29 (55.12	6.13 6.05	4.88 4.95)	6.53d (10.8),	2.64m,	7.42s
5f	71	80	208—209[g]	$C_{16}H_{15}NO_4S$	60.41 (60.56	4.75 4.77	4.48 4.41)	7.02—7.78m,[h]		9.23s

a) Yield from 2-oxoalkanoic acid and benzyl carbamate or *p*-toluenesulfonamide. b) Yield from the hydrolysis of **2** or **3**. c) Colorless needles from chlorobenzene. d) Colorless needles from cyclohexane–benzene. e) Colorless prisms from chloroform. f) Colorless prisms from benzene. g) Colorless prisms from 50% ethanol. h) Overlapped with phenyl protons.

of (*Z*)-**2** and **3** were identical with those of **4** and **5** respectively.

The yields, physical constants, and NMR spectral data of **2**—**4** and **5** are listed in Tables 1 and 2.

Coupling of (Z)-DHA with L-*α-Amino Acid.* As has been reported in the preceding communication,[11] Compound **4** as a carboxyl component was subjected to a reaction with the appropriate L-α-amino acid (Gly, Ala, Ser, Leu, Met, Cys(benzyl), Phe, Trp, and Tyr) esters (methyl and ethyl). As expected, the equimolar coupling of N-protected DHA with the amino acid ester by either the mixed anhydride (MA) method (in the presence of triethylamine and ethyl chloroformate in THF–chloroform at 0 °C for 1 h), the DCC method (in the presence of dicyclohexylcarbodiimide and triethylamine in chloroform at 0 °C for 3 h), or the usual azide method occurred to give Cbz-(*Z*)-dehydroaminoacylamino acid esters (**6**) as colorless syrup or crystals in *ca.* 70% yields.

On the other hand, N-protected L-α-amino acid (Leu, Cys(benzyl), and Phe) was first coupled with (*Z*)-**1** as an amine component by the similar DCC method to

TABLE 3. Cbz–(*Z*)-ΔAA–L-AA–OR (**6**)

ΔAA–L-AA–OR	Yield (%)	Mp/°C	Formula	Found (Calcd), %			NMR spectrum, δ in $CDCl_3$			$[\alpha]_D^{24}$ [f]
				C	H	N	Olefinic proton (J_{Hz})	α-Proton (J_{Hz})	NH	
ΔAla–Ser–OMe	43	syrup	$C_{15}H_{18}N_2O_6$	55.97 (55.89	5.62 5.63	8.88 8.69)	6.08d (2.0), 5.34d	4.57dd (9.0, 2.6),	7.62	−15.0
ΔBut–Gly–OEt	82	106—107[b]	$C_{16}H_{20}N_2O_5$	60.11 (59.99	6.21 6.29	8.71 8.75)	6.69q (7.0),	4.07d (5.2),	6.99s 7.11d	
ΔBut–Ala–OEt	65	syrup	$C_{17}H_{22}N_2O_5$	60.97 (61.06	6.51 6.63	8.19 8.38)	6.55q (7.0),	4.62m,	6.78s 6.91d	
ΔBut–Phe–OEt	61	95—96[c]	$C_{23}H_{26}N_2O_5$	67.25 (67.30	6.46 6.39	6.88 6.83)	6.54q (7.0),	4.95m,	6.65s 6.81d	−0.2
ΔBut–Tyr–OMe	71	syrup	$C_{22}H_{24}N_2O_6$	63.99 (64.06	5.68 5.83	7.01 6.79)	6.46q (7.2),	4.81m,	6.63s 6.78d	
ΔBut–Trp–OMe	72	147—148[e]	$C_{24}H_{25}N_3O_5$	65.91 (66.19	5.90 5.79	10.11 9.65)	6.40q (7.2),	4.86m,	6.63s 6.78d	+6.3
ΔLeu–Leu–OEt	67	74—75[c]	$C_{22}H_{32}N_2O_5$	65.38 (65.32	8.22 7.97	7.14 6.93)	6.25d (10.2),	4.58dt (6.0, 8.0),	6.67s 6.72d	−29.1
ΔLeu–Cys–OEt Bzl	83	syrup	$C_{26}H_{32}N_2O_5S$	64.59 (64.45	6.99 6.66	5.81 5.78)	6.32d (10.0),	4.81m,	6.55s 7.19d	−41.3
ΔPhe–Gly–OEt	85	118—119[b]	$C_{21}H_{22}N_2O_5$	66.00 (65.95	5.84 5.80	7.27 7.33)	7.31s,	4.09d (5.2),	6.95s 7.21t	
ΔPhe–Ala–OEt	85	116—117[b]	$C_{22}H_{24}N_2O_5$	66.57 (66.65	6.15 6.10	7.01 7.01)	7.27s,	4.68m,	6.87s 7.17d	+21.8
ΔPhe–Met–OMe	61	81—82[d]	$C_{23}H_{26}N_2O_5S$	62.51 (62.43	5.81 5.92	6.25 6.33)	7.35s,	4.89m,	6.95s	−30.9

a) Yield by the DCC method. b) Colorless needles from CCl_4. c) Colorless prisms from diisopropyl ether. d) Colorless prisms from diethyl ether. e) Colorless needles from ethyl acetate. f) Measured in ethanol (*c* 1.0).

TABLE 4. Boc– AND Cbz–L-AA–(*Z*)-ΔAA–OR (**7**)

L-AA–ΔAA–OR	Yield[h] (%)	Mp/°C	Formula	Found (Calcd), %			NMR spectrum, δ in $CDCl_3$			$[\alpha]_D^{27}$ [g]
				C	H	N	Olefinic proton (J_{Hz})	α-Proton (J_{Hz})	NH	
Phe–ΔBut–OEt[a]	67	61—62[c]	$C_{20}H_{28}N_2O_5$	62.99 (63.81	7.44 7.50	7.31 7.44)	6.86q (7.6),	4.60dt (7.5, 7.0),	5.36d 7.81s	−18.9
Phe–ΔVal–OEt	72	150—151[d]	$C_{21}H_{30}N_2O_5$	64.27 (64.59	8.00 7.74	7.45 7.17)	—	4.50dt (7.5, 7.0),	5.24d 7.58s	−20.2
Phe–ΔnorVal–OEt[a]	46	96—97[e]	$C_{21}H_{30}N_2O_5$	64.70 (64.59	7.80 7.74	7.02 7.17)	6.76t (7.5),	4.58dt (7.5, 7.0),	5.30d 7.72s	−19.4
Phe–ΔnorLeu–OEt[a]	55	82—83[e]	$C_{22}H_{32}N_2O_5$	65.12 (65.32	7.72 7.97	6.73 6.93)	6.83t (7.5),	4.58dt (7.5, 7.0),	5.33d 7.76s	−19.5
Phe–ΔLeu–OEt[a]	57	94—95[e]	$C_{22}H_{32}N_2O_5$	65.50 (65.32	7.81 7.97	6.71 6.93)	6.58d (10.0),	4.56dt (7.5, 7.0),	5.33d 7.61s	−20.6
Cys–ΔnorLeu–OEt[a] Bzl	56	79—80[f]	$C_{23}H_{34}N_2O_5S$	61.40 (61.31	7.51 7.61	6.10 6.22)	6.86t (7.0),	4.46dt (7.5, 7.0),	5.54d 7.90s	−28.1
Leu–ΔLeu–OEt[b]	39	98—99[e]	$C_{22}H_{32}N_2O_5$	65.20 (65.32	8.09 7.97	7.21 6.93)	6.48d (10.0),	4.36m,	5.80d 7.86s	−7.4

a) Protected with the Boc group. b) Protected with the Cbz group. c) Colorless needles from hexane. d) Colorless needles from carbon tetrachloride. e) Colorless needles from cyclohexane. f) Colorless prisms from diisopropyl ether. g) Measured in methanol (*c* 1.0). h) Yield by the DCC method.

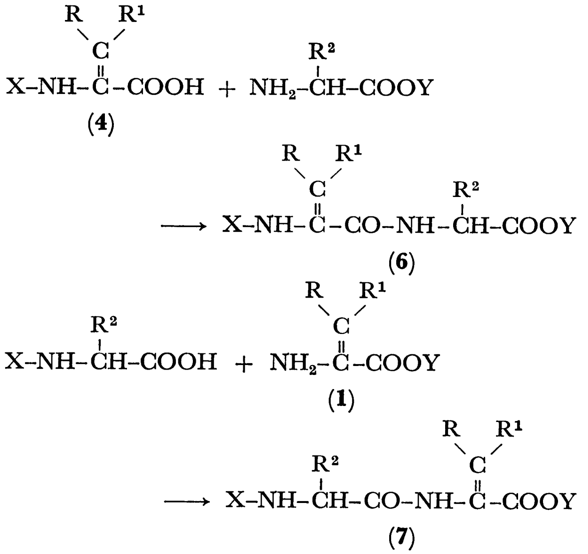
give N-protected L-leucyl-, benzylcysteinyl-, or phenylalanyl-(*Z*)-dehydroamino acid esters (**7**) as colorless crystals in *ca.* 56% yields. However, the coupling of the α-dehydrophenylalanine ester with N-protected amino acid scarcely proceeded at all because of the weak basicity of the amino group in the amine component.

In the IR spectral data, the characteristic differences between **6** and **7** could not be recognized. The presence of secondary amide absorption bands in the 1620—1670 and 1520—1560 cm^{-1} regions indicated the formation of the peptide bond. Moreover, the absorption bands of NH (3200—3400 cm^{-1}), ester carbonyl (1735—1750 cm^{-1}), and carbon-carbon double bond (1640—1685 cm^{-1}) functions are constituent with the assignment of the dehydrodipeptide structure.

The chemical shifts and the coupling constants of **6** and **7** were assigned as is shown in Tables 3 and 4. In the NMR spectrum of **6**, the signals in the δ 4.07—4.95 region as multiplets and in the δ 6.72—7.21 region as doublets are attributable to methine and NH protons of the α-AA residue, while the signals at δ 5.34 and in the 6.08—7.35 and 6.55—6.99 regions are attributable as singlets to vinyl, olefinic, and NH protons of the ΔAA residue respectively. On the other hand, in the case of **7**, the corresponding signals of the α-AA residue appeared in the δ 4.36—4.60 as double triplets and in the δ 5.24—5.80 region as doublets, while those of the ΔAA residue appeared in the δ 6.48—6.86 and 7.58—7.90 regions as singlets.

The configurations of **6** and **7** were readily determined to have (*Z*)-geometry, since the chemical shifts and the spectral patterns of the DHA residue in **6** and **7** were quite similar to the those of the corresponding (*Z*)-configurational **2**—**5**. Accordingly, it was found that the (*Z*)-configuration of DHA residue has been maintained during the peptide formation reaction.

The yields, physical constants, and NMR spectral data of **6** and **7** are listed in Tables 3 and 4.



$$\mathrm{X\text{-}NH\text{-}}\overset{\overset{\mathrm{R}\;\;\mathrm{R^1}}{\mathrm{C}}}{\overset{\|}{\mathrm{C}}}\mathrm{\text{-}COOH} + \mathrm{NH_2\text{-}}\overset{\mathrm{R^2}}{\overset{|}{\mathrm{C}}}\mathrm{H\text{-}COOY}\quad(\mathbf{4})$$

$$\longrightarrow \mathrm{X\text{-}NH\text{-}}\overset{\overset{\mathrm{R}\;\;\mathrm{R^1}}{\mathrm{C}}}{\overset{\|}{\mathrm{C}}}\mathrm{\text{-}CO\text{-}NH\text{-}}\overset{\mathrm{R^2}}{\overset{|}{\mathrm{C}}}\mathrm{H\text{-}COOY}\quad(\mathbf{6})$$

$$\mathrm{X\text{-}NH\text{-}}\overset{\mathrm{R^2}}{\overset{|}{\mathrm{C}}}\mathrm{H\text{-}COOH} + \mathrm{NH_2\text{-}}\overset{\overset{\mathrm{R}\;\;\mathrm{R^1}}{\mathrm{C}}}{\overset{\|}{\mathrm{C}}}\mathrm{\text{-}COOY}\quad(\mathbf{1})$$

$$\longrightarrow \mathrm{X\text{-}NH\text{-}}\overset{\mathrm{R^2}}{\overset{|}{\mathrm{C}}}\mathrm{H\text{-}CO\text{-}NH\text{-}}\overset{\overset{\mathrm{R}\;\;\mathrm{R^1}}{\mathrm{C}}}{\overset{\|}{\mathrm{C}}}\mathrm{\text{-}COOY}\quad(\mathbf{7})$$

α-Amino acid residues = Gly, Ala, Ser, Leu, Met, Cys-(benzyl), Phe, Tyr, and Trp. α-Dehydroamino acid residues = ΔAla, ΔBut, ΔVal, ΔnorVal, ΔnorLeu, ΔLeu, and ΔPhe. X = Cbz and Boc groups. Y = Me and Et.

Scheme 2.

Experimental

All the melting points are uncorrected. The IR spectra were recored 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. The specific rotations were measured in a 0.5 dm using a JASCO DIP-4 Polarimeter (Japan Spectroscopic Co., Ltd.).

Preparation of **2**. *Procedure A*: A solution of ethyl 2-oxoalkanoate (50 mmol), benzyl carbamate (60 mmol), and $POCl_3$ (3 ml) in dry benzene (70 ml) was refluxed for 4 h. To the reaction solution we then added benzene at room temperature, after which the resultant solution was washed with saturated aqueous $NaHCO_3$ and three times with water. The benzene layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a syrupy residue, which was purified on a silica gel column, using benzene as the eluent, to give **2** as a colorless viscous oil or colorless crystals.

Procedure B: Into a solution of (*Z*)-**1** (20 mmol) and pyridine (30 mmol) in dry diethyl ether (14 ml), we stirred Cbz-Cl (20 mmol), drop by drop, under cooling. After stirring at room temperature for 6 h, more diethyl ether (40 ml) was added to the reaction solution. The pyridinium salt thus deposited was filtered off, and the filtrate was successively washed with chilled 3 M HCl, saturated aqueous $NaHCO_3$, and water, and finally dried over anhydrous Na_2SO_4. The syrupy residue thus obtained was similarly worked up to give **2**.

Preparation of **3**. *Procedure A*: In a manner analogous Procedure A described for **2**, the solution of ethyl 2-oxoalkanoate (50 ml), *p*-toluenesulfonamide (60 mmol), and $POCl_3$ (3 ml) in dry benzene (60 ml) was worked up for 9 h to give **3** as colorless crystals.

Procedure B: Into a solution of (*Z*)-**1** (20 mmol) in dry pyridine (14 ml) we stirred Tos-Cl (20 mmol) under cooling. After stirring at room temperature for 12 h, the excess pyridine was evaporated under reduced pressure. The syrupy residue thus obtained was dissolved in ethyl acetate (40 ml), and then the resultant solution was similarly worked up to give **3**.

Preparation of **4**. *Procedure A*: A solution of 2-oxoalkanoic acid (50 mmol), benzyl carbamate (50 mmol), and PTS (2 g) in dry benzene (70 ml) was refluxed for *ca.* 4 h, by which time the theoretical amount of water has separated out. After the solution had then been allowed to stand at room temperature, the crystals deposited were filtered. The filtrate was further extracted twice with saturated aqueous $NaHCO_3$ and the aqueous layer was washed once with ethyl acetate. The resultant aqueous layer was acidified to pH 2 with 6 M HCl and extracted well with ethyl acetate. The organic layer was washed with 20% aqueous NaCl and then dried over anhydrous Na_2SO_4. After the removal of the ethyl acetate, more crystals were obtained. The recrystallization of the combined crystals from chlorobenzene or cyclohexane–benzene gave **4** as colorless needles.

In an analogous manner, pyruvic acid (250 mmol) was treated with benzyl carbamate (100 mmol) in the absence of PTS to give **4a**.

Procedure B: A solution of **2** (10 mmol) in dioxane (20 ml) was treated with 1 M NaOH (30 ml) with stirring at *ca.* 35 °C for 2 h. After cooling with ice water, the reaction solution was acidified to pH 2 with 3 M HCl, and the resultant solution was extracted three times with ethyl acetate (30 ml). The extracts were washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure to give **4**.

Preparation of 5. *Procedure A*: A solution of 2-oxoalkanoic acid (50 mmol), *p*-toluenesulfonamide (50 mmol), and PTS (2.4 g) was worked up exactly according to Procedure A above to give **5** as colorless prisms.

Procedure B: The hydrolysis of **3** (10 mmol) with 1 M NaOH (30 ml) in dioxane (20 ml) was done exactly according to Procedure B above to give **5**.

Preparation of 6. *Procedure A*: Into a solution of **4** (20 mmol) and triethylamine (20 mmol) in dry THF (60 ml) we vigorously stirred ethyl chloroformate (20 mmol), below −15 °C. After stirring had been continued for 15 min, a solution of an appropriate α-amino acid ester (20 mmol) in dry $CHCl_3$ (60 ml) was stirred into the resultant solution at 0 °C. After stirring at 0 °C for 1.5 h and then at room temperature for a further 12 h, the reaction solution was concentrated under reduced pressure to give a residual syrup, which was subsequently dissolved in a mixture of ethyl acetate (300 ml) and water (150 ml). The resulting organic layer was successively washed with 4% aqueous $NaHCO_3$, water, 10% aqueous citric acid, and water, and finally dried over anhydrous Na_2SO_4. The evaporation of the ethyl acetate under reduced pressure gave crude crystals or a syrup, subsequently purified on a silica gel column, using benzene as the eluent, to give **6**. Cbz–ΔLeu–Leu–OEt; yield, 52%. Cbz–Δphe–Gly–OEt; yield, 56%. Cbz–ΔBut–Ala–OEt; yield, 50%.

Procedure B: Into a solution of **4** (10 mmol) and an appropriate α-amino acid ester (10 mmol) in DMF (10 ml) we stirred DCC (11 mmol), portion by portion, under cooling. After the mixture had been stirred at room temperature for 12 h, the urea deposited was filtered off and washed well with ethyl acetate. The filtrate was poured into water (70 ml), and the aqueous solution was extracted twice with ethyl acetate (60 ml). The combined extracts were successively washed with 2% aqueous HCl, water, saturated aqueous $NaHCO_3$ and water, and finally dried over anhydrous Na_2CO_3. The evaporation of the ethyl acetate under reduced pressure gave crystals or a syrup, subsequently purified on a silica gel column, using benzene–ethyl acetate (4 : 1 v/v), to give **6**. See Table 3.

Procedure C: Into a solution of ethyl α-(Cbz-amino)cinnamate (**2f**; 5 mmol) in ethanol (20 ml) we stirred hydrazine hydrate (5.5 mmol) at room temperature. After the mixture had been stirred for 5 h, the ethanol was evaporated to give a residual syrup. To a solution of the syrup obtained in 1 M HCl (15 ml) and ethyl acetate (20 ml) we added a solution of $NaNO_2$ (5.5 mmol) in water (12 ml) by stirring below 0 °C. After stirring had been continued for 30 min, chilled ethyl acetate (15 ml) was added to the reaction solution and the resulting organic layer was dried over anhydrous Na_2SO_4 at 0 °C. Finally, to the resultant chilled solution we added a solution of Gly–OEt (6 mmol) in dry diethyl ether (16 ml), and then the solution was stirred at 0 °C for 12 h and then at room temperature for 12 more h. The reaction solution was washed successively with 2 M HCl, 3% aqueous $NaHCO_3$, and water, and then dried over anhydrous Na_2SO_4. The subsequent evaporation of the solvent gave Cbz–ΔPhe–Gly–OEt in a 61% yield.

Preparation of 7. *Procedure A*: Into a solution of Boc–L-AA (20 mmol) in dry THF (60 ml) and triethylamine (20 mmol) we vigorously stirred ethyl chloroformate (20 mmol) at −15 °C. After stirring had been continued for 5 min, a solution of (*Z*)-**1** (20 mmol) in dry $CHCl_3$ (50 ml) was stirred into the resultant solution. The reaction solution was then worked-up exactly according to Procedure A above to give **7**. Boc–Phe–ΔBut–OEt; yield, 33%. Boc–Phe–ΔLeu–OEt; yield, 34%.

Procedure B: Into a solution of (*Z*)-**1** (10 mmol) and an appropriate N-protected L-AA (10 mol) in CH_2Cl_2 (10 ml) we vigorously stirred DCC (11 mmol), portion by portion, below 0 °C. After stirring had been continued at room temperature for 20 h, the reaction solution was worked-up exactly according to Procedure B above to give **7**. See Table 4.

References

1) T. Kitagawa, T. Tamura, and H. Taniyama, *J. Biochem.*, **81**, 1757 (1977).

2) "Bioactive Peptides Produced by Microorganisms,"ed by H. Umezawa, T. Takita, and T. Shiba, Kodansha, Tokyo (1978).

3) Y. Shimohigashi and N. Izumiya, *Yuki Gosei Kyokaishi*, **36**, 1023 (1978).

4) D. G. Doherty, J. E. Tietzmann, and M. Bergmann, *J. Biol. Chem.*, **147**, 617 (1943).

5) M. L. English and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, **83**, 1464 (1978).

6) A. Srinivasan, R. W. Stephenson, and R. K. Olsen, *J. Org. Chem.*, **42**, 2253 (1977).

7) D. H. Rich and J. P. Tam, *Tetrahedron Lett.*, **1975**, 211.

8) S. Nomoto, A. Sano, and T. Shiba, *Tetrahedron Lett.*, **1979**, 521.

9) C. Shin, M. Masaki, and M. Ohta, *J. Org. Chem.*, **32**, 1860 (1967).

10) H. Poisel, *Chem. Ber.*, **110**, 942 (1977).

11) C. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Tetrahedron Lett.*, **1979**, 1049.

12) In this paper, the symbol Δ indicates a double bond of the α-position in the DHA or DHA residue.

13) C. Shin, Y. Yonezawa, and J. Yoshimura, *Chem. Lett.*, **1976**, 1095.

14) C. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, **52**, 1657 (1979).

15) C. Shin, M. Fujii, and J. Yoshimura, *Tetrahedron Lett.*, **1971**, 2499.