SYNTHETIC STUDIES ON LEUPEPTINS AND THEIR ANALOGS

BUNJI SHIMIZU, AKIO SAITO, AKIRA ITO and KYOKO TOKAWA

Central Research Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo, Japan

KENJI MAEDA and HAMAO UMEZAWA

Institute of Microbial Chemistry, Shinagawa-ku, Tokyo, Japan

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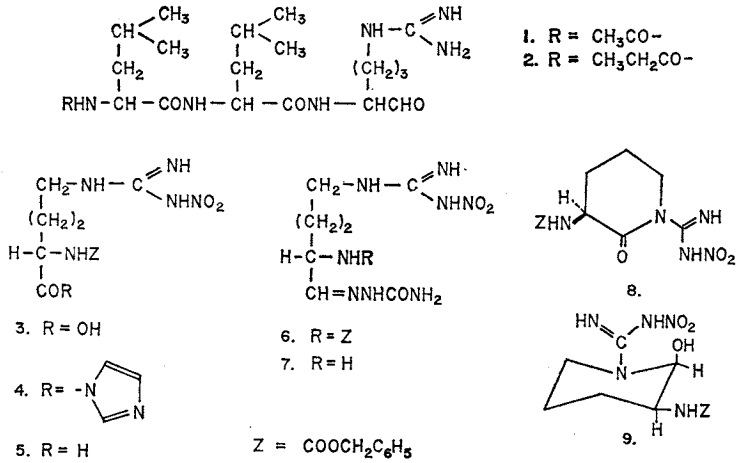
An imidazolide (**4**) obtained from carbobenzoxy-N^G-nitro-L-arginine (**3**) was reduced with lithium aluminum hydride to give carbobenzoxy-N^G-nitro-L-argininal (**5**) in good yield. The aldehyde (**5**) was converted into N^G-nitro-L-argininal semicarbazone (**7**) which was coupled with an active ester of various acylated leucines or isoleucines and then deblocked, giving leupeptins and their analogs, as listed in Table 1. Antiplasmin activities of these compounds are discussed.

Leupeptins, fermentation products of various strains of *Actinomycetes*,[1] showed strong antiplasmin activity[2] and were identified as being a mixture of two major components, leupeptin Ac-LL (acetyl-L-leucyl-L-leucyl-DL-argininal, **1**) and leupeptin Pr-LL (propionyl-L-leucyl-L-leucyl-DL-argininal, **2**), and their analogs containing isoleucine or valine in place of one or two leucine residues of **1** or **2**.[3,4] Chemical synthesis of leupeptin Ac-LL and Pr-LL was carried out by the oxidation of the corresponding argininol derivatives[4] to confirm the proposed structures. The analogously prepared isoleucine or valine variants of **1** or **2** have been shown to have similar interesting biological activities[5]. We present herein an improved method of preparing these peptide aldehyde derivatives along with a synthesis of leupeptins and their analogs.

STAAB *et al.* reported that carboxylic acid imidazolides are easily converted to the corresponding aldehydes by reduction with lithium aluminum hydride in an anhydrous organic solvent[6]. Carbobenzoxy-N^G-nitro-L-arginine (**3**) was treated with carbodiimidazole and the resulting imidazolide (**4**) was reduced with two equivalents of lithium aluminum hydride in tetrahydrofuran at −20°C for 40 minutes, giving carbobenzoxy-N^G-nitro-L-argininal (**5**) as crystals in 70 % yield without significant damage to the protecting groups. The argininal derivative (**5**) thereby obtained was characterized as a crystalline semicarbazone (**6**) which afforded N^G-nitro-L-argininal semicarbazone (**7**) on treatment with hydrogen bromide in acetic acid. *t*-Amyloxycarbonyl-N^G-nitro-L-arginine was analogously converted into the corresponding aldehyde in 51 % yield. This reduction proceeds through a direct attack of hydride on the imidazolide carbonyl group; however, in view of the fact that the activation of the carboxyl

Chart 1.



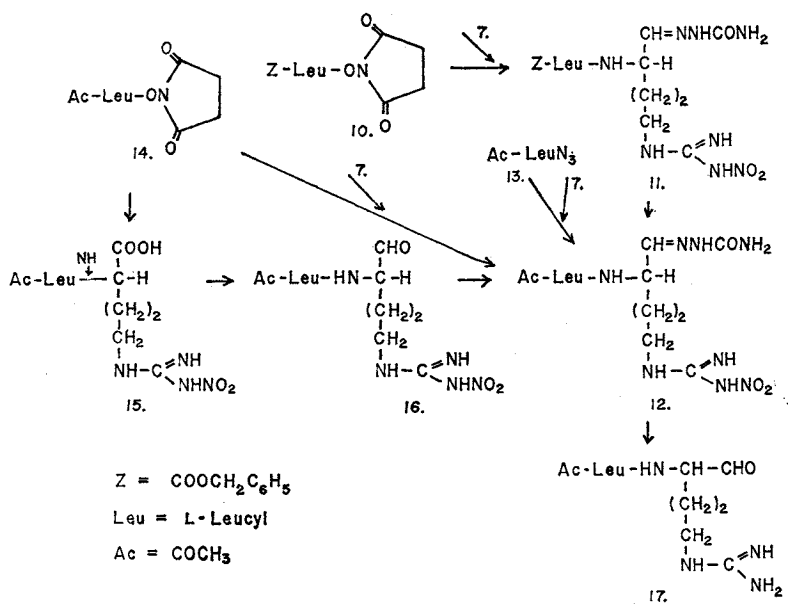
group of N^G-nitroarginine (**3**) results in formation of a δ-lactam (**8**),[7] a reduction of the formed lactam (**8**) into the aldehyde (**5**) might also be involved in this reaction. Further investigation of this problem will be presented in our forthcoming paper.

The structure of the aldehyde (**5**) thus obtained requires brief comment. The infrared spectrum of **5** did not show the presence of a free aldehyde carbonyl group. In addition, the nuclear magnetic resonance (NMR) spectrum of **5** (hexadeuteriodimethyl sulfoxide, 60 Ms), which also supported the absence of a free aldehyde, suggests cyclization of **5** into the carbinolamine (**9**); further, it reveals a doublet centering at δ 6.25 with J=4.0 Hz and a quartet at δ 5.8 with J=4.0 and *ca.* 3 Hz. The doublet is assignable to the hydroxyl proton of **9**, because it disappeared upon addition of deuterium oxide and shows a marked lower-field chemical shift similar to that of the hydroxyl proton on C-1 of α-D-glucopyranose (δ 6.15, J=4.5 Hz).[8] The quartet would be due to a proton residing on the same carbon as the hydroxyl group, because it collapsed into a doublet upon addition of deuterium oxide and these data are consistent with those of 5-acylaminoaldopyranoses[9] (δ 5~6, $J_{1,2}$=3~3.5 Hz) structurally related to **9**. Furthermore, its small coupling constant, J=*ca.* 3 Hz, due to the coupling with its vicinal methine proton probably signified a *cis* (axial-equatorial)-relationship[10] of the hydroxyl and the carbobenzoxyamino groups as shown in Chart 1.

Oxidation of the aldehyde (**5**) with potassium permanganate in the usual manner gave an optically-pure N^G-nitro-L-arginine (**3**) along with the δ-lactam[7] (**8**) which was easily converted into the optically-pure methyl ester of carbobenzoxy-N^G-nitro-L-arginine by treatment with methanol. These facts indicated that the aldehyde (**5**) is optically pure and hardly any racemization occurred during the reduction of the imidazolide (**4**).

Starting from N^G-nitro-L-argininal semicarbazone (**7**), the synthesis of acetyl-L-leucyl-L-argininal, a dipeptide analog of leupeptins, was first attempted and the following preliminary study was carried out (Chart 2). Carbobenzoxy-L-leucine was converted into its hydroxysuccinimide ester (**10**) by the method of WÜNSCH *et al.*[11] and **10** was coupled with the semicarbazone (**7**), giving carbobenzoxy-L-leucyl-N^G-

Chart 2.



nitro-L-argininal semicarbazone (**11**) in a good yield. Removal of the terminal protecting group from **11** and N-acetylation with acetic anhydride yielded acetyl-L-leucyl-N^G-nitro-L-argininal semicarbazone (**12**) along with a further acetylated byproduct having N^G-acetyl-N^G-nitroargininal group. Because of ubiquitous byproduct, we coupled the semicarbazone (**7**) with acetyl-L-leucine as follows. In the usual manner, methyl acetyl-L-leucinate was converted into an azide[12] (**13**) which was treated with **7** to give a crystalline dipeptide semicarbazone (**12**). On the other hand, acetyl-L-leucine was converted into its hydroxysuccinimide ester (**13**), which was coupled with **7**, affording the same semicarbazone (**12**) as obtianed by the azide method. These facts indicated that the latter method does not involve any significant recemization in the leucine moiety during the coupling reaction. Thereafter, the latter method was generally used in syntheses of leupeptins and other analogs because the procedure is easier and the yields are better. Furthermore, the hydroxysuccinimide ester (**14**) of acetyl-L-leucine was coupled with N^G-nitro-L-arginine and the resulting dipeptide (**15**) was converted into its imidazolide which when treated with lithium aluminum hydride afforded acetyl-L-leucyl-N^G-nitro-L-argininal (**16**) as an amorphous powder. The crystalline semicarbazone (**12**) of **16** was identical with the sample obtained as above.

The dipeptide semicarbazone (**12**) thus obtained was treated with liquid hydrogen fluoride at 0°C for 30 minutes according to the procedure of SAKAKIBARA *et al.*[13] giving acetyl-L-leucyl-L-argininal semicarbazone (**17**) which was further treated with formalin and hydrochloric acid to give acetyl-L-leucyl-L-argininal hydrochloride (**18**) as amorphous powder.

Next, leupeptin Ac-LL (**1**), Pr-LL (**2**) and their valyl or isoleucyl variants as shown in Table 1 were also synthesized from the corresponding acylated dipeptides.

by coupling of their hydroxysuccinimide esters with the argininal semicarbazone (**7**) and successive removal of the protecting groups. In addition, acetyl-L-leucyl-L-leucyl-D-argininal, an epimer of leupeptin Ac-LL (**1**) was prepared from N^{G}-nitro-D-argininal semicarbazone in an analogous way.

Table 1. Antiplasmin activity of leupeptins and their analogs.

No.	Compound	Antiplasmin activity (ID_{50}) μg/ml
1	Ac-L-Leu-L-Leu-L-argininal HCl (**1**)	4.5
2	Ac-L-Leu-L-Leu-D-argininal HCl	70.0
3	Pr-L-Leu-L-Leu-L-argininal HCl (**2**)	6.0
4	Pr-L-Val-L-Leu-L-argininal HCl	4.0
5	Ac-L-Val-L-Leu-L-argininal HCl	5.0
6	Pr-L-Val-L-Ileu-L-argininal HCl	43.0
7	Ac-L-Val-L-Ileu-L-argininal HCl	33.0
8	Ac-L-Leu-L-argininal HCl	19.0
9	Ac-L-Leu-L-Leu-DL-argininal HCl (natural product)	8.6

Similar to carbobenzoxy-N^{G}-nitro-L-argininal (**5**), these synthetic leupeptin hydrochlorides did not exhibit a marked aldehyde absorption in their infrared or NMR spectra and they also show broad absorptions at δ *ca.* 6.5 due to hydroxyl group and at δ 5.2 due to a methine proton in their NMR spectra. There was presumably still some ambiguity regarding the optical purity of the argininal moiety. Therefore, the following study on synthetic leupeptin Ac-LL (**1**) was carried out: **1** was oxidized with potassium permanganate to give an acid which, after purification using Dowex 1 (OH^- form), was completely hydrolyzed by refluxing with 6N hydrochloric acid. The resulting mixture of two ninhydrin-positive components, the Rf values of which were identical with those of leucine and arginine by paper chromatography was transferred to the following test. The total quantity of arginine in the mixture was determined by SAKAGUCHI's method[14] and the quantity of L-arginine by a bioassay with *Streptococcus faecalis* according to the method of HENDERSON *et al.*[15] and also by an enzymatic method with L-arginine-decarboxylase.[16] The result showed that the L-arginine content was 85±5%. On the other hand, the L-arginine content in the acid derived from the epimeric acetyl-L-leucyl-L-leucyl-D-argininal hydrochloride was determined as 7%. Conclusively, the leupeptins thus synthesized contain a small amount of the argininal epimer. Further purification of these leupeptins and analogs were not carried out, because this presented formidable difficulties.

Antiplasmin activity of leupeptins and their analogs synthesized are listed in Table 1. As compared with the activity of leupeptin Ac-LL (**1**), that of its D-epimer is quite low. The replacement of the N-terminal leucine residue by valine did not affect the activity, while the substitution of the second leucine residue decreased the activity to some extent.

Experimental*

Carbobenzoxy-N^{G}-nitro-L-argininal (**5**) and its semicarbazone (**6**): To a solution of carbobenzoxy-N^{G}-nitro-L-arginine (**3**) (14.15 g) in tetrahydrofuran (80 ml) was added carbodiimidazole (7.0 g) and the resulting solution was stirred at 10°C for 20 minutes and then

* Melting points are not corrected. NMR spectra were recorded on a Varian A-60 spectrometer. Thin-layer chromatography (TLC) was performed on TLC-plates silica Gel F_{254} pre-coated (E. Merck AG, layer thickness, 0.25 mm) and detection of spots was carried out by UV-irradiation or spraying RYDON-SMITH reagent.[17] Solvents were removed by a rotating flash evaporator at diminished pressure and usually at 35~50 °C.

cooled in a dry-ice bath. To the reaction mixture was added $LiAlH_4$ (3.34 g) in tetrahydrofuran (120 ml) over a period of 30 minutes maintaining the temperature at $-10°\sim-15°C$ and the reaction mixture was stirred for 20 minutes at the same temperature. After decomposition of excess reagent by addition of 2 N HCl (120 ml), the solvent was evaporated and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, then dried (Na_2SO_4), and chromatographed over silica gel (80 g). The fractions eluted with $CHCl_3$ were evaporated and the residue was recrystallized from EtOH, giving a δ-lactam (**8**) (0.1 g) mp 143~146°C, $[\alpha]_D^{22}-2°$ (*c* 2.5, DMF) which was identical with an authentic sample.[7]

The fractions eluted with $CHCl_3$-MeOH (39 : 1, v/v) on evaporation followed by recrystallization of the residue from EtOAc gave **5** (9.50 g, 70.1 %), mp 122~124°C, $[\alpha]_D^{22}-1.6°$ (*c* 0.73, MeOH).

Anal. Calcd. for $C_{14}H_{19}N_5O_5$: C 49.84, H 5.68, N 20.76.
Found: C 49.81, H 5.93, N 20.45.

A solution of **5** (8.68 g) in 75 % EtOH (12 ml) was treated with semicarbazide·HCl (3.0 g) and sodium acetate (4.0 g) at 60°C for 15 minutes. The precipitate which separated on cooling was collected and recrystallized from EtOH to give **6** (9.14 g), mp 107~109°C, $[\alpha]_D^{22}-11.2°$ (*c* 2.84, DMF).

Anal. Calcd. for $C_{15}H_{22}N_8O_5\cdot C_2H_5OH$: C 46.35, H 6.41, N 25.45.
Found: C 46.22, H 6.44, N 25.74.

N^G-Nitro-L-argininal semicarbazone (**7**): A solution of **6** (32.73 g) in 32 % HBr in AcOH (550 ml) was stirred at room temperature for 40 minutes and dry ether (21 ml) was added to the solution. Precipitates were collected and washed with dry ether. Treatment of the cake thus obtained with methanol gave HBr salt of **7** (25.5 g) as pale yellow crystals, mp 176~180°C. To a solution of the HBr salt of **7** in H_2O (170 ml) triethylamine (7.3 g) was added. Thus, **7** (17.6 g) was obtained as white crystals, mp 186.5~188°C (decomp.).

Anal. Calcd. for $C_7H_{16}N_8O_3$: C 32.30, H 6.20, N 43.06.
Found: C 32.51, H 6.35, N 43.40.

t-Amyloxycarbonyl-N^G-nitro-L-argininal: Using the work-up as described in the case of **5**, *t*-amyloxycarbonyl-N^G-nitro-L-arginine was converted into the argininal derivative, amorphous powder, $[\alpha]_D^{22}-0.5°$ (*c* 0.8, DMF), in 51 % yield.

Anal. Calcd. for $C_{12}H_{23}N_5O_5$: C 45.42, H 7.30, N 22.07.
Found: C 45.15, H 7.45, N 21.88.

The argininal formed a semicarbazone, mp 168~169°C.

Anal. Calcd. for $C_{13}H_{26}N_8O_5$: C 41.70, H 7.00, N 29.93.
Found: C 41.43, H 7.16, N 29.68.

Oxidation of carbobenzoxy-N^G-nitro-L-argininal (**5**): To a stirred solution of **5** (2.02 g) in acetone (50 ml) was added a solution of $KMnO_4$ (516 mg) in acetone (50 ml) over a period of 30 minutes at room temperature. The reaction mixture was stirred for 30 minutes and then 0.2 N HCl (20 ml) was added. The solvent was evaporated; the precipitate formed was extracted with $CHCl_3$, and then the aqueous layer was extracted with EtOAc. The $CHCl_3$ solution was washed with H_2O, dried (Na_2SO_4) and then evaporated to afford a syrup. Treatment of the syrup with *n*-hexane - EtOAc gave the δ-lactam (**8**). Chromatography of the mother liquor gave a further crop of **8** (280 mg). A solution of **8** (250 mg) in MeOH (10 ml) was refluxed for 15 minutes. On cooling to room temperature, unchanged **8** (5 mg) was filtered off and the filtrate was evaporated *in vacuo* to afford a syrup which was crystallized from AcOEt to give optically pure crystals of carbobenzoxy-N^G-nitro-L-arginine methyl ester (255 mg), mp 108~111°C, $[\alpha]_D^{22}-12.7°$ (*c* 0.8, MeOH), which was identical with the sample prepared from carbobenzoxy-N^G-nitro-L-arginine in the usual manner.

The AcOEt extract was washed with H_2O, dried (Na_2SO_4), and then evaporated *in vacuo*

Table 2. Physical data of acyl dipeptides

No.	Compound	mp	$[\alpha]_D^{22}$ (c in MeOH)	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
4	Ac-L-Val-L-LeuOH	189~191°C	−58.0° (0.6)	$C_{13}H_{24}O_4N_2$	57.33	8.88	10.29	57.10	8.74	9.93
5	Pr-L-Val-L-LeuOH	197~199°C	−62.7° (0.6)	$C_{14}H_{26}O_4N_2$	58.72	9.15	9.78	58.61	9.26	9.54
6	Ac-L-Val-L-IleuOH	187~200°C	−35.3° (0.6)	$C_{13}H_{24}O_4N_2$	57.33	8.88	10.29	57.33	9.00	10.23
7	Pr-L-Val-L-IleuOH	193.5~194.5°C	−50.7° (0.6)	$C_{14}H_{26}O_4N_2$	58.72	9.15	9.78	58.60	9.26	9.64

Table 3. Physical data of hydroxysuccimide esters of acyl peptides

No.	Compound R=$C_4H_4O_2N$	mp	Formula	Analysis					
				Calcd.			Found		
				C	H	N	C	H	N
1	Ac-L-Leu-L-LeuOR	145.5~147°C	$C_{18}H_{29}O_6N_3$	56.38	7.62	10.96	56.13	7.70	10.77
3	Pr-L-Leu-L-LeuOR	139~141°C	$C_{19}H_{31}O_6N_3$	57.41	7.86	10.57	57.71	8.00	10.36
4	Ac-L-Val-L-LeuOR	174~177°C	$C_{17}H_{27}O_6N_3$	55.27	7.37	11.38	54.86	7.40	11.17
5	Pr-L-Val-L-LeuOR	168~170°C	$C_{18}H_{29}O_6N_3$	56.38	7.62	10.96	56.70	7.83	10.83
6	Ac-L-Val-L-IleuOR	157~159°C	$C_{17}H_{27}O_6N_3$	55.27	7.37	11.38	55.19	7.64	11.47
7	Pr-L-Val-L-IleuOR	146~148°C	$C_{18}H_{29}O_6N_3$	56.38	7.62	10.96	56.53	7.77	10.79
8	Ac-L-LeuOR	118°C	$C_{12}H_{18}O_5N_2$	53.30	6.72	10.37	53.23	6.69	10.66

Table 4. Physical data of acyl $N^{\overset{G}{=}}$-nitroargininal semicarbazones

No.	Semicarbazone	mp (decomp.)	$[\alpha]_D^{22}$ (c in DMF)	Formula	Analysis					
					Calcd.			Found		
					C	H	N	C	H	N
1	Ac-L-Leu-L-Leu-$N^{\overset{G}{=}}$-nitro-L-argininal	amorphous	−22.7° (1.2)	$C_{21}H_{40}O_6N_{10}$	47.71	7.63	26.50	47.52	7.81	26.43
2	Ac-L-Leu-L-Leu-$N^{\overset{G}{=}}$-nitro-D-argininal	130~137°C	−6.5 (0.5)	$C_{21}H_{40}O_6N_{10}$	47.71	7.63	26.50	47.50	7.85	26.80
3	Pr-L-Leu-L-Leu-$N^{\overset{G}{=}}$-nitro-L-argininal	amorphous	−27° (1.04)	$C_{22}H_{42}O_6N_{10}\cdot H_2O$	47.13	7.55	24.97	47.36	7.75	24.80
4	Ac-L-Val-L-Leu-$N^{\overset{G}{=}}$-nitro-L-argininal	165~170°C	−27° (0.89)	$C_{20}H_{38}O_6N_{10}\cdot 3/2\ C_2H_5OH$	46.95	8.85	23.88	47.40	8.87	23.74
5	Pr-L-Val-L-Leu-$N^{\overset{G}{=}}$-nitro-L-argininal	190°C	−24.7° (1.13)	$C_{21}H_{40}O_6N_{10}\cdot CH_3OH$	47.12	7.91	24.99	47.30	7.89	24.74
6	Ac-L-Val-L-Ileu-$N^{\overset{G}{=}}$-nitro-L-argininal	amorphous	−24.6° (0.94)	$C_{20}H_{38}O_6N_{10}\cdot 1/2\ H_2O$	45.88	7.51	26.76	45.57	7.50	26.62
7	Pr-L-Val-L-Ileu-$N^{\overset{G}{=}}$-nitro-L-argininal	220~223°C	−15.6° (0.60)	$C_{21}H_{40}O_6N_{10}\cdot H_2O$	46.12	7.75	25.63	46.42	7.53	25.53
8	Ac-L-Leu-$N^{\overset{G}{=}}$-nitro-L-argininal	208~210°C	−3.1° (1.05)	$C_{15}H_{29}O_5N_9$	43.37	7.03	30.35	43.50	7.24	30.35

to afford a syrup (957 mg) containing carbobenzoxy-$N^{G}_{=}$-nitro-L-arginine as a major component. The syrup was dissolved in 32% HBr-AcOH (20 ml) and the resulting solution was stirred at room temperature for 40 minutes. The precipitate obtained by addition of dry ether (100 ml) was collected and washed with ether. The precipitate was dissolved in H_2O and chromatographed over an ion-exchange resin column (Dowex 1×2, 100~200 mesh, HCO_3^- form, 20 ml). The fractions eluted with 0.1 M NH_4HCO_3 were evaporated to dryness and recrystallization of the residue from aqueous EtOH gave $N^{G}_{=}$-nitro-L-arginine (450 mg), mp 145°C, $[\alpha]_D^{22}+25°$ (*c* 1.5, 2 N HCl), which was identified with an authentic sample.

Acetyl-L-leucyl-$N^{G}_{=}$-nitro-L-argininal semicarbazone (**12**):

(i) To a solution of acetyl-L-leucylhydrazide[12] (0.76 g) in H_2O (5 ml) and AcOH (1 ml) was added a solution of sodium nitrite (0.7 g) in H_2O (0.9 ml) with stirring. The resulting oil was extracted with ether (6×8 ml) and the combined extracts were dried ($MgSO_4$). The solution of the azide (**13**) thus obtained was added dropwise to a solution of **7** (1.04 g) in a mixture of DMF (80 ml) and DMSO (80 ml) with vigorous stirring and cooling in an ice bath. After 48-hour standing, the solvent was evaporated *in vacuo* at a bath temperature of 40~50°C. The residue was triturated with ether and the resulting crystals were recrystallized from EtOH-AcOEt, giving **12** (0.7 g). The physical data are given in Table 4.

(ii) To a solution of acetyl-L-leucine[12] (17.32 g) and N-hydroxysuccinimide (11.51 g) in dioxane (300 ml) at 0°C was added 20.6 g of dicyclohexylcarbodiimide. The resulting solution was stirred at 4°C overnight. Precipitates were removed by filtration and the filtrate was concentrated *in vacuo*. After filtration of the separated solids, the concentrate was evaporated to dryness and the residue was triturated with ether. The resultant crystals were collected and recrystallized from *iso*-PrOH to give an ester (**14**) (11.14 g) whose physical data are given in Table 2.

A mixture of **14** (1.352 g) and **7** (1.302 g) in DMF (5 ml) was stirred at 40°C for 2.5 hours. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in EtOH (10 ml). To the solution was added a large amount of ether to afford precipitates which were dissolved in EtOH again. On addition of AcOEt, the resulting solid was collected, washed with AcOEt and then dried. Thus, **12** (2.075 g) was obtained and identified with the sample obtained by the azide method by mixed mp, infrared spectrometry and thin-layer chromatography.

(iii) To a stirred solution of $N^{G}_{=}$-nitro-L-arginine

Table 5. Physical data of synthesized leupeptins and analogs

No.	Hydrochloride	mp	$[\alpha]_D^{22}$ (*c* in MeOH)	Formula	Analysis									
					Calcd.				Found					
					C	H	N	Cl	C	H	N	Cl		
1	Ac-L-Leu-L-Leu-L-argininal	165~175°C	−60.7° (1.30)	$C_{20}H_{38}O_4N_6HClH_2O$	49.93	8.59	17.47	7.37	49.89	8.86	17.73	7.35		
2	Ac-L-Leu-L-Leu-D-argininal	175~185°C	−26.2° (0.69)	$C_{20}H_{38}O_4N_6HClH_2O$	49.93	8.59	17.47	7.37	50.14	8.90	17.50	7.40		
3	Pr-L-Leu-L-Leu-L-argininal	180~185°C	−61.6° (0.61)	$C_{21}H_{40}O_4N_6HClH_2O$	50.95	8.76	16.98	7.16	51.07	8.89	16.99	7.15		
4	Ac-L-Val-L-Leu-L-argininal	100°C	−58.4° (0.88)	$C_{19}H_{36}O_4N_6HClH_2O$	48.81	8.41	17.99	7.59	48.25	8.51	17.33	7.58		
5	Pr-L-Val-L-Leu-L-argininal	amorphous	−58.6° (0.83)	$C_{20}H_{38}O_4N_6HClC_2H_5OH$	51.95	8.80	16.48	6.97	51.41	8.57	16.43	6.53		
6	Ac-L-Val-L-Ileu-L-argininal	amorphous	−64.6° (0.84)	$C_{19}H_{36}O_4N_6HClH_2O$	48.81	8.41	17.99	7.59	48.83	8.20	18.03	7.09		
7	Pr-L-Val-L-Ileu-L-argininal	amorphous	−63.4° (0.93)	$C_{20}H_{38}O_4N_6HClC_2H_5OH$	51.95	8.80	16.48	6.97	52.09	8.53	16.55	5.80		
8	Ac-L-Leu-L-argininal	165~175°C	−20.3° (0.71)	$C_{14}H_{27}O_3N_5HClH_2O$	45.71	8.22	19.03	9.64	45.63	8.31	18.73	9.63		

(2.19 g) and triethylamine (2.02 g) in H_2O (20 ml) was added 2.7 g of N-hydroxysuccinimide ester (**14**) in 10 ml of tetrahydrofuran. The resulting solution was stirred at room temperature for 2 hours. To the reaction mixture was added 30 ml of Dowex 50 (H^+ form) and after filtration, the mixture was evaporated to dryness. The residue was dissolved again in a small amount of EtOH and 50 ml of ether was added. The resultant precipitate was also dissolved in EtOH. On addition of AcOEt, the solid formed was collected and washed with ether and then dried. Thus, acetyl-L-leucyl-N^G-nitro-L-arginine (**15**) (3.60 g) was obtained as amorphous powder, $[\alpha]_D^{22}-16°$ (*c* 1.23, MeOH).

Anal. Calcd. for $C_{14}H_{26}N_6O_6$: C 44.91, H 7.00, N 22.44.
Found: C 44.58, H 7.21, N 22.30.

In a manner similar to the synthesis of **5**, **15** (1.50 g) was treated with carbodiimidazole (0.72 g) in tetrahydrofuran (15 ml) and the resulting solution was reduced with $LiAlH_4$ (370 mg). The product was chromatographed over a carbon column (30 ml) and the fractions eluted with 50 % MeOH on evaporation gave acetyl-L-leucyl-N^G-nitro-L-argininal (**16**) (603 mg) as amorphous powder which formed the same semicarbazone (**12**).

Acetyl-L-leucyl-L-argininal (**17**) hydrochloride: A mixture of **12** (831 mg) and anisole (0.8 ml) was dissolved in 12 ml of liquid hydrogen fluoride and the resulting solution was stirred at 0°C for 30 minutes. The reaction mixture was evaporated *in vacuo* to afford a syrup which was dissolved in H_2O (30 ml) and adjusted the pH to *ca.* 4.5 by addition of 1 M $NaHCO_3$. After washing with $CHCl_3$ the solution was concentrated *in vacuo* to *ca.* 20 ml. To the solution were added 1 N HCl (2 ml) and formalin (0.75 ml) and the resulting solution was stirred at room temperature for 2 hours. The reaction mixture was neutralized with 1 M $NaHCO_3$ and then chromatographed on a carbon column (Wako Pure Chem. Co., 50 ml). After being washed with H_2O, the column was eluted with 0.001 N HCl in 70 % MeOH. Fractions were monitored by thin-layer chromatography and evaporated under 40°C to dryness. Thus, a hydrochloride of **17** was obtained as powder, whose physical data are given in Table 5.

Syntheses of leupeptins and their analogs: Acetyl- and propionyl-L-leucyl-L-leucines were prepared as described in our preceding paper.[4] Other acylated dipeptides were also synthesized analogously and are listed in Table 2. Hydroxysuccinimide esters of these peptides prepared in a manner similar to the synthesis of the acetyl-L-leucine ester (**14**) were prepared and their physical data are described in Table 3. Analogous coupling of these esters with **7** gave argininal semicarbazones as listed in Table 4, and the protecting groups were removed, giving leupeptins and their analogs as described in Table 5. Carbobenzoxy-N^G-nitro-D-arginine used for synthesis of acetyl-L-leucyl-L-leucyl-D-argininal was prepared by the known method.[18]

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