Coulometric Procedure.—Fifty ml. of buffer solution was added to the coulometer cell and deoxygenated for about 10 minutes; the mercury was then introduced and the solution electrolyzed at a potential more negative than at which the electrolysis was to be run, until the current fell to its minimum value, generally 1 to 2 ma. The mercury was removed; a known volume of a stock solution containing the electroactive species added; nitrogen was again bubbled through the solution; the mercury reintroduced; the coulometer connected (the hydrogen–oxygen coulometer was saturated with the gases prior to use); and electrolysis at the desired controlled potential started. Nitrogen was passed continuously through the cell during electrolysis.

Solutions appropriate for spectrophotometry were prepared by dilution with water to an appropriate volume followed by *p*H measurement. The identical concentration of buffer and *p*H were employed in the reference cell.

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[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE, PITTSBURGH, PENNSYLVANIA]

Insulin Peptides. III. Synthesis of a Protected Nonapeptide Containing the C-Terminal Sequence of the B-Chain of Insulin[1]

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The protected heptapeptide N-carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester was prepared by stepwise elongation of the peptide chain from the amino end. Decarbobenzoxylation of this compound and coupling of the resulting product with N$^{\alpha}$-carbobenzoxy-N$^{\omega}$-nitro-L-arginylglycine *p*-nitrophenyl ester gave the nonapeptide N$^{\alpha}$-carbobenzoxy-N$^{\omega}$-nitro-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester. This protected nonapeptide contains the C-terminal amino acid sequence of the B-chain of insulin.

Degradative studies by Sanger and co-workers[3] led to elucidation of the amino acid sequence of insulin and the postulation of a complete structure for this hormone. In this structure the amino acid sequence. . .-arginyl-glycylphenylalanyl-phenylalanyl-tyrosyl-threonyl-prolyl-lysyl-alanine occupies the C-terminal position of the B-chain of the insulin molecule.

In continuation of our studies of synthetic polypeptides with amino acid sequences found in insulin,[4,5] we undertook the synthesis of a protected nonapeptide (VII) embodying the above mentioned amino acid sequence of the C-terminal region of the insulin B-chain.

The synthesis of this peptide was accomplished by a combination of two principal synthetic approaches, *viz.*, "stepwise elongation" and "fragment condensation."[4] The protected C-terminal heptapeptide,[6] N-carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (VI), was prepared by stepwise elongation of the peptide chain from the amino end. The appropriate N-carbobenzoxy amino acids which served as the "carboxyl component" in each step were activated either by the use of N,N′-dicyclohexylcarbodiimide[7] or by conversion to the corresponding *p*-nitrophenyl esters.[8] In the latter case, aminolysis of the *p*-nitrophenyl esters was carried out in dimethylformamide for 24 hr. The reaction mixture was then treated with 1 *N* NH_4OH to destroy the excess *p*-nitrophenyl ester and the product isolated in the manner described in the experimental section. The yields of the various synthetic steps ranged from 71 to 96% of the theoretical. Leucine amino peptidase (LAP) digestion of the decarbobenzoxylated heptapeptide ester followed by paper chromatography revealed the presence of ninhydrin positive components with R_f values corresponding to the expected amino acids. Ninhydrin reactive components with R_f values corresponding to any of the intermediates were absent. This suggests that the digestion was complete and indicates[9] that the optical homogeneity of the constituent amino acids was preserved during the synthesis of the heptapeptide fragment. The protected dipeptide, N$^{\alpha}$-carbobenzoxy-N$^{\omega}$-nitro-L-arginylglycine,[10] which contains the amino acid sequence found in the N-terminal region of the nonapeptide, was converted to its *p*-nitrophenyl ester and subsequently coupled with the decarbobenzoxylated heptapeptide to give the desired protected nonapeptide N$^{\alpha}$-carbobenzoxy-N$^{\omega}$-nitro-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-

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(2) On leave from Tohoku College of Pharmacy, Sendai, Japan.

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tyrosyl - L - threonyl - L - prolyl - N^{ϵ} - tosyl - L-lysyl-L-alanine methyl ester (VII) in a 67% yield.

As mentioned above, the optical homogeneity of the heptapeptide was established by complete digestion with LAP. Since this peptide was coupled to a C-terminal glycine dipeptide, a step wherein racemization could not occur, it is concluded that the optical purity of the constituent amino acids in the protected nonapeptide was preserved during the synthetic processes.

N^{α}-Carbobenzoxy-N^{ϵ}-tosyl-L-lysine *p*-nitrophenyl ester[11] was coupled with alanine methyl ester to give the crystalline N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (I) in 94% yield. Decarbobenzoxylation of this dipeptide ester by treatment with HBr in acetic acid and coupling of the resulting product which was neutralized with triethylamine with N-carbobenzoxy-L-proline *p*-nitrophenyl ester[8] yielded the crystalline N-carbobenzoxy - L - prolyl - N^{ϵ} - tosyl - L - lysyl - L-alanine methyl ester (II) in a yield varying from 40–70%. A by-product was isolated during this step, in a yield varying from 5–10%, which on the basis of analytical data obtained, proved to be the diketopiperazine of N^{ϵ}-tosyl-L-lysyl-L-alanine. However, when the protected dipeptide I was decarbobenzoxylated by hydrogenation over a palladium on charcoal catalyst in the presence of HCl and the resulted dipeptide ester hydrochloride was mixed with N-carbobenzoxy-L-proline *p*-nitrophenyl ester *followed by* one equivalent of triethylamine, no by-product could be detected. The yield of the product by this procedure was 71%.

Catalytic hydrogenation of N-carbobenzoxy-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester and coupling of the resulting deblocked product with N-carbobenzoxy-L-threonine[12] by the carbodiimide procedure afforded the tetrapeptide N-carbobenzoxy - L - threonyl - L - prolyl - N^{ϵ} - tosyl - L - lysyl-L-alanine methyl ester (III) in 85% yield. This tetrapeptide ester was decarbobenzoxylated by catalytic hydrogenation and the deblocked peptide was condensed with N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester[8] to give the pentapeptide N-carbobenzoxy-O-benzyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (IV) in 96% yield.

Coupling of N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester[13] with the product obtained by catalytic hydrogenation of N-carbobenzoxy-O-benzyl - L - tyrosyl - L - threonyl - L - prolyl - N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester yielded the hexapeptide N - carbobenzoxy - L - phenylalanyl-L - tyrosyl - L - threonyl - L - prolyl - N^{ϵ} - tosyl-L-lysyl-L-alanine methyl ester (V). Hydrogenolysis over a palladium on charcoal catalyst was employed to decarbobenzoxylate the protected hexapeptide (V). The ensuing hexapeptide ester was condensed with N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester to give the protected heptapeptide N-carbobenzoxy-L-phenylalanyl-L-phenylalanyl - L - tyrosyl - L - threonyl - L -prolyl-N^{ϵ}-tosyl-L-lysyl-L-alanine methyl ester (VI) in 78% yield. The optical homogeneity of this peptide was discussed previously. Its chemical purity was established by elemental analysis and using the decarbobenzoxylated derivative by paper chromatography and amino acid analysis. The peptide ester hydrochloride which was obtained by catalytic hydrogenation of the protected heptapeptide (VI) was homogeneous when analyzed by paper chromatography using the ninhydrin and Pauly reagents to develop the chromatograms. Amino acid analysis of an acid hydrolysate of this homogeneous material using a Beckman–Spinco analyzer showed the expected composition. As can be seen from the amino acid ratios obtained (see Experimental section), the tosyl group was split almost quantitatively from the lysine during acid hydrolysis. This is a rather unexpected finding since with other N^{ϵ}-tosyl-lysine containing peptides,[14] the N^{ϵ}-tosyl-lysine remained practically intact during acid hydrolysis. The possibility then exists that the particular sequence found in the above peptide labilizes the N^{ϵ}-tosyl linkage.

N^{α} - Carbobenzoxy - N^{ω} - nitro - L - arginylglycine[10] was converted to its *p*-nitrophenyl ester in the usual manner. This ester was then allowed to react with the decarbobenzoxylated heptapeptide ester to give the desired protected nonapeptide N^{α}-carbobenzoxy-N^{ω}-nitro-L-arginylglycyl - L - phenylalanyl - L - phenylalanyl - L - tyrosyl-L - threonyl - L - prolyl - N^{ϵ} - tosyl - L - lysyl - L-alanine methyl ester (VII) in 67% yield. The stereochemical homogeneity of this peptide was discussed previously. Its chemical purity was established by elemental analysis and by paper chromatography of the decarbobenzoxylated derivative. In the latter case a single, sharp spot was obtained indicating the presence of a single component.

The chemical homogeneity of all the other intermediate peptides was also established by elemental analysis of the protected derivatives and by paper chromatography of the deblocked compounds.

The over-all scheme which was employed for the construction of the protected nonapeptide is summarized in Chart I.

Experimental

Capillary melting points were determined for all compounds and are corrected.

For paper chromatography, the protected amino acids and peptides were deblocked either with HBr in acetic acid or by catalytic hydrogenation in the presence of HCl. The resulting hydrobromides or hydrochlorides were chromatographed on paper, Whatman #1, using the Partridge system[15] and the R_f values are reported. The reported yields were calculated on the basis of the carboxyl component used.

N^{α}-Carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-L-alanine Methyl Ester (I).—L-Alanine methyl ester hydrochloride (11.4 g.) was dissolved in dimethylformamide (200 ml.) and triethylamine (11.2 ml.) was added followed by N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysine *p*-nitrophenyl ester (46.0 g.). After standing overnight at room temperature the solution was diluted with 1 *N* NH_4OH (10 ml.), stirred for 1 hr. and

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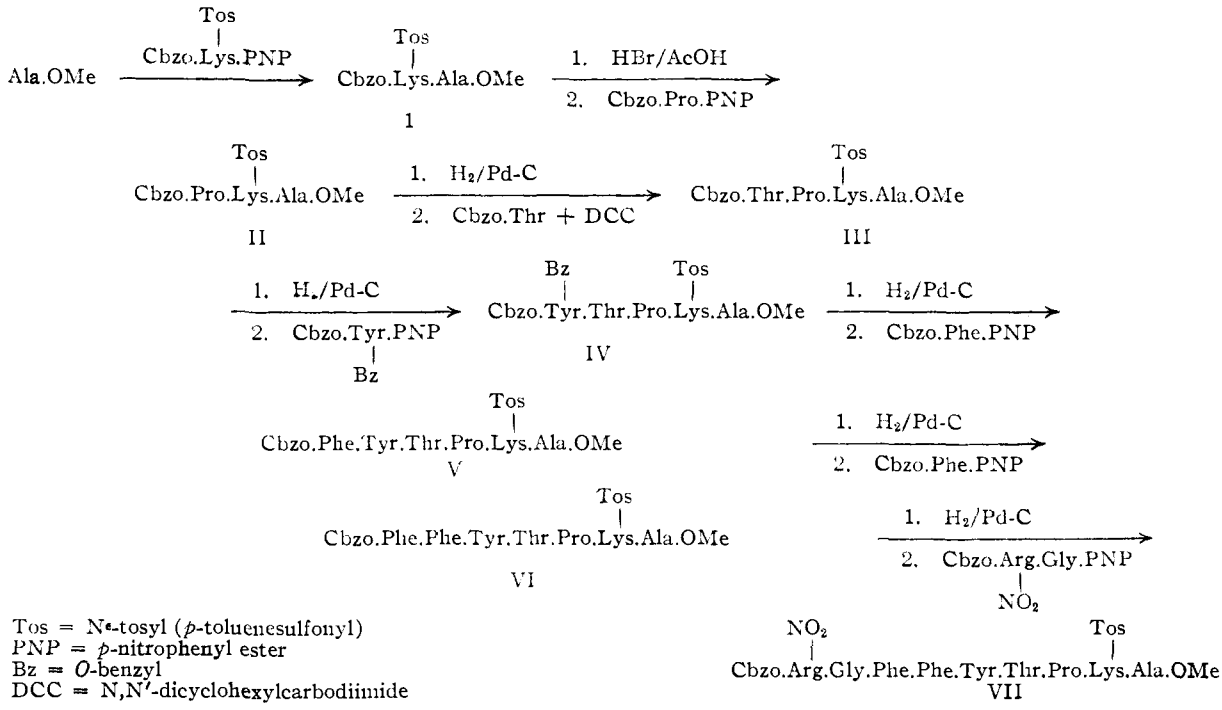
CHART I



Tos = N$^{\epsilon}$-tosyl (*p*-toluenesulfonyl)
PNP = *p*-nitrophenyl ester
Bz = *O*-benzyl
DCC = N,N′-dicyclohexylcarbodiimide

mixed with ethyl acetate (1000 ml.) and water (500 ml.). The organic layer was washed successively with 1 *N* NH_4OH, water, 1 *N* HCl and water. On removal of the ethyl acetate a crystalline residue remained which was recrystallized from aqueous ethanol; wt. 38.8 g. (94%), m.p. 137°, $[\alpha]^{30}D$ −7.7° (*c*, 1.0, dimethylformamide); R_f (hydrobromide) 0.72 [lit.[6] m.p. 133–134°, $[\alpha]^{23}D$ −20.8° (*c*, 2.0, methanol)].

Anal. Calcd. for $C_{25}H_{33}N_3O_7S$: C, 57.7; H, 6.41; N, 8.1. Found: C, 57.6; H, 6.28; N, 8.1.

N-Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester (II). A.—N$^{\alpha}$-Carbobenzoxy-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (6.7 g.) was dissolved in 2 *N* HBr in acetic acid (36 ml.). After 40 minutes at room temperature dry ether (400 ml.) was added and the precipitated semi-solid hydrobromide was triturated several times with ether and dried over KOH *in vacuo*. To a solution of this product in dimethylformamide (50 ml.), triethylamine (1.9 ml.) was added followed by N-carbobenzoxy-L-proline *p*-nitrophenyl ester (4.2 g.). After standing overnight at room temperature the solution was diluted with 1 *N* NH_4OH (2 ml.), stirred 1 hr. and subsequently was poured into ice-cold 1 *N* NH_4OH (300 ml.). The precipitate was filtered off and washed successively with 1 *N* NH_4OH, water, 1 *N* HCl and water. The crude product was suspended in warm ethanol (100 ml.); the insoluble material (P) was filtered off and treated as described subsequently. The filtrate was concentrated to dryness *in vacuo* and the residue was crystallized from aqueous ethanol. The yield varied from 40–70% (3.1–5.6 g.); m.p. 129°, $[\alpha]^{30}D$ − 34.3° (*c*, 1.1, dimethylformamide); R_f (hydrochloride) 0.86 [lit.[6] m.p. 125–127°, $[\alpha]^{22}D$ −52.8° (*c*, 2 methanol)].

Anal. Calcd. for $C_{30}H_{40}N_4O_8S$: C, 58.4; H, 6.55; N, 9.1. Found: C, 58.4; H, 6.77; N, 9.1.

Diketopiperazine of N$^{\epsilon}$-tosyl-lysyl-alanine.—The insoluble material (P) was crystallized from aqueous acetic acid. The yield generally varied from 5–10%, m.p. 240°, $[\alpha]^{30}D$ −20.1° (c. 1.16 dimethylformamide). Ninhydrin reaction was negative. The infrared spectrum of this product indicated the absence of ester- and oxycarbonylamide-groups. Acid hydrolysis with 6 *N* HCl and paper chromatography of the hydrolysate revealed the presence of alanine, lysine and a trace of N$^{\epsilon}$-tosyl-lysine. The molecular weight of this product, determined by the isothermo distillation method was found to be 311. All these data indicate that the substance in question is the diketopiperazine of N$^{\epsilon}$-tosyl-lysyl-alanine.

Anal. Calcd. for $C_{16}H_{23}N_3O_4S$: C, 54.4; H, 6.57; N, 11.9. Found: C, 54.9; H, 6.70; N, 12.1.

The calculated molecular weight is 353.4.

B.—I (12.6 g.) was dissolved in methanol (70 ml.) containing 1 *N* HCl (27 ml.) and hydrogenated over 10% palladium–charcoal catalyst (1.2 g.) until evolution of carbon dioxide ceased. The catalyst was removed by filtration and the filtrate evaporated *in vacuo*. To a solution of the residue in dimethylformamide (50 ml.) N-carbobenzoxy-L-proline *p*-nitrophenyl ester (7 g.) was added *followed* by triethylamine (3.4 ml.). After 24 hr. the reaction mixture was diluted with 1 *N* NH_4OH (5 ml.), stirred for 1 hr. and then mixed with ethyl acetate (500 ml.) and water (100 ml.). The organic layer was washed successively with 1 *N* NH_4OH, water, 1 *N* HCl and water and concentrated to dryness *in vacuo*. The residue was crystallized from aqueous ethanol, wt. 10.7 g. (71%), m.p. 129°.

N-Carbobenzoxy-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester (III).—N-Carbobenzoxy-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (18.1 g.) was hydrogenated for 8 hr. over 5% palladium-charcoal catalyst (2.0 g.) in methanol (100 ml.) containing 1 *N* HCl (24 ml.). The catalyst was removed by filtration and the solvent removed under reduced pressure. The remaining product was dried by the addition of methanol followed by evaporation under reduced pressure. The residue (R_f = 0.86) was dissolved in methylene chloride (100 ml.) containing triethylamine (4.1 ml.) and cooled at 0°; N-carbobenzoxy-L-threonine (7.3 g.) was added to this solution followed by N,N′-dicyclohexylcarbodiimide (6.0 g.). After 24 hr. the precipitated N,N′-dicyclohexylurea was filtered off and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and water. The organic layer was washed successively with 1 *N* acetic acid, water, 1 *N* NH_4OH and water and concentrated to a small volume *in vacuo*. Addition of petroleum ether resulted in the precipitation of the tetrapeptide ester; wt. 18.4 g. (85%). The amorphous product sintered at 67° and melted over a wide range (82–100°); $[\alpha]^{30}D$ −31.8°, (*c*, 1.0 dimethylformamide); R_f (hydrochloride), 0.73 [lit.[6] m.p. 87–90°; $[\alpha]^{22}D$ −56.2 (*c*, 2 methanol)].

Anal. Calcd. for $C_{34}H_{47}N_5O_{10}S$: C, 56.8; H, 6.61; N, 9.7. Found: C, 56.6; H, 6.71; N, 9.5.

N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine Methyl Ester (IV).—N-Carbobenzoxy-L-threonyl-L-prolyl-N$^{\epsilon}$-tosyl-L-lysyl-L-alanine methyl ester (10.0 g.) was hydrogenated for 8 hr.

in the presence of 5% palladium–charcoal catalyst (1.0 g.) in methanol (50 ml.) containing 1 *N* HCl (15 ml.). The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in dimethylformamide (60 ml.) and cooled at 0°. To this solution triethylamine (2 ml.) was added followed by N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester (7.3 g.). After 24 hr. the reaction mixture was diluted with 1 *N* NH_4OH (3 ml.), stirred for 1 hr. and subsequently mixed with ethyl acetate (400 ml.) and water (150 ml.). The organic layer was washed with 1 *N* NH_4OH, water, 1 *N* HCl and water again. On removal of the ethyl acetate *in vacuo* and reprecipitation of the residue from methanol–ethyl acetate–petroleum ether (1:6:30), 13.0 g. (96%) of product was obtained; m.p. 153°, $[\alpha]^{30}D$ −37.7° (*c*, 1.0 dimethylformamide); R_f (hydrochloride), 0.79.

Anal. Calcd. for $C_{50}H_{62}N_6O_{12}S$: C, 61.8; H, 6.45; N, 8.7. Found: C, 62.1; H, 6.61; N, 8.4.

N-Carbobenzoxy-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine Methyl Ester (V).—N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine methyl ester (12.6 g.) was dissolved in methanol (50 ml.) containing 1 *N* HCl (14 ml.) and hydrogenated for 8 hr. in the presence of 5% palladium–charcoal catalyst (1.2 g.). The catalyst was filtered off and the filtrate was concentrated to dryness. To a solution of the residue in dimethylformamide (60 ml.), triethylamine (1.7 ml.) was added followed by N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester (5.1 g.). The reaction mixture was allowed to stand at room temperature for 24 hr., diluted with 1 *N* NH_4OH (3 ml.), stirred for 1 hr. and poured into cold 1 *N* NH_4OH (300 ml.). The precipitated product was isolated by filtration, washed with 1 *N* NH_4OH, water, 1 *N* HCl and water again. On reprecipitation from a mixture of methanol–ethyl acetate–petroleum ether (1:6:30), 9.4 g. (76%) of product was obtained; m.p. 165–167°; $[\alpha]^{30}D$ − 46.0° (*c*, 1.3 dimethylformamide); R_f (hydrochloride), 0.88.

Anal. Calcd. for $C_{52}H_{65}N_7O_{13}S$: C, 60.7; H, 6.37; N, 9.5. Found: C, 60.1; H, 6.38; N, 9.4.

N-Carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine Methyl Ester One Half Hydrate (VI).—N-Carbobenzoxy-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine methyl ester (12.0 g.) was decarbobenzoxylated by catalytic dehydrogenation in the usual manner over 5% palladium catalyst (1.2 g.) in methanol (150 ml.) containing 1 *N* HCl (12.7 ml.). The hexapeptide ester hydrochloride obtained was dissolved in dimethylformamide (40 ml.) containing triethylamine (1.5 ml.). To this solution N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester (5.0 g.) was added. After 24 hr. the reaction mixture was diluted with 1 *N* NH_4OH (3 ml.), stirred 1 hr. and poured into ice-cold 1 *N* NH_4OH (300 ml.). The precipitated product was washed in the usual manner and reprecipitated from aqueous acetic acid; wt. 9.9 g. (72%); m.p. 172–175°, $[\alpha]^{30}D$ −37.3° (*c*, 1.1 dimethylformamide); R_f (hydrochloride), 0.90, single ninhydrin and Pauly positive spot. For the anhydrous peptide it was reported[6] m.p. 199–203°.

Anal. Calcd. for $C_{61}H_{74}N_8O_{14}S \cdot 0.5H_2O$: C, 61.9; H, 6.40; N, 9.5. Found: C, 61.9; H, 6.50; N, 9.5.

The protected heptapeptide ester was decarbobenzoxylated by catalytic hydrogenation in the usual manner. Amino acid analysis of the resulted peptide ester hydrochloride by a Beckman–Spinco analyzer, Model 120, after acid hydrolysis showed the expected composition, expressed as molar ratios: $phe_{2.2}tyr_{1.0}thr_{1.0}pro_{1.0}lys_{0.9}ala_{1.1}$. The heptapeptide ester hydrochloride was also digested with LAP. Paper chromatography of the digest revealed the presence of six ninhydrin positive components with R_f's 0.62, 0.46, 0.25, 0.34, 0.74 and 0.28 identical with the R_f's of authentic samples of phenylalanine, tyrosine, threonine, proline, N$^\epsilon$-tosyl-lysine and alanine, respectively.

N$^\alpha$-Carbobenzoxy-N$^\omega$-nitro-L-arginylglycine *p*-Nitrophenyl Ester.—To a pre-cooled solution of N$^\alpha$-carbobenzoxy-N$^\omega$-nitro-L-arginylglycine (7.5 g.) in dimethylformamide (12 ml.) and tetrahydrofuran (15 ml.) was added *p*-nitrophenol (2.7 g.) followed by dicyclohexylcarbodiimide (3.8 g.). After 30 minutes at 0° and 4 hr. at room temperature the N,N′-dicyclohexylurea which separated was filtered off and the filtrate concentrated to one half of its original volume *in vacuo*. Addition of ether to the remaining solution resulted in the precipitation of an oily product which was solidified on reprecipitation from ethanol five times and drying *in vacuo;* wt. 7.1 g. (75%); m.p. 90–95°, $[\alpha]^{28}D$ −4.8° (*c*, 1.68 dimethylformamide).

Anal. Calcd. for $C_{22}H_{25}N_7O_9$: C, 49.7; H, 4.75; N, 18.5. Found: C, 49.4; H, 5.28; N, 18.9.

N$^\alpha$-Carbobenzoxy-N$^\omega$-nitro-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine Methyl Ester One and One Half Hydrate (VII).—N-Carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N$^\epsilon$-tosyl-L-lysyl-L-alanine methyl ester (7.8 g.) was decarbobenzoxylated by catalytic hydrogenation for 10 hr. over 5% palladium–charcoal catalyst (1.5 g.) in methanol (70 ml.) containing 1 *N* HCl (10 ml.). The resulted heptapeptide ester hydrochloride was dissolved in dimethylformamide (50 ml.) containing triethylamine (1.0 ml.) and cooled at 0°; N$^\alpha$-carbobenzoxy-N$^\omega$-nitro-L-arginylglycine *p*-nitrophenyl ester (3.7 g.) was added to this solution. After 24 hr. at room temperature the reaction mixture was diluted with 1 *N* NH_4OH (5 ml.), stirred for 1 hr. and poured into ice-cold 1 *N* NH_4OH (300 ml.). The precipitated product was washed in the usual manner and reprecipitated from methanol–ether; wt. 7.0 g. (67%), m.p. 185–195°, $[\alpha]^{30}D$ − 31.9° (*c*, 1.07, dimethylformamide). Paper chromatography of the decarbobenzoxylated (HBr/AcOH) nonapeptide ester showed only a single spot (R_f = 0.77) as revealed by ultraviolet quenching and ninhydrin and Pauly reactions.

Anal. Calcd. for $C_{69}H_{88}N_{14}O_{15}S \cdot 1.5H_2O$: C, 56.7; H, 6.20; N, 13.4. Found: C, 56.4; H, 6.40; N, 13.1.

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