CROATICA CHEMICA ACTA CCACAA 54 (2) 233—240 (1981)

CCA-1279

YU ISSN 0011-1643
UDC 547.466.46
Original Scientific Paper

Polyfunctional Lysine Containing Tri- and Tetra-peptides

V. Škarić, J. Makarević, and Đ. Škarić

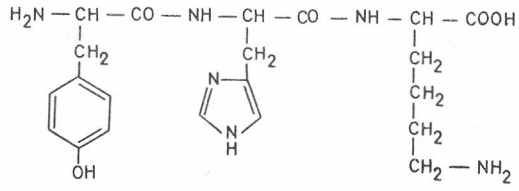
Laboratory of Stereochemistry and Natural Products, »Ruđer Bošković« Institute, 41001 Zagreb, Croatia, Yugoslavia

Received July 10, 1980

The synthesis of glycyl-L-histidyl-L-lysine, glycyl-L-lysyl-L-histidine, L-tyrosyl-L-histidyl-L-lysine, *N*-,*N*-ε,-*N*-ε-tribenzyloxycarbonyl derivative of L-histidyl-L-lysyl-L-lysine methyl ester, L-histidyl-L-tyrosyl-L-lysine, and L-tyrosyl-L-histidyl-L-lysyl-L-lysine by adoption of the azide coupling approach is described.

Hypothalamic peptides continue to be a topic of very great interest[1]. The difficulties in identifying and isolating all protein hormones and releasing factors from anterior pituary glands are not only due to their minute quantities in tissue but also to bioassay problems. Synthesis and assay of oligopeptide analogues of these biologically important molecules should allow a better molecular interpretation of their physiology. This is especially true for the releasing hormone peptides because they can be expected to guide us even in the design of antagonists of greater interest than the hormone themselves. Looking back, fundamental advances in endocrinology and physiology often became possible after an active peptide had been made available synthetically in large quantity and adequate purity.

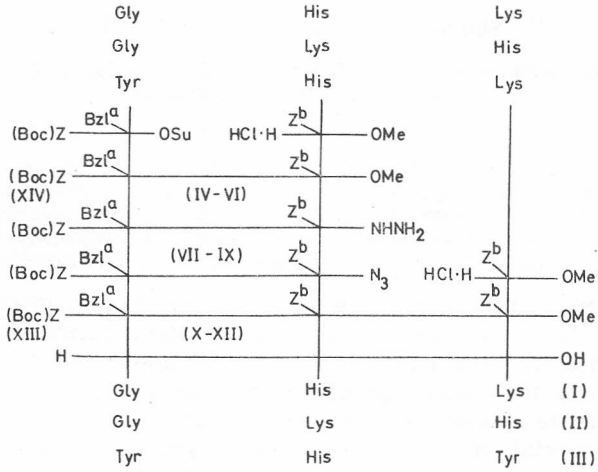
Some of the lysine containing tripeptide growth promoting factors have recently been isolated from human serum and identified as glycyl-L-histidyl-L-lysine[2–4] (I) and glycyl-L-lysyl-L-histidine[1] (II). Synthetic tripeptide I, obtained by a solid-phase method[2], also possessed the protective and growth stimulating properties of a factor normally present in human serum. In recent years, largely as a result of our continuing search for biologically active oligopeptides[5–7], we have synthesized the lysine containing tripeptides I and II by classical fragment condensation in order to compare them with the polyfunctional L-tyrosyl-L-histidyl-L-lysine (III) in biological functions related to those of growth hormones. Results reported earlier[2–4] indicated that



III

normal human serum contains a highly polar tripeptide which stimulates essential biosynthetic effects of serum in mammalian cell cultures. Preliminary biological testing with tripeptides I, II, and III revealed that the polyfunctional tripeptide III to stimulate, even in nanometric concentrations, the in vitro incorporation of labelled sulphate into embryonic chick cartilage[8,9], without the presence of mediating serum factors. It was also established that tripeptide III inhibited the incorporation of ^{3}H-thymidine into lymphocytes.

SCHEME 1



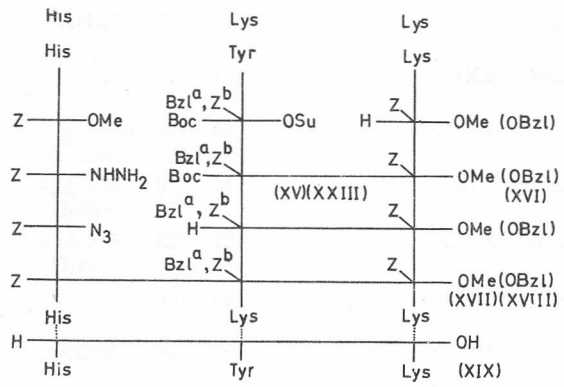
Z = benzyloxycarbonyl, Bzl = benzyl, OSu = hydroxysuccinimide, Gly = glycine, His = L-histidine, Lys = L-lysine, Tyr = L-tyrosine, [a] *O*-protection of Tyr only, [b] *N*-ε--protection of Lys only.

Scheme 1 outlines the syntheses of tripeptides I, II, and III. The benzyloxycarbonyl derivatives of glycyl-L-histidine[10,11] (IV), glycyl-*N*-ε-benzyloxycarbonyl-L-lysine[12] (V), and *O*-benzyl-L-tyrosyl-L-histidine (VI) methyl esters were first converted into their corresponding hydrazides VII[10,13], VIII[12], IX and then to their active azides. The imidazole nitrogen of histidine was kept unprotected. The azides, prepared from hydrazides VII and IX, were then coupled[10] with the *N*-ε-benzyloxycarbonyl-L-lysine methyl ester[14] to yield *N*-benzyloxycarbonyl--glycyl-L-histidyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (X) and *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-histidyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XII), respectively. The azide, obtained from hydrazide VIII, in reaction with L-histidine methyl ester produced *N*-benzyloxycarbonyl-glycyl--*N*-ε-benzyloxycarbonyl-L-lysyl-L-histidne methyl ester (XI).

The *N*-protected tripeptide methyl esters X, XI, and XII were first saponified to the corresponding *N*-protected tripeptide acids and their *N*-debenzyloxycarbonyls removed by hydrogenolysis to give the free tripeptides I, II, and III. The sequence of tripeptide I, as its *N*-*t*-butoxycarbonyl derivatives XIII was also built up by elongation of *N*-*t*-butoxycarbonyl-glycyl-L-histidine methyl ester (XIV).

In order to prepare polyfunctional tripeptides in which the histidyl unit was followed by two trifunctional amino acids the *N*-*t*-butoxycarbonyl derivatives of *N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine

SCHEME 2



[a] *O*-protection of Tyr only, [b] *N*-ε-protection of Lys only.

methyl ester[15] (XV) and *O*-benzyl-L-tyrosyl-*N*-ε-benzyloxycarbonyl-L-lysine benzyl ester (XVI), being first *N*-deprotected by trifluoroacetic acid, was reacted with the freshly prepared *N*-benzyloxycarbonyl-L-histidine azide[13] under coupling conditions known to lead to minimal racemization. Scheme 2 summarizes the synthetic pathways for *N*-benzyloxycarbonyl-L-histidyl-*N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XVII) and *N*-benzyloxycarbonyl-L-histidyl-*O*-benzyl-L-tyrosyl-*N*-ε-benzyloxycarbonyl-L--lysine benzyl ester (XVIII), the latter being blocked with hydrogenolytically removable *N*-benzyloxycarbonyl and *O*-benzyl groups. Thus, the hydrogenolysis of XVIII in ethanol over a mixture of 10% Pd/C and Pd-black catalyst produced L-histidyl-L-tyrosyl-L-lysine (XIX) in high yields. If acetic acid (2% v/v) was added the reactions was accelerated.

SCHEME 3

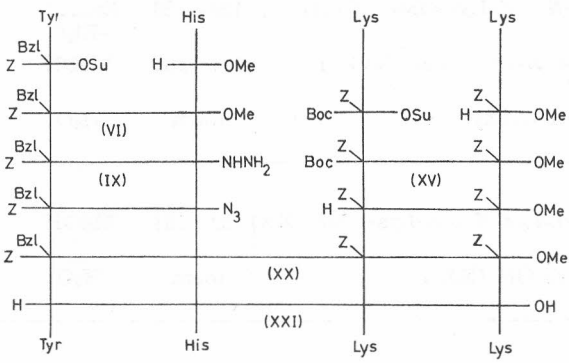


TABLE I

Compound	M. p./°C	Solvent	R_F	Yield %	Mol. weight
			Dipeptides		
Z-Tyr-Bzl-His-OMe (VI)	115—117	EtOAc	0.40	78	556.6
Z-Tyr-Bzl-His-$NHNH_2$ (IX)	207—210	MeOH		85	556.6
N-ε-Z-Boc-Lys-*N*-ε-Z-Lys-OMe (XV)	108—110 (95—97)[14]	EtOAc-*n*-hexane	0.50	93	656.76
Boc-Tyr-Bzl-*N*-ε-Z-Lys-OBzl (XVI)	117—119	EtOAc-Et_2O	0.65	62.6	723.84
Boc-Tyr-Bzl-His-OMe (XXII)	121—123	EtOAc-Et_2O	0.70	77	522.58
Boc-Tyr-Bzl-*N*-ε-Z-Lys-OMe (XXIII)	165—166	EtOAc	0.87	91.6	647.74
Z-Tyr-Bzl-*N*-ε-Z-Lys-OMe (XXIV)	152—154	EtOAc	0.85	81	681.76
Z-Tyr-Bzl-*N*-ε-Z-Lys-$NHNH_2$ (XXV)	169—172	EtOH	0.60	88	681.76
			Tripeptides		
H-Gly-His-Lys-OH (I)	foam	H_2O		83/87*	358.40
H-Gly-Lys-His-OH (II)	foam	H_2O		74/89*	358.40
H-Tyr-His-Lys-OH (III)	foam	H_2O		100/94*	446.50
Z-Gly-His-*N*-ε-Z-Lys-OMe (X)	165—166	EtOH	0.70	75	622.70
Z-Gly-*N*-ε-Z-Lys-His OMe (XI)	125—128	EtOAc-Et_2-O-*n*-hexane	0.65	70	622.66
Z-Tyr-Bzl-His-*N*-ε-Z-Lys-OMe (XII)	162—165	60% EtOH	0.33	70	836.91
Boc-Gly-His-*N*-ε-Z-Lys-OMe (XIII)	158—160	EtOH	0.40	59	588.65
Z-His-*N*-ε-Z-Lys-*N*-ε-Z-Lys-OMe (XVII)	125—126	EtOAc-Et_2O	0.38	34.2	827.91
Z-His-Tyr-Bzl-*N*-ε-Z-Lys-OBzl (XVIII)	187—189	MeOH	0.55	70	894.99
H-His-Tyr-Lys-OH (XIX)	foam	H_2O		95	446.50
			Tetrapeptides		
Z-Tyr-Bzl-His-*N*-ε-Lys-*N*-ε-Z-Lys-OMe (XX)	182—184	MeOH	0.40	88	1081.90
H-Tyr-His-Lys-Lys-OH (XXI)	foam	H_2O		59/93*	574.67

TABLE I (Cont.)

Formula	*Anal.* Calc'd: Found: %C	%H	%N	ν_{max}/cm^{-1}
$C_{31}H_{32}N_4O_6$	66.89 66.71	5.80 6.01	10.07 10.28	3333, 1754, 1721, 1656, 1613, 1543, 1515, 1236br, 740, 697
$C_{30}H_{32}N_6O_5$	64.73 64.52	5.80 5.49	15.10 14.83	3378, 1704, 1661, 1639, 1534br, 1513br, 1263br, 1233br, 740, 694
$C_{34}H_{48}N_4O_9$	62.17 62.20	7.37 7.60	8.53 8.82	3444, 1777, 1695, 1668, 1537br, 1257, 754, 693
$C_{42}H_{49}N_3O_8$	69.68 69.57	6.82 6.97	5.81 6.12	3408, 1728, 1684, 1648, 1608, 1533br, 1519br, 1510, 1240, 790, 689
$C_{28}H_{34}N_4O_6$	64.35 64.08	6.56 6.35	10.72 10.91	3333, 1748, 1678, 1647, 1613, 1250br, 736, 695
$C_{36}H_{45}N_3O_8$	66.75 66.59	7.00 7.13	6.49 6.76	3333, 1736, 1678, 1647, 1608, 1585, 1534, 1508, 1252br, 734, 694
$C_{39}H_{43}N_3O_8$	68.70 68.94	6.36 6.65	6.16 6.18	3333, 1727, 1684, 1642, 1582, 1527br, 1244br, 735, 694
$C_{38}H_{43}N_5O_7$	66.94 66.70	6.36 6.66	10.27 10.50	3333, 1689, 1634, 1536br, 1508br, 1282br, 1258br, 1230br, 742, 695
$C_{14}H_{24}N_6O_4 \cdot H_2O$	46.91 46.53	7.31 7.70	23.45 23.03	
$C_{14}H_{24}N_6O_4 \cdot H_2O$	46.91 46.58	7.31 7.66	23.45 23.09	
$C_{21}H_{30}N_6O_5$	56.49 56.19	6.77 7.11	18.82 18.57	
$C_{31}H_{38}N_6O_8$	59.79 59.61	6.15 6.38	13.50 13.70	3390, 1748, 1695, 1653, 1639, 1536br, 1266br, 1250br, 757, 738, 698
$C_{13}H_{38}N_6O_8$	59.79 59.92	6.15 6.33	13.50 13.75	3413, 1748, 1692, 1639, 1534br, 1247br, 755, 698
$C_{45}H_{50}N_6O_9 \cdot H_2O$	64.64 64.66	6.26 6.47	10.04 10.28	3333, 1748br, 1695, 1639, 1538br, 1515br, 740, 696
$C_{28}H_{40}N_6O_8$	57.13 57.17	6.85 6.67	14.28 14.57	3390, 1739, 1689. 1653, 1634, 1527br, 1269br, 1250br, 752, 748, 702
$C_{43}H_{53}N_7O_{10}$	62.38 62.67	6.45 6.60	11.84 12.03	3322, 1745br, 1689, 1675, 1538br, 1261br, 732, 694
$C_{51}H_{54}N_6O_9$	68.43 68.57	6.08 6.21	9.39 9.77	3289, 1715, 1672, 1631, 1539br, 1504br, 1252br, 736, 683
$C_{21}H_{30}N_6O_5$	56.49 56.21	6.77 7.09	18.82 18.53	
$C_{59}H_{68}N_8O_{12}$	65.55 65.41	6.34 6.61	10.37 10.47	
$C_{27}H_{42}N_8O_6$	56.43 56.28	7.37 7.59	19.50 19.37	3417, 1738br, 1711br, 1688, 1649, 1531br, 1513br, 1260br, 733, 688

* Syponification/Hydrogenolysis

A similar series of reactions (Scheme 3) using the dipeptide derivatives VI and XV furnished *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-histidyl-*N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XX). The saponification of this tetrapeptide derivative followed by hydrogenolysis in ethanol gave L-tyrosyl-L-histidyl-L-lysyl-L-lysine (XXI).

The *N*-benzyloxycarbonyl-dipeptide methyl ester IV—VI and *N*-*t*-butoxycarbonyl-dipeptide esters XV and XVI, used for tri- and tetra-peptide syntheses, were conveniently prepared by means of the *N*-hydroxysuccinimide esters[16,17] of *N*-(*O*)-protected amino acids. The *N*-*t*-butoxycarbonyl-*O*-benzyl derivatives of L-tyrosyl-L-histidine methyl ester (XXII) and L-tyrosyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XXIII) and *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XXIV) were also prepared to be used in the synthesis of further analogs which will be the subject of a forthcoming paper.

EXPERIMENTAL

Melting points, uncorrected were taken on a Kofler hot stage. IR spectra were recorded in potassium bromide pellets using a Perkin-Elmer Infracord model 137. R_F values were measured by silica gel TLC [Merck, HF_{254}, type 60; developed in methylene chloride-methanol (9 : 1)]. The products were rendered visible by use of a ninhydrin spray. *t*-Butoxycarbonyl and benzyloxycarbonyl-amino acids *N*-hydroxysuccinimide esters were purchased from »Fluka«, Chemical Co., Buchs, CH.

N-*Benzyloxycarbonyl- (or* N-t-*butoxycarbonyl-) Peptide Methyl (or Benzyl) Esters. General procedures*

Dipeptide derivatives VI, IX, XV, XVI, XXII—XXV. — A suspension of *N*-hydroxysuccinimide ester of glycine or *O*-benzyl-L-tyrosine as its *N*-benzyloxycarbonyl or *N*-*t*-butoxycarbonyl derivative, or the *N*-hydroxysuccinimide ester of *N*-*t*-butoxycarbonyl-*N*-ε-benzyloxycarbonyl-L-lysine (1 mmol) in dimethylformamide (10—20 ml), dioxane (6.5 ml) or 1,2-dimethoxyethane (10 ml) was treated with the hydrochloride of L-histidine methyl ester or *N*-ε-benzyloxycarbonyl-L-lysine methyl (or benzyl) ester (1.1 mmol) in the presence of triethylamine (1.2 mmol) as outlined in Schemes 1 and 2. Triethylamine hydrochloride was filtered off and the filtrate stired at 70 °C for 5 h at room temperature for 24 h and then evaporated to dryness at 30 °C and 10^{-2} mm Hg. The residue was dissolved in 10% citric acid and partitioned with ethylacetate. The ethylacetate extract was washed with 0.2 mol dm^{-3} $NaHCO_3$, water, and then evaporated to dryness. The products crystallized in 62—91.6% yields (see Table I).

Hydrazides of Dipeptide Derivatives IX, XXV. — To a solution of *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-histidine methyl ester (VI) or *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XXIV) (1 mmol) in anhydrous methanol (5 ml) hydrazine monohydrate (1 ml) was added. The mixture was kept at room temperature for 24 h or refluxed for 1 h. The product precipitated in 85—88% yields (see Table I).

Tripeptide Derivatives I—III, X—XIII, XVII—XIX. — To a 0 °C cooled solution of the hydrazide of *N*-benzyloxycarbonyl- (or *N*-*t*-butoxycarbonyl-) glycyl-L-histidine (IV, XIV), *N*-benzyloxycarbonyl-glycyl-*N*-ε-benzyloxycarbonyl-L-lysine (V), *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosyl-L-histidine (VI), or *N*-benzyloxycarbonyl-L-histidine (1 mmol) in 1 mol dm^{-3} HCl (3 ml) ethylacetate (5 ml), and sodium nitrite (1 mmol) dissolved in water (0.5 ml) was added. The mixture was stirred for 5 min and then treated with 50% potassium carbonate (2.5 ml). The ethylacetate layer, containing generated azide, combined with the freshly prepared ethylacetate solution (3 ml) of *N*-ε-benzyloxycarbonyl-L-lysine methyl ester, L-histidine methyl ester, *N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester, or *O*-benzyl-L-tyrosyl-*N*-ε-benzyloxycarbonyl-L-lysine benzyl ester, (1 mmol) (see Scheme 2). The mixture was kept at 0 °C for 1—4 days. A precipitate separated which was

extracted with ethylacetate. The ethylacetate extract was washed with 5% citric acid, 0.2 mol dm^{-3} $NaHCO_3$, and water, and then evaporated to dryness under reduced pressure giving the products in 34.2—75% yields. For details see Table I.

Tetrapeptide Derivative XX. — The hydrazide of *N*-benzyloxycarbonyl-*O*-benzyl--L-tyrosyl-L-histidine (0.66 mmol) in 1 mol dm^{-3} HCl (2 ml) and ethylacetate (5 ml) was treated with sodium nitrite (0.66 mmol) in water (0.3 ml). The azide produced was added to *N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (0.66 mmol). The mixture was kept for 4 days and treated as described for the synthesis of the tripeptide derivatives. It gave *N*-benzyloxycarbonyl-*O*-benzyl--L-tyrosyl-L-histidyl-*N*-ε-benzyloxycarbonyl-L-lysyl-*N*-ε-benzyloxycarbonyl-L-lysine methyl ester (XX) in 88% yield (see Table I).

Deprotection of Tri- and Tetra-peptide Derivatives. General procedure

The *N*-benzyloxycarbonyl tripeptide X—XII or tetrapeptide XX as its methyl ester (1 mmol) was dissolved in methanolic 1 mol dm^{-3} NaOH (1.5 ml), stirred at room temperature for 4 h, and then diluted with water (18 ml). This solution was acidified with 1 mol dm^{-3} HCl and left at 0 °C for 16 h. The *N*-benzyloxycarbonyl tripeptide either precipitated or was obtained after evaporation to dryness under reduced pressure in 59—100% yields. The free acid obtained (0.33 mmol) or *N*-benzyloxycarbonyl-tripeptide XVIII (as benzyl ester), previously purified from anhydrous ethanol was dissolved in ethanol (25 ml) and hydrogeneated over 10% Pd/C (20 mg) and Pd-black (60 mg) until evolution of carbon dioxide was complete. The catalyst was filtered off and the filtrate repeatedly lyophilized and dissolved in water to yield chromatographycally pure glycyl-L-histidyl-L-lysine (I), glycyl-L-lysyl--L-histidine (II), L-tyrosyl-L-histidyl-L-lysine (III), L-histidyl-L-tyrosyl-L-lysine (XIX), and L-tyrosyl-L-histidyl-L-lysyl-L-lysine (XXI) (see Table I).

Acknowledgements. We thank Mrs. A Poturić for her technical assistance and Mrs. R. Herman for the microanalyses.

REFERENCES

1. A. V. Schally, D. H. Coy, and C. A. Meyers, *Ann. Rev. Biochem.* **47** (1978) 89.
2. L. Pickart, L. Thayer, and M. M. Thaler, *Biochem. Biophys. Res. Commun.* **54** (1973) 562.
3. D. H. Schlesinger, L. Pickart, and M. M. Thaler, *Experientia* **33** (1977) 324.
4. L. Pickart and M. M. Thaler, *Nature New Biology* **243** (1973) 85.
5. V. Škarić, B. Katušin-Ražem, B. Šimunić, and Đ. Škarić, *Croat. Chem. Acta* **47** (1975) 603.
6. V. Škarić, M. Kovačević, and Đ. Škarić, *J. Chem. Soc. Perkin Trans. 1* (1976) 1199.
7. V. Škarić, M. Topić-Bulić, and Đ. Škarić, *Croat. Chem. Acta* **51** (1978) 347.
8. M. Božović, H. Boström, and L. Božović, *Experientia* **26** (1970) 1194.
9. M. Božović, L. Božović, Đ. Škarić, and V. Škarić, YU-Pat. P-144/1976 (Pliva).
10. R. F. Fischer and R. R. Whetstone, *J. Amer. Chem. Soc.* **76** (1954) 5076.
11. R. Badielo, G. Vidali, and A. Marzoto, *Gazz. Chim. Ital.* **94** (1964) 332.
12. B. F. Erlanger and E. Brand, *J. Amer. Chem. Soc.* **73** (1951) 4025.
13. I. R. W. Holley and E. Sondheimer, *J. Amer. Chem. Soc.* **76** (1954) 1326.
14. T. Shiba and T. Taneko, *Bull. Soc. Chem. Japan* **33** (1960) 1721.
15. I. L. Mar'yash and V. A. Shibnev, *Izv. Akad. Nauk SSSR, Ser. Khim.* (1972), 1858.
16. C. Sorg, E. Rüde, and O. Westhal, *Ann.* **734** (1970) 180.
17. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.* **86** (1964) 1839.

SAŽETAK

Polifunkcionalni tri- i tetra-peptidi s lizinom kao komponentom

V. Škarić, J. Makarević i Đ. Škarić

Opisana je sinteza glicil-L-histidil-L-lizina, glicil-L-lizil-L-histidina, L-tirozil-L--histidil-L-lizina, *N*-,*N*-ε,-*N*-ε-tribenziloksikarbonil derivata L-histidil-L-ližil- -lizin metil estera, L-histidil-L-tirozil-L-lizina i L-tirozil-L-histidil-L-lizil-L-lizina primjenom azidne metode.

LABORATORIJ ZA STEREOKEMIJU
I PRIRODNE SPOJEVE
INSTITUT »RUĐER BOŠKOVIĆ«
41001 ZAGREB

Prispjelo 10. srpnja 1980.