A New Synthesis of L-Glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine

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Received September 24, 1962

The hexapeptide, L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine, occurring in adrenocorticotropins and melanotropins, has been synthesized by a stepwise procedure beginning with the COOH-terminal glycine. Bioassay results showed that the hexapeptide is active as a melanocyte-stimulating and lipolytic agent. The following peptides were synthesized in crystalline form for the first time: L-tryptophylglycine *t*-butyl ester, N^G-tosyl-L-arginyl-L-tryptophylglycine *t*-butyl ester, carbobenzoxy-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine *t*-butyl ester, N^α-carbobenzoxy-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine *t*-butyl ester, N^α-carbobenzoxy-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine, *N*-*t*-butyloxycarbonyl-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine *t*-butyl ester, and γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanine-N^G-tosyl-L-arginyl-L-tryptophylglycine.

In connection with our previous synthesis[1,2] of the nonadecapeptide corresponding to the first nineteen amino acid residues of adrenocorticotropins (ACTH), we have reported a synthesis of the protected hexapeptide, N^α-carbobenzoxy-γ-benzyl-L-glutamyl-Im-benzyl-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine. Since this hexapeptide is a key peptide fragment for the synthesis of adrenocorticotropically and melanotropically (MSH) active products, it was deemed desirable to improve the synthesis by obtaining high yields and crystalline intermediates at each synthetic step. The present paper presents a new synthesis[3] of L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (IX), by means of a stepwise procedure from the COOH-terminal residue.

Fig. 1 outlines the synthetic steps for each intermediate product, with its yield, obtained in the course of the synthesis of IX; the heavy underline indicates that the product had been obtained in crystalline form. Carbobenzoxyglycine *t*-butyl ester[6a] was hydrogenated and then crystallized as the hydrochloric acid salt.[6b] This salt was treated with triethylamine to liberate the free base and then condensed with carbobenzoxy-L-tryptophan by the dicyclohexylcarbodiimide (DCCI) procedure.[7] The protected dipeptide (I) failed to crystallize, but its free base (II) did crystallize on hydrogenation; II was then allowed to react, *via* DCCI, with crystalline N^α-carbobenzoxy-N^G-tosyl-L-arginine[8] to produce the amorphous protected tripeptide (III). The free base of the tripeptide ester (IV) crystallized on hydrogenation. This tripeptide ester was next condensed with carbobenzoxy-L-phenylalanine by the N-ethyl-5-phenylisoxazolium 3′ sulfonate procedure[9] to obtain the crystalline protected tetrapeptide (V). The tetrapeptide was hydrogenated and allowed to react with the azide of N^α-carbobenzoxy-L-histidine hydrazide[10] to produce the protected pentapeptide VI. After countercurrent distribution in the toluene system[2] to remove azide decomposition products, VI was crystallized from methanol solution; VI was further characterized by treatment with trifluroacetic acid to obtain the crystalline N^α-carbobenzoxy-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine. After hydrogenation, VI was then coupled with the crystalline *p*-nitrophenyl ester of N^α-*t*-butyloxycarbonyl-γ-benzyl-L-glutamic acid to produce the crystalline protected hexapeptide (VII).

The procedure outlined above for the synthesis of VII gave an over-all yield, based on the hydrochloride of glycine *t*-butyl ester, of approximately 30% of crystalline product. In the azide coupling step to obtain VI, an excess of the azide was used. In a normal azide coupling, an excess of peptide base is preferable in order to minimize the danger of Curtius rearrangement. However, an excess of azide was used to insure complete reaction of the tetrapeptide base; countercurrent distribution was shown to be an effective procedure in separating VI from contaminating decomposition products from excess N^α-carbobenzoxy-L-histidine azide. The protected hexapeptide VII was treated with trifluoroacetic acid to remove the *t*-butyloxycarbonyl and *t*-butyl ester groups, and then reduction in sodium in liquid ammonia[11] was performed to remove the γ-benzyl ester and tosyl groups. The resulting free peptide was purified by zone electrophoresis on starch. The purified product, IX, was submitted to paper chromatography in the solvent system consisting of *n*-butyl alcohol pyridine-acetic acid–water (15:10:3:12, v./v.), and to electrophoresis on paper in buffers of pH 3.6, 6.5, and 11.0; results of these experiments indicated that IX behaves as a homogeneous substance. Moreover, when IX was treated with leucine aminopeptidase[12] (LAP), it was digested completely to its constituent amino acids. Quantitative analyses of the digest by the procedure of Spackman, *et al.*,[13] in the Spinco amino acid analyzer gave a composition in molar ratios which is consistent

(1) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T.-b. Lo, and J. Ramachandran, *J. Am. Chem. Soc.*, **82**, 5760 (1960).

(2) C. H. Li, J. Meienhofer, E. Schnabel, D. Chung, T.-b. Lo, and J. Ramachandran, *ibid.*, **83**, 4449 (1961).

(3) Kappeler[4] reported the synthesis of the glutaminyl analogue of this hexapeptide by a different route. Later, the same investigator in collaboration with Schwyzer[5] described the synthesis of IX by another scheme.

(4) H. Kappeler, *Helv. Chim. Acta*, **44**, 476 (1961).

(5) R. Schwyzer and H. Kappeler, *ibid.*, **44**, 1991 (1961).

(6) (a) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960); (b) While this manuscript was being prepared, it was noted that Taschner and co-workers [E. Taschner, C. Wasielewski, and J. F. Biernat, *Ann.*, **646**, 119 (1961)] have also prepared this compound by a different method.

(7) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(8) J. Ramachandran and C. H. Li, *J. Org. Chem.*, **27**, 4006 (1962).

(9) R. B. Woodward, R. A. Olafson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

(10) W. Holley and E. Sondheimer, *ibid.*, **76**, 1326 (1954).

(11) V. du Vigneaud and O. K. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).

(12) R. L. Hill and E. L. Smith, *ibid.*, **228**, 577 (1957).

(13) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

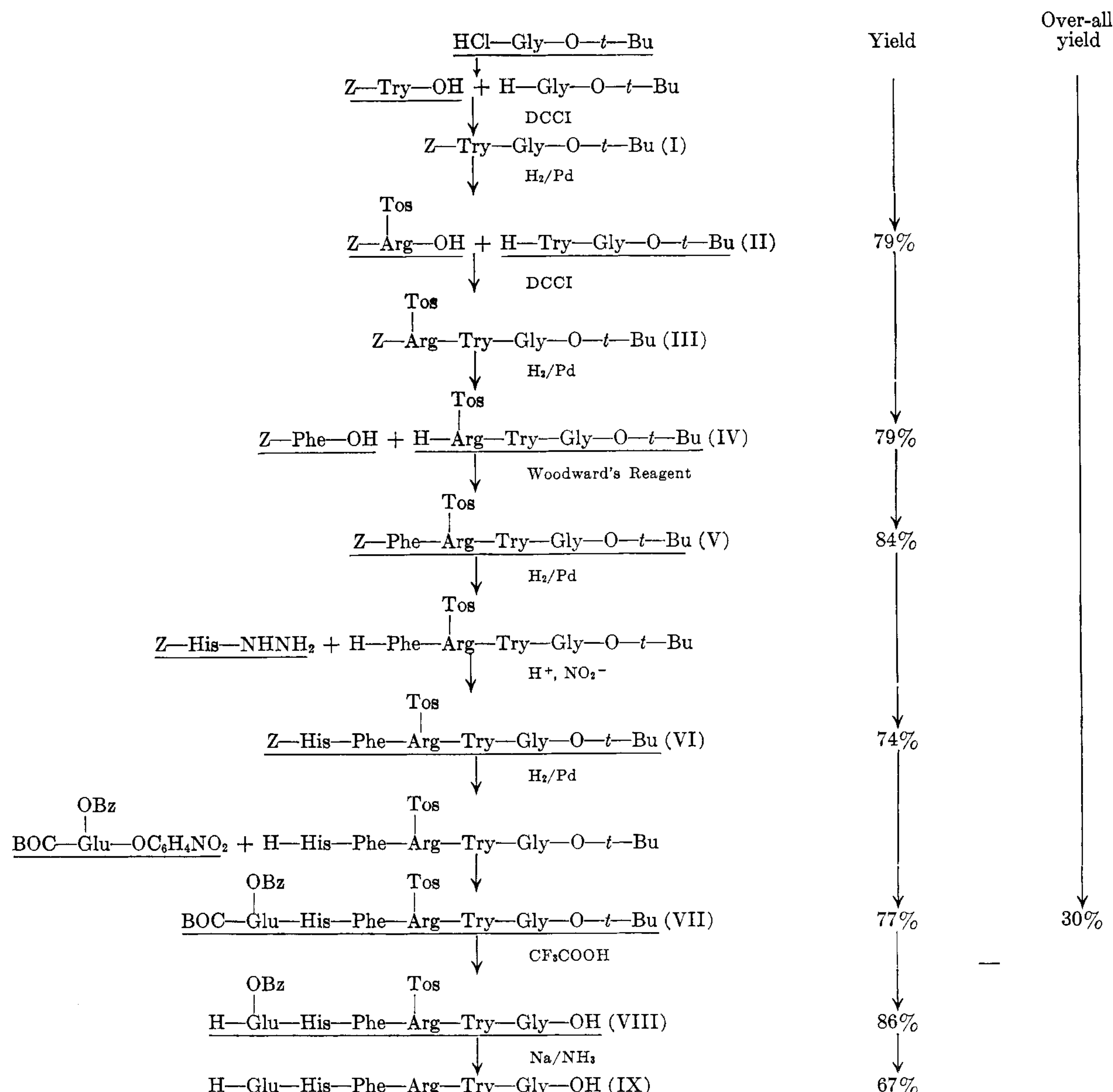

Fig. 1.—Outline of the synthesis of L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl glycine; Z, carbobenzoxy; Bz, benzyl; Tos, p-toluenesulfonyl; BOC, t-butyloxycarbonyl; t-Bu, t-butyl.

with the theoretical calculated values (molecular weight 830.9): $Glu_{1.06}$, $His_{0.97}$, $Phe_{1.02}$, $Arg_{1.08}$, $Try_{0.97}$, $Gly_{0.95}$.

Peptide IX was assayed for melanocyte-stimulating activity by the *in vitro* frog skin method,[14a] and on the basis of change in the melanophore index in hypophysectomized Rana pipiens.[14b] It was found that melanotrophic activities obtained by these two procedures (2×10^5 units per gram; a single dose of two micrograms caused a change in melanophore index from 1+ to 3+ within one hour in hypophysectomized frogs) were almost identical to values reported earlier[15] for the hexapeptide, glycine-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine. In agreement with previous conclusion,[15] it appears that glutamic acid can be replaced by glycine in IX without alteration of the melanocyte-stimulating potency.

By *in vitro* assay for lipolytic activity with perirenal fat pads of rabbits, IX was found to be active[16] in releasing non-esterified fatty acids with a calculated mean effective dose of twenty-three micrograms. Under the same conditions, the synthetic pentapeptide, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine, had only one-fiftieth of the lipolytic activity of IX.

(14) (a) K. Shizume, A. B. Lerner, and T. B. Fitzpatrick, *Endocrinology*, **54**, 533 (1954); (b) L. T. Hogben and D. Slome, *Proc. Roy. Soc.* (London), **B108**, 10 (1931).

(15) E. Schnabel and C. H. Li, *J. Biol. Chem.*, **235**, 2010 (1960).

(16) A. Tanaka, B. T. Pickering, and C. H. Li, *Arch. Biochem. Biophys.*, in press.

Experimental[17]

Glycine t-Butyl Ester Hydrochloride.—Carbobenzoxyglycine t-butyl ester, 10.61 g., (40 mmoles) was prepared according to

(17) All melting points were performed on a Fisher-Johns melting point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. All samples for microanalyses were dried in an Abderhalden drying pistol with phosphorus pentoxide at 77° for 16 hr. at 0.3-mm. pressure. Paper chromatography was carried out on Whatman no. 1 filter paper at room temperature; the solvents used were 1-butanol–acetic acid–water (BAW) in the ratio 4:1:1 and sec-butanol–10% ammonia (SBA) in the ratio of 85:15; the location of peptide spots was revealed by either the ninhydrin reagent, the Ehrlich reagent,[18] the Pauly reagent,[19] or the chlorine method.[20]

(18) J. Smith, *Nature*, **171**, 43 (1953).

(19) F. Sanger and H. Tuppy, *Biochem. J.*, **49**, 463 (1951).

(20) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).

the procedure of Anderson and Callahan[6] and hydrogenated in 150 ml. of methanol with palladium (from 2 g. of palladium chloride) as catalyst for 2 hr. in a Vibro-mixer.[21] The catalyst was removed, and to the filtrate 36.5 ml. of 1.1 *N* hydrochloric acid was added. After evaporation to dryness *in vacuo* (20–25°), the flask containing the ester hydrochloride was kept in a desiccator *in vacuo* overnight over phosphorus pentoxide and sodium hydroxide pellets. The residue was dissolved in a small amount of methanol and crystallized by the addition of ether. After recrystallization from the same solvent system, 4.03 g. (60.5%), of glycine *t*-butyl ester hydrochloride were obtained[6b]; m.p. 137–140° $R_{f\,BAW}$ = 0.6.

Anal. Calcd. for $C_6H_{14}N_1O_2Cl$ (167.7): C, 43.0; H, 8.41; N, 8.35. Found: C, 42.9; H, 8.22; N, 8.42.

Nα-Carbobenzoxy-L-tryptophylglycine *t*-Butyl Ester (I).—Glycine *t*-butyl ester hydrochloride, 3.60 g. (21.4 mmoles), was suspended in 100 ml. of ethyl acetate and then cooled to 0° in an ice bath. A 3.0-ml. aliquot of triethylamine (21.4 mmoles) was added and the resulting suspension was stirred at 0°. Then 6.59 g. of carbobenzoxy-L-tryptophan (19.5 mmoles)[22] and 4.02 g. of N,N′-dicyclohexylcarbodiimide[7] (19.5 mmoles) were added and the mixture stirred for 1 hr. at 0° and then overnight at room temperature. The precipitate, dicyclohexylurea (DCU), was filtered off and the ethyl acetate solution was washed successively with 0.5% acetic acid, water, 1 *M* sodium carbonate, and water, and then dried over anhydrous sodium sulfate. The dried ethyl acetate solution was then evaporated to dryness *in vacuo* (20–25° bath). The residue was redissolved in acetone, more dicyclohexylurea was filtered off, and the acetone was evaporated *in vacuo* (20–25°). The original precipitate was washed with water to remove triethylamine hydrochloride and then filtered and dried. The total weight of dicyclohexylurea was 3.80 g. (87%); m.p. 235°. The Nα-carbobenzoxy-L-tryptophylglycine *t*-butyl ester was isolated as a glassy material; wt., 8.24 g. (94%), m.p. 60–70°. It resisted all attempts at crystallization. $[\alpha]^{25}D$ −19.6° (*c* 1, methanol) $R_{f\,BAW}$ = 0.83; $R_{f\,SBA}$ = 0.81; Ehrlich, chlorine positive, single spot.

Anal. Calcd. for $C_{25}H_{29}O_5N_3$ (451.5): C, 66.5; H, 6.47; N, 9.31. Found. C, 66.2; H, 6.51; N, 9.60.

L-Tryptophylglycine *t*-Butyl Ester (II).—Nα-Carbobenzoxy-L-tryptophylglycine *t*-butyl ester, 4.80 g. (10.6 mmoles), was dissolved in 100 ml. methanol. Palladium from 1 g. of palladium chloride was added and the peptide was decarbobenzoxylated by hydrogenation in a Vibro-mixer. When no more carbon dioxide could be detected, the catalyst was filtered and the methanol was evaporated *in vacuo* (20–25°). The peptide II was then crystallized from ethyl acetate-petroleum ether to give 2.84 g. (84%); m.p. 94–97°; $[\alpha]^{25}D$ + 2.3° (*c* 1, methanol).

Anal. Calcd. for $C_{17}H_{23}O_3N_3$ (317.4): C, 64.3; H, 7.30; N, 13.2. Found: C, 63.9; H, 7.51; N, 13.6.

Nα-Carbobenzoxy-NG-tosyl-L-arginyl-L-tryptophylglycine *t*-Butyl Ester(III).—Nα-Carbobenzoxy-NG-tosylarginine,[8] 4.62 g., (10.0 mmoles), was dissolved in 200 ml. of warm acetonitrile. The solution was cooled to room temperature, 3.59 g. of II (11.3 mmoles) were then added, and the solution then cooled to 0°. Dicyclohexylcarbodiimide in the amount of 2.06 g. (10. mmoles) was added with stirring at 0° for 1 hr., and the mixture was then placed overnight in the refrigerator at 4°. The dicyclohexylurea was filtered and the acetonitrile evaporated *in vacuo* (20–25°). The residue was redissolved in ethyl acetate and then washed with 0.5% acetic acid, water, 1 *M* sodium carbonate, water, and then the washed material was dried over anhydrous sodium sulfate. It was precipitated from ethyl acetate–petroleum ether to yield 6.40 g. (84%). The peptide resisted all attempts at crystallization. It was found to be homogeneous by paper chromatography. $R_{f\,BAW}$ = 0.82, $R_{f\,SBA}$ = 0.75, positive to Ehrlich regent and chlorine. $[\alpha]^{25}D$ −25.1° (*c* 1, methanol).

Anal. Calcd. for $C_{38}H_{47}O_8SN_7$ (761.9): C, 59.9; H, 6.22; N, 12.9. Found: C, 59.6; H, 6.01; N, 12.7.

NG-Tosyl-L-arginyl-L-tryptophylglycine *t*-butyl ester (IV).—Nα - Carbobenzoxy - NG - tosyl - L - arginyl - L - tryptophylglycine *t*-butyl ester, 6.40 g., (8.4 mmoles) was dissolved in 150 ml. of methanol and then catalytically hydrogenated with palladium (from 1 g. of palladium chloride) as above. The catalyst was filtered off and the methanol evaporated *in vacuo* (20–25°). The peptide was crystallized from ethyl acetate; wt., 4.94 g. (79% over-all from the dicyclohexylcarbodiimide coupling step), m.p. 156–157°; $[\alpha]^{25}D$ +8.1° (*c* 1, methanol).

Anal. Calcd. for $C_{30}H_{41}O_6N_7S_1$ (627.8): C, 57.4; H, 6.58; N, 15.6. Found: C, 57.2; H, 6.71; N, 15.5.

Carbobenzoxy-L-phenylalanyl-NG-tosyl-L-arginyl-L-tryptophylglycine *t*-Butyl Ester (V).—Carbobenzoxy-L-phenylalanine,[23] 5.23 g. (17.5 mmoles), was dissolved in 280 ml. of acetonitrile and then cooled to 0°. Triethylamine, 2.45 ml. (17.5 mmoles), and Woodward's reagent K,[9,24] 4.45 g. (17.5 mmoles), were added with stirring for 1 hr. at 0°. Then 10.24 g. of IV (16.0 mmoles) was added and the mixture stirred at room temperature overnight. The acetonitrile was evaporated *in vacuo* (20–25° bath) and the residue was dissolved in ethyl acetate–water (200:100 ml.). The ethyl acetate was then washed twice with water, twice with cold 1% citric acid, once with water, four times with 5% sodium bicarbonate, and then with water until the aqueous wash was neutral. The ethyl acetate layer was washed with saturated sodium chloride and then dried over anhydrous sodium sulfate. The ethyl acetate was evaporated *in vacuo* (20–25° bath), the dried residue was treated with fresh, dry ethyl acetate (150 ml.), and the peptide crystallized. Wt., 12.17 g. (84%); m.p., 160–162°; $[\alpha]^{25}D$ +18.8° (*c* 1, methanol).

Anal. Calcd. for $C_{47}H_{56}N_8O_9S$ (909.1): C, 62.1; H, 6.21; N, 12.3. Found: C, 62.1; H, 6.30; N, 12.6.

L-Phenylalanyl-NG-tosyl-L-arginyl-L-tryptophylglycine *t*-Butyl Ester—Carbobenzoxy-L-phenylalanyl-NG-tosyl-L-arginyl-L-tryptophyl glycine *t*-butyl ester, 3.85 g. (4.22 mmoles), was hydrogenated in 150 ml. of methanol with palladium (from 2 g. of palladium chloride). The catalyst was filtered off and the methanol evaporated *in vacuo* to give a glassy material, wt. 3.1 g. (95%). Single spot positive to ninhydrin and the Ehrlich reagent. $R_{f\,BAW}$ = 0.70.

Nα-Carbobenzoxy-L-histidyl-L-phenylalanyl-NG-tosyl-L-arginyl L-tryptophylglycine *t*-Butyl Ester (VI).—An ice cold ethyl acetate solution of Nα-carbobenzoxy-L-histidine azide prepared from 2.57 g. of the hydrazide (8.5 mmoles) as described by Holley and Sondheimer[10] was added to an ice cold solution of 3.1 g. of L-phenylalanyl - NG - tosyl - L - arginyl - L - tryptophylglycine *t*-butyl ester (4.1 mmoles) in 20 ml. ethylacetate. In a few minutes the pentapeptide ester started to precipitate. The reaction mixture was stirred at 0°C. for 48 hr., and for 12 hr. at room temperature. The ethyl acetate was evaporated *in vacuo* at 0°, and the remaining amorphous material was dissolved in 20 ml. of methanol and added dropwise with stirring to 700 ml. of ether to precipitate the pentapeptide. After filtering and drying, 4.78 g. of crude material were obtained. Paper chromatography in the system BAW showed one Ehrlich positive spot with R_f = 0.80 and three Pauly positive spots with R_f = 0.25, 0.65, and 0.80. For further purification the material was subjected to countercurrent distribution in the system consisting of toluene–chloroform–methanol–water (5:5:8:2). After 100 transfers, the desired pentapeptide was identified by ultraviolet absorption at 280 mμ as the material in tubes 41–61 (*K* = 0.96). In paper chromatography, this material showed a single spot that was positive to the Pauly and Ehrlich reagents and migrated with $R_{f\,BAW}$ = 0.80. Tubes 61–71 contained material that showed two Pauly-positive spots in paper chromatography: R_f = 0.25 and 0.65. The contents of tubes 41–61 were pooled and evaporated *in vacuo* (20–25°). The oily residue was dissolved in 30 ml. of methanol, and water was added until the beginning of a slight turbidity. After the solution had stood for 1 week at 0°, the pentapeptide ester crystallized, and was collected and dried. Yield: 3.74 g. (78%), m.p. 152–160°. The product was then recrystallized from the same solvent system, m.p. 158–160°; $[\alpha]^{25}D$ −32.6° (*c* 1, methanol)

Anal. Calcd. for $C_{53}H_{63}O_{10}N_{11}S_1 \cdot 1/2\ H_2O$ (1055.2): C, 60.3; H, 6.15; N, 14.6. Found: C, 60.3; H, 6.50; N, 14.3.

Nα-Carbobenzoxy-L-histidyl-L-phenylalanyl-NG-tosyl-L-arginyl L-tryptophylglycine.—The above protected pentapeptide ester VI (200 mg.) was dissolved in 2 ml. of trifluoroacetic acid in a nitrogen atmosphere. After being allowed to stand at room temperature for 15 min., ether was added to precipitate the product. The precipitate was washed well with ether to remove all excess trifluoroacetic acid, filtered, and then dried. The peptide acid was crystallized from dimethylformamide–ether to yield 154 mg. (73%), m.p. 162–164°, $R_{f\,SBA}$ = 0.16; $[\alpha]^{25}D$ −20.7 (*c* 1, methanol).

Anal. Calcd. for $C_{49}H_{55}O_{10}N_{11}S_1 \cdot CF_3COOH$ (1104.1): C, 55.5; H, 5.11; N, 14.0. Found. C, 55.8; H, 5.51; N, 14.4.

(21) A. G. Für Chemie Apparateban, Zürich, Model E1.

(22) E. L. Smith, *J. Biol. Chem.*, **175**, 39 (1948).

(23) W. Grassmann and E. Wünsch, *Ber.*, **91**, 462 (1958).

(24) *N*-Ethyl-5-phenylisoxazolium 3′-sulfonate, Pilot Chemicals, Inc. Watertown 72, Mass.

N-*t*-Butyloxycarbonyl-γ-benzyl-L-glutamic Acid *p*-Nitrophenyl Ester.—γ-Benzyl-L-glutamic acid,[25] 2.37 g. (10 mmoles), and MgO, 0.80 g. (20 mmoles), were suspended in 40 ml. of 50% dioxane and stirred for 1 hr. at room temperature. Then 3.0 g. of *t*-butyloxycarbonyl azide[26,27] (21 mmoles) was added and the mixture was stirred at 45–50° for 6 hr. The mixture was then poured into 250 ml. of ice cold water and some insoluble material (MgO) was filtered off. The aqueous solution was then extracted with 150 ml. of ethyl acetate (three times) to remove excess *t*-butyloxycarbonyl azide. The ethyl acetate washings were then washed twice with 20 ml. of 7.5% sodium bicarbonate and once with 50 ml. of water. The combined aqueous extracts were cooled at 0°, and the pH was adjusted to 3 with 10% citric acid (approximately 40 ml.). The acidified solution was then saturated with sodium chloride and extracted twice with 150 ml. of ethyl acetate. The ethyl acetate was washed with saturated sodium chloride, and then dried over anhydrous sodium sulfate. The ethyl acetate was evaporated *in vacuo* (20–25° bath) to leave the N-*t*-butyloxycarbonyl-γ-benzyl-L-glutamic acid as an oil, homogeneous in paper chromatography, $R_{f\,BAW} = 0.90$; $R_{f\,SBA} = 0.58$ (chlorine detection). Wt., 1.54 g. (46%), 4.5 mmole. The oil was dissolved in 20 ml. of ethyl acetate, cooled to 0°, and then 0.63 g. of *p*-nitrophenol (4.5 mmoles) and 0.9 g. of dicyclohexylcarbodiimide (4.5 mmoles) were added. The mixture was stirred at 0° for 2 hr. It was then placed in the refrigerator (4°) overnight. The dicyclohexylurea was filtered off, [wt. 0.77 g. (75%)] and the ethyl acetate was evaporated *in vacuo* (20–25° bath). The *p*-nitrophenyl ester was crystallized from ethyl acetate–petroleum ether (20–30 ml.) to yield 0.53 g. (52%), m.p. 120–121°; $[\alpha]^{25}D$ −32.7° (*c* 1, methanol).

Anal. Calcd. for $C_{23}H_{26}N_2O_8$ (458.3): C, 60.3; H, 5.68; N, 6.11. Found: C, 60.3; H, 5.90; N, 6.32.

N-*t*-Butyloxycarbonyl-γ-benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine *t*-Butyl Ester (VII). N^{α}-carbobenzoxy - L - histidyl - L - phenylalanyl - N^G - tosylarginyl - L - tryptophylglycine *t*-butyl ester, 6.28 g. (6.0 mmoles), was dissolved in 150 ml. of methanol and catalytically hydrogenated with palladium from 2 g. of palladium chloride until no more carbon dioxide was detectable; $R_{f\,BAW} = 0.60$. The palladium was filtered off and the methanol was evaporated *in vacuo* (20–25° bath). The residue was dissolved in 50 ml. of acetonitrile and 3 ml. of dimethylformamide. A 3.04-g. sample of *N*-butyloxycarbonyl-γ-benzylglutamic acid *p*-nitrophenyl ester (6.6 mmoles) was added and the mixture was stirred for 2 days at room temperature. During the course of the reaction, the mixture became gelatinous, and more acetonitrile (approximately 50 ml.) was added to insure good stirring and mixing. After the 2 days of stirring, the solvents were evaporated *in vacuo* (20–25° bath) and the residue treated with a large volume of ether. The resulting precipitate was filtered, washed well with ether, and then dried. Weight of crude VII was 6.8 g. (93%). The product was then crystallized from warm methanol to yield 5.7 g. (77%), m.p. 162–164°. $[\alpha]^{25}D$ −22.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{62}H_{78}O_{13}N_{12}S_1$ (1231.4): C, 60.5; H, 6.38; N, 13.7. Found: C, 60.2; H, 6.43; N, 13.7.

γ-Benzyl-L-glutamyl-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophylglycine (VIII).—The above protected hexapeptide, 0.62 g. (0.5 mmole), was dissolved in 2.0 ml. of trifluoroacetic acid in a nitrogen atmosphere. The solution was allowed to stand at room temperature for 15 min. and then added to 50 ml. of ether to precipitate the desired peptide. The precipitate was thoroughly washed with ether to remove excess trifluoroacetic acid, and then crystallized from the slow evaporation of a methanol solution to give 0.56 g. (86%), m.p. 165–168° (dec.); $[\alpha]^{25}D$ +12.0° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{53}H_{62}O_{11}N_{12}S_1\cdot(CF_3COOH)_2\cdot CH_3OH$ (1335.3): C, 52.2; H, 5.13, N. 12.6. Found: C, 52.1, H, 5.53; N, 13.0.

L-Glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (IX).—The ditrifluoroacetate salt of VIII, 0.534 g., was dissolved in 100 ml. of liquid ammonia, and small pieces of sodium were added at the temperature of the boiling point of liquid ammonia (−33°) until the blue color persisted for 30 min.[11] The ammonia was then allowed to evaporate and the residue was dried completely in a vacuum desiccator over concentrated sulfuric acid. The residue was then desalted on an IRC-50 column, eluted with pyridine–acetic acid–water buffer (30:4:66), and then lyophylized to yield 0.300 g. (90%) of the crude free hexapeptide. A 50-mg. sample of this material was purified by zone electrophoresis on starch in 0.05 *M* sodium carbonate and run for 24 hr. at 200 volts to yield 37 mg. (74%) of a product that was homogeneous in paper chromatography in the BAW, SBA, and 1-butanol–pyridine–acetic acid–water (15:10:3:12) systems, and in paper electrophoresis in buffers of pH 3.6, 6.5, and 11.0. It appeared as a single spot, positive to ninhydrin, the Ehrlich, Pauly, and Sakaguchi reagents, and chlorine; $[\alpha]^{25}D$ −17.3° (*c* 1, in acetic acid).

Anal. Calcd. for $C_{39}H_{50}N_{12}O_9\cdot CH_3COOH\cdot H_2O$ (909.0): C, 54.2; H, 6.21; N, 18.5. Found: C, 54.3; H, 6.78; N, 18.7.

LAP Digest of the Hexapeptide.—The above hexapeptide, 0.8 mg., was dissolved in 0.5 ml. of tri(hydroxymethyl)aminomethane (TRIS)buffer, (pH 8.5, 0.01 *M* Mg^+) and 0.008 ml. of a LAP solution (1 mg. of Worthington LAP, lot no. 5917 in 0.2 ml. of water) was added and the solution was incubated at 37° for 24 hr. Amino acid analysis by the Spinco amino acid analyzer[13] gave the following molar ratios: $Glu_{1.06}His_{0.97}Phe_{1.02}Arg_{1.08}Try_{0.97}Gly_{0.95}$.

Acknowledgment.—This work was supported in part by grants from the National Institutes of Health and the United States Public Health Service (G-2907) and the Upjohn Company. We wish to acknowledge the able technical assistance of Eugene Racz, Katalin Tarczy-Hornoch, and C. W. Jordan, Jr.

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Preparation and Reactions of Triphenyltinlithium

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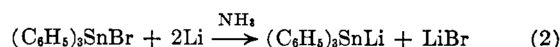
Received July 2, 1962

The preparation, properties, and reactions of triphenyltinlithium with water, triphenyltin fluoride, tri-*n*-butyl phosphate, ethyl iodide, benzyl chloride, bromobenzene, chlorotriphenylsilane, chlorotrimethylsilane, and carbon dioxide are described.

The preparation of triphenyltinlithium has been described in the literature by several investigators. In 1950, G. Wittig[2a] reported its preparation from phenyllithium and diphenyltin, as well as from triphenyltin bromide and metallic lithium in liquid ammonia. Gilman and Rosenberg[2b] later reported the preparation of triphenyltinlithium from stannous chloride and phenyllithium. In this manner the intermediate preparation of diphenyltin was eliminated. Blake, Coates, and

$$C_6H_5Li + (C_6H_5)_2Sn \longrightarrow (C_6H_5)_3SnLi \quad (1)$$



$$(C_6H_5)_3SnBr + 2Li \xrightarrow{NH_3} (C_6H_5)_3SnLi + LiBr \quad (2)$$

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