[Chem. Pharm. Bull. 23(12)3106—3113(1975)]

UDC 547.466.1.04 : 547.281.04

Synthetic Study of Peptide Aldehydes[1)]

Akira Ito, Rieko Takahashi, Chieko Miura, and Yoshihiko Baba

Central Research Laboratories, Sankyo Co., Ltd.[2)]

(Received February 24, 1975)

Some of the leupeptin Ac-LL analogs, in which the argininal moiety was replaced by aromatic aminoaldehydes, were synthesized starting from benzyloxycarbonyl-α-amino-aldehyde semicarbazones derived from benzyloxycarbonyl amino acid esters by the reduction with diisobutylaluminum hydride. The racemization of the aldehyde part of the purified analogs was examined using the analytical ion exchange chromatography of diastereomers produced by the condensation of L-aspartic acid with aminoalcohols, which were obtained by the sodium borohydride reduction of the synthesized peptide aldehydes followed by acid hydrolysis. The results demonstrate that racemization occurs only to a limited extent, when these starting materials are used and careful purifications of the products are carried out.

A microbial product, leupeptin Ac-LL, which shows strong inhibition to various proteinases such as plasmin, trypsin and papain, was discovered and its structure was identified as Ac-L-Leu-L-Leu-argininal.[3,4)] It was also chemically synthesized from Ac-L-Leu-L-Leu-L-argininol by sulfoxide-carbodiimide oxidation method by Pfitzner and Moffatt.[5)] In an improved synthesis by Shimizu, *et al.*,[6)] benzyloxycarbonyl (Cbz)-N^G-nitro-L-arginine imidazolide was reduced to Cbz-N^G-nitro-L-argininal with lithium aluminum hydride and the aldehyde thus obtained was converted into N^G-nitro-L-argininal semicarbazone, which was coupled with Ac–L–Leu–L–Leu–ONSu. By essentially the same way, some analogs of leupeptin Ac–LL modifying its argininal residue to aromatic aminoaldehydes were also synthesized and these analogs were shown to be strong inhibitors of chymotrypsin, in contrast with leupeptin Ac-LL which inhibited trypsin but not chymotrypsin.[7)] However, racemization of C-terminal amino-aldehyde moiety during the synthesis was not examined in this work.

In our previous paper, it was reported that Cbz-L-amino acid methyl or ethyl esters were reduced with diisobutylaluminum hydride [$(i\text{-Bu})_2$AlH] to give the corresponding Cbz-α-amino-aldehydes which were converted directly into their semicarbazones without racemization.[9)] The optically pure semicarbazones thus obtained were expected to be good starting materials in peptide aldehyde synthesis. Then we applied this attempt to synthesize leupeptin Ac-LL analogs (**4a**—**c**). In the present parer, we describe in detail the syntheses of Ac-L-Leu-L-Leu-X (X=L-phenylalaninal, D-phenylalaninal, L-tyrosinal and L-tryptophanal) by this procedure and estimate the degree of racemization in the aldehyde part using the method we developed.

First, Cbz-aromatic amino acid methyl or ethyl esters {Cbz–L–Phe–OMe (**1a**), Cbz-D-Phe-OMe (**1a'**), Cbz-L-Tyr-OEt (**1b**) and Cbz-L-Trp-OMe (**1c**)} were reduced to the correspond-

1) Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, **5**, 2485 (1966), *ibid.*, **6**, 362 (1967), *ibid.*, **11**, 1726 (1972).
2) Location: *1-2-58, Hiromachi, Shinagawa-ku, Tokyo.*
3) T. Aoyagi, T. Takeuchi, A. Matsuzaki, K. Kawamura, S. Kondo, M. Hamada, K. Maeda and H. Umezawa, *J. Antibiotics* (Tokyo), **22**, 283 (1969).
4) K. Kawamura, S. Kondo, K. Maeda and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 1902 (1969).
5) K.E. Pfitzner and J.G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5661, 5670 (1965).
6) B. Shimizu, A. Saito, A. Ito, K. Tokawa, K. Maeda and H. Umezawa, *J. Antibiotics* (Tokyo), **25**, 515 (1972).
7) A part of the results were preliminarily communicated.[8)]
8) A. Ito, K. Tokawa, and B. Shimizu, *Biochem. Biophys. Res. Commun.*, **49**, 343 (1972).
9) A. Ito, R. Takahashi, and Y. Baba, *Chem. Pharm. Bull.* (Tokyo), **23**, 3081 (1975).

ing Cbz-α-aminoaldehydes with $(i\text{-Bu})_2AlH$ and the aldehyde parts were protected as their semicarbazones. The resulting semicarbazones (**2a—c**) were purified by silica gel chromatography. The Cbz group was removed by hydrogenolysis. The α-aminoaldehyde semicarbazones thus obtained were coupled with Ac–L–Leu–L–Leu–N_2H_3[10] by the azide method by

Table I. Physical Constants and Elemental Analysis of Cbz-Amino Acid Esters

Compound	Yield (%)	mp (°C)	$[\alpha]_D^t$ (*c*, MeOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
Cbz-L-Phe-OMe (**1a**)	87	oil	−15.6[19] (1.02)	$C_{18}H_{19}O_4N$	69.01 (68.99)	6.21 (6.11)	4.25 (4.47)
Cbz-D-Phe-OMe (**1a'**)	85	oil	+15.2[19] (0.64)	$C_{18}H_{19}O_4N$	68.82 (68.99)	6.29 (6.11)	4.36 (4.47)
Cbz-L-Tyr-OEt (**1b**)	88	50—51[a]	−7.6[19] (1.00)	$C_{19}H_{21}O_5N\cdot H_2O$	63.28 (63.14)	6.30 (6.42)	3.82 (3.88)
Cbz-L-Trp-OMe (**1c**)	84	oil	−11.4[20] (1.10)	$C_{20}H_{20}O_4N_2$	67.98 (68.17)	5.90 (5.73)	8.17 (7.93)

a) lit.[b] mp 88—91°.
b) E. Wuensch and J. Jentsch, *Chem. Ber.*, **97**, 2490 (1964)

Table II. Physical Constants and Elemental Analysis of Cbz-α-Aminoaldehyde Semicarbazones

Semicarbazone	Yield (%)	mp (°C)	$[\alpha]_D^t$ (*c*, MeOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
Cbz-L-phenylalaninal (**2a**)	53	132—134	−4.1[20] (1.01)	$C_{18}H_{20}O_3N_4\cdot 1/4H_2O$	62.63 (62.67)	6.01 (5.99)	16.47 (16.24)
Cbz-D-phenylalaninal (**2a'**)	50	132—134	+4.1[20] (1.02)	$C_{18}H_{20}O_3N_4$	63.29 (63.51)	5.94 (5.92)	16.69 (16.46)
Cbz-L-tyrosinal (**2b**)	42	171—173	+5.0[20.5] (3.02)	$C_{18}H_{20}O_4N_4$	60.90 (60.66)	5.72 (5.66)	15.82 (15.72)
Cbz-L-tryptophanal (**2c**)	38	87— 91	+14.4[17] (1.76)	$C_{20}H_{21}O_3N_5$	63.12 (63.31)	5.78 (5.58)	18.09 (18.46)

Table III. Physical Constants and Elemental Analysis of Cbz-Aminoalcohols

Compound	Yield (%)	mp (°C)	$[\alpha]_D^t$ (*c*, EtOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
Cbz-L-phenylalaninol	28	90—92	−41.5[20] (1.4)	$C_{17}H_{19}O_3N$	71.48 (71.56)	6.81 (6.71)	4.92 (4.91)
Cbz-D-phenylalaninol	26	90—92	+41.5[21] (0.61)	$C_{17}H_{19}O_3N$	71.67 (71.56)	6.53 (6.71)	4.86 (4.91)
Cbz-L-tyrosinol	31	92—96	−38.4[20] (2.0)	$C_{17}H_{19}O_4N\cdot 1/4H_2O$	66.66 (66.76)	6.37 (6.43)	4.75 (4.58)
Cbz-L-tryptophanol	25	95—98	−38.0[20] (2.2)	$C_{19}H_{20}O_3N_2$	69.93 (70.35)	6.14 (6.22)	8.50 (8.64)

10) H. Saeki, Y. Shimada, N. Kawakita, B. Shimizu, E. Ohki, K. Maeda, and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 163 (1973).

Honzl and Rudinger.[11] The resulted tripeptide aldehyde semicarbazones (**3a—c**) were treated with formalin and hydrochloric acid except for **3c** to give Ac–L–Leu–L–Leu–aromatic amino-aldehydes (**4a, 4a′, and 4b**). In the case of **3c**, hydrochloric acid caused undesirable side reaction. Tests by acetic acid, trichloroacetic acid and trifluoroacetic acid also gave unsatisfactory results. On treatment with thioglycolic acid and formalin, **3c** could be converted into **4c** without a formation of complex side products.

On purification by silica gel chromatography, some of the Cbz-α-aminoaldehydes considerably racemized through keto-enol tautomerism.[9] To avoid to contact with silica gel, these peptide aldehydes (**4a—c**) were purified by chromatography on Sephadex LH-20 eluted with methanol.

In our previous study, the racemization of argininal residue of synthesized leupeptin Ac-LL was determined by biological assay using *Streptococcus faecalis*, after the oxidation of argininal residue to arginine followed by acid hydrolysis.[6] In order to obtain more precise information on racemization, we tried to estimate both L- and D-isomers only by chemical way utilizing the separation method of diastereomers on ion exchange column chromatography. The peptide aldehydes were subjected to a series of reactions leading to the formation of L-aspartyl-L(and/or D)-aminoalcohol. In each reaction step, no isolation of the product was carried out. A peptide aldehyde was reduced to the corresponding peptide alcohol with sodium borohydride ($NaBH_4$). The peptide alcohol thus obtained was hydrolyzed to a mixture of L-leucine and

Table IV. Physical Constants and Elemental Analysis of Ac-L-Leu-L-Leu-aminoaldehyde Semicarbazones

Semicarbazone[a]	Yield (%)	mp (°C)	$[\alpha]_D^t$ (*c*, MeOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
R_1-L-phenylalaninal (**3a**)	73	129—131	$-49.7^{20.5}$(1.02)	$C_{24}H_{38}O_4N_6$	60.87 (60.73)	8.33 (8.07)	17.35 (17.71)
R_1-D-phenylalaninal (**3a′**)	69	123—126	$-16.3^{20.5}$(1.01)	$C_{24}H_{38}O_4N_6$	60.71 (60.73)	8.20 (8.07)	17.36 (17.71)
R_1-L-tyrosinal (**3b**)	62	149—153	-38.3^{17}(1.57)	$C_{24}H_{38}O_5N_6 \cdot H_2O$	56.90 (56.67)	7.93 (7.93)	16.44 (16.53)
R_1-L-tryptophanal (**3c**)	66	133—136	-25.5^{17}(2.01)	$C_{26}H_{39}O_4N_7 \cdot H_2O$	58.83 (58.74)	7.66 (7.77)	18.31 (18.44)

a) R_1=Ac-L-Leu-L-Leu

Table V. Physical Constants and Elemental Analysis of Ac-L-Leu-L-Leu-aminoaldehydes

Compound[a]	Yield (%)	mp (°C)	$[\alpha]_D^t$ (c, MeOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
R_1-L-phenylalaninal (**4a**)	60	153—156	-75.6^{17}(1.24)	$C_{23}H_{35}O_4N_3 \cdot 1/2H_2O$	64.41 (64.76)	8.51 (8.51)	10.02 (9.85)
R_1-D-phenylalaninal (**4a′**)	62	168—172	-34.4^{17}(0.90)	$C_{23}H_{35}O_4N_3 \cdot 3/4H_2O$	64.18 (64.08)	8.39 (8.54)	9.61 (9.75)
R_1-L-tyrosinal (**4b**)	57	103—107	-67.5^{19}(0.79)	$C_{23}H_{35}O_5N_3 \cdot 1/2H_2O$	62.25 (62.42)	8.40 (8.20)	9.69 (9.50)
R_1-L-tryptophanal (**4c**)	34	98—101	-69.2^{17}(0.62)	$C_{25}H_{36}O_4N_4 \cdot 5/4H_2O$	62.54 (62.67)	7.84 (8.10)	11.61 (11.70)

a) R_1=Ac-L-Leu-L-Leu

11) J. Honzl and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2333 (1961).

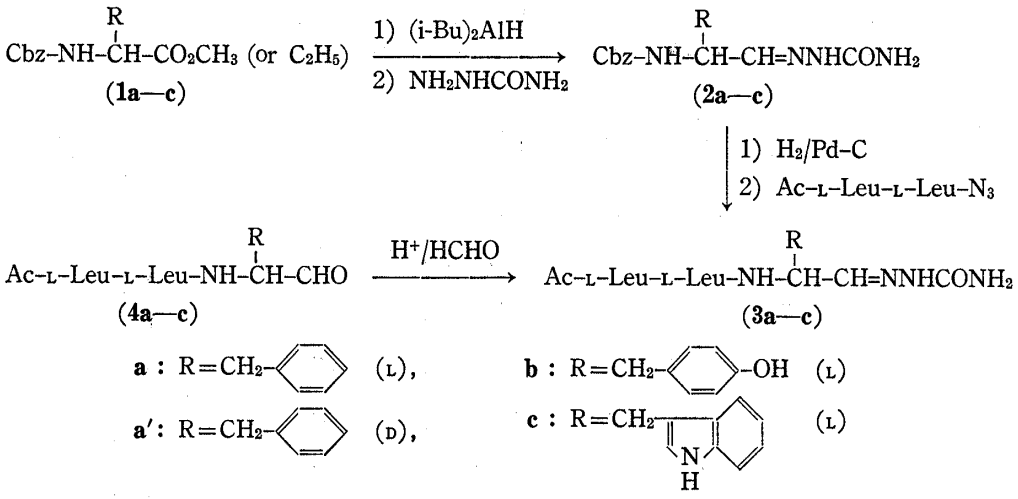

Chart 1

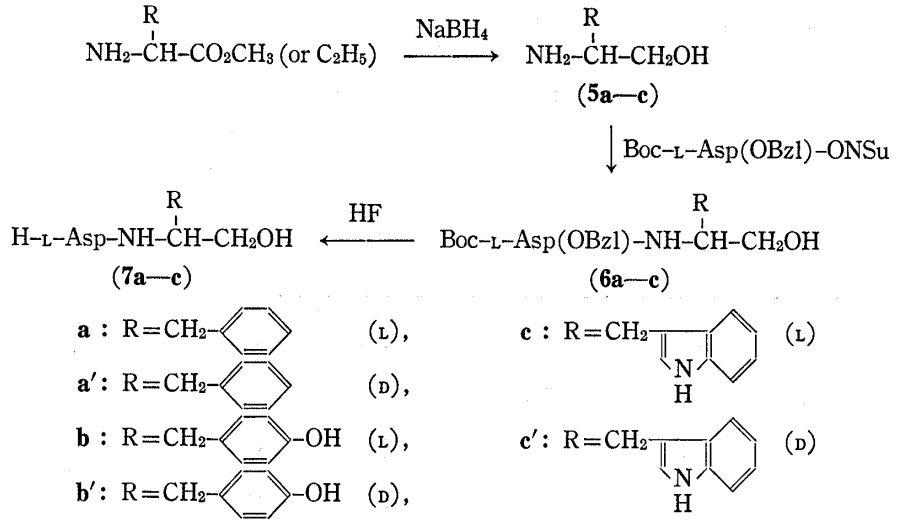

Chart 2

aminoalcohol by 6N hydrochloric acid in an evacuated sealed tube for 24 hr at 110°. In the case of Ac-L-Leu-L-Leu-L-tryptophanol, hydrolysis was carried out in constant boiling hydrochloric acid containing thioglycolic acid (5%) to protect tryptophanol from destruction. The resulting hydrolyzed mixture was reacted with a large excess of Boc-L-Asp(OBzl)-ONSu[12] and then treated with hydrogen fluoride (HF) at 0° for 30 min according to the procedure of Sakakibara, *et al.*[13] The reaction mixture, mainly consisted of L-aspartyl aminoalcohol and L-aspartyl amino acid, was submitted to a column of amino acid analyzer preliminarily calibrated with L-aspartyl-L-aminoalcohol and L-aspartyl-D-aminoalcohol.

As standard compounds for the present purpose, pairs of diastereomeric L-aspartyl aminoalcohols {L-aspartyl-L(and D)-phenylalaninol, L-aspartyl-L(and D)-tyrosinol and L-aspartyl-L(and D)-tryptophanol} were synthesized as shown in Chart 2.

Optically pure aminoalcohols (**5a—c**), starting materials, were prepared by the reduction of the corresponding α-amino acid ester hydrochlorides with $NaBH_4$ according to the method reported by Seki, *et al.*[14] These aminoalcohols (**5a—c**) were reacted with Boc-L-Asp(OBzl)-

12) D.A. Laufer and E.R. Blout, *J. Am. Chem. Soc.*, **89**, 1246 (1967).
13) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965).
14) H. Seki, K. Koga, H. Matsuo, S. Ohki, I. Matsuo, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 995 (1965).

Table VI. Physical Constants and Elemental Analysis of Aminoalcohol Oxalates

Oxalate	Yield (%)	mp (°C) (decomp.)	$[\alpha]_D^t$ (c, H_2O)	Formula	Analysis (%) Found (Calcd.) C	H	N
L-phenylalaninol[a]	73	171—173	-14.0^{25}(1.01)	$C_{11}H_{15}O_5N$	54.88 (54.77)	6.01 (6.27)	5.65 (5.81)
D-phenylalaninol	70	172—173	$+14.3^{25}$(1.15)	$C_{11}H_{15}O_5N$	54.62 (54.77)	6.15 (6.27)	5.69 (5.81)
L-tyrosinol	40	227—228	-17.8^{20}(0.99)	$C_{10}H_{14}O_4N$	56.33 (56.59)	6.50 (6.65)	6.66 (6.60)
D-tyrosinol	32	225—227	$+17.1^{20}$(1.21)	$C_{10}H_{14}O_4N$	56.40 (56.59)	6.54 (6.65)	6.73 (6.60)
L-tryptophanol[b]	61	203—205	-22.4^{20}(1.03)	$C_{13}H_{16}O_5N_2$	55.73 (55.71)	5.77 (5.75)	10.10 (10.00)
D-tryptophanol	67	202—203	$+22.1^{20}$(1.02)	$C_{13}H_{16}O_5N_2$	55.36 (55.71)	5.76 (5.75)	10.15 (10.00)

a) lit.[14] mp 173° (decomp.), $[\alpha]_D^{24}$ −24.1 (c=0.850, H_2O)
b) lit.[14] mp 206.5° (decomp.), $[\alpha]_D^{18.5}$ −25.1 (c=0.976, H_2O)

Table VII. Physical Constants and Elemental Analysis of Boc-L-Asp(OBzl)-aminoalcohol

Compound[a]	Yield (%)	mp (°C)	$[\alpha]_D^t$ (c, EtOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
R_2-L-phenylalaninol (**6a**)	76	120—123	-40.1^{25}(2.15)	$C_{25}H_{32}O_6N_2$	65.95 (65.77)	6.98 (7.07)	6.19 (6.14)
R_2-D-phenylalaninol (**6a'**)	73	90— 91.5	$+13.6^{25}$(2.17)	$C_{25}H_{32}O_6N_2$	65.88 (65.77)	7.02 (7.02)	6.21 (6.14)
R_2-L-tyrosinol (**6b**)	75	138—141	-40.5^{19}(2.12)	$C_{25}H_{32}O_7N_2$	63.59 (63.54)	6.82 (6.83)	5.93 (5.93)
R_2-D-tyrosinol (**6b'**)	70	150—153	$+14.1^{19}$(2.39)	$C_{25}H_{32}O_7N_2$	63.67 (63.54)	6.56 (6.83)	6.23 (5.93)
R_2-L-tryptophanol (**6c**)	70	50— 54	-33.7^{19}(1.95)	$C_{27}H_{33}O_6N_3 \cdot 1/2H_2O$	64.47 (64.27)	6.55 (6.79)	8.10 (8.33)
R_2-D-tryptophanol (**6c'**)	72	104—107	$+13.2^{19}$(1.79)	$C_{27}H_{33}O_6N_3 \cdot 1/4H_2O$	64.89 (64.85)	6.64 (6.75)	8.31 (8.40)

a) R_2=Boc-L-Asp(OBzl)

Table VIII. Physical Constants and Elemental Analysis of L-Aspartyl-aminoalcohols

Compound	Yield (%)	mp (°C)	$[\alpha]_D^t$ (c, AcOH)	Formula	Analysis (%) Found (Calcd.) C	H	N
L-Asp-L-phenylalaninol (**7a**)	68	218—219	-29.1^{20}(0.65)	$C_{13}H_{18}O_4N_2$	58.73 (58.63)	6.61 (6.81)	10.65 (10.52)
L-Asp-D-phenylalaninol (**7a'**)	74	196—198	$+47.2^{20}$(1.12)	$C_{13}H_{18}O_4N_2 \cdot H_2O$	54.57 (54.92)	7.06 (7.09)	9.64 (9.85)
L-Asp-L-tyrosinol (**7b**)	65	208—209	-24.1^{19}(0.69)	$C_{13}H_{18}O_5N_2$	55.27 (55.31)	6.23 (6.43)	10.09 (9.92)
L-Asp-D-tyrosinol (**7b'**)	63	204—206	$+43.5^{19}$(1.68)	$C_{13}H_{18}O_5N_2 \cdot H_2O$	51.90 (51.99)	6.68 (6.71)	9.58 (9.33)
L-Asp-L-tryptophanol (**7c**)	51	188—189	-24.4^{19}(0.54)	$C_{15}H_{19}O_4N_3 \cdot H_2O$	55.67 (55.72)	6.48 (6.55)	12.61 (13.00)
L-Asp-D-tryptophanol (**7c'**)	53	185—189	$+47.8^{20}$(0.50)	$C_{15}H_{19}O_4N_3 \cdot H_2O$	55.49 (55.72)	6.40 (6.55)	12.75 (13.00)

Table IX. Elution Conditions of L-Aspartyl-aminoalcohols

Compound	Column size (cm)	Sodium citrate buffer		Elution volume (ml)
		pH	Ionic strength (N) (Na^+)	
L-Asp-L-phenylalaninol	0.9×12	4.25	0.2	55
L-Asp-D-phenylalaninol	0.9×12	4.25	0.2	63
L-Asp-L-tyrosinol	0.9×50	4.25	0.2	177
L-Asp-D-tyrosinol	0.9×50	4.25	0.2	195
L-Asp-L-tryptophanol	0.9×12	5.28	0.2	58
L-Asp-D-tryptophanol	0.9×12	5.28	0.2	44

Table X. The Percentage of the Racemization of the Aldehyde Part in the Synthesized Peptide Aldehydes

Compound	Racemization %
Ac-L-Leu-L-Leu-L-phenylalaninal (**4a**)	1.3
Ac-L-Leu-L-Leu-D-phenylalaninal (**4a'**)	1.3
Ac-L-Leu-L-Leu-L-tyrosinal (**4b**)	0
Ac-L-Leu-L-Leu-L-tryptophanal (**4c**)	0

ONSu to give Boc-L-Asp(OBzl)-aminoalcohols (**6a—c**) in good yields. To remove the protecting groups, the L-aspartyl-aminoalcohol derivatives (**6a—c**) were treated with anhydrous HF.

A diastereomeric mixture of L-aspartyl-L(and D)-aminoalcohols was separated completely on a column of amino acid analyzer packed with spherical Dowex 50 resin at 50° under the conditions described in Table IX. Using this result, the racemization of aldehyde part in peptide aldehydes (**4a—c**) could be detected accurately with a small amount of sample (about 5 μmoles). This method might be extended to apply for other peptide aldehydes if proper conditions for the elution are selected.

The hydrochloric acid hydrolyzates of peptide alcohols, which were derived from the synthesized peptide aldehydes (**4a—c**) by $NaBH_4$ reduction, were coupled with Boc-L-Asp(OBzl)-ONSu and then treated with HF. The reaction mixture thus obtained was applied on an analytical ion exchange column. The amounts of L-aspartyl-L-aminoalcohol and L-aspartyl-D-aminoalcohol were calculated from the integrated areas of each peak. The presence of L-aspartyl amino acid in the reaction mixture did not interfere with the measurement of L-aspartyl-aminoalcohols because of the weak adsorption of acidic L-aspartyl amino acid on ion exchange column under the conditions given in Table IX. Percentage of the racemization of the aldehyde part in peptide aldehydes was calculated by the following equation:

$$\frac{\text{L-aspartyl-D(or L)-aminoalcohol}}{\text{(L-aspartyl-L-aminoalcohol)} + \text{(L-aspartyl-D-aminoalcohol)}} \times 100$$

As shown in Table X, little racemization was observed in the aldehydes parts of the synthesized peptide aldehydes (**4a—c**). This result indicates that Cbz-α-aminoaldehyde semicarbazones, which were prepared immediately after $(i\text{-Bu})_2AlH$ reduction of the corresponding amino acid esters, were suitable as starting materials in the synthesis of peptide aldehydes and that these peptide aldehydes could be purified without racemization by the column chromatography on Sephadex LH-20 free from keto-enol tautomerism.

Experimental

Melting points are not corrected. Thin-layer chromatography was performed on silica gel (Kieselgel G, Merk). Optical rotations were measured by a Perkin Elmer 141 automatic polarimeter in 1 dm tubes. The diastereomers of L-aspartyl-aminoalcohols were separated with a Hitachi amino acid analyzer (Model

KLA-2) with spherical Dowex 50 resin. For column chromatography, Silica Gel (B) (Iwai Kagaku Co., Ltd.) was used. Activated charcoal for chromatography was obtained from Wako Chemical Industries, Ltd. $(i\text{-Bu})_2AlH$ was obtained from Mitsuwa's Pure Chemical Co., Ltd. as *n*-hexane solution. $NaBH_4$ was purchased from Teika Sangyo Co., Ltd. H-L-Phe–OEt·HCl and H-L-Tyr–OEt·HCl were obtained from Protein Research Foundation. D-Phenylalanine, D-tyrosine and D-tryptophan were purchased from Takara Kohsan Co., Ltd.

Starting Materials——The optically active amino acid methyl or ethyl ester hydrochlorides were prepared by the esterification of the appropriate amino acids by the usual method using abs. MeOH (or EtOH) and dry HCl or with abs. MeOH and $SOCl_2$ in the similar manner described by Brenner, *et al.*[15] The syntheses of the following compounds were reported previously: H-L-Phe–OMe·HCl,[16] H-D-Phe–OMe·HCl,[17] H-L-Trp–OMe·HCl,[18] H-D-Trp–OMe·HCl.[19]

H-D-Tyr-OEt·HCl——The title compound was prepared by the usual method using abs. EtOH and dry HCl. Recrystallization from EtOH–ether gave a colorless needles in 89% yield. mp 163—165°. $[\alpha]_D^{26.5°}$ (c= 2.0, EtOH). *Anal.* Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.55; N, 5.70; Cl, 14.43. Found: C, 53.98; H, 6.47; N, 5.96; Cl, 14.45.

General Preparation of Cbz-Amino Acid Methyl or Ethyl Ester (1a—c) (Table I)——The preparation of Cbz-L-Trp-OMe (**1c**) was described in the previous paper.[9] Cbz-L-Phe–OMe (**1a**), Cbz-D-Phe–OMe (**1a'**) and Cbz-L-Tyr–OEt (**1b**) were also synthesized from the corresponding amino acid ester hydrochlorides in essentially the same way.

General Preparation of Cbz-α-Aminoaldehyde Semicarbazones (2a—c) (Table II)——Cbz-amino acid methyl or ethyl ester (**1a—c**) was reduced to the corresponding α-aminoaldehyde in the similar manner described previously by the present authors.[9] To a solution of Cbz-amino acid ester (**1a—c**) (30 mmoles) in anhyd. toluene (300 ml) cooled to about −50° in a dry-ice-acetone bath, 1.76M $(i\text{-Bu})_2AlH$ solution (40 ml) in *n*-hexane was added dropwise over a period of 1 hr, with vigorous stirring, in the stream of dry argon. After stirring for additional 30 min, excess reagent was decomposed by careful addition of 2N HCl (200 ml). In the case of **1c**, 10% citric acid was used for decomposition of excess $(i\text{-Bu})_2AlH$. The resulting reaction mixture was allowed to warm to approximately 0° and the organic layer was separated. The aqueous layer was extracted twice with AcOEt (100 ml). The organic layers were combined, washed with H_2O, dried over Na_2SO_4 and then evaporated to dryness below 40° (bath temp.). A solution of the crude Cbz-α-aminoaldehyde in 70% EtOH (90 ml) was treated with semicarbazide hydrochloride (3.3 g) and sodium acetate (2.7 g) at 80° for 5 min. The solvent was evaporated and the residue was extracted with AcOEt. The AcOEt extract was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*, giving a syrup. This syrup was purified by chromatography on silica gel (120 g) eluted with $CHCl_3$–MeOH (100: 1, v/v) in **2a** and **2a'**. In the case of **2c**, elution was carried out with $CHCl_3$–MeOH (100: 5, v/v). **2b** was purified by recrystallization from MeOH–$CHCl_3$. The mother liquor left after collection of **2b** was evaporated and the residue was chromatographed on silica gel (100 g) using AcOEt as eluent. In these purification stages, a small amount of (**1a—c**), the starting material, was recovered. Cbz-aminoalcohol which was probably produced by a further $(i\text{-Bu})_2AlH$ reduction of Cbz-α-aminoaldehyde was also obtained. Physical constants and yields of these alcohols are listed in Table III.

General Preparation of Ac-L-Leu-L-Leu-α-Aminoaldehyde Semicarbazones (3a—c) (Table IV)——Cbz-α-aminoaldehyde semicarbazone (**2a—c**) (11 mmoles) was hydrogenated in the presence of 5% Pd-C (0.8 g) in MeOH (100 ml) containing AcOH (0.8 ml) for 3 hr. The catalyst was filtered off and the filtrate was concentrated to dryness *in vacuo*. The resulting α-aminoaldehyde semicarbazone acetate was not purified further at this stage. The acetate was dissolved in dimethylformamide (DMF) (30 ml) and triethylamine (1.54 ml) was added. On the other hand, to a stirred solution of Ac-L-Leu-L-Leu-N_2H_3[10] (3.0 g, 10 mmoles) in DMF (70 ml) cooled to about −50° in a dry-ice-acetone bath, 3.4N HCl in dioxan (11.8 ml) and isoamyl nitrite (1.55 ml) were added. When the temperature of the reaction mixture was kept at −20° for 20 min, the hydrazine test became negative.[20] After being neutralized with triethylamine (5.6 ml), the above described α-aminoaldehyde semicarbazone solution was added to the azide solution. The mixture was stirred at 4° overnight and the solvent was evaporated *in vacuo*. The residue was triturated with H_2O and the resulting powder was recrystallized from MeOH–ether.

General Preparation of Ac-L-Leu-L-Leu-α-Aminoaldehydes (4a—c) (Table V)——To a solution of **3a—b** (0.6 mmole) in MeOH (12.5 ml), 0.5N HCl (6.0 ml) and 37% formalin (1.3 ml) were added. After standing

15) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).
16) R.A. Boiassonnas, S. Guttmann, P.A. Jaquenoud, and J.P. Waller, *Helv. Chim. Acta*, 39, 1421 (1956); H. Schwarz, F.M. Bumpus and I.H. Page, *J. Am. Chem. Soc.*, **79**, 5697 (1957).
17) B.F. Erlanger, H. Sachs, and E. Brand, *J. Am. Chem. Soc.*, **76**, 1806 (1954).
18) E. Abderhalden and M. Kempe, *Z. Physiol. Chem.*, **52**, 207 (1907); R.A. Boiassonnas, S. Guttmann, R.L. Hugenin, P.A. Jaquenoud, and E. Sandrin, *Helv. Chim. Acta*, **41**, 1867 (1958).
19) M. Shinitzky and M. Fridkin, *Eur. J. Biochem.*, **9**, 176 (1969).
20) H. Ertel and L. Horner, *J. Chromatog.*, **7**, 268 (1962).

at room temperature for 2 hr, the reaction mixture was diluted with H_2O (20 ml) and the solution was extracted twice with AcOEt (40 ml). The AcOEt extract was washed with sat. aqueous NaCl, dried over Na_2SO_4 and evaporated below 40° (bath temp.) to leave an amorphous powder. The powder was purified by chromatography on LH-20 (1.5 × 90 cm) using MeOH as eluent. Since the tryptophanal moiety of **4c** was decomposed by HCl, the following method was adopted to convert the peptide aldehyde semicarbazone into the peptide aldehyde. To a solution of **3c** (0.39 mmole) in MeOH (10 ml) containing 37% formalin (0.5 ml), thioglycolic acid (1.39 ml) was added and the reaction mixture was allowed to stand overnight at room temperature. Then the mixture was neutralized with 1N $NaHCO_3$ (20 ml) and extracted twice with AcOEt (30 ml). The AcOEt extract was washed with H_2O, dried over Na_2SO_4 and evaporated below 40° (bath temp.) to leave a colorless syrup. This syrup was purified by chromatography on LH-20 (1.5 × 94 cm) using MeOH as eluent.

General Preparation of Optically Pure Aminoalcohols (5a—c) (Table VI)——The optically pure amino acid methyl or ethyl ester hydrochlorides were reduced to the corresponding aminoalcohols with $NaBH_4$ in essentially the same way reported by Seki, *et al.*[14] The aminoalcohols were characterized as their oxalates.

L-Tryptophanol (5c)——A solution of H-L-Trp-OMe·HCl (10 g) in 75% EtOH (180 ml) was added dropwise to a solution of $NaBH_4$ (7.0 g) in 75% EtOH (120 ml). The resulting mixture was refluxed for 5 hr. EtOH was evaporated *in vacuo* and the aqueous solution thus obtained was applied on a column of activated charcoal (2.4 × 30 cm). After being washed thoroughly with H_2O (200 ml), the column was eluted with 5% AcOH. Removal of the solvent gave an colorless oil (6.0 g), which was converted into acid oxalate.

General Preparation of Boc-L-Asp(OBzl)-Aminoalcohol (6a—c) (Table VII)——To an ice-cooled and stirred solution of optically pure aminoalcohol oxalate (3 mmoles) in DMF (15 ml), triethylamine (0.42 ml) and Boc-L-Asp(OBzl)-ONSu (1.26 g) were added. After stirring for an additional 4 hr at room temperature, the solvent was removed *in vacuo*. The residual oil was dissolved in the mixture of H_2O (20 ml) and AcOEt (70 ml). The organic layer was further washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel (30 g) using $CHCl_3$ containing MeOH (0.5—1.0%) as eluent.

General Preparation of L-Asp-Aminoalcohol (7a—c) (Table VIII)——A mixture of Boc-L-Asp(OBzl)-aminoalcohol (**6a—c**) (1 mmole) and anisole (0.5 ml) was dissolved in anhyd. HF (10 ml) and the resulting solution was stirred at 0° for 30 min. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in H_2O (20 ml). After being washed with ether, the aqueous layer was applied on a column of Dowex 1-x4 (acetate form, 2 × 10 cm) and the column was eluted with H_2O. The necessary fraction monitored by thin-layer chromatography was concentrated *in vacuo* to leave a crystalline mass, which was recrystallized from aq. MeOH or aq. EtOH. In the case of **6c** and **6c'**, thioglycolic acid (0.1 ml) was also added on HF treatment to protect tryptophanol from decomposition.

General Procedure for the Determination of Racemization Extent of the Aldehyde Part in the Synthesized Peptide Aldehyde (4a—c)——The synthesized peptide aldehyde (**4a—c**) (about 5 μmoles) was weighed into a Pyrex test tube (15 × 150 mm) and dissolved in EtOH (1.0 ml). $NaBH_4$ (1.0 mg) in a weighing cup was dissolved in EtOH (0.6 ml) and this solution was combined with the above solution of the peptide aldehyde. After the reaction mixture was allowed to stand at room temperature for 30 min, the excess $NaBH_4$ was decomposed with AcOH (0.1 ml) and the solvent was evaporated from the test tube *in vacuo*. The hydrolysis of the peptide alcohol thus prepared was performed at 110° for 24 hr with 6N HCl (1.0 ml) in this tube sealed and evacuated. The hydrolyzate was washed into a 20 ml flask and evaporated *in vacuo*. In order to remove a trace of HCl, the residue was dissolved in H_2O (2.0 ml) and evaporated to dryness five times. To a solution of this hydrolyzate in 0.1N $NaHCO_3$ (2.0 ml), a solution of Boc-L-Asp(OBzl)-ONSu (60 mg) in dioxan (2.0 ml) was added. After standing overnight at room temperature, the reaction mixture was neutralized with AcOH and evaporated *in vacuo*. A suspension of this residue in MeOH (3.0 ml) was transferred into a difuron cylinder and the solvent was evaporated thoroughly *in vacuo*. After the addition of anisole (0.2 ml), the reaction mixture was treated with HF (5 ml) at 0° for 30 min. In the case of **4c**, thioglycolic acid (0.2 ml) was also added. Most of HF was removed under reduced pressure. To the residue in the difuron cylinder dried completely over KOH pellets, 0.2N sodium citrate buffer (pH 2.2) was added. After filtration, a part of the filtrate was applied to a column of amino acid analyzer. Elution conditions are given in Table IX.