is now more reasonably assigned to the parallel amide II vibration of the α-helix. A weak shoulder is observed around 1660 cm.$^{-1}$ which may be assigned to the random conformation in the amorphous region. Infrared spectra similar to those of horsehair have also been observed for elephant hair[29] and epidermis.[30]

Summary

(1) The amide I and II frequencies of the α-helix, the parallel-chain pleated sheet, the antiparallel-chain pleated sheet, and the random conformations of polypeptides have been explained in terms of vibrational interactions between adjacent peptide groups in the chain and through hydrogen bonds. (2) Parallel-chain pleated sheet conformations can be distinguished from antiparallel-chain pleated sheet conformations since the former exhibit the parallel amide I band at 1645 cm.$^{-1}$, while the latter show the band at 1695 cm.$^{-1}$. The perpendicular amide I band (*ca.* 1630 cm.$^{-1}$) and the parallel amide II band (*ca.* 1525 cm.$^{-1}$) are common to both these conformations. (3) Using the above criteria many extended polypeptide chains (except β-keratin) were found to be in the antiparallel-chain pleated sheet conformation. (4) The random coil conformation shows the amide I and II bands *ca.* 1660 and *ca.* 1535 cm.$^{-1}$, respectively. (5) Both the parallel and perpendicular amide I bands of the α-helix are observed at *ca.* 1650 cm.$^{-1}$ whereas the parallel and perpendicular amide II bands are observed at *ca.* 1520 and *ca.* 1550 cm.$^{-1}$, respectively. (6) The directions of the amide I and II transition moments of the α-helix of poly-γ-benzyl-L-glutamate were estimated to be inclined from the helix axis by 29–34° and 75–77°, respectively. (7) On the basis of the infrared spectra–conformation correlations described in this paper it has been possible to revise the interpretation of the amide I and II bands of several proteins.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On the Peptides of L-Lysine[1,2]

BY BASILIKI BEZAS AND LEONIDAS ZERVAS

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By carbobenzoxylation of N^{ϵ}-monobenzylidene-L-lysine (I) followed by acidification benzaldehyde is split off and pure α-monocarbobenzoxy-L-lysine (IIIa) is formed. By the action of dry hydrogen bromide dissolved in glacial acetic acid on N^{ϵ}-trityl-N^{α}-carbobenzoxy-L-lysine methyl ester only the carbobenzoxy group is eliminated and crystalline dihydrobromide of pure N^{ϵ}-trityl-L-lysine methyl ester (V) is formed; on treatment of N^{α},N^{ϵ}-ditrityl-L-lysine methyl ester with two equivalents of hydrogen chloride in methanol only the N^{α}-trityl group is split off and the crystalline dihydrochloride of the same N^{α}-trityl derivative V is formed. Compounds III and V are valuable intermediates for the preparation of different types of lysine peptides, III for the synthesis of ϵ-peptides and V for the synthesis of α-as well as mixed α,ϵ-peptides of lysine. Several such peptides were prepared in this way.

Introduction

It has been demonstrated that in some natural products, as in biocytin[3] and bacitracin,[4,5] the ϵ-amino group of L-lysine participates in the formation of an amide bond. However, until recently, no evidence was available for such a peptide linkage in proteins[6] involving the ϵ-amino group of L-lysine. The isolation of N^{ϵ}-(glycyl-α-glutamyl)-L-lysine after partial hydrolysis of collagen[7] suggests that, at least in some proteins, L-lysine may serve as a branching point of the polypeptide chain. For the elucidation of this unusual facet of protein structure and for the synthesis of bacitracin analogs, the availability of α- and ϵ-peptides, as well as of mixed α,ϵ-peptides of L-lysine would be very useful.

The preparation of α-peptides of L-lysine starting with N^{ϵ}-carbobenzoxy-L-lysine[8a] or N^{ϵ}-formyl-L-lysine[8b] presents no difficulty. ϵ-Carbobenzoxy-L-lysine was first prepared by selective decarbobenzoxylation[8a] of dicarbobenzoxy-L-lysine, and subsequently by direct carbobenzoxylation of the L-lysine copper complex.[9] The latter method of protection of the α-amino group served in a few cases for the introduction of amino acid residues directly at the ϵ-position.[10,11] A rather tedious way for the synthesis of ϵ-peptides is through the use of N^{α}-tosyl-L-lysine as starting material,[7,12] prepared in turn from N^{ϵ}-carbobenzoxy-L-lysine. After the

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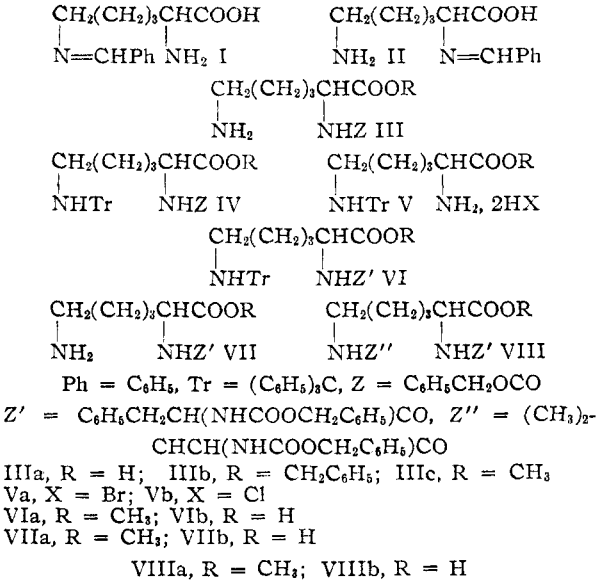
incorporation of carbobenzoxylamino acids in the free ϵ-position of the tosyl derivative, all the protective groups are removed by reduction with sodium in liquid ammonia. To the author's knowledge, no reports of the synthesis of mixed α,ϵ-peptides of L-lysine have appeared.

In the present investigation general methods for the synthesis of α- and ϵ-peptides as well as mixed α,ϵ-peptides of L-lysine, having different amino acids at the α- and ϵ-positions, are presented. As starting material, monobenzylidene-L-lysine[13] (I), which on the basis of spectroscopic and chemical data seemed to be N^{ϵ}-benzylidene derivative,[14] was used. By carbobenzoxylation of I in alkaline solution for 5–6 minutes at -5 to $-10°$ followed by acidification, benzaldehyde was split off and a pure monocarbobenzoxy-L-lysine (IIIa) was obtained in good yield. Its physical properties were different from those of the N^{ϵ}-derivative,[8a,9] it does not yield a significant amount of carbon dioxide with ninhydrin, and can thus be assigned the N^{α}-structure. Carbobenzoxylation of I in the usual manner (0–5°, 20–30 minutes) results in the formation of a mixture of monocarbobenzoxy-L-lysines, with the α-derivative predominating. It is obvious that in the alkaline solution a slow isomerization of I to II takes place to some extent.

By coupling the benzyl ester IIIb with the corresponding carbobenzoxyamino acid *via* the mixed anhydride[15] or the carbodiimide[16] method, followed by catalytic hydrogenation, chromatographically and optically pure ϵ-peptides (*e.g.*, L-phenylalanyl, glycyl and L-valyl peptides) were isolated. By an identical procedure a tripeptide, N^{ϵ}-(L-phenylalanyl)-L-lysyl-L-tyrosine, was prepared.

By tritylation[17] of IIIc the corresponding N^{α}-carbobenzoxy-N^{ϵ}-trityl-L-lysine methyl ester (IV) was obtained. Surprisingly, upon treatment of IV with an excess of dry hydrogen bromide, only the α-carbobenzoxy group was split off and crystalline N^{ϵ}-trityl-L-lysine methyl ester dihydrobromide (Va) was formed; this could then be transformed to the corresponding crystalline dihydrochloride Vb. The formation of dihydrohalogenides is to be expected, since the N-tritylation does not fully suppress the ability of the amino group to form salts in inert media.[17] The formation of Vb by the action of excess hydrochloric acid on N^{α},N^{ϵ}-dityrityl-L-lysine methyl ester has been reported.[18] It was not isolated in pure condition (no analysis was presented); even so, it served for the synthesis of some α-peptides.[18] By selective detritylation of N^{α},N^{ϵ}-ditrityl-L-lysine methyl ester with 2 equiv. of methanolic hydrochloride[17] at room temperature pure crystalline ϵ-monotrityl derivative Vb and trityl methyl ether[17] were isolated in good yield; Vb can now serve as an easily available



Ph = C_6H_5, Tr = $(C_6H_5)_3C$, Z = $C_6H_5CH_2OCO$

Z' = $C_6H_5CH_2CH(NHCOOCH_2C_6H_5)CO$, Z'' = $(CH_3)_2$-$CHCH(NHCOOCH_2C_6H_5)CO$

IIIa, R = H; IIIb, R = $CH_2C_6H_5$; IIIc, R = CH_3

Va, X = Br; Vb, X = Cl

VIa, R = CH_3; VIb, R = H

VIIa, R = CH_3; VIIb, R = H

VIIIa, R = CH_3; VIIIb, R = H

starting material for the preparation of pure α-peptides of L-lysine. For instance, N^{α}-(L-phenylalanyl)-L-lysine was prepared by coupling carbobenzoxy-L-phenylalanine with Vb, saponifying the resulting product VIa to the free acid VIb, detritylating by means of acetic acid to VIIb and finally removing the carbobenzoxy group by catalytic hydrogenation; alternatively, the special detritylation step can be omitted in this case, since all the protecting groups in VIb can be removed in one step by catalytic hydrogenation.[17] Otherwise compound VIIb can also be prepared, although in lower yield, by coupling directly I with carbobenzoxy-L-phenylalanine as described in the Experimental section.

The crystalline N^{ϵ}-trityl-L-lysine ester Vb is also an ideal starting material for the preparation of mixed α,ϵ-peptides of L-lysine, since the trityl group can be removed selectively and without any interference to other protecting groups. In this manner N^{α}-L-phenylalanyl-N^{ϵ}-L-valyl-L-lysine was prepared by detritylation of VIa with acetic acid to VIIa and coupling at the unmasked ϵ-amino group with carbobenzoxy-L-valine. From the resulting tripeptide derivative VIII, the free tripeptide was obtained after saponification followed removal of both the carbobenzoxy groups by catalytic hydrogenation.

Experimental

Prior to analysis[19] the free peptides were dried at 78° in high vacuum over phosphorus pentoxide; other compounds were dried at room temperature.

Analysis of the ϵ-dipeptides of lysine, as well as the tripeptide ϵ-phenylalanyl-lysyl-tyrosine for the presence of free α-amino acid by the Van Slyke manometric ninhydrin–CO_2 method proceeded with liberation of carbon dioxide (103–106%, instead of 100%).

The R_f-values of the peptides were determined by ascending chromatography on Whatman No. 1 paper in 1-butanol–acetic acid–water–pyridine[20]; in all cases only a single ninhydrin-positive spot ensued.

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N^{ϵ}-Benzylidene-L-lysine (I).—To the chilled solution of 9.1 g. (0.05 mole) of L-lysine monohydrochloride in 25 ml. of 2 *N* lithium hydroxide, 5.3 ml. of freshly distilled benzaldehyde was added. After shaking for a very short time, the benzaldehyde disappeared and compound I precipitated out. After standing in the refrigerator for several hours the crystals were filtered off, washed with alcohol and dried over phosphorus pentoxide; yield 10 g. (82%), m.p. 206–208° dec. (reported[13,14] 205–207°).

N^{α}-Carbobenzoxy-L-lysine (IIIa).—In 100 ml. of *N* sodium hydroxide, chilled to −5°, 23.4 g. (0.1 mole) of I was dissolved. Immediately after, and while the temperature of the solution was kept between −5 and −10°, 150 ml. of 1 *N* sodium hydroxide, precooled to −5°, and 17 ml. of freshly prepared carbobenzoxychloride were added in two portions and over a period of 5 to 6 minutes under vigorous stirring. The mixture was stirred for 10 minutes more at −5 to −10°. Stirring was further continued at room temperature for another 10 minutes, concd. hydrochloric acid (25 ml.) was added and the mixture was heated for 5 minutes at 50° and extracted twice with ether. The water layer was adjusted to *p*H 6.2[21] and concentrated *in vacuo* to a volume of approximately 100 ml. After standing in the ice-box for many hours, the crystals were filtered off and washed with a small quantity of cold water; yield 20 g. (71%), m.p. 232–233°, m.p. 235–237° after recrystallization from water, $[\alpha]^{25}D$ −12.2° (*c* 5.6 in 0.2 *N* hydrochloric acid).

Anal. Calcd. for $C_{14}H_{20}O_4N_2$ (280.3): C, 60.0; H, 7.2; N, 10.0. Found: C, 59.8; H, 7.3; N, 9.9.

N^{α}-Carbobenzoxy-L-lysine Benzyl Ester (IIIb).—A mixture of 2.8 g. (0.01 mole) of IIIa, 1.8 g. (0.01 mole) of benzenesulfonic acid and 15 ml. of benzyl alcohol was heated on a steam-bath until they were completely dissolved. Carbon tetrachloride was added, and the mixture was concentrated by distillation on a steam-bath. The removal of the solvent and the addition of fresh carbon tetrachloride was continued until the distillate was free of water. Upon adding anhydrous ether and cooling in the refrigerator, the crystalline benzenesulfonate of IIIb separated out; yield 5 g. (94%), m.p. 90–92°. After recrystallization from ethanol–ether the m.p. was raised to 96–98°, $[\alpha]^{25}D$ −17.5° (*c* 5.6 in methanol).

Anal. Calcd. for $C_{27}H_{32}O_7N_2S$ (528.6): N, 5.3. Found: N, 5.2.

N^{ϵ}-Glycyl-L-lysine.—To a solution of 2.65 g. (0.005 mole) of benzenesulfonate of IIIb in 20 ml. of pure tetrahydrofuran and 1.4 ml. of triethylamine, 1.05 g. carbobenzoxyglycine and 1.1 g. of N,N′-dicyclohexylcarbodiimide were added. The urea derivative began to precipitate within a few minutes; it was filtered off (1 g.), after the solution had been left at room temperature for 12 hours. The filtrate was evaporated to dryness, the residue dissolved in 150 ml. of ethyl acetate and the solution washed successively with dilute hydrochloric acid, potassium hydrogen carbonate and water, dried over sodium sulfate and concentrated to a volume of 50 ml. On standing in the refrigerator, 2 g. (72%) of N^{α}-carbobenzoxy-N^{ϵ}-(carbobenzoxyglycyl)-L-lysine benzyl ester separated out, m.p. 95–98°. After recrystallization from ethyl acetate or alcohol the m.p. was 98–100°.

Anal. Calcd. for $C_{31}H_{35}O_7N_3$ (561.6): C, 66.4; H, 6.25; N, 7.5. Found: C, 66.4; H, 6.4; N, 7.4.

The suspension of 2.2 g. (0.004 mole) of the above substance in a mixture of 40 ml. of ethanol–acetic acid (1:1) was hydrogenated in the presence of palladium black catalyst. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in water and evaporated again to dryness. Upon dissolving the remainder in hot abs. alcohol (20 ml.) and 2 drops of water, and standing in the refrigerator, 0.8 g. (77%) of ε-glycyl-L-lysine monoacetate was obtained, m.p. 205–207°, $[\alpha]^{25}D$ −6.8° (*c* 5.5 in water), R_f 0.18 (reported[10,22] m.p. 198–200°).

Anal. Calcd. for $C_{10}H_{21}O_6N_3$ (263.3): C, 45.6; H, 8.0; N, 16. Found: C, 45.8; H, 7.7; N, 15.9.

When this hydrogenation was carried out in the presence of one equivalent of hydrochloric acid, ε-glycyl-L-lysine monohydrochloride was obtained; yield 93%, m.p. 239–240° after recrystallization from hot ethanol and a few drops of water, $[\alpha]^{22}D$ −7.5° (*c* 3.2 in water).

Anal. Calcd. for $C_8H_{16}O_3N_3Cl$ (239.7): C, 40.1; H, 7.6; N, 17.5. Found: C, 40.0; H, 7.6; N, 17.5.

N^{ϵ}-L-Valyl-L-lysine. N^{α}-Carbobenzoxy-N^{ϵ}-(carbobenzoxy-L-valyl)-L-lysine benzyl ester was first prepared in the same way as that described for the comparable glycyl derivative; the yield in pure substance (recrystallized from abs. ethanol) was 50%, m.p. 157–159°.

Anal. Calcd. for $C_{34}H_{41}O_7N_3$ (603.7): N, 6.95. Found: N, 7.1.

The free peptide was prepared by catalytic hydrogenolysis (Pd) of the above compound in acetic acid solution, and was isolated as the acetate; yield, 90%, m.p. 208–210° (after recrystallization from ethanol), $[\alpha]^{20}D$ +27.8° (*c* 3.2 in water), R_f 0.36.

Anal. Calcd. for $C_{18}H_{27}O_5N_3$ (305.4): N, 13.7. Found: N, 13.8.

N^{ϵ}-L-Phenylalanyl-L-lysine.—A solution of 1.55 g. (0.005 mole) of carbobenzoxy-L-phenylalanine and 0.7 ml. of triethylamine in 10 ml. of dry chloroform was cooled to −5°, and 0.47 ml. of ethyl chloroformate was added. After 15 minutes at −5°, the solution was added to a solution of 2.2 g. of benzenesulfonate of IIIb in 10 ml. of chloroform and 0.7 ml. of triethylamine. The reaction was allowed to proceed at room temperature for 5 hours. The chloroform solution was washed successively with dilute hydrochloric acid, potassium hydrogen carbonate, water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. Complete removal of traces of chloroform was ensured by the addition of some methanol, and evaporation again to dryness. The crystalline residue **N^{α}-carbobenzoxy-N^{ϵ}-(carbobenzoxy-L-phenylalanyl)-L-lysine benzyl ester** was triturated with ether, filtered off, and recrystallized from ethanol; yield 2 g. (60%), m.p. 153–155°.

Anal. Calcd. for $C_{38}H_{41}O_7N_3$ (651.7.): C, 70.05; H, 6.3; N, 6.45. Found: C, 70.05; H, 6.5; N, 6.3.

The catalytic (Pd) hydrogenolysis of this compound in ethanol solution containing one equivalent of 1 *N* HCl, followed by concentration of the solution to dryness *in vacuo*, yielded 71% of crystalline peptide monohydrochloride, m.p. 216–218° after recrystallization from ethanol, $[\alpha]^{25}D$ +50.0° (*c* 4.9 in water), R_f 0.46.

Anal. Calcd. for $C_{15}H_{24}O_3N_3Cl$ (329.8): C, 54.6; H, 7.3; N, 12.7. Found: C, 54.65; H, 7.3; N, 12.7.

N^{α}-Carbobenzoxy-N^{ϵ}-(carbobenzoxy-L-phenylalanyl)-L-lysine.—The corresponding benzyl ester, prepared as described above (2.6 g., 0.004 mole), was dissolved in a mixture of 20 ml. of dioxane, 20 ml. of methanol and 2.5 ml. of 5 *N* sodium hydroxide. After standing at room temperature for 12 hours, the solution was diluted with water and concentrated *in vacuo* at room temperature until most of the dioxane and methanol were removed. Traces of unsaponified material were filtered off. Upon acidification, 2 g. (89%) of the product were obtained; needles, m.p. 166–168° (after recrystallization from alcohol), $[\alpha]^{22}D$ +4.7° (*c* 5.5 in methanol).

Anal. Calcd. for $C_{31}H_{35}O_7N_3$ (561.6): N, 7.5. Found: N, 7.4.

N^{ϵ}-L-Phenylalanyl-L-lysyl-L-tyrosine. N^{α}-Carbobenzoxy-N^{ϵ}-(carbobenzoxy-L-phenylalanyl)-L-lysyl-L-tyrosyl benzyl ester was first prepared by coupling the preceding acid, m.p. 166–168°, with L-tyrosine benzyl ester by the carbodiimide method as described above; yield 50%, m.p. 173–174° after recrystallization from ethanol.

Anal. Calcd. for $C_{47}H_{50}O_9N_4$ (814.6): N, 6.9. Found: N, 6.8.

The above tripeptide derivative (0.815 g., 0.001 mole) was catalytically (Pd) hydrogenated in ethanol–water (2:1) in the presence of 1 equiv. of hydrochloric acid, as usual. The filtrate was evaporated to dryness *in vacuo;* upon adding abs. acetone and standing in the refrigerator, 0.4 g. (80%) of the hydrochloride of the peptide was obtained, m.p. 120°, R_f 0.82.

(21) If carbobenzoxychloride is added at longer time intervals and if the temperature is above −5°, N^{ϵ}-carbobenzoxy-L-lysine which is sparingly soluble in water precipitates out in varying but small quantities; in such cases, the ε-isomer is removed by filtration and the isolation of the α-isomer from the filtrate continues as described.

(22) The specific rotation of this compound given by D. M. Theodoropoulos in *J. Org. Chem.*, **23**, 140 (1958), is in error (private communication of Dr. D. Theodoropoulos).

Anal. Calcd. for $C_{24}H_{33}O_5N_4Cl$ (493): C, 58.45; H, 6.7; N, 11.35. Found: C, 58.4; H, 6.7; N, 11.3.

N^{ϵ}-Trityl-L-lysine Methyl Ester (V). a. By Selective Decarbobenzoxylation of IV.—Into 15 ml. of abs. methanol, precooled at −10°, 0.8 ml. of freshly distilled thionyl chloride was dissolved and, keeping the temperature of the solution at −10°, 2.8 g. (0.01 mole) of IIIa was added with vigorous stirring. As soon as IIIa was completely dissolved, the solution was left at room temperature for 2 hours. It was then refluxed for 30 minutes and evaporated to dryness. The hydrochloride of IIIc which was formed (sirup, crystallizing after standing in a desiccator for several days) was dissolved in a mixture of chloroform (30 ml.) and triethylamine (3 ml.). To this solution 2.8 g. of trityl chloride was added and, after standing at room temperature for 12 hours, the solution was washed twice with water, dried over sodium sulfate, and evaporated to dryness. The sirupy residue (IV) was mixed with 80 ml. of 10% hydrogen bromide in acetic acid. Upon adding 125 ml. of dry ether and standing for 24 hours at room temperature, a mixture of crystals and sirup separated out; the precipitate was triturated with ether, dissolved in a mixture of acetone–methanol, evaporated to dryness and redissolved in acetone. Upon standing at room temperature for 3–4 days, crystalline Va separated out; yield 2.8 g. (50%), m.p. 138–140° after recrystallization from methanol–acetone.

Anal. Calcd. for $C_{26}H_{32}O_2N_2Br_2$ (564.4): N, 4.95; Br, 28.3. Found: N, 4.8; Br, 28.5.

Decarbobenzoxylation of IV and formation of Va was also accomplished by dissolving IV in 50 ml. of chloroform, bubbling in dry hydrogen bromide for 15 minutes, and leaving the solution at room temperature for 12 hours. The yield was almost the same as in the previously-described method.

b. By Selective Detritylation of N^{α},N^{ϵ}-Ditrityl-L-lysine Methyl Ester.—To a solution of 2.3 g. (0.01 mole) of L-lysine methyl ester dihydrochloride in 40 ml. of chloroform and 6.2 ml. of triethylamine, 5.6 g. (0.02 mole) of trityl chloride was added. After standing at room temperature for 24 hours, the solution was washed twice with water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. Complete removal of the chloroform was ensured by the addition of methanol and evaporation again to dryness. The sirupy residue, consisting of N^{α},N^{ϵ}-ditrityl-L-lysine methyl ester, was dissolved in 20 ml. of 1 *N* HCl in absolute methanol; on standing at room temperature for 30–60 min., the N^{α}-trityl group was split off and trityl methyl ether (2.4 g., 90%, m.p. 83°) separated out. The filtrate was evaporated *in vacuo* to dryness, and the sirupy residue was titrurated with ether. The remainder was dissolved in 50 ml. of hot absolute acetone. After standing in the refrigerator for 3–4 days, Vb separated out; yield 3 g. (65%), prisms, m.p. 152–153°. The substance was purified by dissolving it in the necessary amount of hot abs. methanol and adding abs. acetone; m.p. 155–157°, $[\alpha]^{20}D$ +12.3° (*c* 3.8 in methanol).

Anal. Calcd. for $C_{26}H_{32}N_2O_2Cl_2$ (475.5): N, 5.9; Cl, 14.9. Found: N, 5.9; Cl, 14.5.

Dihydrochloride Vb has also been prepared from Va by dissolving Va in water, adding potassium hydrogen carbonate, extracting with ether and bubbling into the ether solution dry hydrogen chloride; the crude product was purified as described above; yield 84%, m.p. 155–157°.

N^{α}-L-Phenylalanyl-L-lysine.—A solution containing 1.5 g. (0.005 mole) of carbobenzoxy-L-phenylalanine, 10 ml. of dry chloroform and 0.7 ml. of triethylamine was cooled to 0°, and 0.45 ml. (0.005 mole) of ethyl chloroformate was added. After 10 min., 2.12 g. of Vb dissolved in 10 ml. of chloroform and 1.4 ml. of triethylamine was added. The coupling proceeded with evolution of CO_2. The solution was left at room temperature for 5 hours and then it was washed successively with dilute acetic acid, potassium hydrogen carbonate and water. It was dried over sodium sulfate and evaporated to dryness *in vacuo*. The sirupy residue is **N^{α}-(carbobenzoxy-L-phenylalanyl)-N^{ϵ}-trityl-L-lysine methyl ester (VIa)**; it was saponified by dissolving it in 5 ml. of *N* methanolic sodium hydroxide. After standing at room temperature for 1 hour, the solution was diluted with 100 ml. of water and acidified with acetic acid. The free acid of the above ester precipitated out; yield 2.3 g. (70%).

Anal. Calcd. for $C_{42}H_{43}O_5N_3$ (669.8): N, 6.3. Found: N, 6.2.

The acid was detritylated by dissolving it (3.35 g., 0.005 mole) in 15 ml. of hot acetic acid and a few drops of water, and heating the solution for 5 minutes at 100°. Upon the subsequent addition of water, triphenylcarbinol separated out (1.25 g., 96%, m.p. 160°); the filtrate was evaporated to dryness *in vacuo*. Upon adding acetone, crystalline, **N^{α}-(carbobenzoxy-L-phenylalanyl)-L-lysine** was obtained; yield 0.95 g. (45%), m.p. 198–200° after recrystallization from ethanol.

Anal. Calcd. for $C_{23}H_{29}O_5N_3$ (427.5): N, 9.8. Found: N, 9.6.

This compound was also obtained by coupling I with N-carbobenzoxy-L-phenylalanyl chloride in a manner similar to the one described for the carbobenzoxylation of I; yield 20%, m.p. 198–200°.

Catalytic hydrogenolysis (Pd) of N^{α}-carbobenzoxy-L-phenylalanyl-L-lysine was performed in the presence of one equivalent of hydrogen chloride. The filtrate was concentrated to dryness *in vacuo;* alcohol was added, and the crystalline hydrochloride of the above dipeptide was obtained; yield 90%, m.p. 273–275°, after recrystallization from alcohol; R_f 0.46, $[\alpha]^{25}D$ +22.2° (*c* 2.5 in water).

Anal. Calcd. for $C_{15}H_{24}O_3N_3Cl$ (329.8): N, 12.7; Cl, 18.7. Found: N, 12.5; Cl, 10.6.

N^{α}-(Carbobenzoxy-L-phenylalanyl)-L-lysine Methyl Ester (VIIa).—A solution of 4.1 g. (0.006 mole) of the sirupy VIa in 4.2 ml. of acetic acid was mixed with 2 ml. of methanolic 4 *N* HCl and 0.2 ml. of water, and the resulting solution was heated for 5 minutes at 100°. Upon addition of ether, the hydrochloride of VIIa separated out; yield 1.7 g. (60%), m.p. 189–190° after recrystallization from methanol–acetone, $[\alpha]^{25}D$ −16.8° (*c* 2.2 in methanol).

Anal. Calcd. for $C_{24}H_{32}O_5N_3Cl$ (478): C, 60.3; H, 6.7; N, 8.8. Found: C, 60.1; H, 6.9; N, 8.6.

N^{α}-L-Phenylalanyl-N^{ϵ}-L-valyl-L-lysine.—A chloroform solution of VIIa is prepared by dissolving 0.96 g. (0.002 mole) of the corresponding hydrochloride in water, adding potassium hydrogen carbonate, extracting with chloroform, and drying over sodium sulfate. Into this chloroform solution, 0.5 g. (0.002 mole) of carbobenzoxy-L-valine and 0.45 g. of N,N′-dicyclohexylcarbodiimide were added. The mixture was left to stand at room temperature for 8 hours. After filtering off the N,N′-dicyclohexylurea (0.4 g., m.p. above 230°) the filtrate was washed successively with dilute hydrochloric acid, potassium hydrogen carbonate, water, and evaporated to dryness *in vacuo*. The crystalline residue (0.75 g., 55%) is **N^{α}-(carbobenzoxy-L-phenylalanyl)-N^{ϵ}-(carbobenzoxy-L-valyl)-L-lysine methyl ester** (VIIIa), m.p. 161–162° after recrystallization from methanol, $[\alpha]^{25}D$ −13.9° (*c* 4.5 in chloroform).

Anal. Calcd. for $C_{37}H_{46}O_8N_4$ (674.8): C, 65.85; H, 6.9; N, 8.3. Found: C, 66.0; H, 6.9; N, 8.45.

To a solution of 1.35 g. (0.002 mole) of this ester in a mixture of 8 ml. of dioxane and 5 ml. of methanol, 2.5 ml. of 1 *N* KOH in methanol was added. After standing at room temperature for 15–20 minutes, the reaction mixture was diluted with 40 ml. of water, and acidified with hydrochloric acid; crystalline **N^{α}-(carbobenzoxy-L-phenylalanyl)-N^{ϵ}-(carbobenzoxy-L-valyl)-L-lysine** (VIIIb) separated out; yield 1.2 g. (90%), m.p. 170–173° after recrystallization from methanol.

Anal. Calcd. for $C_{36}H_{44}O_8N_4$ (660.8): N, 8.5. Found: N, 8.3.

This last compound was catalytically (Pd) hydrogenated in water–alcohol suspension in the presence of 1 equiv. of hydrochloric acid. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in alcohol; upon adding acetone, the monohydrochloride of the above tripeptide separated out; yield 0.67 g. (80%), m.p. 150–153°, $[\alpha]^{25}D$ +30.9° (*c* 2.4 in water), R_f 0.62. The substance is hygroscopic.

Anal. Calcd. for $C_{20}H_{33}O_4N_4Cl$ (429): C, 55.9; H, 7.75; N, 13.05; Cl, 8.25. Found: C, 55.1; H, 7.6; N, 12.85; Cl, 8.2.