[ursprünglich 610 mg (98%)] werden in 2 ml Äthanol gelöst und unter Eiskühlung mit 0,22 ml 65proz. Salpetersäure in 1 ml Äthanol versetzt. Das Nitrat kristallisiert sogleich aus. Nach 2 Std. Stehen im Eisschrank werden die Kristalle abgenutscht und getrocknet: 286 mg (36,6%) Nitrat, Smp. 152–155°; nach weiterem 2maligem Kristallisieren aus Äthanol 180 mg (22,7%), Smp. 166–166,5° (Zers.) [nach der 2. Kristallisation, Smp. 165–166° (Zers.)]. Gemäss der optischen Drehung ($[\alpha]_D = +76{,}8° \pm 3°$ ($c = 1{,}022$, Wasser)) besteht das Salz aus 91% **18**-Nitrat und 9% **19**-Nitrat[11]). Die Substanz unterscheidet sich im NMR.-Spektrum nicht von reinem Pilocarpinnitrat. Zur gas-chromatographischen Untersuchung[12]) wird aus einer Probe die Base freigesetzt. Die Analyse ergibt 93,2 ± 1% **18** und 6,8 ± 1% **19**.

LITERATURVERZEICHNIS

[1] *A. R. Battersby & H. T. Openshaw*, in *R. H. F. Manske & H. L. Holmes*, «The Alkaloids», Vol. III, 201; New York 1953; *R. C. Elderfield*, «Heterocyclic Compounds», Vol. 5, 235; New York 1957; *H.-G. Boit*, «Ergebnisse der Alkaloid-Chemie bis 1960», Berlin 1961.

[2] *H. W. Voigtländer & W. Rosenberg*, Arch. Pharm. *292*, 579 (1959).

[3] *R. K. Hill & S. Barcza*, Tetrahedron *22*, 2889 (1966).

[4] *N. A. Preobrashenski, A. N. Poljakowa & W. A. Preobrashenski*, Izv. Akad. Nauk SSSR *1936*, 983 [Chem. Zbl. *1937* II, 998]; *A. N. Dey*, J. chem. Soc. *1937*, 1057.

[5] *A. G. Natradze & E. E. Mikhlina*, J. gen. Chemistry USSR *17*, 1718 (1947) [Chem. Abstr. *42*, 2967 (1948)].

[6] *N. A. Preobrashenski, M. E. Maurit & G. V. Smirnova*, Doklady Akad. Nauk SSSR, *81*, 613 (1951) [Chem. Abstr. *47*, 4345 (1953)].

[7] *G. V. Chelintsev & V. A. Fish*, J. gen. Chem. USSR, *11*, 459 (1941) [Chem. Abstr. *35*, 6591 (1941)]; *R. Burtles, F. L. Pyman & J. Roylance*, J. chem. Soc. *127*, 581 (1925).

[8] *J. K. Mehrota & A. N. Dey*, J. Indian chem. Soc. *38*, 971 (1961).

[9] *F. L. Pyman*, J. chem. Soc. *101*, 530 (1912).

[10] *A. M. Poljakowa, W. A. Preobrashenski & N. A. Preobrashenski*, J. gen. Chemistry USSR, *9*, 1402 (1939) [Chem. Zbl. *1940* I, 869]; *N. A. Dryamova, S. I. Zav'yalov & N. A. Preobrashenski, ibid. 18*, 1733 (1948) [Chem. Abstr. *43*, 2625 (1949)].

[11] *W. Oberhänsli*, private Mitteilung.

[12] *R. G. Jones*, J. Amer. chem. Soc. *71*, 644 (1949).

[13] *R. G. Jones & K. C. McLaughlin*, J. Amer. chem. Soc. *71*, 2444 (1949).

[11]) Pilocarpinnitrat [aus reinem Pilocarpinhydrochlorid (*Siegfried*) hergestellt]: Smp. 173,5–174,0° (Zers.); $[\alpha]_D = +81{,}0° \pm 3°$ ($c = 1{,}618$, Wasser). Isopilocarpinnitrat (*Koch-Light*): Smp. 158–160°; $[\alpha]_D = +34{,}3° \pm 3°$ ($c = 1{,}804$, Wasser).

[12]) GC. mit *Perkin-Elmer* 900-Gerät ausgeführt (3% OV 17, Gas-Chrom. Q, 80/100 mesh, Länge 3 m, Durchmesser 2,2 mm).

109. Antigen Synthesis: The Preparation of Selected Dodecapeptide Carriers with Systematically Altered Structures by a Two-Phase Method

by **C. H. Schneider** and **W. Wirz**

Institute for Clinical Immunology, Inselspital, 3008 Bern

(28. 2. 72)

Summary. The synthesis of a series of dodecapeptide ethyl esters containing L-leucine, L-alanine, glycine and L-lysine is described. These peptides are suitable carriers for the preparation of haptenic conjugates to be used in antigenicity studies. The strategy for preparing intermediary hexapeptides has been called a two-phase approach since the elongation of the peptide chain is

carried out stepwise in a water-immiscible organic phase, whereas removal of side-products and excess reagent is effected by means of a suitably adapted aqueous phase. The method allows an efficient production of relatively large amounts of fully defined peptide antigens.

During the last decade it has become increasingly apparent that in addition to proteins and large polysaccharides, simpler and smaller molecules can function as complete antigens. The use of polymers containing a restricted number of randomly distributed amino acids has been particularly extensive and led to a number of valuable generalizations regarding the influence of various molecular parameters such as charge, shape and (in a restricted sense) composition on antigenic properties [1]. In contrast, comparatively small, but rigorously defined peptide antigens have only occasionally been studied and little use has been made of the possibility to synthesize modified structures for additional immunological investigation.

It seems quite certain that fully defined peptide antigens, together with a selection of their analogues, will prove their value as incisive instruments for studies on induction of the immunological responses as well as on elicitation of the various immunological reactions. Progress along these lines appears to depend however on two major methodological achievements. One is related to the high sensitivity required in assessing weak immunogenic effects. From available evidence it can be predicted that a number of small peptides will be only weakly immunogenic, yet important immunogenicity differences may be expected within this class of antigens. Thanks to the introduction of versatile and highly sensitive radioimmunological techniques, the general problem of measuring very low titered antisera has been solved in principle [2], and it may be added that also highly sensitive biological tests for antibody detection have become available, notably a test based on the immunospecific inactivation of bacteriophages [3].

The second advance required consists in a considerable simplification of methodology in peptide synthesis. Only with syntheses that do not depend on the tedious isolation and purification of intermediates, a sufficient number and variety of peptide antigens may be expected to become available. Recently, two types of approach by-passing the classical isolation of intermediates have become prominent in studies on ribonuclease synthesis, namely the solid-phase synthesis of *Merrifield* [4] and the N-carboxyanhydride method [5]. Both procedures are eminently promising, but at the same time seem to exhibit a number of drawbacks for the purposes of the present series of syntheses. We therefore adopted a strategy which can be characterized as a two-phase method which makes use of two immiscible solvents in conjunction with suitable classical techniques for coupling and protecting amino acids and peptides in such a manner that no intermediate of the growing peptide chain needs isolation.

We here describe the synthesis of a selection of related dodecapeptides suitable for conjugation with various haptenic groups by this two-phase approach. In principle coupling by means of amino acid activated esters was performed stepwise from the C-terminus in such a way that the growing peptide chain could be kept dissolved in a water-immiscible organic phase (phase one), whereas excess reagent and side-products were removed by washing the organic phase with aqueous extractants (phase two). Ideally, no further purification beyond the extraction of the organic phase is necessary. Deprotection steps depend on the groups to be removed. In the case of the

Boc-protection, deprotection was usually achieved by transferring the peptide into trifluoroacetic acid. The deprotected peptide was converted into the acetate with the aid of a "Dowex-2" column in the acetate-form and then retransferred into a water-immiscible organic phase for the next coupling.

In our experience, amino acid N-hydroxysuccinimide esters [6] proved highly valuable. N-Hydroxysuccinimide, the by-product of acylation with these esters is quite soluble in water. Excess reagent can be hydrolysed and extracted from the organic phase with sodium carbonate solution. Couplings proceed smoothly and give good yields. Indeed, purification of peptide intermediates beyond simple extraction of the organic phase seemed rarely required after acylations with these esters.

Instead of activated ester procedures, other couplings affording by-products extractable by aqueous phases could be used, notably the N-ethyl-5-phenyl-isoxazolium-3'-sulfonate procedure [7] or methods using water-soluble carbodiimides. Indeed it seems that *Sheehan*'s group first used a two-phase approach by preparing oligopeptides in methylene chloride with 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride as condensing reagent without isolation of intermediates [8]. In our hands this method could be used alternatively, particularly for the smaller peptides. Relatively long reaction times were required however, and with some peptides, purification was less easy and yields were lower than after N-hydroxysuccinimide ester coupling. As an example, the synthesis of Boc-Lys(*Z*)-Ala-Leu-Lys-(*Z*)-Gly-OEt by means of carbodiimide is given in the experimental part.

It is evident that the two-phase approach resembles the solid phase method of *Merrifield* in certain aspects but differs in others. Its main limitation derives from the fact that suitable water-immiscible solvent systems are available for syntheses of oligopeptides up to the octapeptide or nonapeptide stage only. Larger peptides will therefore have to be made by fragment condensation. Since progress in the field of peptide condensations is quite promising this is not necessarily a drawback. The main advantages of the present method are ease of control at each step and the possibility of interruption of the monotonous synthetic procedure for insertion of additional purification if and when required. Since the preparation of the present sequences is not inherently troublesome, these assets of the method may not be clearly apparent in this work. They were however a major motive for developing and adopting the two-phase approach instead of the solid-phase procedure. It should be kept in mind that the antigenic peptides synthesized and to be synthesized do not *a priori* possess a known biological activity which could be used as a guide in the final purification, and therefore the problem of maintaining a high product quality throughout all the intermediary stages under all circumstances appeared quite essential.

The syntheses of the dodecapeptides described here were performed in order to have an initial stock of defined carriers to which defined numbers of various haptenic groups could be conjugated in defined positions at defined intervals. Initially a free choice of amino acids to be used in the sequence could be made. We selected L-lysine to serve with its ε-amino function as a general point of haptenic attachment. The positions between the lysine units were occupied by L-alanine, L-leucine and glycine. It seemed economical to prepare a number of hexapeptides by the two-phase method

and to combine them to dodecapeptides. Glycine was used as the C-terminal acid in all hexapeptides, thus allowing application of a variety of condensation methods even under conditions were racemization of a similarly situated optically active amino acid would be expected. In our hands removal of the protecting ester from the terminal carboxyl function by alkaline hydrolysis and subsequent condensation of the deprotected hexapeptide with dicyclohexyl-carbodiimide in the presence of

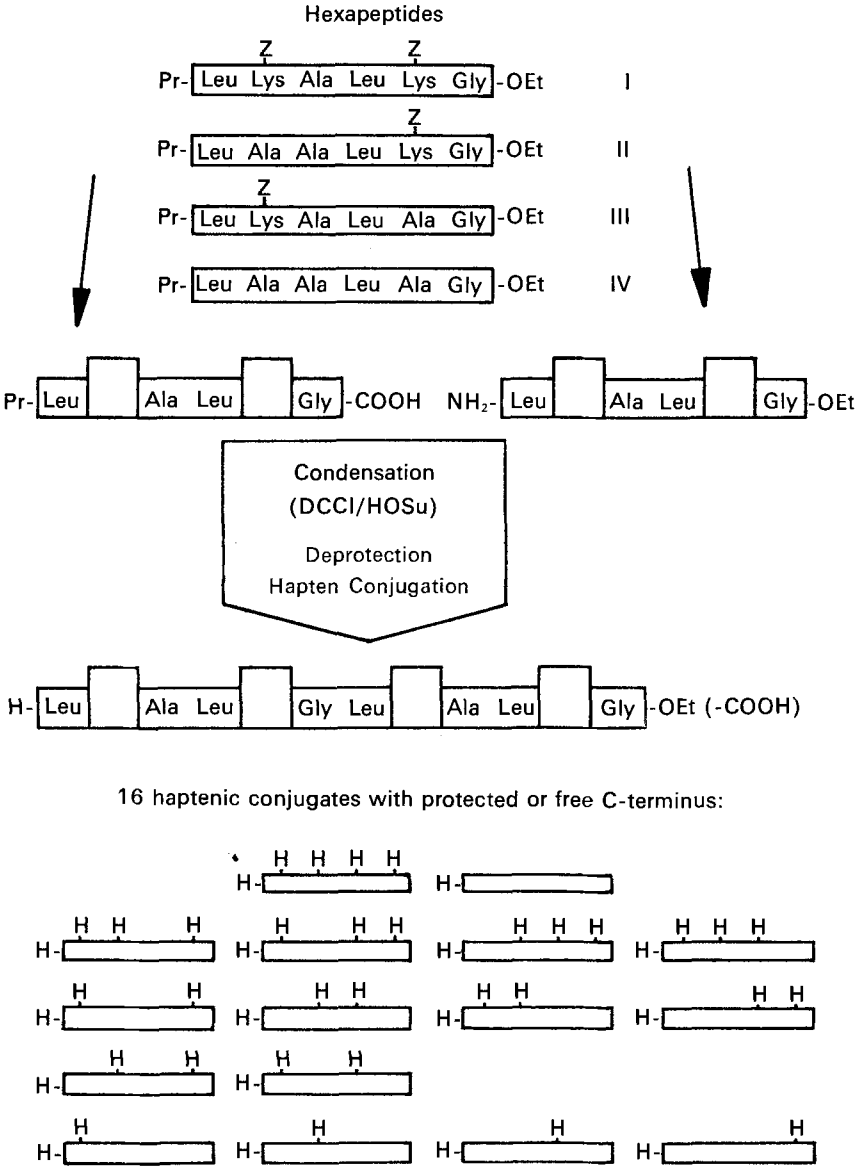

Fig. 1. *Synthesis of a series of dodecapeptides with systematically altered haptenic arrangement*

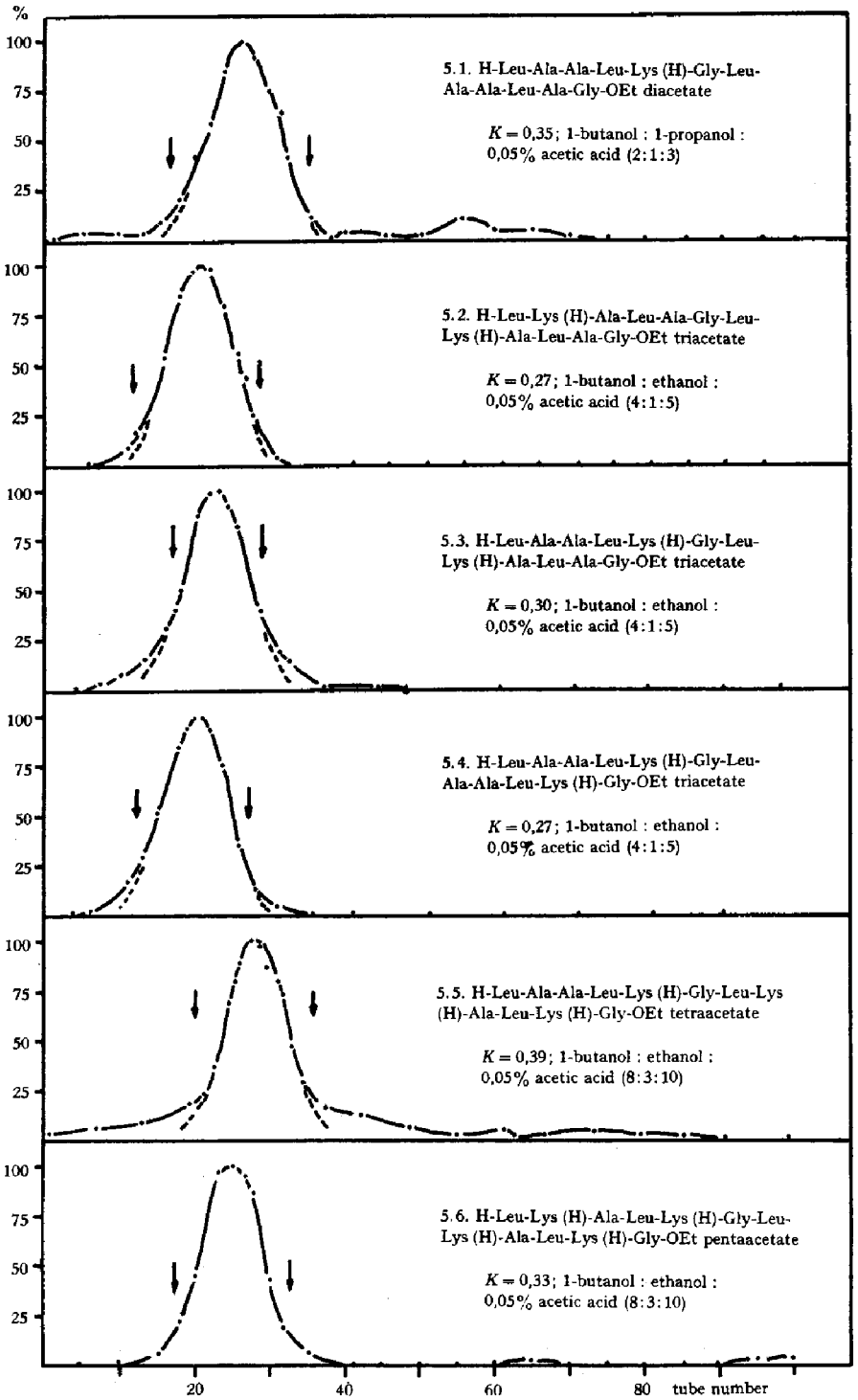

Fig. 2. *Countercurrent distribution of dodecapeptide esters*

Distributions of 100 transfers were carried out with dodecapeptide aliquots of 150–250 mg. (For details see "Experimental part".) They are shown in per cent of the residual weight in the peak tube. Only the residual weight in the lower phase was determined throughout. The residue in the upper phase was controlled additionally from tube 70–100. Where the calculated curve does not coincide with the experimental curve of the main component, its outline is shown by dotted lines. After recovery of the main component the relative ratios of the amino acids based on a value of 2,00 for glycine were estimated. Optical rotations and electrophoretic mobilities were also determined.

Peptide 5.1.: Amino acid analysis: Gly:Ala:Leu:Lys 2.0:5.0:3.9:1.0; $[\alpha]_D^{25} = -73.4°$ ($c = 1$, H_2O); PE: single zone at 2.0 cm (cathodic) after 4 h.

Peptide 5.2.: Amino acid analysis: Gly:Ala:Leu:Lys, 2.0:3.9:4.0:2.1; $[\alpha]_D^{25} = -73.5°$ ($c = 1$, H_2O); PE: single zone at 2.9 cm (cathodic) after 4 h.

Peptide 5.3.: Amino acid analysis: Gly:Ala:Leu:Lys, 2.0:3.9:4.0:1.9; $[\alpha]_D^{25} = -79.3°$ ($c = 1$, H_2O); PE: single zone at 2.4 cm (cathodic) after 4 h.

Peptide 5.4.: Amino acid analysis: Gly:Ala:Leu:Lys, 2.0:4.0:4.1:2.2; $[\alpha]_D^{25} = -74.1°$ ($c = 1$, H_2O); PE: single zone at 2.5 cm (cathodic) after 4 h.

Peptide 5.5.: Amino acid analysis: Gly:Ala:Leu:Lys, 2.0:3.0:3.9:2.9; $[\alpha]_D^{25} = -72.3°$ ($c = 1$, H_2O); PE: single zone at 2.9 cm (cathodic) after 4 h.

Peptide 5.6.: Amino acid analysis: Gly:Ala:Leu:Lys, 2.0:2.1:4.1:4.0; $[\alpha]_D^{25} = -62{,}6°$ ($c = 1$, H_2O); PE: single zone at 3.0 cm (cathodic) after 4 h; PC: Rf 0.38, zone with tailing (ninhydrin), solvent F.

N-hydroxysuccinimide was reasonably satisfactory. Other condensations have therefore not yet been studied in detail.

A general outline of the syntheses and haptenic conjugates obtainable is depicted in Fig. 1. A portion of each of the four protected hexapeptides I–IV, prepared by the two-phase method, is deprotected at the N-terminal end and a second portion of each protected peptide is freed from the C-terminal ester protection by hydrolysis. Pairs of the partially deprotected hexapeptides are condensed with dicyclohexyl-carbodiimide/hydroxysuccinimide [9] to give a series of protected dodecapeptides. Subsequent deprotection of all amino functions affords dodecapeptide carriers suitable for attachment of various haptenic groups. A total of sixteen carriers differing in the number and distribution of their haptenic attachment points may be prepared. The possible conjugates based on a $Leu^1Lys^2Lys^5Lys^8Lys^{11}$-pentaamino carrier, a Leu^1-monoamino carrier, four tetraamino carriers, six triamino carriers and four diamino carriers are schematically represented in Fig. 1. Very simple means for additional variation of haptenic arrangement will be the use of permanent (non-haptenic) protection on the N-termini of the dodecapeptides and further to employ the C-terminal carboxyl as an additional site for haptenic conjugation. In this way a variety of highly defined haptenic conjugates become available in a relatively efficient manner.

We have prepared six protected carriers namely the Leu^1Lys^5-diamino, the Leu^1-Lys^2Lys^5-triamino, the $Leu^1Lys^5Lys^8$-triamino, the $Leu^1Lys^5Lys^{11}$-triamino, the Leu^1-$Lys^5Lys^8Lys^{11}$-tetraamino and the $Leu^1Lys^2Lys^5Lys^8Lys^{11}$-pentaamino carrier. These peptides could be synthesized with reasonable overall yield and analytically pure compounds were obtained by simply using liquid extraction, in some instances with one or two reprecipitations. Liberation of the amino functions was achieved with HBr in glacial acetic acid [10], affording free dodecapeptide esters which required little additional purification as shown by their characterization by paper chromatography, electrophoresis and by *Craig* countercurrent distribution. It is to be noted

that the countercurrent distributions presented in Fig. 2 exhibit one major peak with a distribution in close agreement with the calculated one. The products occasionally separated from the main component were mostly ninhydrin-negative and would thus not be revealed by the usual paper chromatographic and electrophoretic techniques.

The carriers are now fitted with penicilloyl and other antigenic determinants and used in immunological studies to be reported elsewhere. It should be noted that other systematically modified antigens can be economically realized by starting from our stock of hexa- or dodeca-peptides. The attachment of two or more different haptenic groups onto the same carrier, and further the enlargement of the dodecapeptides to defined multiples, seems very promising.

Experimental Part

General remarks. – *Abbreviations:* Pr: protecting group, unspecified; Z: benzyloxycarbonyl-; Boc: *t*-butyloxycarbonyl-; –OSu: N-succinimidoxy-; HOSu: N-hydroxysuccinimide; DCCI: dicyclohexyl-carbodiimide; TFA: trifluoroacetic acid; DMF: dimethylformamide; TLC: thin-layer chromatography; PC: paper chromatography; PE: paper electrophoresis.

Materials: Aminoacid derivatives and coupling reagents were obtained from *Fluka AG.*, Buchs, Switzerland. 1-Ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (m.p. 112,5 to 114.5°) was from the *Ott Chemical Company*, Muskegon, Mich.

Methods: After the coupling step the peptide solutions were extracted 3 times with about equal volumes of each of the following: 1N acetic acid, H_2O, 0.5M K_2CO_3 and again H_2O. For batches of about 5 mmoles an extractant volume of 30 ml per extraction was satisfactory. The reaction solutions containing DMF were finally extracted 5 to 6 times with water in order to remove most of the DMF from the organic phase. The first water extracts were reextracted with small volumes of CH_2Cl_2 in those cases. After the last extraction, the organic layers were dried with Na_2SO_4 and evaporated in vacuo. In some of the runs a continuous liquid-liquid extraction was used in place of the batchwise procedure. This was achieved by injecting large volumes of extractants through a nozzle in the form of small droplets into the organic phase. This method appears to improve the quality of the product and is suitable for automatic operation[1]).

For ion-exchange, a 2 cm × 20 cm column of purified *Dowex*-2X4, 20–50 mesh, was used for portions above one mmole of deprotected peptide salt. Peptide intermediates to be converted into the acetate were usually dissolved in ethanol/water 1:1 to give less than 1% solutions. These solutions, followed by liberal amounts of solvent, were then passed through the column in the acetate form. A similar procedure was used to obtain free deprotected peptides from the column in the OH^--form. Whenever required, removal of solvents was performed on a rotary evaporator in vacuo below 35°. Intermediates were dried in vacuo (0.2 Torr) over P_2O_5 to constant weight. Most preparations were carried out more than once and reference to additional runs is made by indicating their yields in per cent as well as in absolute amounts. Melting points were determined in capillary tubes and are corrected. Optical rotations were measured in a *Perkin-Elmer* polarimeter 141.

TLC was performed with 100 μg samples on silica gel plates containing a fluorescence indicator (*Merck* F_{254}). PC was carried out on *Schleicher & Schuell* Nr. 2043 B mgl paper. Usually the circular technique (radius of paper: 8.5 cm) and a sample size of 25 μg was employed. The following chromatographic solvent systems were used:

solvent A: $CHCl_3$/methanol 95:5
solvent B: 1-butanol/acetic acid/H_2O 8:1:1
solvent C: $CHCl_3$/methanol 97.5:2.5
solvent D: 1-butanol/acetic acid/H_2O 4:1:5
solvent E: 1-butanol/ethanol/0.05% acetic acid 4:1:5
solvent F: 1-butanol/ethanol/0.05% acetic acid 8:3:10.

[1]) *C. H. Schneider & W. Wirz*, to be published.

PE was carried out on a *Spinco* paper electrophoresis cell, model R, at approximately 10 V/cm for 240 min in 0.05M phosphate buffer pH 7.4. Substance spots on TCL plates and on paper were detected with ninhydrin, by exposure to iodine vapour (I_2) or by examination in ultraviolet light at 254 nm (UV.).

For estimation of the relative amino acid ratios, the peptides were hydrolysed in 6N HCl in a nitrogen atmosphere or in vacuo (0.05 Torr) at 110° for 24 h and analysed on a *Spinco* amino acid analyser, model 120 or on a *Beckman* Unichrom analyser.

For countercurrent distribution lyophilized samples of deprotected dodecapeptide esters were dissolved in 20 to 30 ml lower phase and introduced into the first tubes of a 100 tube *Craig* apparatus which contains 10 ml of lower phase in each tube and transfers the same volume of upper phase. Distributions were carried out very slowly (mostly only 1 to 2 transfers per hour) below 10°. This seemed necessary because heavy emulsions with long separation times formed in several tubes. Distributions (Fig. 2) were obtained from analysis of residual weights [11]. The phases containing the main portion of the peptides (between arrows in Fig. 2) were removed and the upper phase was extracted with 3 equal volumes of 0.05% acetic acid. The lower phase, combined with the extracts, was concentrated to a small volume below 25°, diluted with water and lyophilized. Before analytical data were measured, the lyophilizate was further dried at 100° (2 h) and at 40° (24 h) at 0,2 Torr over P_2O_5.

1. Hexapeptide I, Boc-L-leucyl-N^{ε}-Z-L-lysyl-L-alanyl-L-leucyl-N^{ε}-Z-L-lysyl-glycine ethyl ester and deprotected derivatives.

1.1. *Boc-Lys(Z)-Gly-OEt:* Ethyl glycinate hydrochloride (3.5 g, 25 mmoles) was suspended in 40 ml CH_2Cl_2 at 0°. After Et_3N (3.50 ml, 25 mmoles) a solution of 11,9 g (25 mmoles) N^{α}-Boc-N^{ε}-Z-Lys-OSu in 110 ml CH_2Cl_2 was added at 0° and the resulting, initially turbid solution was stirred at room temperature for 5 h. Obtained: 10.5 g (90%) of glassy residue (other yields: 4.3 g, 93%; 4.35 g, 93.5%; 4.05 g, 87%). TLC: Rf 0.87 (UV., I_2), solvent A.

1.2a. *H-Lys(Z)-Gly-OEt acetate:* The dipeptide of 1.1. (10.5 g, 22.6 mmoles) was kept in 15 ml TFA at room temperature for 40 min. The TFA was removed and the residue converted into the acetate. Lyophilization of the eluate from the ion-exchange column gave an oily residue which was dissolved in 300 ml H_2O and freed from a slight turbidity by filtration. Lyophilization of the filtrate afforded 9.4 g (98%) (other yields: 4.0 g, 100%; 4.0 g, 100%). TLC: Rf 0.56 (ninhydrin), solvent B.

1.2b. *H-Lys(Z)-Gly-OEt hydrochloride:* The dipeptide of 1.1. (4.3 g, 9.2 mmoles) was dissolved in 40 ml warm ethyl acetate and slowly mixed at 0° with 40 ml 5N HCl in ethyl acetate. After $2^1/_2$ h at 4° the reaction solution had solidified. The white crystals were filtered and washed on the filter with 60 ml of cold ethyl acetate. Obtained: 3.1 g (83%). TLC: Rf 0.67 (ninhydrin), methanol. The mother liquor contained 0.3 g of chromatographically inhomogeneous material.

1.3. *Boc-Leu-Lys(Z)-Gly-OEt:* To the dipeptide of 1.2a. (1.50 g, 3.53 mmoles), dissolved in 30 ml DMF 1.15 g (3.50 mmoles), Boc-Leu-OSu in 10 ml CH_2Cl_2 and thereafter 0.495 ml Et_3N were added and the slightly turbid solution was stirred at room temperature for 4 h. Extractions of the reaction solution were carried out after addition of 120 ml CH_2Cl_2. Obtained: 1.86 g (91%) yellowish crystals (other yields: 3.4 g, 96%; 4.3 g, 86%; 5.0 g, 92%, reaction in CH_2Cl_2-DMF (1:2)). TLC: Rf 0.77 (UV., I_2), solvent A.

1.4a. *H-Leu-Lys(Z)-Gly-OEt acetate:* The tripeptide of 1.3. (5.0 g, 8.65 mmoles) was dissolved in 5 ml TFA at room temperature. After 20 min TFA was evaporated and the residue was converted into the acetate. The eluate from the ion-exchange column was taken to dryness, the residue was dissolved in 60 ml H_2O, filtered, and the filtrate was lyophilized: 4.3 g (92%) yellowish powder (other yields: 3.3 g, 83%). TLC: Rf 0.55 (UV., I_2), solvent B.

1.4b. *H-Leu-Lys(Z)-Gly-OEt hydrochloride:* To the tripeptide of 1.3. (0.55 g) in 3 ml ethyl acetate, 5 ml 5N HCl in ethyl acetate was slowly added at 0°. After 4 h at 4° the solution was taken to dryness. Obtained: 460 mg (94%). TLC: Rf 0.65, traces at Rf 0.17, 0.42 (ninhydrin), solvent B.

1.5. *Boc-Ala-Leu-Lys(Z)-Gly-OEt:* The tripeptide of 1.4a. (2.1 g, 3.9 mmoles) and 0.56 ml Et_3N were added to 20 ml CH_2Cl_2. To the solution Boc-Ala-OSu (1.15 g, 4.0 mmoles) in 25 ml CH_2Cl_2 was added at 0° with stirring, which was continued for 5 h. Obtained: 2.45 g (97%) of

white residue (other yields: 4.9 g, 94%; 3.65 g, 92%). TLC: Rf 0.85, traces at Rf 0.94 and 0.60 (UV., I_2), solvent A.

1.6. *H-Ala-Leu-Lys(Z)-Gly-OEt acetate:* The tetrapeptide of 1.5. (4.47 g, 6.88 mmoles) was kept in 5 ml TFA at room temperature for 30 min. TFA was removed and the residue was converted into the acetate. After evaporation of the eluate from the ion-exchange column the residue was taken up in 80 ml H_2O and lyophylized. Obtained: 4.0 g (95%) of white powder (other yields: 1.9 g, 82%; 3.20 g, 91%). TLC: Rf 0.74 (ninhydrin), solvent B.

1.7. *Boc-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt:* To 3.20 g (5.25 mmoles) tetrapeptide of 1.6. in 50 ml CH_2Cl_2, Et_3N (0.75 ml) and 2.50 g (5.25 mmoles) N^{α}-Boc-N^{ε}-Z-Lys-OSu in 10 ml CH_2Cl_2 were added at 0°. Within 5 min a gelatinous mass appeared which could be dissolved by adding 15 ml DMF. The clear solution was stirred at room temperature for 5 h and diluted with 100 ml CH_2Cl_2 before extraction. Obtained: 4.6 g (95%). TLC: Rf 0.83 (UV., I_2), solvent A.

1.8. *H-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt acetate:* The pentapeptide of 1.7. (4.5 g, 4.93 mmoles) was added to 7.5 ml TFA. After 40 min at room temperature the TFA was evaporated and the residue was converted into the acetate. The material from the ion-exchange column was obtained as a slightly water-soluble residue after removal of the eluting solvent. It was suspended in 50 ml H_2O and lyophylized: 3.9 g (91%). TLC: Rf 0.53 (UV., I_2, ninhydrin), solvent B.

1.9. *Boc-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt:* The pentapeptide of 1.8. (3.9 g, 4.5 mmoles) was suspended in 30 ml CH_2Cl_2 and dissolved by adding 60 ml DMF. Et_3N (0.65 ml) and 1.5 g (4.57 mmoles) Boc-Leu-OSu in 60 ml DMF were added and the clear solution was stirred for $5^1/_2$ h at room temperature. Before extraction 300 ml CH_2Cl_2 were added. After the first extraction with 0,5 N K_2CO_3 separation of crystals started. The solution was therefore concentrated in vacuo to one third of its original volume and the crystals were filtered off: 4.30 g (94%). TLC: Rf 0.84 (UV., I_2), no spots with ninhydrin, solvent A. A sample was recrystallized with little loss from ethanol-H_2O: m.p. 218–221°; $[\alpha]_D^{25} = -24.7°$ ($c = 1$, DMF).

$C_{52}H_{80}N_8O_{13}$ (1025.3) Calc. C 60.92 H 7.87 N 10.93% Found C 60.83 H 7.96 N 11.04%

1.10. *Boc-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OH:* The hexapeptide of 1.9. (1.35 g, 1.32 mmoles) was dissolved in 30 ml warm DMF and mixed with 20 ml dioxane, 50 ml ethanol and 50 ml 0,5 N NaOH. The clear solution was kept for 90 min at 40°, concentrated to about one fourth of its original volume until the mixture solidified. Ice-cold 1 N citric acid (100 ml) was added and the white precipitate was collected by filtration. It was reprecipitated from DMF-H_2O: 1.20 g (92%) m.p. 177–180°. TLC: Rf 0.44 (UV., I_2), no spots with ninhydrin, solvent B. A small sample was reprecipitated with little loss from DMF-H_2O: m.p. 180–183°.

$C_{50}H_{76}N_8O_{13} \cdot H_2O$ Calc. C 59.16 H 7.74 N 11.04%
(1015.2) Found ,, 59.04 ,, 7.81 ,, 10.99%

1.11. *H-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt:* The hexapeptide of 1.9. (1.8 g, 1.75 mmoles) was dissolved in 5 ml TFA. After 30 min TFA was removed in vacuo and the residue was dissolved in 40 ml DMF and 120 ml ethanol. The solution was passed through a Dowex-2 column in the OH'-form and eluted with DMF/ethanol 1:5. The eluate was concentrated to 70 ml and used in further synthetic work. A small portion was taken to dryness. TLC: Rf 0.63 (UV., I_2, ninhydrin), trace at Rf 0.20 (ninhydrin), solvent B.

1.12. *Boc-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt by carbodiimide coupling* [8]. – *H-Ala-Leu-Lys(Z)-Gly-OEt hydrochloride:* Boc-Ala-Leu-Lys(Z)-Gly-OEt (13.1 g, 20.2 mmoles), prepared by the carbodiimide method using 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide was dissolved in 100 ml ethyl acetate and 120 ml CH_2Cl_2 and slowly mixed with 250 ml 3.2 N HCl in ethyl acetate. The solution was stirred at 4° for $2^1/_4$ h. The solvent was evaporated and the residue was dissolved in 220 ml H_2O. The solution was extracted with two portions of 30 ml CH_2Cl_2 each, concentrated to 100 ml and lyophilized: 11.8 g (100%) of white powder. TLC: Rf 0.76 (ninhydrin), solvent B.

Boc-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt: To a suspension of the above deprotected tetrapeptide (11.8 g, 20.2 mmoles) in 100 ml CH_2Cl_2, 2.1 g Et_3N in 20 ml CH_2Cl_2 was added with stirring and after 10 min 17.0 g (30.3 mmoles) N^{α}-Boc-N^{ε}-Z-Lys-OH dicyclohexylamine salt in 100 ml CH_2Cl_2 and 6,0 g (31.3 mmoles) 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride in 100 ml CH_2Cl_2 were added. The solution was stirred at room temperature for 18 h. Obtained after extractions: 20 g of yellow, viscous solid. It was reprecipitated from acetone/CH_2Cl_2 1:1. TLC: Rf 0.81

and 0.69 (UV., I_2), solvent A. Reprecipitation from ethanol gave 8.3 g (45%) product (other yields: 0.70 g, 76%). TLC: Rf 0.82 (UV., I_2), solvent A. A small sample was reprecipitated with little loss from ethanol/ether: m.p. 184–188°; $[\alpha]_D^{25} = -27.6°$ ($c = 2$, propanol).

$C_{46}H_{69}N_7O_{12}$ (912.11) Calc. C 60.58 H 7.63 N 10.75% Found C 60.21 H 7.45 N 10.45%

2. Hexapeptide II, Boc-L-leucyl-L-alanyl-L-alanyl-L-leucyl-N$^\varepsilon$-Z-L-lysyl-glycine ethyl ester and deprotected derivatives.

2.1. *Boc-Ala-Ala-Leu-Lys(Z)-Gly-OEt:* To 4.0 g (6.57 mmoles) of tetrapeptide 1.6. in 50 ml CH_2Cl_2 0.95 ml Et_3N and 1.88 g (6.57 mmoles) Boc-Ala-OSu in 40 ml CH_2Cl_2 were added and the solution was stirred for 5 h at room temperature. Obtained: 4.63 g (98%) of colourless glass (other yields: 1.53 g, 91%; 2.15 g, 95%). TLC: Rf 0.26, trace at the origin (UV., I_2), no spots with ninhydrin, solvent C.

2.2. *H-Ala-Ala-Leu-Lys(Z)-Gly-OEt acetate:* The pentapeptide of 2.1. (1.53 g, 2.13 mmoles) was kept in 5 ml TFA for 20 min. TFA was evaporated and the residue was converted into the acetate. The eluate of the ion-exchange column was taken to dryness, the residue was dissolved in 100 ml H_2O and the solution was lyophilized after removal of some turbidity by filtration: 1.31 g (91%) of white powder (other yields: 3.95 g, 90%). TLC: Rf 0.65, trace at 0.44 (ninhydrin), solvent B.

2.3. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-OEt:* The pentapeptide of 2.2. (3.95 g, 5.81 mmoles) was dissolved in 150 ml DMF and mixed with 0.85 ml Et_3N and 1.90 g (5.80 mmoles) Boc-Leu-OSu in 40 ml CH_2Cl_2. The clear solution was stirred for 16 h at room temperature. Before extraction 200 ml CH_2Cl_2 were added. Obtained: 4.70 g (97%) of glassy residue (other yields: 1.43 g, 89%). TLC: Rf 0.46 (UV., I_2), solvent A. The residue was washed with hot acetone and precipitated with little loss from ethanol-ether: m.p. 213–215°; $[\alpha]_D^{25} = -26.8°$ ($c = 1$, DMF).

$C_{41}H_{67}N_7O_{11}$ (834.03) Calc. C 59.04 H 8.10 N 11.76% Found C 58.69 H 8.19 N 11.53%

2.4. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-OH:* The hexapeptide of 2.3. (1.62 g, 1.95 mmoles) was dissolved in 100 ml dioxane at 50°. After 50 ml ethanol, 35 ml 0.5 N NaOH was added and the solution was kept at 37° for 90 min. After concentration to 25 ml, 8 ml H_2O and subsequently 100 ml 1 N citric acid were added. After 30 min the white precipitate was filtered off in the cold: 1.16 g (74%) (other yields: 2.0 g, 71%; 0.55 g, 68%). TLC: Rf 0.68 (UV., I_2), solvent B. A small sample was reprecipitated with little loss from ethanol/ethyl acetate 1:6 with hexane: m.p. 170–173° (dec.); $[\alpha]_D^{25} = -22.7°$ ($c = 1$, DMF).

$C_{39}H_{63}N_7O_{11}$ (805.97) Calc. C 58.12 H 7.88 N 12.17% Found C 58.00 H 7.75 N 12.21%

2.5. *H-Leu-Ala-Ala-Leu-Lys(Z)-Gly-OEt:* The hexapeptide of 2.3. (210 mg, 0.252 mmole) was kept in 1 ml TFA at room temperature for 20 min. The solution was then taken to dryness and the residue was dissolved in 100 ml ethanol and passed through a small column of Dowex-2 in the OH^--form. The eluate afforded after evaporation: 170 mg (92%) of white residue. TLC: Rf 0.69, trace at the origin (UV., I_2), solvent B.

3. Hexapeptide III, Pr-L-leucyl-N$^\varepsilon$-Z-L-lysyl-L-alanyl-L-leucyl-L-alanyl-glycine ethyl ester and deprotected derivatives.

3.1. *Boc-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OEt:* Starting from ethyl glycinate hydrochloride the synthesis proceeded stepwise in CH_2Cl_2 by the two-phase method similar to the syntheses of hexapeptides I and II. In four cycles of coupling, extraction and N-terminal deprotection the following amino acid derivatives were successively added: Boc-Ala-OSu, Boc-Leu-OSu, Boc-Ala-OSu and N$^\alpha$-Boc-N$^\varepsilon$-Z-Lys-OSu. The intermediate yields in several batches of 5–10 mmoles were for the dipeptide 96, 94, 89% (protected), 96, 94, 85% (deprotected), for the tripeptide 93, 92, 92% (protected), 92, 93, 79% (deprotected), for the tetrapeptide 90, 93, 89% (protected), 98, 98, 95% (deprotected), and for the pentapeptide 85, 84, 98% (protected), 96, 93, 97% (deprotected).

All intermediate residues were tested by TLC; none required additional purification. Finally, the deprotected H-Lys(Z)-Ala-Leu-Ala-Gly-OEt acetate (2.11 g, 3.10 mmoles) in 55 ml DMF was mixed with 0.44 ml Et_3N and 1.02 g (3.10 mmoles) Boc-Leu-OSu in 27 ml CH_2Cl_2 and left at room temperature for 16 h. Before extraction 0.25 l CH_2Cl_2 was added. Obtained: 2.10 g (81%) (other yields: 1.3 g, 92%; 1.2 g, 84%). TLC: Rf 0.77 (UV., I_2), solvent A. The material was reprecipitated with little loss from ethanol-ether and from ethanol-H_2O: m.p. 214–217°. A small sample was

washed with hot acetone and reprecipitated from ethanol-H_2O: m.p. 217–218°; $[\alpha]_D^{25} = -25{,}6°$ ($c = 1$, DMF).

$C_{41}H_{67}N_7O_{11}$ (834.03) Calc. C 59.04 H 8.10 N 11.76% Found C 58.69 H 7.91 N 11.79%

3.2. *Z-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OEt:* To 500 mg (0.73 mmole) H-Lys(Z)-Ala-Leu-Ala-Gly-OEt in 30 ml CH_2Cl_2 and 20 ml DMF was added 0,11 ml Et_3N and 266 mg (0,735 mmole) Z-Leu-OSu in 15 ml CH_2Cl_2. The clear solution was stirred for $4^1/_2$ h at room temperature. The residue obtained after extractions was washed with cold acetone: 506 mg (80%). TLC: Rf 0.62 (UV., I_2), solvent A. The material was precipitated with little loss from ethanol/ether and from ethanol/1,2-dichloroethane 1:1 with ether: m.p. 219–221°; $[\alpha]_D^{25} = -22.5°$ ($c = 1$, DMF).

$C_{44}H_{65}N_7O_{11}$ (868.05) Calc. C 60.88 H 7.55 N 11.29% Found C 60.68 H 7.65 N 11.02%

3.3. *Z-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OH:* To 300 mg (0.346 mmole) hexapeptide of 3.2., dissolved in 25 ml warm dioxane, 2 ml 1 N HCl was added and the solution was kept at 90° for 90 min. It was concentrated to about 8 ml and mixed with 40 ml H_2O. After 1 h the white precipitate was collected in the cold: 270 mg (93%). TLC: Rf 0.63 (UV., I_2), no spots with ninhydrin, solvent B. The material was reprecipitated with little loss from ethanol-ether: m.p. 237–239°.

$C_{42}H_{61}N_7O_{11}$ (840.00) Calc. C 60.06 H 7.32 N 11.67% Found C 59.85 H 7.40 N 11.49%

3.4. *Boc-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OH:* The hexapeptide of 3.1. (500 mg, 0.6 mmol) was dissolved in 40 ml warm dioxane. Ethanol (10 ml) and 10 ml 0.5 N NaOH were added and the solution was kept at 40° for 90 min. After concentration to about 8 ml the turbid solution was cleared by adding 10 ml H_2O. Mixing with 30 ml 1 N citric acid afforded a precipitate which was collected after 30 min. The precipitate was dissolved in 1 N NaOH and reprecipitated with citric acid. Precipitation from ethanol-H_2O yielded 341 mg (70%) (other yields: 0.55 g, 56%). M.p. 179–181° (dec.). A reprecipitation from ethanol did not significantly raise the m.p. (180–182°, dec.). TLC: Rf 0.82 (UV., I_2), no spots with ninhydrin, solvent B; $[\alpha]_D^{25} = -24.4°$ ($c = 1$, DMF).

$C_{39}H_{63}N_7O_{11}$ (805.97) Calc. C 58.12 H 7.88 N 12.17% Found C 58.04 H 7.90 N 12.01%

3.5. *H-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OEt:* The hexapeptide of 3.1. (1.0 g, 1.2 mmoles) was kept in 5 ml TFA for 30 min. After removal of most of the TFA the residue was dissolved in 100 ml ethanol and passed through a Dowex-2 column in the OH^--form. The eluate was taken to dryness: 870 mg (98%). TLC: Rf 0.65, trace at 0.28 and at the origin (ninhydrin), solvent B.

A second batch of hexapeptide (1.32 g) was deprotected as above. The residue after TFA evaporation was dissolved in 200 ml ethanol/H_2O 1:1 and passed through the ion-exchange column. The eluate was concentrated in vacuo to 40 ml, mixed with 0.5 ml Et_3N and extracted with CH_2Cl_2. The extracts were twice washed with water, dried with Na_2SO_4 and yielded after evaporation of the solvent 778 mg (67%) of white powder. TLC: Rf 0.63 (ninhydrin), solvent B.

4. Hexapeptide IV, Pr-L-leucyl-L-alanyl-L-alanyl-L-leucyl-L-alanyl-glycine ethyl ester and deprotected derivatives.

4.1. *Z-Leu-Ala-Ala-Leu-Ala-Gly-OEt:* Starting from 15 mmoles ethyl glycinate hydrochloride the synthesis proceeded in CH_2Cl_2 by the two-phase method. In four cycles of coupling, extraction and deprotection, Boc-Ala-OSu, Boc-Leu-OSu, Boc-Ala-OSu and again Boc-Ala-OSu were successively added to the growing peptide chain, each time in equimolar amounts. Yields were above 90%; only the coupling of Boc-Ala-OSu to the deprotected tripeptide gave 83% and on repetition 88%. Intermediate control by TLC was satisfactory in all cases. Finally, Z-Leu-OSu was coupled to the deprotected pentapeptide in CH_2Cl_2/DMF 4:1, affording the desired hexapeptide in 85% yield. The residue obtained after the usual extractions and after evaporation of the solvent was precipitated with little loss from a mixture of ethanol/DMF 9:1 by addition of H_2O. M.p. 254–256°. TLC: Rf 0.45 (UV., I_2), solvent A; $[\alpha]_D^{25} = -25.7°$ ($c = 1$, DMF).

$C_{33}H_{52}N_6O_9$ (676.81) Calc. C 58.56 H 7.74 N 12.42% Found C 58.70 H 7.83 N 12.28%

4.2. *H-Leu-Ala-Ala-Leu-Ala-Gly-OEt:* To 4.0 g (8.20 mmoles) of the pentapeptide H-Ala-Ala-Leu-Ala-Gly-OEt acetate, obtained according to 4.1., in 120 ml CH_2Cl_2 and 30 ml DMF, 1.15 ml Et_3N and 2.69 g (8.20 mmoles) Boc-Leu-OSu in 30 ml CH_2Cl_2 were added and the solution was kept at room temperature for 4 h. 5.0 g (95%) resulted of a residue of Boc-Leu-Ala-Ala-Leu-Ala-Gly-OEt. TLC: Rf 0.50 (UV., I_2), no spots with ninhydrin, solvent A.

An aliquot (2.0 g, 3.12 mmoles) of the protected hexapeptide was kept in 5 ml TFA for 30 min. After removal of most of the TFA the crystalline residue was dissolved in 300 ml 95% ethanol and passed through Dowex-2 in the OH^--form. Evaporation of the eluate left: 1.65 g (97%) of white crystalline residue (other yield: 1.2 g, 88%). TLC: Rf 0.39 (ninhydrin), solvent B.

4.3. *Z-Leu-Ala-Ala-Leu-Ala-Gly-OH:* To 2.0 g (2.96 mmoles) hexapeptide of 4.1. in 40 ml DMF and 120 ml dioxane 15 ml 1N HCl was added. A clear solution was obtained above 60°. The solution was kept at 90° for 120 min, concentrated to about 5 ml and mixed with 100 ml H_2O. After 30 min at 0° the white precipitate was collected: 1.78 g (93%). TLC: Rf 0.62 (UV., I_2), no spots with ninhydrin, solvent B.

5. Dodecapeptides. – *Condensation of hexapeptides:* To a 1.5 mmol-portion of hexapeptide deprotected at the C-terminus in about 40 ml DMF a 5–10% molar excess of hexapeptide deprotected at the N-terminus in 45 ml DMF was added. After addition of 1.84 mmoles of N-hydroxysuccinimide the solution was cooled to $-15°$ and mixed with 1.67 mmoles of DCCI [9]. The turbid solution was stirred at $-15°$ for 24 h, diluted with 45 ml DMF and the now clear solution was stirred for another 24 h at room temperature. Cooling to $-15°$ separated the N,N'-dicyclohexylurea which was filtered off after 2 h. The filtrate was concentrated to 30 ml and mixed with 270 ml H_2O and 5 ml 1N acetic acid. The precipitate was collected on a filter after about 20 min at 0° and dried over P_2O_5 in vacuo. It was twice extracted with hot ethanol (120 ml altogether) and dissolved in 55 ml hot DMF. The hot solution was filtered and the filtrate was precipitated with 450 ml H_2O at about 40°. After cooling the mixture to 0° the precipitate was collected and dried (yield usually between 50 and 70%) and a sample was extensively dried at 100° for analysis.

N-deprotection: To a 1 mmol-portion of protected dodecapeptide 3 ml glacial acetic acid were added, followed by 15 ml hydrobromic acid in glacial acetic acid (33%) at 4° until after about 30 min a clear solution was obtained. The solution was mixed with 120 ml ether and the resulting precipitate was centrifuged at 0° and thrice washed with ether on the centrifuge. After drying, the solid was dissolved in 55 ml H_2O and passed through a small column of Dowex-2 in the acetate form. The eluate was lyophilized to give the acetates of the dodecapeptide esters in yields above 80%.

5.1. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-Leu-Ala-Ala-Leu-Ala-Gly-OEt and deprotected dodecapeptide ester:* The protected dodecapeptide was obtained by condensing peptide of 2.4. with peptide of 4.2.: m.p. 290–293° (dec.); $[\alpha]_D^{25} = -11.4°$ ($c = 1$, DMF).

$C_{64}H_{107}N_{13}O_{17}$ (1330.6) Calc. C 57.77 H 8.11 N 13.68% Found C 58.06 H 8.33 N 13.77%

Deprotected peptide: PC: Rf 0.75, single zone (ninhydrin), solvent D.

5.2. *Boc-Leu-Lys(Z)-Ala-Leu-Ala-Gly-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OEt and deprotected dodecapeptide ester:* Condensation of peptide of 3.4. with peptide of 3.5. afforded the protected dodecapeptide: m.p. 274–276° (dec.); $[\alpha]_D^{25} = -10.7°$ ($c = 1$, DMF).

$C_{75}H_{120}N_{14}O_{19}$ (1521.9) Calc. C 59.19 H 7.94 N 12.88% Found C 59.40 H 7.74 N 12.66%

Deprotected peptide: PC: Rf 0.49, single zone (ninhydrin), solvent F.

5.3. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-Leu-Lys(Z)-Ala-Leu-Ala-Gly-OEt and deprotected dodecapeptide ester:* Condensation of peptide of 2.4. with peptide of 3.5. afforded protected dodecapeptide: m.p. 285–288°; $[\alpha]_D^{25} = -13.8°$ ($c = 1$, DMF).

$C_{75}H_{120}N_{14}O_{19}$ (1521.9) Calc. C 59.19 H 7.94 N 12.88% Found C 59.11 H 7.86 N 12.78%

Deprotected peptide: PC: Rf 0.43 and 0.35 (trace) (ninhydrin), solvent E.

5.4. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-Leu-Ala-Ala-Leu-Lys(Z)-Gly-OEt and deprotected dodecapeptide ester:* Condensation of peptide of 2.4. with peptide of 2.5. afforded a product: m.p. 281–284°; $[\alpha]_D^{25} = -10.3°$ ($c = 1$, DMF).

$C_{75}H_{120}N_{14}O_{19}$ (1521.9) Calc. C 59.19 H 7.94 N 12.88% Found C 59.05 H 8.05 N 12.90%

Deprotected peptide: PC: Rf 0.56, zone with tailing (ninhydrin), solvent E.

5.5. *Boc-Leu-Ala-Ala-Leu-Lys(Z)-Gly-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt and deprotected dodecapeptide ester:* Condensation of peptide of 2.4. with peptide of 1.11. afforded a product: m.p. 277–280° (dec.); $[\alpha]_D^{25} = -12.8°$ ($c = 1$, DMF).

$C_{86}H_{133}N_{15}O_{21}$ (1713.1) Calc. C 60.30 H 7.83 N 12.26% Found C 60.50 H 7.74 N 12.37%

Deprotected peptide: PC: Rf 0.55, sharp zone (ninhydrin), solvent F.

5.6. *Boc-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-Leu-Lys(Z)-Ala-Leu-Lys(Z)-Gly-OEt and deprotected dodecapeptide ester:* Condensation of peptide of 1.10. with peptide of 1.11. afforded the protected dodecapeptide: m.p. 282–284° (dec.); $[\alpha]_D^{25} = -15.6°$ ($c = 1$, DMF).

$C_{97}H_{146}N_{16}O_{23}$ (1904.3) Calc. C 61.12 H 7.73 N 11.76% Found C 61.14 H 7.70 N 11.84%

Deprotected peptide: PC: Rf 0.6–0.75, broad zone (ninhydrin), solvent F.

We are indebted to Dr. *K. Vogler* (*F. Hoffmann-La Roche & Co., AG*, Basel) for elementary and amino-acid analyses, to Dr. *K. Eder* (Ecole de chimie, Genève) for elementary analyses, to Mr. *P. Schilt* (Landwirtschaftliche Forschungsanstalten, Liebefeld) for amino-acid analyses and to Mrs. *Ch. Schaper* for technical assistance.

This work has been supported in part by the *Swiss National Foundation for Scientific Research* and the *Emil Barell Foundation* of *F. Hoffmann-La Roche Ltd.*, Basel, Switzerland.

BIBLIOGRAPHY

[1] *P. H. Maurer*, Progr. Allergy *8*, 1 (1964); *M. Sela*, Adv. Immunol. *5*, 29 (1966); *M. Sela*, Science *166*, 1365 (1969).

[2] *R. S. Farr*, J. infect. Diseases *103*, 239 (1958); *J. J. Mulligan, A. G. Osler & E. Rodriguez*, J. Immunol. *96*, 324 (1966).

[3] *J. Haimovich, E. Hurwitz, N. Novik & M. Sela*, Biochim. biophysica Acta *207*, 115, 125 (1970).

[4] *R. B. Merrifield*, Science *150*, 178 (1965); *B. Gutte & R. B. Merrifield*, J. Amer. chem. Soc. *91*, 501 (1969).

[5] *R. Hirschmann, R. G. Strachan, H. Schwam, E. F. Schoenewaldt, H. Joshua, B. Barkenmeyer, D. F. Veber, W. J. Paleveda, T. A. Jacob, T. E. Beesley & R. G. Denkewalter*, J. org. Chemistry *32*, 3415 (1967); *R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Paleveda, H. Schwam, H. Barkemeyer, B. H. Arison, D. F. Veber, R. G. Denkewalter & R. Hirschmann*, J. Amer. chem. Soc. *90*, 3254 (1968); *R. G. Denkewalter, D. F. Veber, F. W. Holly & R. Hirschmann, ibid. 91*, 502 (1969).

[6] *G. W. Anderson, J. E. Zimmermann & F. Callahan*, J. Amer. chem. Soc. *85*, 3039 (1963).

[7] *R. B. Woodward, R. A. Olofson & H. Mayer*, J. Amer. chem. Soc. *83*, 1010 (1961); *R. A. Olofson & Y. L. Marino*, Tetrahedron *26*, 1779 (1970).

[8] *J. C. Sheehan, J. Preston & P. A. Cruickshank*, J. Amer. chem. Soc. *87*, 2492 (1965).

[9] *F. Weygand, D. Hoffmann & E. Wünsch*, Z. Naturforsch. *21b*, 426 (1966); *E. Wünsch & F. Drees*, Chem. Ber. *99*, 110 (1966).

[10] *D. Ben-Ishai & A. Berger*, J. org. Chemistry *17*, 1564 (1952).

[11] *L. C. Craig*, "Analytical Methods of Protein Chemistry", p. 121, *P. Alexander & R. J. Block*, Eds., Pergamon, London 1960.