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Homologous Hexapeptides

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The *N*-hydroxysuccinimide esters of N-tritylated tripeptides II—V were coupled with hydroacetates of tripeptide esters VII—IX to correspoding *N*-tritylated hexapeptide esters XV—XXI. The saponification of so obtained compounds followed by detritylation afforded pentaglycyl-β-alanine (XXXIII), pentaglycyl-γ-aminobutyric acid (XXXIV), diglycyl-β-alanyl-diglycyl-β-alanine (XXXV), diglycyl-β-alanyl-diglycyl-γ-aminobutric acid (XXXVI), diglycyl--γ-aminobutyryl-diglycyl-γ-aminobutyric acid (XXXVII), diglycyl--γ-aminobutyryl-glycyl-β-alanyl-γ-aminobutyric acid (XXXVIII), and glycyl-β-alanyl-γ-aminobutyryl-glycyl-β-alanyl-γ-aminobutyric acid (XXXIX).

There have been many peptides prepared containing several of simpler α-amino acids, but relatively few which include β- and γ-amino acids. This is especially true for peptides of γ-aminobutyric acid. However, carnosine, anserine, balenine and ophtidine[1,2] as constituents of muscles, γ-L-glutamyl-β--alanine as constituent of iris bulbs and the seeds of *Lunaria annua,* γ-glutamyl-β-aminopropionitrile in *Lathyrus adoratus* as toxic factor[3], homologues of glutathione in *Phaseolus aureus*[4] and many biologically active peptides containing β-alanine and γ-aminobutyric acid were recognized many years ago.

The polypeptides containing α-, β-, or γ-amino acids should reflect the inherent features to such homologizations. In the present work glycine, β-alanine and γ-aminobutyric acid alternatively inserted in the hexapeptide chain were described.

The common strategy for the synthesis of H-$(Gly)_5$-βAla-OH (XXXIII), H-$(Gly)_5$-γAbu-OH (XXXIV), H-(Gly-Gly-β$Ala)_2$-OH (XXXV), H-$(Gly)_2$-βAla--$(Gly)_2$-γAbu-OH (XXXVI), and H-(Gly-Gly-γ$ABu)_2$-OH (XXXVII) is demonstrated by Scheme 1. The synthesis of H-$(Gly)_2$-γAbu-Gly-βAla-γAbu-OH (XXXVIII) is outlined in the Scheme 2 and the preparation of H-(Gly-βAla--γ$Abu)_2$-OH (XXXIX) in the Scheme 3.

The condensation of N-hydroxysuccinimide esters of N-tritylated glycine[5], β-alanine and peptides I—V with hydrochlorides of glycine thyl ester[6], β--alanine ethyl ester[7], methyl ester of γ-aminobutyric acid[8] and hydroacetates of methyl (ethyl) esters of peptides VI—IX is a particularly satisfactory procedure in the syntheses of di-, tri- and hexapeptides containing glycine, β--alanine, and γ-aminobutyric acid.

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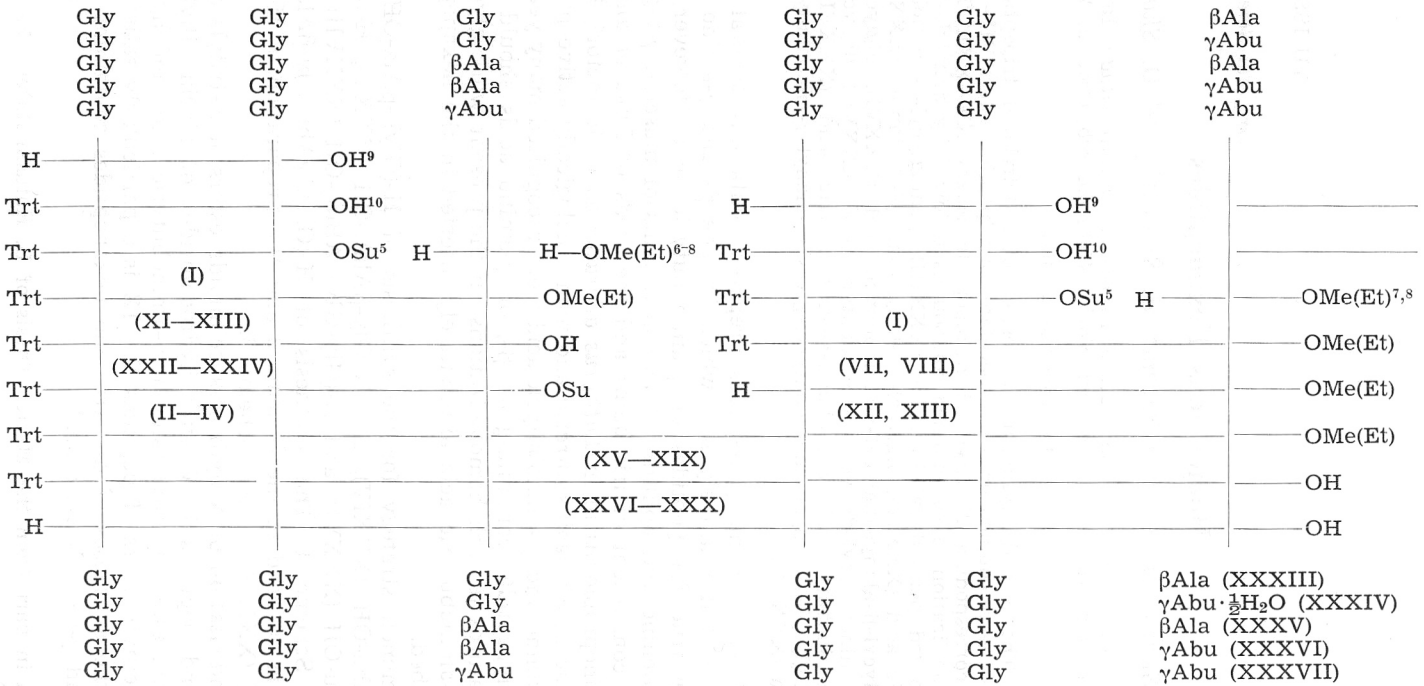
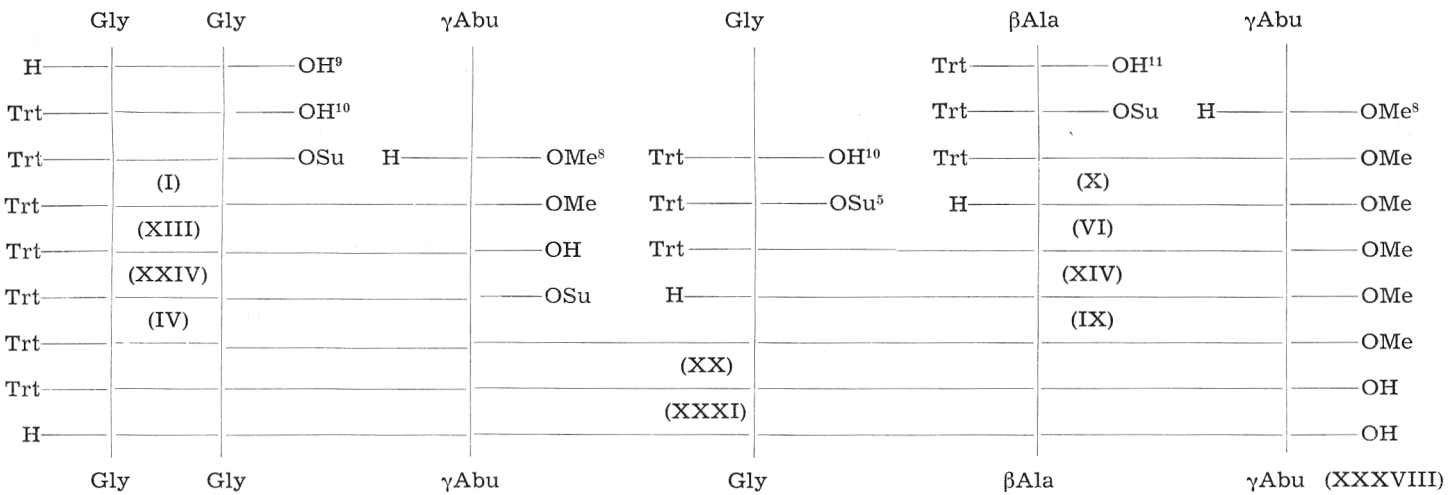
* Taken in part from the Master Thesis of B. Katušin-Ražem, Faculty of Science, Zagreb.

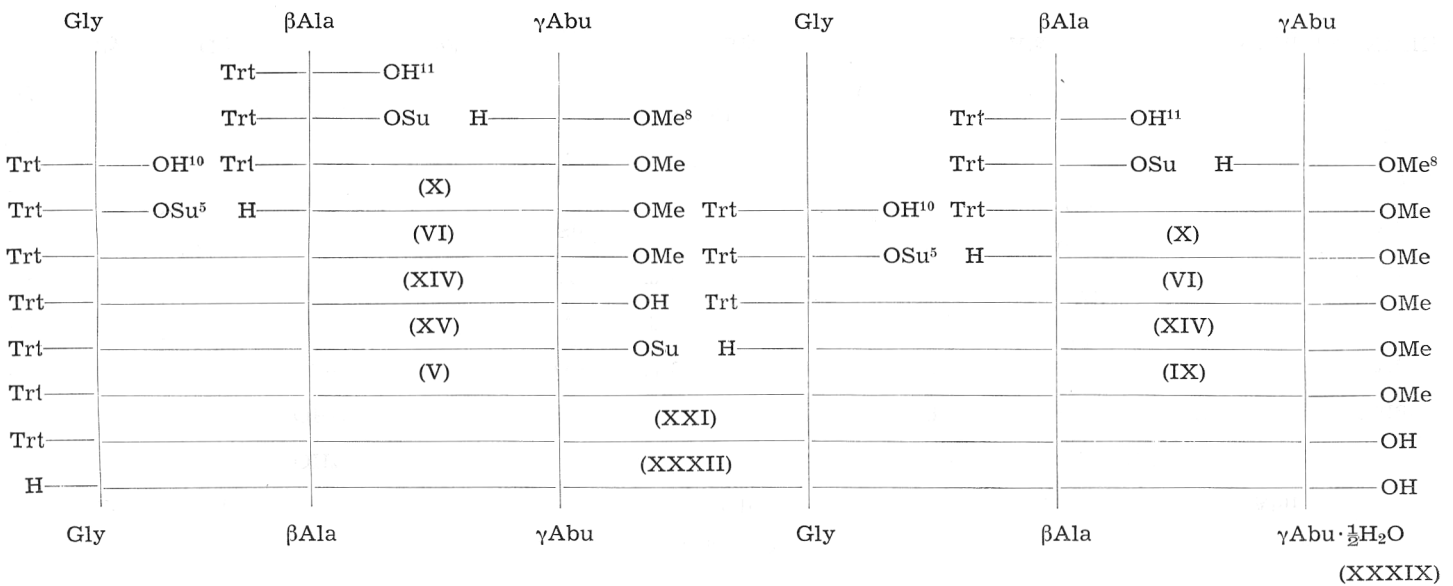
Scheme 1


Gly Gly Gly Gly Gly
Gly Gly Gly Gly Gly
Gly Gly βAla βAla γAbu
H
Trt
Trt
Trt
Trt
Trt
Trt
H
OH⁹
OH¹⁰
OSu⁵
(I)
(XI—XIII)
(XXII—XXIV)
(II—IV)
H—OMe(Et)⁶⁻⁸
OMe(Et)
OH
OSu
(XV—XIX)
(XXVI—XXX)
Gly Gly Gly Gly Gly
Gly Gly Gly Gly Gly
βAla γAbu βAla γAbu γAbu
H
Trt
Trt
Trt
H
OH⁹
OH¹⁰
OSu⁵
(I)
(VII, VIII)
(XII, XIII)
H
OMe(Et)⁷,⁸
OMe(Et)
OMe(Et)
OMe(Et)
OH
OH
Gly Gly Gly Gly Gly
Gly Gly Gly Gly Gly
Gly Gly βAla βAla γAbu
Gly Gly Gly Gly Gly
Gly Gly Gly Gly Gly
βAla (XXXIII)
γAbu·½H₂O (XXXIV)
βAla (XXXV)
γAbu (XXXVI)
γAbu (XXXVII)

Scheme 2


Gly
Gly
γAbu
Gly
βAla
γAbu
H
Trt
Trt
Trt
Trt
Trt
Trt
H
OH9
OH10
OSu
(I)
(XIII)
(XXIV)
(IV)
H
OMe8
OMe
OH
OSu
Trt
Trt
Trt
H
OH10
OSu5
(XX)
(XXXI)
Trt
Trt
H
OH11
OSu
(X)
(VI)
(XIV)
(IX)
H
OMe8
OMe
OMe
OMe
OMe
OMe
OH
OH
Gly
Gly
γAbu
Gly
βAla
γAbu (XXXVIII)

Scheme 3


Gly
βAla
γAbu
Trt
OH[11]
OSu
H
OMe[8]
OMe
(X)
OH[10]
OSu[5]
(VI)
(XIV)
OH
(XV)
(V)
(XXI)
(XXXII)
γAbu·½H₂O
(XXXIX)

A modified dicyclohexylcarbodiimide method[5] has been used for the synthesis of the N-hydroxysuccinimide esters of the *N*-tritylated glycine, β-alanine, and peptides I—V at room temperature in ethylacetate or dioxane. The saponification of *N*-trityl, methyl (ethyl) esters XI—XXI in 1 M sodium (potassium) hydroxide yielded *N*-tritylpeptides XXII—XXXII as hygroscopic materials. The detritylation of XXVI—XXXII with 50% acetic acid afforded hexapeptides XXXIII—XXXIX in very high yields.

EXPERIMENTAL

Melting points, uncorrected were taken on a Kofler hot stage. The ir spectra were recorded in potassium bromide pellets using a Perkin-Elmer Infracord model 137. Mass spectra were measured with a Varian MAT CH-7 spectrometer. The R_f values were recorded on silica gel t. l. c. (Merck, HF_{254}, type 60). The products were rendered visible by use of a ninhydrin spray, by treatment with iodine vapour, by uv illumination, and by chloro-*o*-toluidine-iodine reagent.

N-Hydroxysuccinimide Esters of N-Tritylpeptides (I—V). — General procedure

To a solution of *N*-tritylpeptide (1 mmol) in anhydrous ethyl acetate (1.5 l) or dioxane (250 ml) *N*-hydroxysuccinimide (1 mmol) and *N,N*-dicyclohexylcarbodiimide (1.5 mmol) were added. The mixture was stirred for 20 h at room temperature and the formed dicyclohexylurea filtered off. The filtrate was evaporated to a residue which crystallized from benzene—*n*-hexane in 79—86% yields (see Table Ia).

Detritylation of N-Tritylpeptide Methyl (Ethyl) Esters. — General procedure

A suspension of *N*-tritylpeptide methyl (ethyl) ester X, XII—XIV (1 mmol) in 50% acetic acid (2 ml) was heated for 10 minutes at 80 °C and then diluted at room temperature with water (40 ml). The triphenylcarbinol was removed by filtration and the filtrate evaporated to a residue. The hydroacetate of peptide methyl (ethyl) esters VI—IX were obtained in 86—91% yields, $R_f \approx 0.2$ [developed in methylene chloride—methanol (8 : 2)]. For details of products see Table Ib.

Methyl (Ethyl) Esters of N-Tritylpeptides (X—XXI). — General procedure

Di- X and tripeptide derivatives XI—XIV. — A suspension of *N*-hydroxysuccinimide ester of *N*-tritylglycine[5], *N*-trityl-β-alanine or *N*-tritylpeptide I (1 mmol) in 1,2-dimethoxyethane (5 ml) was treated by strirring with the hydroacetate of methyl ester of dipeptide VI, hydrochloride of glycine ethyl ester[6], β-alanine ethyl ester[7] and methyl ester of γ-aminobutyric acid[8] (1 mmol) respectively in the presence of triethylamine (1 mmol) for 3 h at room temperature. After addition of water and isolation of crude products, crystalization from chloroform—*n*-hexane afforded di- X and tripeptides XI—XII in 73—80% yields, $R_f \approx 0.7$ [developed in methylene chloride—methanol (8 : 2)] (see Table Ic).

Hexapeptide derivatives XV—XXI. — A solution of *N*-hydroxysuccinimide ester of *N*-trityltripeptide II—V (1 mmol) in *N,N*-dimethylformamide (17 ml) was treated with the hydroacetate of tripeptide alkyl ester VII—IX (1 mmol) respectively. The mixture containing methyl ester (VIII or IX) was stirred for 20 h at 80 °C and only 4 h at 80 °C and 16 h at room temperature when ethyl ester (VII) was treated. The solution was evaporated to dryness under reduced pressure (10^{-3} mmHg) and crystallized from methanol—ether—*n*-hexane, unless otherwise stated, yields 80—85%, $R_f \approx 0.5$ [developed in methylene chloride—methanol (8 : 2)]. For details see Table Ic.

N-Tritylpeptides (XXII—XXXII). — General procedure

A solution of methyl ester of *N*-tritylpeptide X, XIII, XIV, XVI, XVIII—XXI (1 mmol) in methanol (10 ml) was treated with 1 M sodium hydroxide (4 ml), stirred for 16 h and then evaporated to dryness. [The saponification of ethyl ester of *N*--tritylpeptide XI, XII, XV, XVII (1 mmol) was performed in methanol (10 ml) with

TABLE I

Compound	M.p/°C	Yield %	Mol. weight	Formula	Anal. Calc'd.: Found: %C	%H	%N	ν_{max}/cm^{-1}
a) N-Hydroxysuccinimid Esters of N-Tritylpeptides								
Trt—GlyGlyGly—OSu (II)	100 (155)	85	528.5	$C_{29}H_{28}N_4O_6$				3290, 1810, 1780, 1730, 1630, 1525(broad), 708
Trt-GlyGlyβAla—OSu (III)	166—168	83	542.5	$C_{30}H_{30}N_4O_6$	66.41 66.20	5.56 5.90	10.33 10.26	3400(broad), 1820, 1780, 1730, 1650(broad), 1525(broad), 708
Trt—GlyGlyγAbu—OSu (IV)	164—169	79	556.6	$C_{31}H_{32}N_4O_6$	66.89 66.77	5.80 6.10	10.07 10.25	3400, 3330, 1810, 1775, 1725, 1670 (broad), 1530(broad), 709
Trt—GlyβAlaγAbu—OSu (V)	100 (150)	86	570.6	$C_{32}H_{34}N_4O_6$				3370, 1815, 1785, 1740, 1650 (broad), 1530(broad), 710
b) Hydroacetates of Peptide Esters								
HOAc·H—βAlaγAbu—OMe (VI)		77.5	60.1+188.2[a]	$C_{10}H_{20}N_2O_5$				3250(broad), 2950, 1725, 1640 (broad), 1545(broad)
HOAc·H—(Gly)$_2$βAla—OEt (VII)	98—102	91.2	60.1+231.2[b]	$C_{11}H_{21}N_3O_6$	45.35 45.39	7.27 7.46	14.43 14.62	3310, 2950(broad), 1725, 1650, 1560(broad
HOAc·H—(Gly)$_2$γAbu—OMe (VIII)	96—98	92.5	60.1+231.2[b]	$C_{11}H_{21}N_3O_6$	45.35 45.08	7.27 7.28	14.43 14.43	3380, 1740, 1640, 1575(broad)
HOAc·H—GlyβAlaγAbu—OMe (IX)	90—96	86.3	60.1+245.2[c]	$C_{12}H_{23}N_3O_6$				3370, 2950(broad), 1740, 1670, 1645, 1550(broad)
c) Methyl (Ethyl) Esters of N-Tritylpeptides								
Trt—βAlaγAbu—OMe (X)	113—116[a]	83	430.5	$C_{23}H_{30}N_2O_3$	75.32 75.06	7.02 7.31	6.51 6.24	3360, 1740, 1640, 1550, 710
Trt—GlyGlyβAla—OEt (XII)	148—151[a]	78	473.5	$C_{28}H_{31}N_3O_4$	71.01 70.92	6.60 6.78	8.86 8.86	3340, 1725, 1640, 1520, 705
Trt—GlyGlyγAbu—OMe (XIII)	146—149[a]	73	473.5	$C_{28}H_{31}N_3O_4$	71.01 70.75	6.60 6.53	8.87 9.10	3340, 1730, 1640, 1510, 710
Trt—GlyβAlaγAbu—OMe (XIV)	177—179[a]	80.5	487.6	$C_{29}H_{33}N_3O_4$	71.43 71.60	6.82 6.88	8.62 8.83	3400, 3310, 1730, 1630, 1515, 712
Trt—(Gly)$_5$βAla—OEt (XV)	172—176	80.5	644.7	$C_{34}H_{40}N_6O_7$	63.34 63.24	6.25 6.36	13.04 13.22	3330(broad), 1730, 1690(sh), 1630(broad), 1520(broad), 702
Trt—(Gly)$_5$γAbu—OMe (XVI)	169—173	84.5	644.7	$C_{34}H_{40}N_6O_7$	63.34 63.28	6.25 6.56	13.04 13.31	3300, 1725, 1680 (sh), 1620 (broad), 1510, 703
Trt—(GlyGlyβAla)$_2$—OEt (XVII)	194—195.5	84.2	658.7	$C_{35}H_{42}N_6O_7$	63.81 63.98	6.43 6.58	12.76 12.69	3350, 1745, 1660, 1640, 1550, 709

[a] Fund M^+, 188. [b] Found M^+, 231. [c] Found M^+, 245.

Compound	M.p/°C	Yield %	Mol. weight	Formula	Anal. Calc'd.: found: %C	%H	%N	ν_{max}/cm^{-1}
Trt—(Gly)$_2$βAla(Gly)$_2$γAbu—OMe (XVIII)	187—188	94.2	658.7	$C_{35}H_{42}N_6O_7$	63.81 63.53	6.43 6.72	12.76 12.85	3375(broad), 1740, 1655(broad), 1550, 704
Trt—(GlyGlyγAbu)$_2$—OMe (XIX)	128—132[b]	85	672.8	$C_{36}H_{44}N_6O_7$	64.27 63.97	6.59 6.74	12.49 12.78	3320(broad), 1730, 1635(broad), 1550(broad), 703
Trt—(Gly)$_2$γAbuGlyβAlaγAbu—OMe (XX)	(95) 157—164	80	704.8	$C_{37}H_{46}N_6O_7 \cdot H_2O$	63.05 63.23	6.86 6.92	11.93 12.05	3340(broad), 1730, 1650(broad), 1535, 707
Trt—(GlyβAlaγAby)$_2$—OMe (XXI)	138—146	81.5	700.8	$C_{38}H_{48}N_6O_7$	65.12 64.85	6.90 6.94	11.99 11.91	3310, 1730, 1635(broad), 1545 broad), 707
d) N-Trityltripeptides								
Trt—GlyGlyβAla—OH (XXIII)	163—166	54	445.3	$C_{26}H_{27}N_3O_4$	70.10 70.26	6.11 6.40	9.43 9.66	3375(broad), 1740(broad), 1655 (broad), 1550(broad), 704
Trt—GlyGlyγAbu—OH (XXIV)	152—155	65	459.5	$C_{27}H_{29}N_3O_4$	70.57 70.36	6.36 6.60	9.14 9.24	3350, 1680(sh), 1640, 1620, 1545 (broad), 705
Trt—GlyβAlaγAbu—OH (XXV)	192—196[a]	73.5	473.6	$C_{28}H_{31}N_3O_4$	71.01 70.88	6.60 6.87	8.87 8.57	3375, 1720, 1650, 1625, 1540, 701
e) Hexa peptides								
H—(Gly)$_5$βAla—OH (XXXIII)	~240 (dec.)	95	374.4	$C_{13}H_{22}N_6O_7$	41.71 41.42	5.92 6.20	22.45 22.44	3350, 1640(broad), 1570(broad)
H—(Gly)$_5$γAbu—OH (XXXIV)	~240 (dec.)	92	397.4	$C_{14}H_{24}N_6O_7 \cdot \frac{1}{2}H_2O$	42.31 42.32	6.34 6.64	21.15 21.68	3510, 3360, 1645, 1570(broad)
H—(GlyGlyβAla)$_2$—OH (XXXV)	248—250	88	388.4	$C_{14}H_{24}N_6O_7$	43.29 43.51	6.23 6.49	21.64 21.35	3350, 1680, 1640, 1560(broad)
H—(Gly)$_2$βAla(Gly)$_2$γAbu—OH (XXXVI)	~240 (dec.)	89	402.4	$C_{15}H_{26}N_6O_7$	44.77 44.89	6.51 6.65	20.89 20.65	3310, 1635(broad), 1550(broad)
H—(GlyGlyγAbu)$_2$—OH (XXXVII)	230—235	90	416.4	$C_{16}H_{28}N_6O_7$	46.14 46.23	6.78 6.69	20.18 20.39	3340, 1680, 1645, 1550(broad)
H—(Gly)$_2$γAbuGlyβAlaγAbu—OH (XXXVIII)	~270 (dec.)	86	430.5	$C_{17}H_{30}N_6O_7$	47.43 47.54	7.03 7.39	19.52 19.61	3400, 1655(broad), 1560(broad)
H—(GlyβAlaγAbu)$_2$—OH (XXXIX)	225—228	90	453.5	$C_{18}H_{32}N_6O_7 \cdot \frac{1}{2}H_2O$	47.67 47.62	7.33 7.50	18.53 18.74	3340, 1650, 1625, 1545

[a] From chloroform — *n*-hexane. [b] From benzene — *n*-hexane.

[a] From acetone — *n*-hexane.

methanolic 1 M potassium hydroxide (2 ml)]. The residue was dissolved in water (50 ml), precooled to 0 °C, and treated with 10% acetic acid to pH = 5. It brought out the precipitation of products which crystallized from ethanol—ether—*n*-hexane in 58—85% yields, $R_f \approx 0.3$ [developed in methylene chloride—methanol (7 : 3)] (see Table Id). The hygroscopic *N*-tritylhexapeptides XXVI—XXXII, m.p. 115—120 °C (XXVI), 112—116 °C (XXVII), 112—115 °C (XXIX), 116—120 °C (XXX), 97—103 °C (XXXI), 131—139 °C (XXXII), showed characteristic ir spectral bands at 3300—3380, 1720—1735, 1645—1665, 1530—1550, and 701—718 cm^{-1}.

N-Trt(GlyGly-βAla)$_2$OH (XXVIII) appeared as monohydrate in 85% yield, m. p. 115—117 °C.

Anal. $C_{33}H_{38}N_6O_7 \cdot H_2O$ (648.7) calc'd.: C 61.10; H 6.22; N 12.96%
found: C 61.07; H 6.33; N 12.91%

Detritylation of N-Tritylhexapeptides. — General procedure

A suspension of *N*-tritylhexapeptide XXVI—XXXII (1 mmol) in 50% acetic acid (2 ml) was detritylated as already described and the hexapeptides XXXIII—XXXIX crystallized from water—ethanol in 86—95% yields, $R_f \approx 0.5$ [developed in methanol—water (7 : 3)] (see Table Ie).

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REFERENCES

1. P. O. Dennnis and P. A. Lorkin, *J. Chem. Soc. C* 1965, 4968.
2. G. Kalyankar and A. Meister, J. *Biol. Chem.* **245** (1970) 2962.
3. P. R. Carnegie, *Biochem. J.* **89** (1963) 459.
4. P. R. Carnegie, *Biochem. J.* **89** (1963) 471.
5. G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.* **86** (1964) 1839.
6. M. Goodman and W. J. McGahren, *Tetrahedron* **23** (1967) 2031.
7. H. Weidel and E. Roithner, *Monatsh. Chem.* **17** (1896) 172.
8. D. L. Garmaise, R. Schwartz, and A. F. McKay, *J. Amer. Chem. Soc.* **80** (1958) 3332; A. Kjeer, and R. Gmelin, *Acta Chem. Scand.* **11** (1957) 577.
9. H. F. Schott, J. B. Larkin, L. B. Rockland, and M. S. Dunn, *J. Org. Chem.* **12** (1947) 490.
10. L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.* **78** (1956) 1359.
11. T S.. Safonova and S. I. Sergievskaya, *Zh. Obshch. Khim.* **34** (1964) 919.

SAŽETAK

Homologni heksapeptidi

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N-Hidroksisukcinimidni esteri *N*-tritiliranih tripeptida II—V u reakciji s hidroacetatima tripeptid-estera VII—IX prelaze u *N*-tritilirane heksapeptid-estere XV—XXI. Saponifikacija tako dobivenih spojeva kao i njihovo detritiliranje daje pentaglicil-β-alanin (XXXIII), pentaglicil-γ-aminomaslačnu kiselinu (XXXIV), diglicil-β--alanil-diglicil-β-alanin (XXXV), diglicil-β-alanil-diglicil-γ-aminomaslačnu kiselinu (XXXVI), diglicil-γ-aminobutiril-diglicil-γ-aminomaslačnu kiselinu (XXXVII), diglicil--γ-aminobutiril-glicil-β-alanil-γ-aminomaslačnu kiselinu (XXXVIII) i glicil-β-alanil--γ-butiril-glicil-β-alanil-γ-aminomaslačnu kiselinu (XXXIX).

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