Proc. Estonian Acad. Sci. Chem., 1993, 42, 1, 1—9
https://doi.org/10.3176/chem.1993.1.01

UDC 547.466.1:547.725:547.156.2

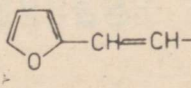
Katrin TRUMMAL, Nadezhda PABERIT,* Juri MITIN,***
Aavo AAVIKSAAR,# and Toomas VÄLIMÄE**

SYNTHESIS OF N-3-(2-FURYL)ACRYLOYLTRIPEPTIDES USING PENTAFLUOROPHENYL ESTERS

Abstract. A series of tripeptides, potential chromophoric substrates for various proteases, FA—*L*—Phe—*L*—*X*—*L*—Ala—OH, where FA is 3-(2-furyl)acryloyl, and *X* is Ala, Val, Leu, Ile, Phe, Thr, α-aminobutyryl, norvalyl, norleucyl, or α-aminocapryl, was synthesized through pentafluorophenyl esters. The synthesis was performed according to the following scheme:

$$Z\text{—}X\text{—OPfp} + H\text{—Ala—OBu}^t \rightarrow Z\text{—}X\text{—Ala—OBu}^t \xrightarrow{H_2/Pd} \rightarrow H\text{—}X\text{—Ala—OBu}^t \xrightarrow{Boc\text{—Phe—OPfp}} Boc\text{—Phe—}X\text{—Ala—OBu}^t \xrightarrow{TFA} \rightarrow H\text{—Phe—}X\text{—Ala—OH} \xrightarrow{FA\text{—OPfp}} FA\text{—Phe—}X\text{—Ala—OH.}$$

The obtained tripeptides were purified by HPLC and column chromatography on silica gel. Their structure and purity were confirmed by elemental analysis and in some cases by amino acid analysis and ^{13}C NMR spectra. The peptides have characteristic UV spectra.

Peptides and derivatives of amino acids containing the N-3-(2-furyl)-acryloyl group,  , can be used as chromophoric substrates for various proteolytic enzymes. The presence of this group with its characteristic UV spectrum adjacent to the bond to be cleaved enables to follow the course of the reaction by means of a convenient and precise spectrophotometric method.

These substrates have been suggested for chymotrypsin, subtilisin [1–3], and carboxypeptidase A [4]. N-3-(2-furyl)acryloyl-glycyl-*L*-leucinamide proposed by Feder [5] has become a standard substrate for thermolysin and other neutral metalloproteases. Dipeptide substrates containing the FA[a] group have been successfully used in the studies of substrate specificity of neutral metalloproteases [6–8].

* Eesti Teaduste Akadeemia Keemilise ja Bioloogilise Füüsika Instituut (Institute of Chemical Physics and Biophysics, Estonian Academy of Sciences). Akadeemia tee 23, EE-0026 Tallinn, Estonia.

** Институт белка Российской Академии наук (Institute of Protein Research, Russian Academy of Sciences). Россия. 142292 Пущино Московской обл.

\# Present address: Eesti Teaduste Akadeemia Eksperimentaalbioloogia Instituut (Institute of Experimental Biology, Estonian Academy of Sciences). EE-3051 Harjumaa, Harku, Estonia.

[a] Abbreviations used: Abu — α-aminobutyryl; Acp — α-aminocapryl (—NHCH(C_6H_{13})CO—); DCC — dicyclohexyl carbodiimide; DCHA — dicyclohexylamine; DMF — dimethylformamide; FA — 3-(2-furyl)acryloyl; Nle — norleucyl; Nva — norvalyl; Pfp — pentafluorophenyl; TEA — triethylamine; TFA — trifluoroacetic acid. Abbreviated designations of other amino acids and their derivatives obey the tentative rules of IUPAC—IUB Commission on Biochemical Nomenclature. Except where specified, the constituent amino acids were all of the *L*-configuration.

Table 1

Properties of the compounds synthesized for the first time

Number	Compound	Yield, %	Method of purification[a, b]	M.p., °C	$[\alpha]_{578}$ (DMF, c1), degree	TLC	
						R_f	Chrom. syst.[b]
1	2	3	4	5	6	7	8
I	Z—*D, L*—Acp—OH	48	Cr, AcOEt/hexane	91—94		0.43	*A*
II	Z—*L*—Acp—OH	80	Cr, hexane	64—67		0.72	*B*
III	Z—Abu—OPfp	82	Cr, ether/hexane	73—74	—25	0.70	*B*
IV	Z—Nva—OPfp	70	Rp, AcOEt/hexane	78—79	—23	0.93	*B*
V	Z—Nle—OPfp	55	CC, SiO_2/*B*	53—55	—20	0.83	*B*
VI	Z—Acp—OPfp	62	Cr, AcOEt/hexane	52—53		0.90	*B*
VII	Boc—Thr—OPfp	72	Cr, AcOEt/hexane	102—105	—2	0.59	*B*
VIII	Z—Abu—Ala—OBu^t	84	CC, Al_2O_3/*B*	79—80	—18	0.46	*B*
IX	Z—Val—Ala—OBu^t	68	Cr, ether/hexane	117—118		0.51	*B*
						0.61	*C*
X	Z—Nva—Ala—OBu^t	43	CC, Al_2O_3/*B*	89—90	—22	0.59	*B*
XI	Z—Leu—Ala—OBu^t	90	CC, Al_2O_3/*B*	73—74	—22	0.59	*B*
XII	Z—Ile—Ala—OBu^t	90	Rp, ether/hexane	111—113	—22	0.49	*D*
XIII	Z—Nle—Ala—OBu^t	82	CC, Al_2O_3/*B*	84—86	—22	0.60	*D*
XIV	Z—Acp—Ala—OBu^t	56	CC, Al_2O_3/*B*	74—76		0.58	*B*
XV	Z—Phe—Ala—OBu^t	84	Cr, hexane/ether	98—101	—22	0.36	*B*
XVI	Boc—Phe—Ala—Ala—OBu^t	72	Cr, AcOEt/hexane	141—142	—18	0.21	*B*
XVII	Boc—Phe—Abu—Ala—OBu^t	61	Cr, ether/hexane	121—124	—24	0.21	*B*
XVIII	Boc—Phe—Val—Ala—OBu^t	82	Cr, hexane	175—178	—26	0.35	*E*
XIX	Boc—Phe—Nva—Ala—OBu^t	55	Cr, ether/hexane	151—152		0.28	*B*
XX	Boc—Phe—Leu—Ala—OBu^t	69	Rp, AcOEt/hexane	114—116		0.23	*B*
XXI	Boc—Phe—Ile—Ala—OBu^t	82	Cr, ether/hexane	125—129	—32	0.25	*B*
XXII	Boc—Phe—Nle—Ala—OBu^t	92	Cr, AcOEt	140—143	—30	0.27	*B*
XXIII	Boc—Phe—Acp—Ala—OBu^t	60	Rp, AcOEt/hexane	105—112		0.22	*B*

Table 1 (continued)

1	2	3	4	5	6	7	8
XXIV	Boc—Phe—Phe—Ala—OBu^t	85	Cr, ether/hexane	146—148		0.39	*E*
XXV	Boc—Phe—Thr—Ala—OH	51	CC, SiO_2/*F*	160—170		0.26	*F*
XXVI	FA—OPfp	72	Cr, hexane-ether 4:1	44—45		0.89	*G*
XXVII	FA—Phe—Ala—Ala—OH	32	HPLC	205—208	—34	0.44	*G*
						0.76	*H*
XXVIII	FA—Phe—Abu—Ala—OH	29	HPLC, CC, SiO_2/*I*	204—211	—33	0.41	*G*
XXIX	FA—Phe—Val—Ala—OH	35	HPLC, CC, SiO_2/*I*	213—220	—18	0.59	*G*
						0.82	*H*
XXX	FA—Phe—Nva—Ala—OH	30	CC, SiO_2/*I*	179—189	—44	0.53	*G*
XXXI	FA—Phe—Leu—Ala—OH	15	HPLC, CC, SiO_2/*I*	128...	—36	0.50	*G*
				163....		0.78	*H*
				189—195[c]			
XXXII	FA—Phe—Ile—Ala—OH	44	HPLC	219—225	—42	0.48	*G*
XXXIII	FA—Phe—Nle—Ala—OH	52	HPLC	182—186	—39	0.50	*G*
XXXIV	FA—Phe—Acp—Ala—OH	65	Rc, AcOEt	198—203	—48	0.46	*G*
XXXV	FA—Phe—Phe—Ala—OH	19	HPLC, CC, SiO_2/*I*	220—227	—68	0.43	*G*
						0.79	*H*
XXXVI	FA—Phe—Thr—Ala—OH	32	HPLC, CC, SiO_2/*I*	168—175	—32	0.56	*G*
						0.78	*H*

[a] Cr — crystallization; Rp — reprecipitation; Rc — recrystallization; CC — preparative column chromatography, sorbent/eluent.
[b] The composition of chromatographic systems see in "Experimental."
[c] The compound melted at 128 °C, crystallized again at 163 °C, and melted at 189—195 °C.

The use of tri- and longer peptide substrates would probably enable to gain a better insight into the mechanism of peptidases. However, only a few examples of the synthesis of longer peptides containing the FA group can be found. The synthesis of a series of FA-tripeptides, substrates of thermolysin, has been described in [9]. Single FA-tripeptides have been synthesized in [10] (a substrate of thermolysin) and [11, 12] (substrates of elastase from *Pseudomonas aeruginosa*). These peptides contained the residues of glycine, alanine, leucine, and phenylalanine in various combinations.

The aim of the present work was to synthesize a number of new tripeptides, containing the FA group, with the formula FA—Phe—*X*—Ala—OH, where *X* is Ala, Abu, Val, Nva, Leu, Ile, Nle, Acp, Phe, or Thr. These peptides can be used as a systematic series of chromophoric substrates for studying the substrate specificity and the mechanism of action of neutral metalloproteinases, chymotrypsin, and other proteolytic enzymes.

In [9, 10, 12] the synthesis of FA-tripeptides was performed using activated N-hydroxysuccinimide esters. The FA group was introduced using 3-(2-furyl)acrylic acid N-hydroxysuccinimide ester with 40—60% yields. The use of *p*-nitrophenyl ester of 3-(2-furyl)acrylic acid has also been described [11], the yields were about 60%.

In the present work peptide bonds were mostly synthesized using activated pentafluorophenyl esters [13]. The synthesis was performed according to the following scheme:

$$Z\text{—}X\text{—OPfp} + H\text{—Ala—OBu}^t \rightarrow Z\text{—}X\text{—Ala—OBu}^t \xrightarrow{H_2/Pd} \rightarrow H\text{—}X\text{—Ala—OBu}^t \xrightarrow{Boc\text{—Phe—OPfp}} Boc\text{—Phe—}X\text{—Ala—OBu}^t \xrightarrow{TFA} \rightarrow H\text{—Phe—}X\text{—Ala—OH} \xrightarrow{FA\text{—OPfp}} FA\text{—Phe—}X\text{—Ala—OH}.$$

The synthesis of the substrate with *X*=Thr was started from Boc—Thr—OPfp.

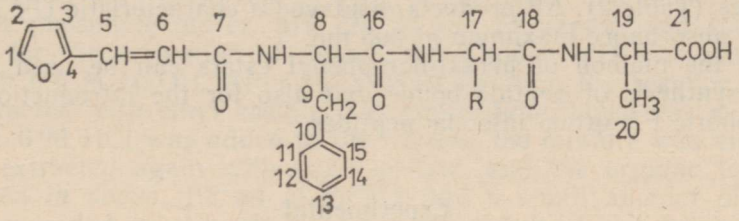
The synthesis of pentafluorophenyl esters of N-acylamino acids was performed from N-acylamino acids and pentafluorophenol with the aid of DCC [13]. The esters synthesized for the first time are characterized in Table 1 (compounds III—VII).

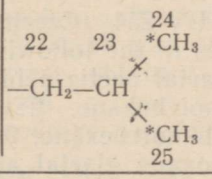
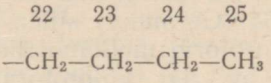
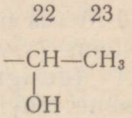
All dipeptides of the type Z—*X*—Ala—OBu^t, as well as Boc—Thr—Ala—OBu^t, were prepared using pentafluorophenyl esters with the exception of the derivative of Ile, which was synthesized *via* N-hydroxysuccinimide ester. Most dipeptides were synthesized and described for the first time (Table 1, compounds VIII—XV).

In the synthesis of the protected tripeptides (Table 1, XVI—XXV) Boc—Phe—OPfp was used as the carboxylic component, and the protecting groups were removed from the resulting tripeptides by treatment with TFA.

At the last stage of the synthesis the chromophoric FA group was introduced into the substrates using pentafluorophenyl ester of 3-(2-furyl)acrylic acid (Table 1, XXVI). All the obtained FA-tripeptides crystallized easily, but as they contained impurities that could not be removed by recrystallization, they were further purified by HPLC technique and column chromatography on silica gel. The obtained products were homogeneous according to HPLC and TLC data, and they are characterized in Table 1 (compounds XXVII—XXXVI). Low yields in some cases were probably caused by the lability of the FA group leading to great losses during purification, especially in HPLC.

Carbon-13 chemical shifts for the peptides FA—Phe—*X*—Ala—OH



	X		
	Leu	Nle	Thr
	R		
			
Carbon atom	Chemical shifts, ppm from internal TMS		
C 1	145.7	145.7	145.5
C 2	115.0	115.0	115.2
C 3	113.2	113.2	113.2
C 4	152.6	152.5	152.3
C 5	129.1	129.1	129.5
C 6	118.9	118.8	118.5
C 7	168.3	168.3	168.3
C 8	56.1	56.1	56.3
C 9	38.7	38.7	38.6
C 10	138.3	138.3	138.9
C 11, 15	129.4	129.3	129.5
C 12, 14	130.3	130.2	130.2
C 13	127.7	127.6	127.8
C 16	173.5	173.5	173.5
C 17	53.0	54.6	59.4
C 18	174.0	173.5	171.3
C 19	50.0	50.0	50.0
C 20	17.8	18.1	18.1
C 21	176.0	176.8	176.1
C 22	41.9	32.8	68.3
C 23	25.6	28.7	19.6
C 24	23.4*	23.3	
C 25	22.1*	14.2	

* assignments can be interchanged

The structure and purity of all FA-tripeptides were confirmed by elemental analysis. Amino acid analysis was performed for the compounds with X=Val (XXIX), Leu (XXXI), and Phe (XXXV). ^{13}C NMR spectra of some peptides (with X=Leu (XXXI), Nle (XXXIII), and Thr (XXXVI)) were obtained. The spectra confirmed their suggested structures (Table 2). All products displayed a characteristic UV spectrum with the absorbance maximum at 305 nm.

Thus the method of pentafluorophenyl esters can be used not only for the synthesis of peptide bonds, but also for the introduction of the chromophoric FA group into the peptides.

Experimental

Materials

Amino acids and their derivatives were obtained from Reanal and Serva, benzyloxycarbonylchloride was purchased from Serva, pentafluorophenol from Reakhim, and DCC from Fluka. Z—Abu—OH, Z—Nva—OH, Z—Nle—OH, and Z—D, L—Acp—OH were synthesized using Z—Cl [14].

Methods

TLC analyses were performed on Silufol UV-254 (Czechoslovakia) and Merck 60 F 254 (Germany) silica gel plates in the following solvent systems (v/v): chloroform:methanol:hexane:glacial acetic acid, 9:0.5:5:0.1 (designated by *A*); chloroform:methanol:hexane, 9:1:10 (*B*); hexane:ethyl acetate, 2:1 (*C*); chloroform:methanol:hexane, 9:1:5 (*D*); chloroform:methanol, 95:5 (*E*); benzene:1,4-dioxane:glacial acetic acid, 9:3:0.4 (*F*); chloroform:methanol, 9:1 (*G*); *n*-butanol:glacial acetic acid:pyridine:water, 15:3:10:12 (*H*); chloroform:methanol, 3:1 (*I*). Ultraviolet light, ninhydrin, and iodide-hypochlorite-*o*-tolidine were used to detect the chromatographic components.

The melting points were determined on a Boetius melting point apparatus (GDR) and are reported uncorrected. Elemental analyses were performed on a Perkin-Elmer 240 B elemental analyser (USA). Optical rotation was determined on a Polamat A polarimeter (GDR) at room temperature; amino acid analyses were carried out on a Biotronik LC 2000 analyser (FRG). High-voltage paper electrophoresis was performed on a OE-201 apparatus (Hungary) in 30% acetic acid. Preparative column chromatography was carried out on Silica gel L40/100 and on Al_2O_3 L5/40 (Chemapol, Czechoslovakia) with the same solvent systems as TLC.

Analytical HPLC was performed on a DuPont liquid chromatograph (USA) using an ODS reverse-phase column. Preparative HPLC was carried out on a Silasorb C-18 (Czechoslovakia) reverse-phase column (3×50 cm) with the flow-rate of 20 ml/min using a DuPont 880 high-pressure pump and a 2138 Uvicord S detector at 276 nm. Linear gradient of 10—50% (v/v) aqueous ethanol was used in chromatographing the FA—Phe—*X*—Ala—OH peptides.

The UV spectra were recorded on a Beckman UV 5260 spectrophotometer (USA). The ^{13}C NMR spectra were recorded at 125.76 MHz on an AM-500 Bruker spectrometer (FRG) at room temperature using CD_3OD as solvent and tetramethylsilane as internal standard. The assignment of ^{13}C chemical shifts, based on the chemical shift data for amino acids and model compounds and carbon-13 signal multiplicities as determined from J-modulated spectra, is straightforward.

Synthesis

Z—*L*—Acp—OH. Z—*D, L*—Acp—OCH_3 was synthesized from Z—*D, L*—Acp—OH as described in [15]. Z—*L*—Acp—OH was obtained from Z—*D, L*—Acp—OCH_3 by papain hydrolysis. 3.5 g of Z—*D, L*—Acp—OCH_3 (oily product) was dissolved in 4 ml of methanol and added to 20 ml of 0.1 M phosphate buffer, pH 7.0. 50 mg of papain and 100 mg of cysteine for the activation of papain were added. pH was maintained at 7 with 0.1 M NaOH. After the end of titration the reaction mixture was extracted with ethyl acetate, and the organic layer was evaporated *in vacuo*. 6 M HCl was added to the residue, the mixture was vigorously shaken, extracted again with ethyl acetate, and the organic layer was evaporated *in vacuo*. 1.5 ml of DCHA and a small amount of hexane were then added to the oily product, and 2.3 g of crystalline Z—*L*—Acp—OH·DCHA was obtained. The latter was dissolved in ethyl acetate, washed with 10% $NaHSO_4$, and twice with water. The solvent was evaporated *in vacuo* and the remaining oil crystallized after it was kept under hexane at 4 °C for a week. 1.3 g of Z—*L*—Acp—OH was obtained.

Z—*X*—Ala—OBu^t (*X*=Ala, Abu, Val, Nva, Leu, Nle, Acp, or Phe). 1 eq. of Z—*X*—OPpf and 1.2 eq. of H—Ala—OBu^t·HCl (Reanal) were dissolved in DMF, 1.25 eq. of TEA was added, and the reaction mixture was stirred at room temperature for 2 hours. Ethyl acetate was then added and the resulting mixture was washed successively with 10% $NaHSO_4$, water, saturated solution of $NaHCO_3$, and twice with water. The organic layer was evaporated with dioxane *in vacuo*. Mostly oily products were obtained and were further treated with hexane for crystallization. In cases when the compounds did not crystallize entirely, the oily products were purified by column chromatography on Al_2O_3.

Boc—Thr—Ala—OBu^t was obtained similarly from Boc—Thr—OPfp and H—Ala—OBu^t·HCl and used without purification as an oily product.

Z—Ile—Ala—OBu^t was prepared in a similar manner from Z—Ile—ONSu (Serva) and HCl·H—Ala—OBu^t.

Boc—Phe—*X*—Ala—OBu^t (*X*=Ala, Abu, Val, Nva, Leu, Ile, Nle, Acp, or Phe). *Z*—*X*—Ala—OBu^t was subjected to catalytic hydrogenolysis with palladium black in dioxane for 1.5—3 hours; the completeness of the reaction was checked by TLC. The purity of the product was verified by electrophoresis. The solvent was evaporated, the remaining oil was dissolved in DMF and coupled with Boc—Phe—OPfp as described above for Z—*X*—Ala—OBu^t. pH of the reaction mixture was adjusted to 8 with TEA.

Boc—Phe—Thr—Ala—OH. Boc—Thr—Ala—OBu^t was kept in TFA at room temperature for 1 hour, then the reaction mixture was evaporated, and the oily product was crystallized by rubbing it with a glass stick in absolute ether. The purity of the product was checked by electrophoresis. The obtained H—Thr—Ala—OH·TFA was coupled with Boc—Phe—OPfp as described above for the synthesis of Z—*X*—Ala—OBu^t.

FA—OPfp. 2.76 g (20 mmol) of 3-(2-furyl)acrylic acid and 4.05 g (22 mmol) of pentafluorophenol were dissolved in 30 ml of the 1:1 (v/v) mixture of ethyl acetate and dioxane, the solution was cooled to +2 °C and, upon stirring, 4.53 g (22 mmol) of DCC was added. After stirring the mixture for 2 hours at 2 °C, precipitate of dicyclohexyl urea was filtered off and washed with ethyl acetate and dioxane. The filtrate was evaporated, the residue was dissolved in ethyl acetate, and an additional amount of urea was filtered off. The solution was evaporated again, the remaining brown oily product was treated with the hexane—

ether mixture (4:1, v/v), the forming light beige crystals were filtered off and washed with the same cooled mixture. The filtrate was evaporated and an additional crystallization was carried out in the same manner. Total yield was 4.41 g (72.5%).

FA—Phe—*X*—Ala—OH. In order to remove Boc— and —OBu^t groups, compounds XVI—XXV were treated with TFA. The obtained products, except for the Thr derivative, crystallized after having been washed or rubbed with absolute ether. The purity of the products was checked by electrophoresis. Then the products were coupled with FA—OPfp.

1 eq. of a tripeptide as TFA salt (with Thr, 1.1 eq.) and 1 eq. of FA—OPfp were suspended in DMF and, upon stirring, 2—3 eqs. of TEA were added to bring pH up to 8. The reagents dissolved gradually, and stirring was continued at room temperature for at least 1.5 hours. After that ethyl acetate was added and the mixture was successively washed twice with 10% $NaHSO_4$ and 2 or 3 times with water. The organic layer was evaporated with dioxane *in vacuo*. The product was either crystalline or an oil that crystallized after it was treated with organic solvents (ethanol, ethyl acetate, or hexane—ether). With *X*=Ile, Nle, Acp, Phe, or Thr, the product partly precipitated upon washing the reaction mixture with aqueous solutions. These precipitates were filtered out, washed with water, and added to the products obtained after the evaporation of organic layers. All the obtained FA-tripeptides were purified by crystallization, HPLC, and column chromatography on silica gel, as shown in Table 1.

Acknowledgements

The authors are grateful to Mrs. K. Kanger and Mrs. T.-M. Laht for carrying out amino acid analyses.

REFERENCES

1. *Bernhard, S. A., Lau, S. J., Noller, H. F.* Spectrophotometric identification of acyl enzyme intermediates. — Biochemistry, 1965, **4**, 6, 1108—1118.
2. *Charney, E., Bernhard, S. A.* Optical properties and the chemical nature of acyl-chymotrypsin linkages. — J. Amer. Chem. Soc., 1967, **89**, 11, 2726—2733.
3. *Noller, H. F., Bernhard, S. A.* Isolation and structural determination of chromophoric acyl peptides from subtilisin enzymes. — Biochemistry, 1965, **4**, 6, 1118—1126.
4. *McClure, W. O., Neurath, H.* The reaction of carboxypeptidase A with chromophoric substrates. — Biochemistry, 1966, **5**, 4, 1425—1438.
5. *Feder, J.* A spectrophotometric assay for neutral protease. — Biochem. Biophys. Res. Commun., 1968, **32**, 2, 326—332.
6. *Feder, J., Schuck, J. M.* Studies on the *Bacillus subtilis* neutral-protease- and *Bacillus thermoproteolyticus* thermolysin-catalyzed hydrolysis of dipeptide substrates. — Biochemistry, 1970, **9**, 14, 2784—2791.
7. *Pank, M., Kirret, O., Paberit, N., Aaviksaar, A.* Hydrophobic interaction in thermolysin specificity. — FEBS Letters, 1982, **142**, 2, 297—300.
8. *Панк М., Кирет О., Паберит Н., Аавиксаар А.* Специфичность нейтральной протеазы *Bacillus brevis* в реакции с дипептидными субстратами. — Изв. АН ЭССР. Хим., 1983, **32**, 3, 157—162.
9. *Blumberg, S., Vallee, B. L.* Superactivation of thermolysin by acylation with amino acid N-hydroxysuccinimide esters. — Biochemistry, 1975, **14**, 11, 2410—2419.
10. *Fukuda, M., Kunugi, S.* Pressure dependence of thermolysin catalysis. — Eur. J. Biochem., 1984, **142**, 3, 565—570.
11. *Poncz, L.* Substrate inhibition of *Pseudomonas aeruginosa* elastase by 3-(2-furyl)-acryloyl-glycyl-*L*-phenylalanyl-*L*-phenylalanine. — Arch. Biochem. Biophys., 1988, **266**, 2, 508—515.

12. *Saulnier, J. M., Curtil, F. M., Duclos, M.-C., Wallach, J. M.* Elastolytic activity of *Pseudomonas aeruginosa* elastase. — Biochim. Biophys. Acta, 1989, **995**, 3, 285—290.
13. *Kisfaludy, L., Löw, M., Nyéki, O., Szirtes, T., Schön, I.* Die Verwendung von Pentafluorphenylestern bei Peptidsynthesen. — Lieb. Ann. Chem., 1973, 9, 1421—1429.
14. *Greenstein, J. P., Winitz, M.* Chemistry of the Amino Acids. John Wiley & Sons, Inc., New York—London, 1961, Chap. 5.
15. *Brenner, M., Huber, W.* Herstellung von α-Aminosäureestern durch Alkoholyse der Methylester. — Helv. Chim. Acta, 1953, **36**, 5, 1109—1115.

Received
September 29, 1992

Katrin TRUMMAL, Nadežda PABERIT, Juri MITIN, Aavo AAVIKSAAR, Toomas VÄLIMÄE

N-3-(2-FURÜÜL)AKRÜLOÜÜLTRIPEPTIIDIDE SÜNTEES PENTAFLUOROFENÜÜLESTRITE MEETODIL

Pentafluorofenüülestrite meetodi abil sünteesiti tripeptiidide rida üldvalemiga FA—*L*—Phe—*L*—*X*—*L*—Ala—OH, kus FA on 3-(2-furüül)akrüloüül ja *X* on Ala, Val, Leu, Ile, Phe, Thr, α-aminobutürüül, norvalüül, norleutsüül või α-aminokaprüül. Sünteesitud peptiidid on kasutatavad proteaaside kromofoorsete substraatidena. Süntees tehti järgmise skeemi kohaselt:

$$Z\text{—}X\text{—OPfp} + H\text{—Ala—OBu}^t \rightarrow Z\text{—}X\text{—Ala—OBu}^t \xrightarrow{H_2/Pd} \rightarrow H\text{—}X\text{—Ala—OBu}^t \xrightarrow{Boc\text{—Phe—OPfp}} Boc\text{—Phe—}X\text{—Ala—OBu}^t \xrightarrow{TFA} \rightarrow H\text{—Phe—}X\text{—Ala—OH} \xrightarrow{FA\text{—OPfp}} FA\text{—Phe—}X\text{—Ala—OH}.$$

Saadud FA-tripeptiidid puhastati kõrgsurvevedelikukromatograafia ja kolonnikromatograafiaga silikageelil. Nende struktuur ja puhtus tõestati elementanalüüsiga, mõnede ühendite puhul ka aminohapete analüüsi ja ^{13}C TMR-spektritega. Sünteesitud peptiididel on iseloomulik UV-spekter.

Катрин ТРУММАЛ, Надежда ПАБЕРИТ, Юрий МИТИН, Аавo ААВИКСААР, Тоомас ВЯЛИМЯЭ

СИНТЕЗ N-3-(2-ФУРИЛ)АКРИЛОИЛТРИПЕПТИДОВ МЕТОДОМ ПЕНТАФТОРФЕНИЛОВЫХ ЭФИРОВ

Методом пентафторфениловых эфиров синтезирована серия трипептидов — потенциальных хромофорных субстратов различных протеаз — с общей формулой FA—*L*—Phe—*L*—*X*—*L*—Ala—OH, где FA — 3-(2-фурил)акрилоил, *X*=Ala, Val, Leu, Ile, Phe, Thr, α-аминобутирил, норвалил, норлейцил или α-аминокаприл. Синтез проведен по схеме:

$$Z\text{—}X\text{—OPfp} + H\text{—Ala—OBu}^t \rightarrow Z\text{—}X\text{—Ala—OBu}^t \xrightarrow{H_2/Pd} \rightarrow H\text{—}X\text{—Ala—OBu}^t \xrightarrow{Boc\text{—Phe—OPfp}} Boc\text{—Phe—}X\text{—Ala—OBu}^t \xrightarrow{TFA} \rightarrow H\text{—Phe—}X\text{—Ala—OH} \xrightarrow{FA\text{—OPfp}} FA\text{—Phe—}X\text{—Ala—OH}.$$

Полученные FA-трипептиды очищены ВЭЖХ и колоночной хроматографией на силикагеле. Их строение и чистота подтверждены элементным анализом, а для некоторых соединений — еще аминокислотным анализом и спектрами ^{13}C-ЯМР. Синтезированные пептиды обладают характерным УФ-спектром.