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Synthesis of the Four Structural Isomeric L-Pentapeptides of Ala. Glu. Lys. Ala. Ala. Sequence: Characterization and Correlation with the L-D-L-D-D-Isomer Related to the Peptidoglycan Peptide Chain

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The synthesis of the four L-pentapeptides containing the Ala. Glu. Lys. Ala. Ala. sequence, which differ with regard to the site of linkage between the glutamyl-lysyl residues, is described. In each case the chain was built by coupling the protected *N*-terminal dipeptide *N*-hydroxysuccinimide ester (II or IV) with the *C*-terminal tripeptide benzyl ester (VIII or X). The sequentially identical L-D-L-D-D-pentapeptide having the γ-carboxy-group of D-glutamic acid residue involved in the peptide bond, was prepared after the same general pattern. Complete deprotection of the isomers in two successive steps gave the free pentapeptides which were characterized as solid di-trifluoroacetate salts by analytical, optical and proton NMR spectral data. The isomers were compared by paper electrophoresis at different pH values. The alanine methyl resonances in the NMR spectra of the α-α L-pentapeptide XVIII and the γ-α L-D-L-D-D-pentapeptide XXII were assigned by observing the shift in spectral lines with varying pH.

INTRODUCTION

In a previous paper[1] from this laboratory it was reported that the incubation of the biotin-requiring mutant of *Brevibacterium divaricatum* with penicillin, or semisynthetic penicillins, led to the accumulation of soluble uncross-linked peptidoglycan fragments in the culture medium. Shortly thereafter the same phenomen was claimed[2,3] to occur in the penicillin-treated cultures of two other bacterial species. In connection with our studies on the structure and properties of the excreted fragments, we became interested in model compounds related to the peptide part of peptidoglycan. Synthetic work in this field has been very intensive in last years, and various peptides possessing unusual structural and stereochemical features encountered in the bacterial cell-wall peptidoglycans were prepared[4], mainly to elucidate the structure of naturally occurring material. However, for some of those compounds only a limited amount of information concerning their physical data and chemical behaviour is available.

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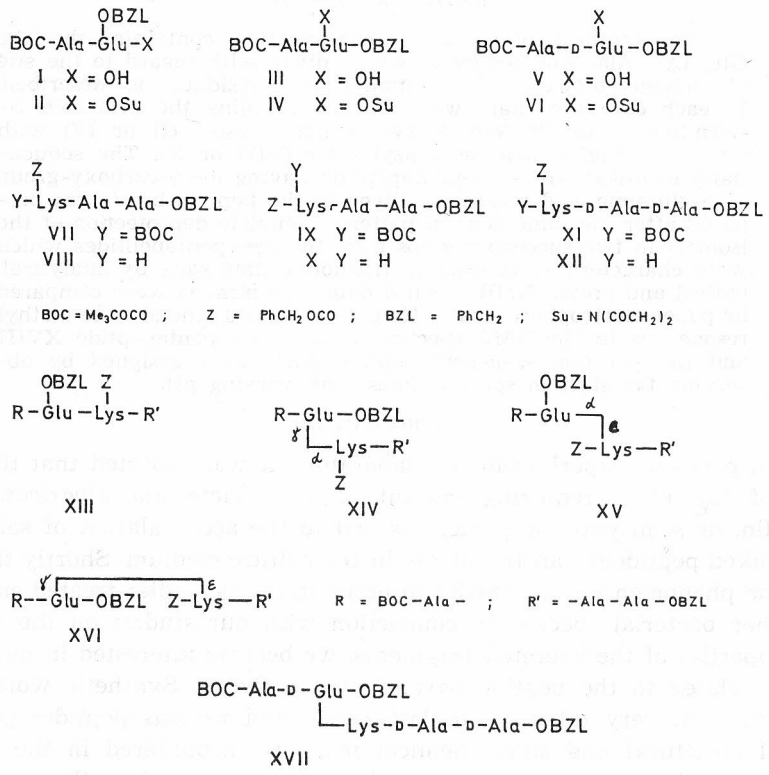
Since we were particularly interested in the differentiation of physical data due to variations in the type of linkage between the isomers possessing the amino acid sequence of the peptide moiety precursor of the cell-wall peptidoglycan, we first undertook the synthesis of the pentapeptide isomers containing the L-Ala. L-Glu. L-Lys. L-Ala. L-Ala. sequence. In the present paper the synthesis of four structural isomers which differ with regard to the site of linkage between the glutamyl-lysyl residues, is described. The sequentially identical L-alanyl-D-γ-glutaml-L-lysyl-D-alanyl-D-alanine[5,6] which is structurally and stereochemically related to the peptide units of some uncross-linked peptidoglycan chains was also prepared and fully characterized. We now report on the synthesis and correlation of some physical data of the above isomeric structures.

RESULTS AND DISCUSSION

The general design for the synthesis of the four fully protected isomeric pentapeptides XIII—XVI is outlined in Scheme 1. In each case the chain was



SCHEME 1.

built by coupling the protected *N*-terminal dipeptide *N*-hydroxysuccinimide ester (II or IV) with the C-terminal tripeptide benzyl ester (VIII or X); *tert*-butoxycarbonyl (BOC) protection was used for the amino group of *N*-terminal alanine, benzyloxycarbonyl protection for the lysine amino function not

involved in the peptide bond, and benzyl groups were used to protect the carboxy-group of *C*-terminal alanine and the 1- or 5-carboxy-group of glutamic acid. The synthesis of the sequentially identical L-D-L-D-D-pentapeptide XVII having the γ-carboxy-group of D-glutamic acid engaged in the peptide bond, was patterned after that of its stereoisomeride XIV; it involved the coupling of the *N*-terminal L-D-dipeptide active ester VI with the *C*-terminal L-D-D-tripeptide benzyl ester XII.

The isomeric dipeptides BOC-Ala-Glu(OBZL)-OH (I) and BOC-Ala-Glu(OH)-OBZL (III) were obtained by coupling *N-tert*-butoxycarbonyl-L-alanine *N*-hydroxysuccinimide ester with 5-and 1-benzyl L-glutamic acid ester, respectively; the products were characterized as crystalline dicyclohexylamine salts. Standard treatment of I and III with *N*-hydroxysuccinimide afforded the corresponding active esters II and IV. Analogously to IV, but using 1-benzyl D-glutamic acid ester, the protected L-D-dipeptide active ester VI[7] was obtained in crystalline form and fully characterized.

Comparison of the NMR spectra in $CDCl_3$ of the above isomers revealed differences in the chemical shift value for the benzyl methylene protons. In the spectrum of the active ester II, having the glutamate benzyl ester group in γ-relationship to the peptide bond, the PhCH_2 singlet (τ 4.91) appeared at a higher field than those of isomeric 1-benzyl active esters IV and VI (τ 4.87 and 4.85). Essentially the same chemical shift difference was observed between the 5-benzyl monoester derivative I and 1-benzyl monoester derivative V. In the spectrum of an artificial 1 : 2.5 mixture of II and IV, the benzyl methylene protons appeared as two well resolved singlets of appropriate relative intensities with chemical shift difference of 3.5 Hz, thus indicating that the observed difference may be of diagnostic use in peptide synthesis involving benzyl glutamate derivatives.

The *C*-terminal components VII and IX were obtained by coupling the *p*-nitrophenyl esters of N^2-*tert*-butoxycarbonyl-N^6-benzyloxycarbonyl-L-lysine and N^2-benzyloxycarbonyl-N^6-*tert*-butoxycarbonyl-L-lysine, respectively with L-alanyl-L-alanine benzyl ester. In our preliminary experiments, a strong dependence of the reactivity of *p*-nitrophenyl esters on the nature of solvents[8] was observed: with ethyl acetate as the solvent both reactions practically failed whereas in dimethylformamide (DMF) the coupling proceeded at a moderate rate. However, in the presence of two equivalents of imidazole the reaction rates increased singnificantly, and high yields (> 70%) of VII and IX were obtained regardless of the solvent used. The imidazole-promoted synthesis of N^2-*tert*-butoxycarbonyl-L-lysyl-D-alanyl-D-alanine benzyl ester[9,10] (XI) was performed analogously to that of VII by using D-Ala-D-Ala-OBZL as the *C*-terminal component.

The NMR spectrum of XI (in $CDCl_3$) revealed, in addition to the two sharp singlets due to the aromatic protons (τ 2.73) and the Me_3C group (τ 8.61), two well separated 2H + 2H singlets (τ 4.88 and 4.95). On the basis of comparison with the spectra of related compounds, the signal at lower field was assigned to the PhCH_2 protons of D-alanine benzyl ester group and that at a higher field to the PhCH_2 protons of the L-lysine N^6-benzyloxycarbonyl group. Assignement of the alanine methyl resonances proved to be difficult because of the overlapping Me_3C signal. However, after the BOC deprotection of XI, the NMR spectrum of N^6-benzyloxycarbonyl-L-lysyl-D-alanyl-D-alanine benzyl ester (XII)

in CD_3OD revealed the alanine methyl groups as two sets of overlapped doublets at τ 8.61 and 8.73 with the coupling constants J = 7 Hz. According to the observation[11] that the benzyl ester aromatic ring causes an upfield shift of the adjacent alanine methyl group, as well as on the basis of comparison of the alanine methyl resonances in Z-Ala-Ala-OMe (τ 8.68 and 8.61, CD_3OD solution) with those in Z-Ala-Ala-OBZL (τ 8.68 and 8.75), the upfield doublet in the NMR spectrum of XII is assigned to the *C*-terminal D-alanine methyl group.

Removal of the BOC protection from the α-amino function of lysine in tripeptides VII and XI and from the ε-amino function in IX, gave the crystalline trifluoroacetate salts of VIII, XII and X, respectively, which were characterized by elemental analysis and optical rotation data.

The coupling of the dipeptide active ester II with the tripeptide components VIII or X led to the fully protected α—α and α—ε pentapeptides XIII and XV, whereas coupling of the dipeptide active ester IV with VIII or X led to the γ—α and γ—ε pentapeptides XIV and XVI, respectively. After the same pattern the protected γ—α L-D-L-D-D-pentapeptide XVII[10] was prepared from VI and XII. The isomeric structures having the α-amino group of lysine involved in the peptide bond (compounds XIII, XIV and XVII) were formed in higher yields than those having the ε-amino function of lysine engaged in the peptide linkage.

The comparison of the NMR spectra of XIII—XVII in $CDCl_3$ and CD_3OD solutions, revealed some structural differences among the isomers. In $CDCl_3$, the methylene benzyl protons of the benzyloxycarbonyl group and two benzyl ester groups appeared as 2 singlets resonating between τ 5.03—4.92 and τ 4.88—4.84; the ratio of the upfield to downfield signal in the spectra of the α—α isomer XIII and the α—ε isomer XV was 2 : 1, whereas the reverse was true for the γ—α isomers XIV and XVII and the γ—ε isomer XVI. Hence, the signals of the Ph*CH*$_2$ protons of benzyloxycarbonyl and glutamate 5-benzyl ester group coincide at higher field and those of the Ph*CH*$_2$ protons of *C*-terminal alanine and glutamate 1-benzyl ester groups at lower field. In CD_3OD, a comparable and even more distinct picture of the Ph*CH*$_2$ signals was displayed by the isomeric structures XIV, XVI and XVII, whereas the spectra of the α—α isomer XIII and the α—ε isomer XV revealed the Ph*CH*$_2$ protons as 3 resolved singlets of equal intensities. The aromatic protons of the Ph*CH*$_2$ groups appeared in all cases mainly as a single peak, except in the $CDCl_3$ spectrum of the α—α isomer XIII in which the protons of 2 phenyl groups (attributed to the benzyloxycarbonyl and glutamate 5-benzyl ester group) were shifted to higher fields relative to their position in the spectra of other isomers. Due to extensive overlapping of the Me_3C signal, the alamine methyl doublets were partly observable only when CD_3OD was used as the solvent; slight differences in chemical shifts of the methyl doublets could be noted when comparing the spectra of the isomers.

Deprotection of XIII—XVII was performed in two successive steps: the benzyloxycarbonyl group and the benzyl ester groups were removed by catalytic hydrogenation and the BOC group on exposure to trifluoroacetic acid. The free pentapeptides XVIII—XXII were isolated as solid di-trifluoroacetate salts and characterized. When kept under anhydrous conditions the products proved to be quite stable for more than six months, however, in aqueous

solutions, decomposition of the L-γ—α isomer XIX and the α—ε isomer XX was notable after few days. On paper chromatography in 3 different solvent systems, the isomers travelled more or less parallel to each other, the differences in mobility being mostly apparent in solvent system *E* (see Experimental part).

As expected, a better resolution of the isomers was achieved by paper electrophoresis (Table I), due to differences in p*K* values between the α- and γ-carboxy-group of glutamic acid and the α- and ε-amino function of lysine.

TABLE I

Electrophoretic mobilities of isomeric L-*pentapeptides at different pH values**

Isomer No.	Glu, Lys type of linkage	Migration distances in cm			
		pH 2.4	pH 4.2	pH 8.5	pH 11.7
XVIII	α—α	—7.1	—1.0	+0.5	+2.0
XIX	γ—α	—6.1	—0.5	+0.7	+2.1
XX	α—ε	—7.0	—1.0	+3.2	+3.5
XXI	γ—ε	—5.8	0	+3.5	+3.5

* Low voltage paper electrophoresis, 12 V/cm. Conditions: 0.2 M acetic acid, pH 2.4, 2 hr; pyridine-acetic acid-water pH 4.2, 3 hr; 0.05 M sodium borate — 0.2 M boric acid buffer, 3 hr; 0.05 M sodium borate — 0.2 M sodium hydroxide buffer, pH 11.7, 3 hr. Colour reaction: ninhydrin.

At pH 2.4, the α—α and α-ε isomers XVIII and XX, having the γ-carboxy-group of glutamic acid residue free, are stronger cations than the isomers XIX and XXI in which the acid strength of the free α-carboxy-group of glutamic acid residue is increased by the adjacent peptide bond[12]. The slower migration of the γ—ε isomer XXI as compared to that of the γ—α isomer XIX can be ascribed to the acidifying effect of the peptide bond on the α-NH_3^+ group of the lysine residue; the effect is less pronounced for the ε-NH_3^+ group in XIX because of the distance of the CONH group. A similar picture of migration rates was observed at pH 4.2, where the γ—α and γ—ε isomers XIX and XXI appear to be very near their isoelectric points. At pH 8.5, the influence of the lysine amino functions is predominant: the α—α and γ—α isomers XVIII and XIX containing the still ionized ε-amino group of lysine are considerably less anionic than XX and XXI in which the lysine α-amino function has already lost its proton. Slight differences between the α—α and γ—α isomers XVIII and XIX, as well as between the α—ε and γ—ε isomers XX and XXI can be again ascribed to the relative proximities of the acidifying peptide bonds. In strongly basic media where all the four isomers behave as true anionic species, the difference in migration between the α- and ε-lysyl bonded isomers is much less pronounced.

The NMR spectra at 60 MHz of D_2O solutions of the pentapeptides XVIII—XXII di-trifluoroacetate salts revealed considerable resemblance. The most notable differences in resonance positions of the alanine methyl protons among the isomers were noted for the second doublet, which according to the results presented below, can be ascribed to the *C*-terminal alanine methyl group. The signal of the ε-CH_2 group of lysine residue was observable as a

TABLE II

Chemical shifts of alanyl methyl protons in pentapeptides XVIII and XXII and related di- and tripeptides in D_2O solutions as a function of pH[a]

Peptide	pH	Chemical shift[b]			Shift difference[c]		
		H-Ala-	-Ala-	-Ala-OH			
	0.6	92.5	83.5	85.5	0	0	—5.5
H-Ala-Glu-Lys-Ala-Ala-OH	1.2	92.0	83.5	85.5	+0.5	0	—5.5
	5.6	92.5	83.5	80.0	0	0	0
XVIII	9.0	79.0	83.0	79.0	+13.5	+0.5	+1.0
	12.5[d]	74.5	82.5	79.0	+18.0	+1.0	+1.0
	0.9	93.5	83.0	86.0	—1.0	—1.0	—6.5
H-Ala-D-Glu	1.4	93.5	83.0	85.5	—1.0	—1.0	—6.0
└Lys-D-Ala-D-Ala-OH	4.5	92.5	82.0	80.5	0	0	—1.0
	6.8	92.5	82.0	79.5	0	0	0
XXII	9.3	79.5	82.0	79.5	+13.0	0	0
	12.1[d]	75.5	81.5	79.5	+17.0	+0.5	0
	1.2	93.5	—	—	—1.0	—	—
H-Ala-D-Glu-OH	4.0	92.5	—	—	0	—	—
	11.5[d]	75.0	—	—	+17.5	—	—
	1.2	93.5	—	—	—0.5	—	—
H-Ala-Glu-OH	3.8	93.0	—	—	0	—	—
	12.7[d]	75.5	—	—	+17.5	—	—
	0.9	—	85.0	86.0	—	—1.0	—6.5
H-Lys-D-Ala-D-Ala-OH	7.8	—	84.0	79.5	—	0	0
	9.0	—	83.5	79.5	—	+0.5	0
	11.0	—	83.5	79.5	—	+0.5	0

[a] Spectra were measured on solutions containing 20—30 mg of the peptide in D_2O (0.5 ml); the pH of the solutions were adjusted with trifluoroacetic acid or NaOH + D_2O and measured with a Radiometer pH meter 26.

[b] Proton NMR spectra were determined on a Varian A-60A spectrometer at probe temperature. Chemical shifts (metyl doublets, J = 7 Hz) are quoted in hertz downfield from internal sodium dimethylsilapentylsulphonic acid (SDSS). H-Ala-, -Ala- and -Ala-OH represent alanyl residues at the *N*-terminal, central and C-terminal position, respectively. The error of measurement was ± 1 Hz.

[c] Up- (+) and downfield (—) shifts of the spectral lines of alanine methyl protons for acidic and basic solutions of the peptide relative to their position in solution containing the peptide in zwitterionic form.

[d] Chemical shift of *CH* quartet of *N*-terminal alanine: 212 Hz.

broad triplet: in the spectra of isomers XVIII, XIX and XXII, which have the lysine ε-amino function unsubstituted, the signal appeared at a higher field as compared to its position in the spectra of XX and XXI, which have the ε-amino group of lysine involved in the peptide bond.

The assignement of the alanine methyl resonances in the α—α L-pentapeptide XVIII and the γ—α L-D-L-D-D-pentapeptide XXII was made by using the Sheinblatt's method[13] based on the fact that the position of the spectral line of a group adjacent to a potentially ionizable group depends on the state of ionisation of that group; accordingly, the resonance positions of the protons on the side chains of amino acids are also influenced by the charged amino and carboxy-groups[13–15]. Hence, in both pentapeptide isomers, the addition of

base should shift the doublet of the *N*-terminal alanine methyl group to higher field, acidification should shift the *C*-terminal alanine methyl signal toward lower field, whereas the signal of the penultimate alanine residue in the peptide chain should be affected only slightly by changes of pH.

The shifts of the spectral lines of alanine methyl groups of XVIII and XXII and the related di- and tripeptides are given in Table II. The shifts observed for the two pentapeptides agree very well with those of the parallely examined reference compounds, as well as with the data reported[15] for some related oligopeptides. The assignement of the α-*CH* proton of *N*--terminal alanine was made by considering the effects of pH changes on the proton resonances appearing in the region obscured by the large HOD peak: in basic solutions the one-proton quartet was strongly shifted upfield relative to its position in the spectra of acidic and neutral solutions of both pentapeptides as well as of the two reference dipeptides. In addition, at pH $>$ 9, an upfield shift (~ 25 Hz) of the lysine ε-CH_2 triplet, due to neutralization of the ε-NH_3^+ group was clearly observable. On the other hand, resolution and assignements of the α-*CH* protons to particular amino acids in the pentapeptide molecules were not possible with the magnet used.

Hence, the chemical shift differences of alanine methyl doublets are large enough to permit assignement of the three alanine residues in the isomeric pentapeptides XVIII and XXII by the above simple NMR procedure. The results suggest that this procedure may be also useful in structure elucidation of similar peptides isolated from natural material.

EXPERIMENTAL

General

Melting points are uncorrected. Organic solutions were dried over anhydrous sodium sulphate, and concentrations were carried out under reduced pressure in a rotary evaporator at minimum temperature. TLC was conducted on plates coated with Kiesel G (Merck), and paper-chromatography and -electrophoresis were performed on Whatman No 1 paper. The homogeneity of the intermediates and end products was ascertained by TLC or paper-chromatography in following solvent systems (v/v): *A*, chloroform-methanol (1 : 1); *B*, ethyl acetate-benzene (1 : 1); *C*, chloroform-acetone (3 : 2); *D*, *n*-butanol-pyridine-acetic acid-water (15 : 10 : 3 : 12); *E*, *iso*-butyric acid-conc. ammonia-water (66 : 2 : 23); *F*, *n*-butanol-acetic acid-water (60 : 15 : 25). Compounds with free amino groups were detected with ninhydrin, *N*-acylated compounds were revealed either by ninhydrin, after spraying the plates with 2% HBr in acetic acid-ethanol (1 : 5) and heating, or by the chlorine-starch--iodine method.

Optical rotations were determined for 1% solutions, if not stated otherwise, at 20—25 °C in the solvent specified. Proton NMR spectra were recorded on a Varian A-60A spectrometer in deuterochloroform and deuteromethanol with tetramethylsilane (TMS) as internal standard, and in deuterium oxide by using sodium dimethylsilapentylsulphonic acid (SDSS) as internal standard, if not stated otherwise.

N-tert-*Butoxycarbonyl*-L-*alanyl*-5-*benzyl*-L-*glutamic Acid* (I) *(Dicyclohexylammonium Salt)*

To a stirred suspension of 5-benzyl-L-glutamic acid (3.92 g, 16.6 mmoles) and sodium hydrogen carbonate (1.39 g, 16.6 mmoles) in water (25 ml), *N*-*tert*-butoxycarbonyl-L-alanine *N*-hydroxysuccinimide ester (2.86 g, 10 mmoles) in 1,2-dimethoxyethane (25 ml) was added at room temperature; after 3 hr of stirring, the mixture was acidified at 0 °C with aqueous 10% citric acid to pH 3. The precipitated 5-benzyl-L--glutamic acid was filtered off, the filtrate was concentrated to about one third of the volume, and the product was extracted with ethyl acetate (3 x). The combined

organic layers were washed with water, dried and concentrated. The remaining oil was dissolved in ethyl acetate (10 ml) and dicyclohexylamine (1.80 g, 10 mmoles) in ethyl acetate (5 ml) was added under stirring; subsequent addition of petroleum ether at 0 °C precipitated the DCHA salt of I (5.6 g, 95%), m. p. 135—137 °C. After crystallization from ethyl acetate-petroleum ether, the product had m. p. 139—141 °C and $[\alpha]_D$ + 1.6° (c 2, DMF).

Anal. $C_{32}H_{51}N_3O_7$ (589.75) calc'd.: C 65.17; H 8.72; N 7.13%
found: C 65.45; H 8.90; N 7.04%

A sample of the above salt was dissolved in precooled aqueous 25% citric acid (5 ml), the solution was extracted with ethyl acetate (3 x), and the combined extracts were washed with water, dried and concentrated. The remaining oil was dissolved in dry ether (5 ml); subsequent addition of petroleum ether at 0° C precipitated the dipeptide I as colourless crystals, m. p. 75—77 °C, $[\alpha]_D$ — 7.6 ° (c 1.7, DMF). NMR data ($CDCl_3$): τ 0.11 (*s*, 1 H, COOH), 2.73 (*s*, 5 H, Ph), 2.88 (*d*, 1 H, J = 8 Hz, NH), 4.92 (*s*, 2 H, $PhCH_2O$), 8.62 (*s*, 9 H, Me_3C), 8.68 (*d*, J = 7 Hz, the downfield peak masked by Me_3C signal, *Me*-CH).

Anal. $C_{20}H_{28}N_2O_7$ (408.44) calc'd.: C 58.81; H 6.91; N 6.86%
found: C 58.58; H 7.10; N 6.65%

N-tert-*Butoxycarbonyl*-L-*alanyl*-5-*benzyl*-L-*glutamic Acid* N-*Hydroxysuccinimide Ester* (II)

The DCHA salt of I (1.47 g, 2.5 mmoles) was treated with citric acid as described above, and to the free peptide in ethyl acetate (5 ml) were added *N*-hydroxysuccinimide (288 mg, 2.5 mmoles) and dicyclohexylcarbodi-imide (DCC, 516 mg, 2.5 mmoles) in ethyl acetate at 0 °C. The mixture was kept at 0 °C for 24 hr whereupon *N,N'*-dicyclohexylurea was filtered off, and the filtrate was washed with 10% citric acid, water, saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The remaining viscous oil (1.2 g, 95%) traveled as a single spot on TLC in solvents *A* and *C*; $[\alpha]_D$ — 21.0 ° (EtOAc). NMR data ($CDCl_3$): τ 2.71 (*s*, 5 H, Ph), 2.88 (*d*, 1 H, J = 8 Hz, NH), 4.91 (*s*, 2 H, $PhCH_2O$), 5.79 (*q*, 1 H, *CH* of Ala), 7.23 (*s*, 4 H, 2 × CH_2 from succinimide), 8.61 (*s*, Me_3C), 8.68 (*d*, 3 H, J = 7 Hz, the downfield peak masked by Me_3C signal, *Me*-CH).

Anal. $C_{24}H_{31}N_3O_9$ (505.51) calc'd.: C 57.02; H 6.18; N 8.31%
found: C 57.25; H 6.40; N 8.38%

N-tert-*Butoxycarbonyl*-L-*alanyl*-1-*benzyl*-L-*glutamic Acid* (III) *(Dicyclohexylammonium Salt)*

Starting with 1-benzyl L-glutamic acid (1.77 g), the procedure described for the preparation of the isomeric dipeptide I was followed to give the DCHA salt of III: 3.55 g, 81.0%, m. p. 157—159 °C, $[\alpha]_D$ — 16.0° (EtOH), $[\alpha]_D$ — 16.8° (DMF).

Anal. $C_{32}H_{51}N_3O_7$ (589.75) calc'd.: C 65.17; H 8.72; N 7.13%
found: C 64.89; H 8.98; N 7.00%

A sample of the above salt was treated with aqueous citric acid as described for I to give the free dipeptide III as a chromatographically homogeneous oil which crystallized subsequently from ether-petroleum ether at 0 °C: m. p 43—49 °C, $[\alpha]_D$ — 22.4 ° (DMF).

Anal. $C_{20}H_{28}N_2O_7$ (408.44) calc'd.: C 58.81; H 6.91; N 6.86%
found: C 58.96; H 6.87; N 6.69%

N-tert-*Butoxycarbonyl*-L-*alanyl*-1-*benzyl*-L-*glutamic Acid* N-*Hydroxysuccinimide Ester* (IV)

To a solution of III (prepared from 3.54 g of III × DCHA salt as described above) in ethyl acetate (15 ml) were added *N*-hydroxysuccinimide (690 mg) and DCC

(1.24 g) in ethyl acetate at 0 °C, and the mixture was further treated as described for II. After work up, the active ester IV was obtained as a viscous oil which crystallized subsequently from chloroform-petroleum ether at 0 °C: m. p. 96—98 °C, $[\alpha]_D$ — 11.7° (EtOAc), yield: 70%. NMR data ($CDCl_3$): τ 2.74 (*s*, Ph), 2.97 (*d*, 1 H, J = 8 Hz, NH), 4.68 (*d*, 1 H, J = 8 Hz, NH), 4.87 (*s*, 2 H, PhCH_2O), 5.91 (*q*, 1 H, *CH* of Ala), 7.25 (*s*, 4 H, 2 × CH_2 from succinimide), 8.61 (*s*, Me_3C), 8.70 (*d*, 3 H, J = 7 Hz, *Me*-CH).

Anal. $C_{24}H_{31}N_3O_9$ (505.51) calc'd.: C 57.02; H 6.18; N 8.31%
found: C 57.01; H 6.18; N 8.38%

N-tert-*Butoxycarbonyl*-L-*alanyl*-*1*-*benzyl*-D-*glutamic Acid* (V)

The DCHA salt of V[7] was prepared as described for III and then treated with aqueous citric acid to give the free dipeptide V as a viscous oil which crystallized from ether-petroleum ether: m.p. 88—89 °C, $[\alpha]_D$ + 9.3° (DMF). Lit.[16]: m.p. 83—84 °C, $[\alpha]_D$ + 18.34° (DMF). NMR data ($CDCl_3$): τ 0.94 (broad, 1 H, COOH), 2.71 (*s*, Ph), 2.99 (*d*, J = 8 Hz, NH), 4.87 (*s*, 2 H, PhCH_2O), 8.60 (*s*, Me_3C), 8.67 (*d*, J = 7 Hz, the downfield peak masked by Me_3C, *Me*-CH).

Anal. $C_{20}H_{28}N_2O_7$ (408.44) calc'd.: C 58.81; H 6.91; N 6.86%
found: C 59.04; H 7.08; N 6.72%

N-tert-*Butoxycarbonyl*-L-*alanyl*-*1*-*benzyl*-D-*glutamic Acid* N-*Hydroxysuccinimide Ester* (VI)

Starting with V (1.74 g), the procedure described for the preparation of the diastereoisomeride IV was used. After work up, the active ester VI was obtained as a solid foam (1.94 g). A sample was crystallized from ethyl acetate-petroleum ether: on standing at 0 °C, crystals with m. p. 84—86 °C and $[\alpha]_D$ — 7.0° (c 0.6, EtOAc) deposited. NMR data ($CDCl_3$): τ 2.70 (*s*, Ph), 3.03 (*d*, 1 H, J = 8 Hz, NH), 4.85 (*s*, 2 H, PhCH_2O), 5.87 (*q*, 1 H, *CH* of Ala), 7.21 (*s*, 4 H, 2 × CH_2 from succinimide), 8.58 (*s*, Me_3C), 8.68 (*d*, 3 H, J = 7 Hz, *Me*-CH).

Anal. $C_{24}H_{31}N_3O_9$ (505.51) calc'd.: C 57.02; H 6.18; N 8.31%
found: C 57.13; H 6.46; N 8.12%

N^2-tert-*Butoxycarbonyl*-N^6-*benzyloxycarbonyl*-L-*lysyl*-L-*alanyl*--L-*alanine Benzyl Ester* (VII)

To *N*-*tert*-butoxycarbonyl-L-alanyl-L-alanine benzyl ester[17] (1.75 g, 5 mmoles) was added cold trifluoroacetic acid (8 ml), and the solution was kept at 0 °C for 30 min and then at ambient temperature for 15 min. Trifluoroacetic acid was evaporated and traces were removed by repeated co-distillation with benzene; the residue was dissolved in DMF (4 ml) and neutralized with *N*-methylmorpholine (0.56 ml) at 0 °C. To this solution were added under stirring at 0 °C N^2-*tert*-butoxycarbonyl-N^6-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester[5] (2.5 g, 5 mmoles) and imidazole (680 mg, 10 mmoles) in DMF (5 ml each). The reaction mixture was kept at 0 °C for 24 hr, whereupon it was diluted with water and extracted with ethyl acetate (3 × 50 ml). The combined extracts were washed with 10% aqueous citric acid, water, saturated aqueous sodium hydrogen carbonate and water, dried and concentrated. The residue was crystallized from hot ethanol to give the fully protected tripeptide VII (2.2 g, 72%), m. p. 132—134 °C. A second crystallization from ethyl acetate-petroleum ether afforded the analytical sample. m. p. 134—136 °C, $[\alpha]_D$ — 17.9° (DMF).

Anal. $C_{32}H_{44}N_4O_8$ (612.70) calc'd.: C 62.72; H 7.24; N 9.15%
found: C 62.48; H 7.45; N 9.26%

N^6-*Benzyloxycarbonyl*-L-*lysyl*-L-*alanine Benzyl Ester* (VIII) *(Trifluoroacetate Salt)*

The foregoing peptide VII (766 mg, 1.25 mmole) was treated with trifluoroacetic acid (5 ml) as described above, and the residue was dissolved in ethyl acetate; sub-

sequent addition of ether at 0 °C precipitated the trifluoroacetate salt of VIII (640 mg, 81.8%), m. p. 165—166 °C, $[\alpha]_D$-8.0° (DMF).

Anal. $C_{29}H_{37}F_3N_4O_8$ (626.62) calc'd.: C 55.58; H 5.95; N 8.94%
found: C 55.59; H 6.20; N 8.94%

N^2-*Benzyloxycarbonyl*-N^6-tert-*butoxycarbonyl*-L-*lysyl*-L-*alanyl*-L-*alanine Benzyl Ester* (IX)

To a solution of L-alanyl-L-alanine benzyl ester in DMF (2 ml), prepared from BOC-Ala-Ala-OBZL (525 mg, 1.25 mmole) as described in the preparation of VII, were added under stirring at 0 °C N^2-benzyloxycarbonyl-N^6-*tert*-butoxycarbonyl-L-lysine *N*-hydroxysuccinimide ester[18] (717 mg, 1.5 mmole) and imidazole (204 mg, 3 mmoles) in DMF (3 ml each). The reaction mixture was further treated as described for the synthesis of VII; crystallization of the product from ethyl acetate-petroleum ether, followed by ethanol, afforded the analytical sample of the tripeptide IX (yield 700 mg, 76%), m. p. 132—133 °C, $[\alpha_D]$ — 19.1° (DMF).

Anal. $C_{32}H_{44}N_4O_8$ (612.70) calc'd.: C 62.72; H 7.24; N 9.15%
found: C 62.62; H 7.28; N 9.40%

N^2-*Benzyloxycarbonyl*-L-*lysyl*-L-*alanyl*-L-*alanine Benzyl Ester* (X) *(Trifluoroacetate Salt)*

The foregoing tripeptide IX (140 mg, 0.23 mmole) was treated with trifluoroacetic acid as described above; after crystallization from ethyl acetate-petroleum ether, the trifluoroacetate salt of X (116 mg, 81%) had m. p. 114—116 °C and $[\alpha]_D$ — 21.0° (c 0.5, DMF).

Anal. $C_{29}H_{37}F_3N_4O_8$ (626.62) calc'd.: C 55.58; H 5.95; N 8.94%
found: C 55.76; H 6.22; N 8.83%

N^2-tert-*Butoxycarbonyl*-N^6-*benzyloxycarbonyl*-L-*lysyl*-D-*alanyl*-D-*alanine Benzyl Ester* (XI)

Starting with *N*-*tert*-butoxycarbonyl-D-alanyl-D-alanine benzyl ester[19] (2.45 g, 7.0 mmoles), the procedure described for the synthesis of VII was used. Crystallization from ethanol gave the L-D-D-tripeptide XI (3.3 g, 77.0%), m. p. 120—122 °C; the analytical sample had 122—124 °C (from ethyl acetate-petroleum ether) and $[\alpha]_D$ + + 9.4° (DMF), $[\alpha]_D$ + 32.7° (MeOH). Lit.[9]: m. p. 118—120 °C, $[\alpha]_D$ + 30.3° (c 0.7, MeOH); lit.[10]: m. p. 110—112 °C, $[\alpha]_D$ + 32.1° (c 1.4, MeOH). NMR data ($CDCl_3$): τ 2.73 (*s*, 10 H, 2 × Ph), 4.88 (*s*, 2 H, PhCH_2O), 4.95 (*s*, 2 H, PhCH_2OCON), 8.61 (*s*, Me_3C), 8.62 and 8.66 (2 *d*, J = 7 Hz, the downfield peaks masked by Me_3C signal, 2 × *Me*-CH).

Anal. $C_{32}H_{44}N_4O_8$ (612.70) calc'd.: C 62.72; H 7.24; N 9.15%
found: C 62.58; H 7.37; N 9.02%

N^6-*Benzyloxycarbonyl*-L-*lysyl*-D-*alanyl*-D-*alanine Benzyl Ester* (XII) *(Trifluoroacetate Salt)*

The foregoing tripeptide XI (1.3 g, 2.12 mmoles) was treated with trifluoroacetic acid (4 ml) as described above to give the trifluoroacetate salt of XII (1.2 g, 90%); the analytical sample had m. p. 145—147 °C (ethyl acetate-ether) and $[\alpha]_D$ + 31.0° (DMF).

Anal. $C_{29}H_{37}F_3N_4O_8$ (626.62) calc'd.: C 55.58; H 5.95; N 8.94%
found: C 55.60; H 6.16; N 8.94%

To a sample (120 mg) of the above salt in methanol (0.5 ml) was added at 0 °C 1 equivalent of triethylamine in ethyl acetate (15 ml); the mixture was stirred for 15 min, the organic phase was washed with water, dried and evaporated, and the

residue was dissolved in methanol-d_4. NMR data (CD_3OD): τ 2.75 (*s*, 10 H, 2 × Ph), 4.90 (*s*, 2 H, PhCH_2O), 4.96 (*s*, 2 H, PhCH_2OCON), 8.61 and 8.70 (2 sets of overlapping doublets, 2 × 3 H, J = 7 Hz, 2 × *Me*-CH).

N-tert-*Butoxycarbonyl*-L-*alanyl*-*5-benzyl*-L-*glutamyl*-N^6-*benzyloxycarbonyl*-L-*-lysyl*-L-*alanyl*-L-*alanine Benzyl Ester* (XIII)

To a solution of the trifluoroacetate salt of VIII (576 mg, 0.92 mmole) in DMF (5 ml) was added *N*-methylmorpholine (0.1 ml, 0.92 mmole) at 0°C. The mixture was stirred for 10 min, the dipeptide active ester II (465 mg, 0.92 mmole) in DMF was added, and stirring was continued for 1 hr at 0 °C. The reaction mixture was kept at 0 °C for 48 hr whereupon it was diluted with water (15 ml), the precipitated product was filtered off, washed with water and ether-petroleum ether (1 : 1) and dried. Crystallization from ethyl acetate-petroleum ether gave the protected pentapeptide XIII (717 mg, 86%), m. p. 161—162 °C. A second crystallisation from ethanol afforded the analytical sample, m. p. 162—163 °C, $[\alpha]_D$ — 19.8° (DMF). NMR data ($CDCl_3$): τ 2.72, 2.80, 2.83 (3 *s*, 15 H, 3 × Ph), 4.84 (*s*, 2 H, PhCH_2O of Ala), 5.03 (*s*, 4 H, 5-PhCH_2O of Glu + PhCH_2OCON of Lys), 8.64 (*s*, Me_3C); (CD_3OD): τ 2.70 (*s*, 15 H, 3 × Ph), 4.88, 4.91, 4.96 (3 × 2 H singlets, PhCH_2O of Ala, 5-PhCH_2O of Glu, PhCH_2OCON), 8.60 (*s*, Me_3C), 8.64 (*d*, the downfield peak masked by Me_3C, *Me*-CH), 8.69 and 8.73 (2 sets of overlapping doublets, 2 × 3 H, J = 7 Hz, 2 × *Me*-CH).

Anal. $C_{47}H_{62}N_6O_{12}$ (903.02) calc'd.: C 62.51; H 6.92; N 9.31%
found: C 62.47; N 6.95; N 9.17%

N-tert-*Butoxycarbonyl*-L-*alanyl*-*1-benzyl*-L-*γ-glutamyl*-N^6-*benzyloxycarbonyl*-L-*-lysyl*-L-*alanyl*-L-*alanine Benzyl Ester* (XIV)

The tripeptide VIII was liberated from its trifluoroacetate salt (1.25 g, 2 mmoles) as described above, and coupled with the dipeptide active ester IV (1.01 g, 2 mmoles) by the same procedure which is described for the synthesis of XIII. Crystallization of the precipitated product from ethanol gave XIV (1.2 g, 66.5%), m. p. 161—163 °C; two further crystallization from DMF-petroleum ether and then from ethanol gave the analytical sample, m. p. 179—181 °C (sintering at 165 °C), $[\alpha]_D$ — 17.9° (DMF). NMR data ($CDCl_3$):τ 2.72 (*s*, 3 Ph), 4.88 (*s*, 4 H, PhCH_2O of Ala + 1-PhCH_2O of Glu), 4.95 (*s*, 2 H, PhCH_2OCON), 8.60 (*s*, Me_3C), 8.68 and 8.70 (overlapping doublets partly masked by Me_3C, 3 × *Me*-CH); (CD_3OD): τ 2.72 (*s*, 3 PH), 4.89 (*s*, 4 H, PhCH_2O of Ala + + 1-PhCH_2O of Glu), 4.95 (*s*, 2 H, PhCH_2OCON), 8.60 (*s*, Me_3C), 8.66 (*d*, 3 H, J = 7 Hz, partly masked by Me_3C, *Me*-CH), 8.70 and 8.76 (2 sets of overlapping doublets, 2 × 3 H, J = 7 Hz, 2 × *Me*-CH).

Anal. $C_{47}H_{62}N_6O_{12}$ (903.02) calc'd.: C 62.51; H 6.92; N 9.31%
found: C 62.66; H 7.17; N 9.53%

N^2-*Benzyloxycarbonyl*-N^6-*[*N-tert-*butoxycarbonyl*-L-*alanyl*-*5-benzyl*-L-*-glutamyl]*-L-*lysyl*-L-*alanyl*-L-*alanine Benzyl Ester* (XV)

The trifluoroacetate salt of X (1.25 g, 2 mmoles) was liberated from its salt and the tripeptide X was coupled with II as described for the isomeric pentapeptide XIII. Crystallization from ethanol gave XV (926 mg, 51.3%); the analytical sample had m. p. 156—158 °C (ethyl acetate-petroleum ether) and $[\alpha]_D$ — 22.0° (DMF). NMR data ($CDCl_3$): τ 2.71 (*s*, 3 Ph), 4.87 (*s*, 2 H, PhCH_2O of Ala), 4.92 (*s*, 4 H, 5-PhCH_2O of Glu + PhCH_2OCON), 8.59 (*s*, Me_3C); (CD_3OD): τ 2.71 (*s*, 3 Ph), 4.88, 4.91, 4.93 (3 × × 2 H singlets, PhCH_2O of Ala, 5-PhCH_2O of Glu, PhCH_2OCON), 8.60 (*s*, Me_3C), 8.69 and 8.74 (2 sets of overlapping doublets, 3 H + 6 H, J = 7 Hz, partly masked by Me_3C, 3 × *Me*-CH).

Anal. $C_{47}H_{62}N_6O_{12}$ (903.02) calc'd.: C 62.51; H 6.92; N 9.31%
found: C 62.31; H 7.00; N 9.33%

N^2-*Benzyloxycarbonyl*-N^6-[N-*tert-butoxycarbonyl*-L-*alanyl*-1-*benzyl*-L-γ--*glutamyl*]-L-*lysyl*-L-*alanine Benzyl Ester* (XVI)

Starting with the trifluoroacetate salt of X (602 mg, 0.96 mmole) and the dipeptide active ester IV (486 mg, 0.96 mmole), the procedure described for the preparation of XIII was used. Crystallization of the precipitated product from a minimal amount of ethanol gave the pentapeptide XVI (430 mg, 50%), and a second crystallization from the same solvent afforded the analytical sample: m. p. 175—177 °C (sintering at 158 °C), $[\alpha]_D$ — 13.3° (DMF). NMR data ($CDCl_3$): τ 2.72 (*s*, 3 Ph), 4.87 (*s*, 4 H, PhCH_2O of Ala + 1-PhCH_2O of Glu), 4.96 (*s*, 2 H, PhCH_2OCON), 8.60 (*s*, Me_3C); (CD_3OD): τ 2.71 (*s*, 3 Ph), 4.88 (*s*, 4 H, PhCH_2O of Ala + 1-PhCH_2O of Glu), 4.97 (*s*, 2 H, PhCH_2OCON), 8.59 (*s*, Me_3C), 8.70 and 8.75 (2 sets of overlapping doublets, 3H + 6 H, J = 7 Hz, partly masked by Me_3C, 3 × *Me*-CH).

Anal. $C_{47}H_{62}N_6O_{12}$ (903.02) calc'd.: C 62.51; H 6.92; N 9.31%
found: C 62.43; H 6.65; N 9.48%

N-tert-*Butoxycarbonyl*-L-*alanyl*-1-*benzyl*-D-γ-*glutamyl*-N^6-*benzyloxycarbonyl*--L-*lysyl*-D-*alanyl*-D-*alanine Benzyl Ester* (XVII)

Starting with the L-D-D-tripeptide XII (931 mg, 1.49 mmole) trifluoroacetate salt and the L-D-dipeptide active ester VI, the procedure described for the preparation of XIII was used. The crude pentapeptide XVII (1.14 g, 85%) had m. p. 154—155 °C and $[\alpha]_D$ + 20.1° (MeOH); two recrystallization from ethanol afforded the analytical sample, m.p. 160—162 °C, $[\alpha]_D$ + 21.6° (MeOH), $[\alpha]_D$ + 12.3° (DMF). Lit.[10]: m. p. 154—156 °C, $[\alpha]_D$ + 18.1° (MeOH). NMR data ($CDCl_3$): τ 2.72 (*s*, 3 Ph), 4.88 (broad singlet, 4 H, 2 × PhCH_2O) and 4.94 (*s*, 2 H, PhCH_2OCON), 8.61 (*s*, Me_3C); (CD_3OD): τ 2.73 (*s*, 3 Ph), 4.90 (broad singlet, 4 H, 2 × PhCH_2O), 4.97 (*s*, 2 H, PhCH_2OCON), 8.59 (*s*, Me_3C), 8.62 (*d*, partly masked by Me_3C, *Me*-CH), 8.69 and 8.74 (2 sets of overlapping doublets, J = 7 Hz, 2 × *Me*-CH).

Anal. $C_{47}H_{62}N_6O_{12}$ (903.02) calc'd.: C 62.51; H 6.92; N 9.31%
found: C 62.31; H 6.96; N 9.48%

Removal of the Protecting Groups of Pentapeptide Isomers XIII—XVII

General Procedure. — To a solution of the protected pentapeptide (476 mg, 0.5 mmole) in acetic acid (5 ml), was adedd 10% Pd/C (Fluka puriss., 100 mg), and the mixture was shaken at room temperature and pressure until the uptake of hydrogen was complete (~ 10 hr, monitoring by TLC in solvent systems *D* and *E*). The catalyst was centrifuged off, washed with acetic acid, and the combined supernatants were evaporated to dryness (0.1 Torr, bath 30 °C). The residue was dissolved at 0 °C in trifluoroacetic acid (4 ml); after 20 min at 0 °C and 10 min at ambient temperature, dry ether was added to the solution, and the precipitate was centrifuged off, washed with dry ether (3 ×) and dried. A second crystallization from trifluoroacetic acid-dry ether, followed by drying *in vacuo* over phosphorous pentoxyde and conc. sulphuric acid, afforded the analytical sample.

Pentapeptides XVIII—XXII di-trifluoroacetate salts were subjected in parallel runs to paper chromatography in solvent systems cited below. NMR spectra of XVIII—XXII di-TFA salts were taken in D_2O with TMS as external standard. Alanine methyl groups invariably produced doublets with J = 7 Hz; in all spectra 2 doublets were observed as 3 peaks.

L-*Alanyl*-L-*glutamyl*-L-*lysyl*-L-*alanyl*-L-*alanine* (XVIII)
(Di-Trifluoroacetate Salt)

Yield: 86%, m. p. 145—150 °C (decomp.), $[\alpha]_D$ — 47.4° (c 1.39, water). R_f (solvent system): 0.17 (*D*); 0.65 (*E*); 0.13 (*F*). NMR data: τ 8.47 and 8.59 (2 *d*, 2 × 3 H, 2 × × *Me*-CH), 8.62 (*d*, 3 H, Me-*CH*), 7.00 (broad, *t*, 2 H, ε-CH_2 of Lys).

Anal. $C_{24}H_{38}F_6N_6O_{12}$ (716.59) calc'd: C 40.22; H 5.35; N 11.73%
found: C 40.47; H 5.39; N 11.88%

L-*Alanyl*-L-γ-*glutamyl*-L-*lysyl*-L-*alanyl*-L-*alanine* (XIX) (Di-*Trifluoroacetate Salt*)

Yield: 83%, m.p. 115—120 °C (decomp.), $[\alpha]_D$ — 36.7° (c 0.79, water). R_f: 0.15 (*D*); 0.56 (*E*); 0.09 (*F*). NMR data: τ 8.46 and 8.57 (2 *d*, 2 × 3 H, 2 × *Me*—CH), 8.61 (*d*, 3 H, *Me*—CH), 6.98 (broad, t, ε—CH_2 of Lys).

Anal. $C_{24}H_{38}F_6N_6O_{12}$ found: C 40.33; H 5.63; N 11.55%

N^6-[L-*Alanyl*-L-*glutamyl*]-L-lysyl-L-*alanyl*-L-*alanine* (XX) (Di-*Trifluoroacetate Salt*)

Yield: 76%, m.p. 110—115 °C (decomp.), $[\alpha]_D$ — 19.8° (c 1.5, water). R_f: 0.19 (*D*); 0.66 (*E*); 0.11 (*F*). NMR data: τ 8.49 and 8.61 (2*d*, 2 × 3 H, 2 × *Me*—CH), 8.59 (*d*, 3 H, *Me*—CH), 6.80 (broad, *t*, 2 H ε—CH_2 of Lys).

Anal. $C_{24}H_{38}F_6N_6O_{12}$ found: C 40.37; H 5.33; N 11.70%

N^6-[L-*Alanyl*-L-γ-*glutamyl*]-L-*lysyl*-L-*alanyl*-L-*alanine* (XXI) (Di-*Trifluoroacetate Salt*)

Yield: 80%, m.p. 125—130 °C (decomp.), $[\alpha]_D$ — 15.1° (water). R_f: 0.16 (*D*); 0.58 (*E*); 0.11 (*F*). NMR data: τ 8.49, and 8.61 (2 *d*, 2 × 3 H, 2 × *Me*—CH), 8.63 (*d*, 3 H, *Me*—CH), 6.85 (broad, *t*, 2 H, ε—CH_2 of Lys).

Anal. $C_{24}H_{38}F_6N_6O_{12}$ found: C 40.04; H 5.48; N 11.84%

L-*Alanyl*-D-γ-*glutamyl*-L-*lysyl*-D-*alanyl*-D-*alanine* (XXII) (Di-*Trifluoroacetate Salt*)

Yield: 85%, m.p. 125—130 °C (decomp.), $[\alpha]_D$ + 17.5° (water). R_f: 0.14 (*D*); 0.49 (*E*); 0.10 (*F*). NMR data: τ 8.44 and 8.57 (2*d*, 2 × 3 H, 2 × *Me*—CH), 8.62 (*d*, 3 H, *Me*—CH), 6.93 (broad, *t*, 2 H, ε—CH_2 of Lys).

Anal. $C_{24}H_{38}F_6N_6O_{12}$ found: C 40.29; H 5.37; N 11.89%

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REFERENCES

1. D. Keglević, B. Ladešić, O. Hadžija, J. Tomašić, Z. Valinger, M. Pokorny, and R. Naumski, *Eur. J. Biochem.* **42** (1974) 389.
2. D. Mirelman, R. Bracha, and N. Sharon, *Biochemistry* **13** (1974) 5045.
3. Z. Tynecka and J. B. Ward, *Biochem. J.* **146** (1975) 253.
4. E. Bricas, in P. G. Katsoyannis (Ed.), *The Chemistry of Polypeptides*, Plenum Press, New York, 1973, pp. 205—235.
5. A. E. Lanzilotti, E. Benz, and L. Goldman, *J. Amer. Chem. Soc.* **86** (1964) 1880.
6. M. C. Khoshla, N. C. Chaturvedi, H. G. Garg, and N. Anand, *Indian J. Chem.* **3** (1965) 111.
7. P. Dezélée and E. Bricas, *Biochemistry* **9** (1970) 823.
8. M. Bodanszky, and K. W. Funk, *J. Org. Chem.* **38** (1973) 1296.
9. P. Lefrancier and E. Bricas, *Bull. Soc. Chim. Biol.* **49** (1967) 1257.
10. A. R. Zeiger and P. H. Maurer, *Biochemistry* **12** (1973) 3387.
11. B. Weinstein and A. E. Pritchard, *J. Chem. Soc. Perkin I*, (1972) 1015.
12. J. P. Greenstein and M. Winitz, *Chemistry of Amino Acids*, J. Wiley and Sons, New York, 1961 Vol. I pp. 435—522.
13. M. Sheinblatt, *J. Amer. Chem. Soc.* **88** (1966) 2845.
14. B. Halpern, L. F. Chew, and B. Weinstein, *J. Amer. Chem. Soc.* **89** (1967) 5051.
15. E. van Gorkom, *Tetrahedron Lett.* (1966) 5433.

16. M. C. Khoshla, N. Chaturvedi, and N. Anand, *Indian J. Chem.* **5** (1967) 237.
17. D. A. Laufer, T. M. Chapman, D. I. Marlborouth, V. M. Vaidya, and E. R. Blout, *J. Amer. Chem. Soc.* **90** (1968) 2696.
18. M. A. Ruttenberg, *J. Amer. Chem. Soc.* **90** (1968) 5598.
19. M. Nieto and H. R. Perkins, *Biochem. J.* **123** (1971) 789.

SAŽETAK

Četiri strukturna izomera L-pentapeptida sekvence Ala. Glu. Lys. Ala. Ala. Sinteza, karakterizacija i korelacija sa izomerom L-D-L-D-D-konfiguracije čija struktura je srodna peptidskim lancima peptidoglikana

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Opisana je sinteza četiri L-pentapeptida sekvence Ala. Glu. Lys. Ala. Ala. koji se međusobno razlikuju obzirom na mjesto peptidske veze između glutamil i lizil ostatka. U svim slučajevima peptidski lanac je dobiven kondenzacijom potpuno protektiranog dipeptid sukcinimid estera (BOC-Ala-Glu(OBZL)-OSu ili BOC-Ala--Glu(OSu)-OBZL), kao *N*-terminalne komponente, sa protektiranim tripeptid benzil esterom (Lys(Z)-Ala-Ala-OBZL ili Z-Lys-Ala-Ala-OBZL), kao *C*-terminalnom komponentom. Po istoj shemi sintetiziran je i odgovarajući pentapeptid L-D-L-D-D-konfiguracije u kojem je γ-karboksil grupa D-glutamil ostatka vezana sa α-amino grupom L-lizil ostatka. Deprotekcija izomernih pentapeptida izvršena je u dva stupnja: 1. katalitičkim hidriranjem, i 2. djelovanjem trifluoroctene kiseline. Slobodni pentapeptidi izolirani su kao di-trifluoracetat soli i karakterizirani analizom, optičkim skretanjem i NMR spektrima. Korelirana je elektroforetska pokretljivost izomera kod različitih pH vrijednosti. Primjenom NMR titracije provedena je asignacija alaninskih ostataka u peptidskom lancu α—α L-izomera XVIII i γ—α L-D-L-D-D--izomera XXII.

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