LINEAR TRI- AND TETRAPEPTIDES ACTING AS PRODRUGS

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Dedicated to Dr Miroslav Protiva on the occasion of his 70th birthday.

Tri- and tetrapeptides with C-terminal 1-amino-1-cycloalkanecarboxylic acid of the general formula X-Ala-Y-OR, where X is Ala, Leu, Phe, Ac-Tyr, Gly-Pro, Ac-Leu-Lys or Ac-Leu-Arg, Y is Acb, Acp or Ach, and R is methyl or ethyl, have been prepared. These peptides containing a rationally chosen N-substituent are cleaved with a suitable enzyme (leucinaminopeptidase, alaninaminopeptidase, chymotrypsin, plasmin or kallikrein) in an aqueous medium. The arising C-terminal dipeptide ester undergoes spontaneous cyclization to give biologically active spirocyclic dipeptide. The prepared short peptides exhibit all functional features of prodrugs.

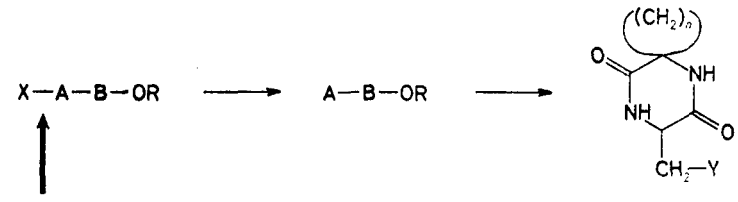
The spirocyclic dipeptide cyclo(alanyl-1-amino-cyclopentane-carbonyl) (VÚFB 15 754; ALAPTIDE) represents a potential drug that significantly influences biological systems in several clinical applications (nootropic acitvity, therapy of skin and mucous membrane lesions)[1]. From the pharmaco-kinetic viewpoint, the relatively low solubility of Alaptide – and thus its low concentration – may have an adverse effect on its transport to and concentration in the target organs and corresponding receptors.

One of the possibilities how to overcome these drawbacks is to design such an inactive precursor of sufficient solubility that would lose the N-terminal additional part (e.g. amino acid, acylamino acid or a short peptide) by action of a specific enzyme system and thus liberate the active compound. This principle of generating active peptide from an inactive precursor has been suggested by us for a series of prodrug-type compounds. The subsequent reactions, liberating the active compound, consist in enzyme-induced generation of C-terminal aminodipeptide ester which is spontaneously cyclized to the corresponding spirocyclic dipeptide (see Scheme 1).

The strategy of synthesis of linear peptides has been based on the cyclization of aminodipeptide esters with a built-in C-terminal 1-amino-1-cycloalkanecarboxylic acid. Therefore, in the final steps we have chosen the method consisting in condensation of the peptide (as the C-active component) with the ester Acb, Acp or Ach*.

* The symbols for amino acids and peptides comply with the suggestion of the IUPAC-IUB Commission on Biochemical Nomenclature[2]. Acb = 1-amino-1-cyclobutanecarboxylic acid; Acp = 1-amino-1-cyclopentanecarboxylic acid; Ach = 1-amino-1-cyclohexanecarboxylic acid. All amino acids are of L-configuration (with the exception of Acb, Acp and Ach).

Only in the case of *IIi* and *IIj* we performed the condensation with C-terminal dipeptide ester (under specific conditions), without any side reaction (i.e. formation of 2,5-dioxopiperazine derivative).



Enzyme catalyzed hydrolysis
of peptide bond

X: Ala, Leu, Phe, Ac-Tyr, Gly-Pro, Ac-Leu-Lys, Ac-Leu-Arg
A: Ala, Phe, Acp
B: Acb, Acp, Ach, Ser
n: 3, 4, 5
Y: H, OH, $CH_2C_6H_5$
R: CH_3, C_2H_5

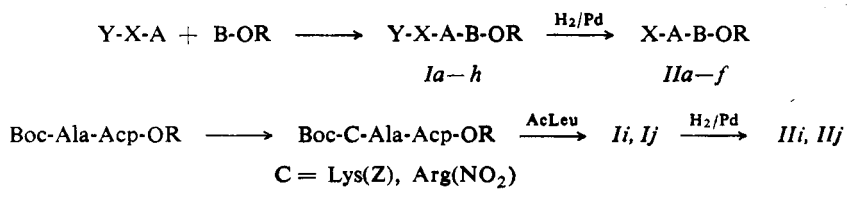
SCHEME 1

The synthesis of prodrug-type peptide was realized by condensation in solution using the carbodiimide method in the presence of N-hydroxy-succinimide or the anhydride method (Et—O—CO—Cl). Of the temporary N-protecting groups we used the benzyloxycarbonyl (Z) or the tert-butyloxycarbonyl (Boc) group, the side functional groups were protected by the nitro group (N^G-arginine) and the acetyl group (OH-tyrosine). The Z- and nitro groups were removed by pressure hydrogenolysis on Pd/C under established optimum conditions (pressure and solvent). The pressure hydrogenolysis was used for simultaneous deblocking of both protecting groups (Z and nitro) in the final step. In most cases, the final products were isolated as crystalline salts (citrates or acetates, see Scheme 2).

The yields and analytical data of the protected intermediates are given in Table I, those of the final products in Table II.

In the set of peptidic precursors we have focussed first of all on the Ala-Acp sequence which affords the desired final cyclic product cyclo(Ala-Acp), i.e. the clinically studied compound Alaptide. We also included peptides with C-terminal 1-amino--1-cycloalkanecarboxylic acid with smaller spirocycle (Acb) and with larger spirocycle (Ach), i.e. *IIa*, *IIb* and *IIc*. However, concurrent and subsequent pharmacological tests have shown that spirocyclic dipeptides with cyclobutane (Acb) or cyclohexane (Ach) ring exhibit no marked biological effect; this is also true for the precursors

Ih and *IIf*. The peptides with N-terminal N-acetyltyrosine moiety, *Ig* and *Ih*, are considered to be target precursors.



I	Y	X	A	B	R	*II*	X	A	B	R
a	Z	Ala	Ala	Acb	Me	*a*	Ala	Ala	Acb	Me
b	Z	Ala	Ala	Acp	Et	*b*	Ala	Ala	Acp	Et
c	Z	Ala	Ala	Ach	Et	*c*	Ala	Ala	Ach	Et
d	Z	Leu	Ala	Acp	Et	*d*	Leu	Ala	Acp	Et
e	Z	Gly-Pro	Ala	Acp	Et	*e*	Gly-Pro	Ala	Acp	Et
f	Z	Phe	Phe	Acp	Et	*f*	Phe	Phe	Acp	Et
g	Ac	Tyr	Ala	Acp	Et					
h	Ac	Tyr	Acp	Ser	Me					
i	Ac-Leu-Lys(Z)		Ala	Acp	Me	*i*	Ac-Leu-Lys	Ala	Acp	Me
j	Ac-Leu-Arg(NO_2)		Ala	Acp	Et	*j*	Ac-Leu-Arg	Ala	Acp	Et

SCHEME 2

The design of N-terminal enzymatically removable peptide sequence (or amino acid) was rationally selected according to the substrate specificity of the followed enzymes: leucine-aminopeptidase (Leu), alanine-aminopeptidase (Ala), chymotrypsin (Phe, Ac-Tyr), dipeptidylaminopeptidase IV (Gly-Pro), plasmin (Ac-Leu-Lys) and kallikrein (Ac-Leu-Arg).

In vitro incubation experiments have shown that Leu-Ala-Acp-OEt was cleaved with leucine-aminopeptidase (Tris-buffer, pH 7·4, 25°C) during 4 to 12 h to give cyclo(Ala-Acp) in a 20% to 60% yield; also the intermediate, free aminodipeptide ester Ala-Acp-OEt was detected. The hydrolysis and spontaneous conversion were followed by thin-layer chromatography in 1-butanol–acetic acid–water (4 : 1 : 1); Leu-Ala-Acp-OEt (R_F 0·44), Ala-Acp-OEt (R_F 0·16) and cyclo(Ala-Acp) (R_F 0·64). Analogous experiments were carried out with chymotrypsin and dipeptidylaminopeptidase IV. Detailed results of these in vitro as well as in vivo (activation with plasmin and kallikrein) experiments will be the subject of another paper.

The cyclization of aminopeptide esters in an aqueous medium is not quite usual. In non-aqueous media these cyclization reactions are alkali- or acid-catalyzed[2]. In this respect, cyclo(His-Pro), exhibiting central nervous system activity[3–6], deserves

attention. Precursor of this cyclic peptide, thyreoliberin[7] (pGlu-His-Pro-NH_2), on reaction with pyroglutamyl aminopeptidase in vivo loses pyroglutamic acid (pGlu) and the C-terminal dipeptide amide undergoes a spontaneous cyclization to give the corresponding 2,5-dioxopiperazine derivative[8,9]. The kinetics of the cyclizations of glycine dipeptide esters into the corresponding cyclic dipeptides in aqueous media has been studied by Purdie and Benoiton[10]. The use of cyclization reactions of peptidic compounds in vivo to give cyclic dipeptides has been published in 1989 by Bundgaard and coworkers[11].

The aim of our investigation is connected with the generally desired economical and aimed pharmacotherapy of various diseases requiring e.g. physiological transport-

TABLE I
Properties of protected peptides

Compound Cryst. solvent[a]	M.p., °C Yield, %	$[\alpha]_D^{20}$[b]	Formula (M.w.)	Calculated/Found %C	 %H	 %N
Ia	101–103	−43·0°	$C_{20}H_{27}N_3O_6$	59·25	6·71	10·36
A	68		(405·5)	58·75	6·96	10·54
Ib	134–136	−39·2°	$C_{22}H_{31}N_3O_6$	60·95	7·21	9·69
A	72		(433·5)	60·72	7·44	9·60
Ic	110–112	−42·2°	$C_{23}H_{33}N_3O_6$ 0·5 H_2O	60·51	7·51	9·20
A	84		(456·6)	60·93	7·46	9·46
Id	104–106	−35·9°	$C_{25}H_{37}N_3O_6$	63·14	7·84	8·83
A	80		(475·6)	63·33	7·86	9·14
Ie	[c]	−65·6°	$C_{26}H_{36}N_4O_7$	60·46	7·02	10·85
A	79		(516.6)	60·83	7·36	10·23
If	93–96	−22·5°	$C_{34}H_{39}N_3O_6$ 0·5 H_2O	68·66	6·78	7·07
B	81		(594·7)	68·13	6·93	7·20
Ig	168–171	−3·1°	$C_{22}H_{30}N_3O_6$ 0·5 H_2O	59·71	7·06	9·50
C	88		(442·5)	60·14	7·24	9·17
Ih	128–132	+8·6°	$C_{21}H_{29}N_3O_7$ 0·5 H_2O	56·75	6·80	9·45
D	51		(444·5)	56·97	7·09	8·86
Ii	202–204	−41·6°	$C_{32}H_{49}N_5O_8$	60·84	7·82	11·09
E	72		(631·8)	60·10	7·63	11·47
Ij	223–225	−11·8°[d]	$C_{25}H_{44}N_8O_8 \cdot H_2O$	49·82	7·69	18·59
E	71		(602·7)	50·35	8·04	17·97

[a] A Ethyl acetate–light petroleum, B toluene–light petroleum, C methanol–ether, D 2-propanol–light petroleum, E methanol–water; [b] in methanol; [c] solid foam; [d] in dimethylformamide.

ation of the active compound into the region of the pathophysiological process without interaction with other, healthy tissue or in cases when a prolonged action is required. Beside higher concentrations of the drug, this method of its synthesizing at the desired site in the organism is of decisive importance because it controls the physiological equilibrium of a given biological system. Pathophysiological processes, e.g. some inflammatory diseases[12-14] but also malignant tumors[15] are accompanied by just such disturbed enzymatic equilibria. By means of feedback, the increased key enzymatic activity controls the formation of the active drug from an inactive intermediate.

The proposed group of linear peptides–precursors represents a new type of potential drugs in which the N-terminal amino acid or peptide part functions as the so-called "peptide destination code number" and its amino acid quality (substrate specificity) is determined by the pertinent enzyme at the target site. Since the key compound, Alaptide, suggests a clinical use we may justifiably assume that the

TABLE II
Properties of free peptides

Compound Cryst. solvent[a]	M.p., °C Yield, %	$[\alpha]_D^{20}$ [b]	Formula[c] (M.w.)	Calculated/Found		
				% C	% H	% N
IIa	55–58	−12·9°	$C_{12}H_{21}N_3O_4 \cdot C_6H_8O_7$	46·62	6·30	9·06
A	78		(463·8)	46·98	7·12	8·48
IIb	108–109	−14·8°	$C_{14}H_{25}N_3O_4 \cdot C_6H_8O_7$	48·84	6·76	8·54
A	76		(491·8)	48·99	6·97	9·21
IIc	155–159	−25·3°	$C_{15}H_{27}N_3O_4 \cdot C_6H_8O_7$	49·86	6·97	8·31
A	58		(505·9)	49·19	6·84	8·49
IId	109–112	−10·9°	$C_{23}H_{39}N_3O_4 \cdot C_6H_8O_7 \cdot 0{\cdot}5\,H_2O$	52·71	7·74	9·22
A	65		(455·7)	52·33	7·30	8·77
IIe	115–118	−62·7°	$C_{18}H_{29}N_4O_5 \cdot C_6H_8O_7 \cdot 0{\cdot}5\,H_2O$	49·37	6·56	9·60
A	62		(583·9)	49·27	6·74	9·37
IIf	94–98	−4·7°	$C_{26}H_{33}N_3O_4 \cdot C_6H_8O_7$	59·68	6·42	6·52
A	48		(644·0)	59·46	6·69	6·38
IIi	212–213	−47·1°	$C_{24}H_{43}N_5O_6 \cdot C_2H_4O_2 \cdot 3\,H_2O$	51·05	8·73	11·45
B	66		(611·8)	50·72	7·96	12·07
IIj	203–205	−32·1°	$C_{25}H_{45}N_7O_6 \cdot C_2H_4O_2 \cdot H_2O$	52·49	8·32	15·87
B	78		(617·8)	52·91	8·12	15·81

[a] A Methanol–ether, B methanol–ethyl acetate; [b] in methanol; [c] $C_6H_8O_7$ citrate, $C_2H_4O_2$ acetate.

mentioned precursors, and an extended set with N-terminal peptidic moiety specific for other enzyme types (e.g. kathepsins), will enhance the interest in this type of prodrugs not only among clinical physicians but also enzymologists and molecular biologists.

EXPERIMENTAL

The melting points were determined on a Kofler block and were not corrected. The samples for analysis were dried in vacuo at 70 Pa over phosphorus pentoxide at 105°C. Compounds with melting points below 120°C were dried at room temperature. The optical rotation measurements were carried out in a Perkin–Elmer polarimeter; the concentration of the solutions varied between 0·2 and 0·3. The evaporation of the solvents from samples was carried out generally at reduced pressure. The standard procedure of treatment of the compound involves dissolving in ethyl acetate and stepwise extraction with 1 M-HCl water, 5% sodium hydrogen carbonate, water, drying over anhydrous sodium sulfate and evaporation. Thin-layer chromatography was carried out on silica gel plates (Kieselgel G, Merck) in system 1-butanol–acetic acid–water, 4 : 1 : 1 (S1) and 1-butanol–acetic acid–pyridine–water, 15 : 3 : 10 : 6 (S2).

Ethyl Benzyloxycarbonylleucyl-alanyl-1-amino-1-cyclopentanecarboxylate (*Id*)

N-Hydroxysuccinimide (2·3 g; 20 mmol) was added to a solution of benzyloxycarbonylleucyl -alanine (6·72 g; 20 mmol) in dimethylformamide (70 ml). After cooling to −5°C, N,N′-dicyclohexylcarbodiimide (4·4 g) was added and the mixture was stirred for 1 h with cooling (−5°C). A solution of ethyl 1-amino-1-cyclopentanecarboxylate, (liberated from the corresponding hydrochloride (3·86 g; 20 mmol) with N-ethylpiperidine (2·8 ml) in dimethylformamide (40 ml) was added and the reaction mixture was stirred at room temperature for 2 h. After 12 h the separated N,N′dicyclohexylurea was filtered off, washed with dimethylformamide and the filtrate was evaporated. The residue was dissolved in ethyl acetate and worked up in the usual manner. Crystallization from ethyl acetate (25 ml) and light petroleum (200 ml) afforded 7·6 g (80%) of the product. The peptides *Ia*—*Ig* were prepared similarly from the corresponding N-terminal dipeptides or the tripeptide Z-Gly-Pro-Ala.

Benzyloxycarbonylglycyl-prolyl-alanine Methyl Ester

To a solution of benzyloxycarbonylglycyl-proline pentachlorophenyl ester (5·55 g; 10 mmol) in dimethylformamide (30 ml) was added alanine methyl ester, liberated from 1·4 g (10 mmol) of the corresponding hydrochloride with N-ethylpiperidine (1·4 ml). After stirring for 5 h and standing for 12 h at room temperature, the solvent was evaporated and the residue was worked up in a standard manner. Crystallization from ethyl acetate and light petroleum afforded 1·95 g (50%) of product, m.p. 103—106°C. An analytical sample was crystallized from the same solvents, m.p. 105—108°C; $[\alpha]_D^{20}$ −100·1° (*c* 0·2, methanol). For $C_{19}H_{25}N_3O_6$ (191·4) calculated: 58·30% C, 6·44% H, 10·74% N; found: 58·79% C, 6·33% H, 10·69% N.

Benzyloxycarbonylglycyl-prolyl-alanine

A solution of benzyloxycarbonylglycyl-prolyl-alanine methyl ester (2·34 g; 6 mmol) in methanol (25 ml) and 1 M-NaOH (9 ml) were stirred at room temperature for 1 h, the mixture was adjusted to pH 5 with 1M-HCl, the methanol was evaporated and the remaining aqueous solution was

acidified to pH 2. The separated crystalline product was filtered and crystallized from methylene chloride (30 ml) and light petroleum (50 ml), yielding 1·8 g (79%) of product, m.p. 144—145°C. $[\alpha]_D^{20}$ −95·6° (*c* 0·2, methanol). For $C_{18}H_{23}N_3O_6$ (377·4) calculated: 57·29% C, 6·14% H, 11·13% N; found: 57·30% C, 6·29% H, 10·90% N.

N,O-Diacetyltyrosyl-alanine Methyl Ester

Ethyl chloroformate (5 ml) was added at −15°C to a solution of N,O-diacetyltyrosine (13·3 g; 50 mmol) and N-ethylpiperidine (7 ml) in tetrahydrofuran (100 ml). After cooling (−15°C) and stirring for 15 min, alanine methyl ester (liberated from 7·0 g (50 mmol) of its hydrochloride by N-ethylpiperidine (7 ml) was added. The mixture was stirred at 0°C for 2 h and then set aside at room temperature for 12 h. The solvent was evaporated and the residue was worked up in the usual manner. Crystallization from 2-propanol–light petroleum gave 10·6 g (60%) of product, m.p. 173—176°C; $[\alpha]_D^{20}$ −7·5° (*c* 0·2, methanol). For $C_{17}H_{22}N_2O_6$ (350.4) calculated: 57·62% C, 6·33% H, 8·00% N; found: 57·97% C, 6·28% H, 7·98% N.

N-Acetyltyrosyl-alanine

N,O-Diacetyltyrosyl-alanine methyl ester (7 g; 20 mmol) in methanol (100 ml) was hydrolyzed with 2M-NaOH (25 ml) for 1 h. The usual work-up furnished noncrystalline product; R_F 0·59 (S1), 0·67 (S2).

Methyl N,O-Diacetyltyrosyl-1-amino-1-cyclopentanecarboxylate

The title compound was prepared by the anhydride method from N,O-diacetyltyrosine and methyl 1-amino-1-cyclopentanecarboxylate in 43% yield. An analytical sample was crystallized from ethanol; m.p. 210—212°C. $[\alpha]_D^{20}$ + 5·7° (*c* 0·2, dimethylformamide). For $C_{20}H_{26}N_2O_6$ (390·5) calculated: 61·52% C, 6·71% H, 7·17% N; found: 61·71% C, 6·89% H, 7·22% N.

N-Acetyltyrosyl-1-amino-1-cyclopentanecarboxylic Acid

A solution of methyl N,O-diacetyltyrosyl-1-amino-1-cyclopentanecarboxylate (7·8 g; 20 mmol) in ethanol (75 ml) and water (50 ml) was made alkaline by addition of 2M-NaOH (40 ml) at room temperature. The course of hydrolysis was followed by electrophoresis. After 3 h the reaction solution was acidified with 2M-HCl to pH 3, the alcohol was evaporated and after 2 h the crystalline product was collected; yield 6·15 g (92%) of product melting at 252—254°C. For $C_{17}H_{22}N_2O_5$ (334·4) calculated: 61·06% C, 6·63% H, 8·38% N; found: 61·08% C, 6·81% H, 8·29% N.

Ethyl Leucyl-alanyl-1-amino-1-cyclopentanecarboxylate Citrate (*IId*)

A suspension of 5% Pd/C catalyst (0·8 g) in toluene (30 ml) was added to a solution of ethyl benzyloxycarbonylleucyl-alanyl-1-amino-1-cyclopentanecarboxylate (7·5 g; 15·8 mmol) in methanol (100 ml). The stirred (2 000 r.p.m.) mixture was hydrogenated in an autoclave at initial hydrogen pressure 2 MPa, at room temperature. After 10 min the autoclave was degassed, the catalyst was filtered off and the filtrate was taken down. The residue was dissolved in methanol (30 ml), the solution was mixed with citric acid (3·3 g) in methanol (20 ml) and the formed solution was gradually mixed with ether (300 ml). After 2 h, the separated citrate was collected on filter, washed with ether and dried in a desiccator over phosphorus pentoxide. Yield 4·7 g (65%), m.p. 109—112°C. An analogous method was used for the preparation of the final compounds *IIa—f* (see Table II).

Methyl N^{α}-Tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-1-amino-1-cyclopentanecarboxylate

Ethyl chloroformate (0·9 ml) was added to a solution of N^{α}-tert-butyloxycarbonyl-N^{ε}-benzyloxycarbonyllysine (9 mmol) and N-ethylpiperidine (1·3 ml) in dimethylformamide (30 ml), precooled to −20°C. After stirring at −15°C for 15 min, a solution of methyl alanyl-1-amino-1-cyclopentanecarboxylate (freshly liberated with N-ethylpiperidine (1·3 ml) at −15°C from the corresponding hydrochloride (230 mg; R_F 0·48 in S1) was added, the reaction mixture was stirred for 2 h at 0°C and then set aside for 12 h at room temperature. The solvent was evaporated and the residue was worked up in the usual manner. Crystallization from ethyl acetate and light petroleum afforded 440 mg (80%) of the product as amorphous foam. $[\alpha]_D^{20}$ −27·9° (*c* 0·2, methanol). For $C_{29}H_{44}N_4O_8 \cdot 2\,H_2O$ (612·6) calculated: 56·86% C, 7·90% H, 9·15% N; found: 57·33% C, 7·28% H, 9·38% N.

Methyl Acetylleucyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-1-amino-1-cyclopentanecarboxylate (*Ii*)

N,N′-Dicyclohexylcarbodiimide (1·6 g) was added at −15°C to a solution of acetylleucine (1·21 g; 7 mmol) and N-hydroxysuccinimide (805 mg) in dimethylformamide (15 ml) and stirred at −5°C for 1 h. To this solution was added a solution of methyl N^{ε}-benzyloxycarbonyllysyl-alanyl-1-amino-1-cyclopentanecarboxylate, liberated with N-ethylpiperidine from its hydrochloride (7 mmol; R_F 0·52 in S1, 0·63 in S2) which in turn was prepared from the corresponding tert-butyloxycarbonyl derivative (430 mg; 7 mmol) by acidolysis with 2·1M-HCl in acetic acid. The next day the reaction solution was taken down, the noncrystalline residue was dissolved in methanol (75 ml) and the product was precipitated with water (50 ml). An analytical sample was prepared analogously (see Table I). A similar procedure was used in the preparation of *Ij*.

Methyl Acetylleucyl-lysyl-alanyl-1-amino-1-cyclopentanecarboxylate (Acetate) (*IIi*)

A suspension of 5% Pd/C (0·2 g) in toluene (6 ml) was added to a mixture of methyl acetylleucyl-N^{ε}-benzyloxycarbonyllysyl-alanyl-1-amino-1-cyclopentanecarboxylate (1·9 g; 3 mmol). methanol (110 ml), toluene (20m l) and glacial acetic acid (15 ml). The hydrogenolysis was carried, out at hydrogen pressure of 1·5 MPa for 1 h. The catalyst was filtered off, the solvent was evaporated and the residue was treated with ethyl acetate. The precipitated product was collected on filter and washed with light petroleum. An analytical sample (see Table II) was obtained by precipitation from methanol and ethyl acetate.

Ethyl N^{α}-Tert-butyloxycarbonyl-N^{G}-nitroarginyl-alanyl-1-amino-1-cyclopentanecarboxylate

This compound was prepared from the corresponding N^{α}-tert-butyloxycarbonyl-N^{G}-nitroarginine analogously as described for the protected lysine tripeptide. Crystallization from ethyl acetate and light petroleum afforded the product in a yield of 62%. An analytical sample was crystallized from ethyl acetate; m.p. 112—115°C. $[\alpha]_D^{20}$ −20·4 (*c* 0·2, methanol). For $C_{22}H_{39}N_7O_8$ (529·6) calculated: 49·89% C, 7·42% H, 18·51% N; found: 50·14% C, 7·35% H, 18·29% N.

Ethyl N^{G}-Nitroarginyl-alanyl-1-amino-1-cyclopentanecarboxylate Hydrochloride

This compound was prepared in 88% yield similarly as described for the noncrystalline lysine tripeptide by acidolysis with 2·1M-HCl in glacial acetic acid followed by precipitation with ether. The hygroscopic product melted at 134—138°C (sintering at 124°C). R_F 0·33 (S1), 0·68 (S2),

$[\alpha]_D^{20}$ + 6·5° (*c* 0·2, methanol). For $C_{17}H_{32}N_6O_4 . H_2O$ (484·0) calculated: 42·19% C, 7·08% H, 20·26% N; found: 42·62% C, 7·06% H, 19·69% N.

Ethyl Acetylleucyl-arginyl-alanyl-1-amino-1-cyclopentanecarboxylate (Acetate) (*IIj*)

The pressure hydrogenation was carried out as described for *IIi* except that the pressure was 6 MPa and the time 3 h. The product was electrophoretically homogeneous, positive to Sakaguchi reagent and negative to ninhydrin (see Table II).

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