the salt in ethyl acetate gave partial separation of the two diastereomers. Fractions with mp 176–178°, $[\alpha]^{27}D$ +36.0° (*c* 1, 95% ethanol, 0.4 g), and mp 168–171°, $[\alpha]^{27}D$ +20.0° (*c* 1, 95% ethanol, 0.2 g) were obtained. Hydrolysis of the two iminium salt fractions gave two liquid ketone fractions with $[\alpha]^{27}D$ +26.4° (*c* 1, 95% ethanol, 0.16 g) and $[\alpha]^{27}D$ −3.4° (*c* 1, 95% ethanol, 0.06 g).

Partial resolution of 3-methyl-5-(*p*-methoxyphenyl)-2-cyclohexen-1-one (IV) was achieved using procedure B.

Pyrrolidine perchlorate (1.6 g, 0.009 mole) and IV (2.0 g, 0.009 mole) in absolute ethanol (10 ml.) were cooled, giving the iminium perchlorate salt (1.8 g, 60%), mp 90–91°. Treatment with potassium *d*-camphor-10-sulfonate converted the iminium perchlorate salt to the *d*-camphor-10-sulfonate salt, mp 171–172°. The diastereomeric mixture (16.6 g) was fractionally recrystallized from acetonitrile–ether giving two salt fractions, mp 174–175°, $[\alpha]^{27}D$ +42.3° (*c* 1, 95% ethanol, 4.7 g.), and mp 140–143°, $[\alpha]^{27}D$ −18.0° (*c* 1, 95% ethanol, 0.3 g). Hydrolysis of the two iminium salt fractions gave two crystalline ketone fractions, mp 58–59°, $[\alpha]^{27}D$ +55.6° (*c* 1, 95% ethanol, 1.8 g), and mp 57–59°, $[\alpha]^{27}D$ −26.8° (*c* 1, 95% ethanol, 0.08 g).

The method for resolution presented above is not a panacea. It is, however, the simplest method to try. It does not involve synthesis of optically active reagents, and regeneration of the carbonyl compound is performed under exceptionally mild conditions. The iminium salts are readily available and generally high melting solids.

Satisfactory analyses were obtained for all new compounds.

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(13) DuPont Teaching Fellow, 1964–1965.
(14) Public Health Service Fellow (5-Fl-GM-21, 687-02), National Institute of General Medical Sciences.

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Insulin Peptides. XI. The Synthesis of the B Chain of Human Insulin and Its Combination with the Natural A Chain of Bovine Insulin to Generate Insulin Activity[1]

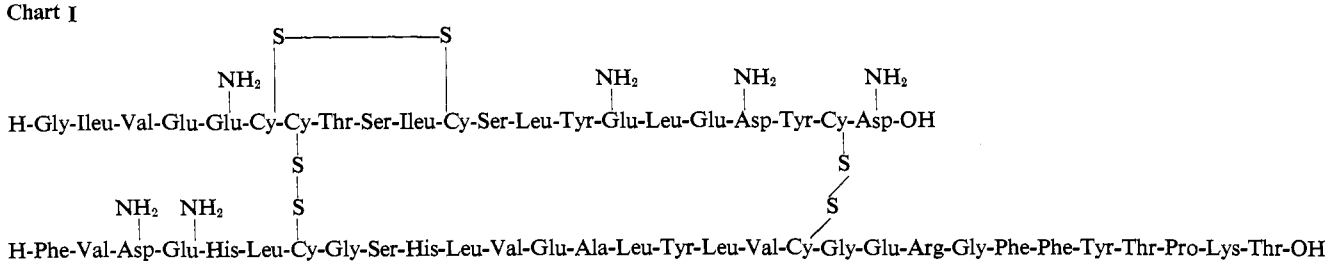
Sir:

In previous communications[2,3] we have reported the synthesis and isolation in the S-sulfonate form of the A and B chains of sheep insulin. Combination experiments between these chains and crossed recombination experiments between the synthetic chains and the natural chains of bovine insulin led to generation of insulin activity. We wish to report now the synthesis and isolation in the S-sulfonate form of the B chain of human insulin. The proposed[4] structure of human insulin is shown in Chart I. The synthetic B chain, upon combination with the natural A chain of bovine insulin, generated insulin activity in an over-all yield ranging from 4 to 8% (based on crystalline bovine insulin). This represents the partial synthesis of a human protein.

N^{α}-Carbobenzoxy-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester [mp 99–101°; $[\alpha]^{27}D$ +1.9° (*c* 10, DMF[5]) (*Anal.* Calcd for $C_{26}H_{35}N_3O_8S$: C, 56.8; H, 6.42; N, 7.7. Found: C, 57.2; H, 6.52; N, 7.9); after hydrogenolysis in the presence of HCl: R_f[6] 0.71], prepared from N^{α}-carbobenzoxy-N^{ϵ}-tosyl-L-lysine p-nitrophenyl ester[7] and threonine methyl ester, was decarbobenzoxylated by catalytic hydrogenation and condensed with N-carbobenzoxy-L-proline *p*-nitrophenyl ester[8] to give N-carbobenzoxy-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (I): mp 125–128°; $[\alpha]^{27}D$ −24.9° (*c* 1.0, DMF) (*Anal.* Calcd for $C_{31}H_{42}N_4O_9S$: C, 57.6; H, 6.55; N, 8.7. Found: C, 57.6; H, 6.67; N, 8.9); after hydrogenolysis in the presence of HCl: R_f[6] 0.68, R_f[9] 4.35 × His. Removal of the carbobenzoxy group from I by hydrogenolysis and coupling of the ensuing product with N-carbobenzoxy-L-threonine[10] by the carbodiimide method[11] yielded N-carbobenzoxy-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (II): mp 84–90°; $[\alpha]^{27}D$ −32.7° (*c* 1, DMF) (*Anal.* Calcd for $C_{35}H_{39}N_5O_{11}S$: C, 56.2; H, 6.60; N, 9.4. Found: C, 56.3; H, 6.56; N, 9.1); for the hydrochloride: R_f[6] 0.72, R_f[9] 5.07 × His. N-Carbobenzoxy-O-benzyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (III) [mp indefinite 88–110°; $[\alpha]^{29}D$ −32.1° (*c* 2, DMF) (*Anal.* Calcd for $C_{51}H_{64}N_6O_{13}S$: C, 61.2; H, 6.44; N, 8.4. Found: C, 61.0; H, 6.43; N, 8.4); after hydrogenolysis in the presence of HCl: R_f[6] 0.73, R_f[9] 2.99 × His] was prepared by coupling N-carbobenzoxy-O-benzyl-L-tyrosine *p*-nitrophenyl ester[8] with the product obtained by catalytic hydrogenation of II. Removal of the carbobenzoxy and benzyl groups from III by hydrogenolysis and coupling of the ensuing product with N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester[12] yielded N-carbobenzoxy-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (IV): mp 139–141°; $[\alpha]^{27}D$ −40.9° (*c* 1, DMF) (*Anal.* Calcd for $C_{53}H_{67}N_7O_{14}S$: C, 60.2; H, 6.38; N, 9.3. Found: C, 60.3; H, 6.42; N, 9.2); for the hydrochloride R_f[6] 0.84, R_f[9] 3.96 × His. Catalytic hydrogenation of IV and reaction of the resulting product with N-carbo-

(1) Presented (P. G. K.) at the National Academy of Sciences Autumn Meeting, Seattle, Wash., Oct 11-13 1965, and at the Brookhaven National Laboratory Symposium on "Structure and Function of Polypeptide Hormones: Insulin," Upton, N. Y., Nov 8–11, 1965.
(2) P. G. Katsoyannis, A. Tometsko, and K. Fukuda, *J. Am. Chem. Soc.*, 85, 2863 (1963).
(3) P. G. Katsoyannis, K. Fukuda, A. Tometsko, K. Suzuki, and M. Tilak, *ibid.*, 86, 930 (1964).
(4) D. S. H. W. Nicol and L. F. Smith, *Nature*, 187, 483 (1960).
(5) DMF stands for N,N-dimethylformamide.
(6) The R_f refers to the Partridge system: S. M. Partridge, *Biochem. J.*, 42, 238 (1948).
(7) M. Bodanszky, J. Meienhofer, and V. du Vigneaud, *J. Am. Chem. Soc.*, 82, 3195 (1960).
(8) M. Bodanszky and V. du Vigneaud, *ibid.*, 81, 5688 (1959).
(9) The R_f refers to the system 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (S. G. Waley and G. Watson, *Biochem. J.*, 55, 328 (1953)), and is expressed as a multiple of the distance traveled by a histidine marker.
(10) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1961, p 895.
(11) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955).
(12) M. Bodanszky and V. du Vigneaud, *ibid.*, 81, 6072 (1959).

Chart I



benzoxy-L-phenylalanine *p*-nitrophenyl ester afforded the heptapeptide derivative N-carbobenzoxy-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (V): mp 163–165°; $[\alpha]^{27}D$ −38.5° (*c* 1, DMF) (*Anal.* Calcd for $C_{62}H_{76}N_8O_{15}S$: C, 61.8; H, 6.36; N, 9.3. Found: C, 61.8; H, 6.40; N, 9.0); for the hydrochloride: R_f^6 0.91, R_f^9 5.1 × His. N-Carbobenzoxyglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (VI) [mp 150–157°; $[\alpha]^{27}D$ −34.3° (*c* 1, DMF) (*Anal.* Calcd for $C_{64}H_{79}N_9O_{10}S$: C, 60.9; H, 6.30; N, 10.0. Found: C, 60.6; H, 6.56; N, 10.0); for the hydrochloride: R_f^6 0.89, R_f^9 4.53 × His] was prepared by treating N-carbobenzoxyglycine *p*-nitrophenyl ester[13] with the product obtained by hydrogenolysis of V. Decarbobenzoxylation of VI by catalytic hydrogenation and coupling of the ensuing product with N^{α}-carbobenzoxy-N^{ω}-tosyl-L-arginine[14] using 2-ethyl-5-phenylisoxazolium 3′-sulfonate[15] as the condensing reagent yielded N^{α}-carbobenzoxy-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (VII): mp 155–160°; $[\alpha]^{27}D$ −12.0° (*c* 2, DMF); (*Anal.* Calcd for $C_{77}H_{97}N_{13}O_{19}S_2$: C, 58.8; H, 6.21; N, 11.6. Found: C, 58.8; H, 6.11; N, 11.7); for the hydrochloride: R_f^6 0.86, R_f^9 4.53 × His. Reaction of N-carbobenzoxy-L-glutamic acid γ-*t*-butyl-α-*p*-nitrophenyl ester[16] with the product obtained by hydrogenolysis of VII gave N-carbobenzoxy-γ-*t*-butyl-L-glutamyl-N^{ω}-tosyl-L-arginylglycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (VIII): mp 200°; $[\alpha]^{28}D$ −22.6° (*c* 2, DMF) (*Anal.* Calcd for $C_{86}H_{112}N_{14}O_{22}S_2$: C, 58.7; H, 6.42; N, 11.2. Found: C, 58.6; H, 6.54; N, 11.1); for the acetate: R_f^6 0.95, R_f^9 4.67 × His; amino acid analysis after acid hydrolysis: $lys_{0.90}arg_{1.0}thr_{2.0}glu_{1.2}pro_{1.0}gly_{1.1}tyr_{0.9}phe_{2.0}$. N-Carbobenzoxy-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycine hydrazide (IX) [mp 285° dec; $[\alpha]^{28}D$ −43.0° (*c* 1, DMF) (*Anal.* Calcd for $C_{46}H_{64}N_8O_9S$: C, 61.0; H, 7.13; N, 12.4. Found: C, 61.1; H, 7.31; N, 12.3] was prepared from the respective ethyl ester[17] upon treatment with hydrazine. Conversion of IX to the corresponding azide and coupling with the product obtained by catalytic hydrogenation of VIII yielded the protected hexadecapeptide N-carbobenzoxy-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-γ-*t*-butyl-L-glutamyl-N^{ω}-tosyl-L-arginyl-glycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine methyl ester (X): mp 255–256°; $[\alpha]^{29}D$ −30.7° (*c* 1, DMF) (*Anal.* Calcd for $C_{124}H_{166}N_{20}O_{29}S_3$: C, 59.7; H, 6.70; N, 11.2. Found: C, 60.1; H, 6.84; N, 11.6); after HBr in trifluoroacetic acid treatment: R_f^6 0.96, R_f^9 2.75 × His; amino acid ratios in acid hydrolysate: $lys_{0.9}arg_{1.0}thr_{1.8}glu_{1.0}pro_{0.9}gly_{2.1}val_{1.0}leu_{2.1}tyr_{1.8}phe_{1.8}$S-benzylcysteine$_{1.0}$.

N-Carbobenzoxy-γ-*t*-butyl-L-glutamyl-L-alanine methyl ester (XI) [mp 102°; $[\alpha]^{28}D$ −15.5° (*c* 1, DMF); lit[18] $[\alpha]^{20}D$ −13° (*c* 1, DMF)], prepared by the reaction of the azide of N-carbobenzoxy-γ-*t*-butyl-L-glutamic acid with L-alanine methyl ester, was converted by the *p*-nitrophenyl ester method to N-carbobenzoxy-L-valyl-γ-*t*-butyl-L-glutamyl-L-alanine methyl ester[18] (XII). Treatment of N^{α}-carbobenzoxy-*im*-benzyl-L-histidyl-L-leucine benzyl ester[19] with hydrazine yielded the respective hydrazide [mp 110–111°; $[\alpha]^{26}D$ −18.9° (*c* 1, acetic acid) (*Anal.* Calcd for $C_{27}H_{34}N_6O_4$: C, 64.0; H, 6.96; N, 16.6. Found: C, 64.0; H, 7.00; N, 16.6], which in turn was converted to the corresponding azide and coupled with the decarbobenzoxylated XII to give N-carbobenzoxy-*im*-benzyl-L-histidyl-L-leucyl-L-valyl-γ-*t*-butyl-L-glutamyl-L-alanine methyl ester (XIII): mp 180–181°; $[\alpha]^{28}D$ −22° (*c* 1, DMF) (*Anal.* Calcd for $C_{45}H_{63}N_7O_{10}$: C, 62.7; H, 7.36; N, 11.4. Found: C, 62.9; H, 7.64; N, 11.3); after HBr in trifluoroacetic acid treatment: R_f^6 0.84, R_f^9 3.5 × His. XIII upon treatment with hydrazine afforded the respective hydrazide (XIV: mp 244°; $[\alpha]^{28}D$ −16.9° (*c* 1, DMF) (*Anal.* Calcd for $C_{44}H_{63}N_9O_9$: C, 61.4; H, 7.36; N, 14.6. Found: C, 61.2; H, 7.22; N, 14.5). XIV was converted to its azide and then condensed with the product obtained by HBr in trifluoroacetic acid treatment of X to give the partially protected heneicosapeptide; this in turn on exposure to alkali and then to HBr in trifluoroacetic acid yielded the C-terminal heneicosapeptide fragment of the B chain *im*-benzyl-L-histidyl-L-leucyl-L-valyl-L-glutamyl-L-alanyl-L-leucyl-L-tyrosyl-L-leucyl-L-valyl-S-benzyl-L-cysteinylglycyl-L-glutamyl-N^{ω}-tosyl-L-arginyl-glycyl-L-phenylalanyl-L-phenylalanyl-L-tyrosyl-L-threonyl-L-prolyl-N^{ϵ}-tosyl-L-lysyl-L-threonine trihydrobromide (XV): mp 267–269°; $[\alpha]^{28}D$ −22.5° (*c* 1, dimethyl sulfoxide) (*Anal.* Calcd for $C_{147}H_{206}N_{27}O_{34}S_3 \cdot 3HBr$: C, 54.6; H, 6.41; N, 11.7; Br, 7.4. Found: C, 54.4; H, 6.40; N, 11.1; Br, 6.80) (in none of the usual chromatographic systems employed in this work did this peptide move from the origin; hence no paper chromatography could be performed); amino acid ratios in acid hydrolysate: $lys_{0.80}arg_{0.80}$-

(13) B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).
(14) J. Ramachandran and C. H. Li, *J. Org. Chem.*, **27**, 4006 (1962).
(15) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).
(16) K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *ibid.*, 87, 611 (1965).
(17) P. G. Katsoyannis and M. Tilak, *ibid.*, **85**, 4028 (1963).
(18) J. Meienhofer, *Z. Naturforsch.*, **19b**, 114 (1964).
(19) D. Thedoropoulos, *Acta Chim. Scand.*, **12**, 2043 (1958).

$thr_{1.80}glu_{2.30}pro_{0.92}gly_{2.0}ala_{1.2}val_{2.08}leu_{2.92}tyr_{1.71}phe_{1.75}$S-benzylcysteine$_{0.75}$ (*im*-benzylhistidine not determined).

N-Carbobenzoxy-L-phenylalanyl-L-valyl-L-asparaginyl-L-glutaminyl-*im*-benzyl-L-histidyl-L-leucyl-S-benzyl-L-cysteinylglycyl-L-serine hydrazide[3] was converted to the respective azide, and this in turn was coupled with XV to give the protected sulfhydryl form of the B chain. The blocking groups were removed by treatment with sodium in liquid ammonia[20] and the deblocked product was converted to the S-sulfonate form and purified by continuous-flow electrophoresis. The S-sulfonate of the B chain thus obtained exhibited on high-voltage paper electrophoresis a single Pauli-positive spot. Amino acid analysis of the synthetic material after acid hydrolysis gave a composition, in molar ratios, which is consistent with the theoretically expected values for the human B chain: $lys_{0.8}his_{2.7}arg_{1.0}thr_{1.0}$[21]$ser_{1.0}glu_{3.5}pro_{0.8}gly_{3.2}ala_{1.5}cys_{1.8}val_{3.5}leu_{4.5}tyr_{1.8}phe_{3.0}$.

Combination experiments between the synthetic human B chain and the natural A chain of bovine insulin[22] generated considerable insulin activity. As judged by the mouse convulsion method, the over-all yield of the hybrid insulin produced was 4 to 8% of theory. The yield is based on the amount of the S-sulfonates of the A and B chains used originally before their conversion to the sulfhydryl form and their oxidation and not on the protein content of the final product. Since crystalline insulin, generated by the combination of synthetic B chain of sheep insulin with the natural bovine A chain, was recently obtained in our laboratory,[1] work is now in progress for the isolation of crystalline insulin generated by combination of synthetic human B chain with the natural bovine A chain.

(20) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, 108, 753 (1935).
(21) Uncorrected.
(22) Prepared from crystalline zinc insulin by a new method: P. G. Katsoyannis and A. Tometsko, unpublished data.
(23) We wish to express our appreciation to Eli Lilly and Co. for carrying out part of the mouse convulsion assays, to Miss Roberta Klimaski for carrying out mouse convulsion assays in our own laboratories, and to Miss Karen Scheibe for the amino acid analyses.
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Insulin Peptides. XII. Human Insulin Generation by Combination of Synthetic A and B Chains[1]

Sir:

In the preceding communication[2] we reported the synthesis of the B chain of human insulin, its isolation in the S-sulfonate form, and its combination with the natural A chain of bovine insulin[3] to generate insulin activity. We wish now to report the synthesis and isolation in the S-sulfonate form of the A chain of human insulin and its combination, either with the natural bovine B chain[3] or with the synthetic human B chain, to generate insulin activity. This last observation appears to represent the first chemical synthesis of a human protein.

The structure of human insulin as proposed by Nicol and Smith[4] is shown in Chart I.

N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-threonyl-L-serine methyl ester [mp 185–187°; $[\alpha]^{27}D$ −16.4° (*c* 1, DMF[5a]) (*Anal.* Calcd for $C_{26}H_{33}N_3O_8S$: C, 57.1; H, 6.03; N, 7.7. Found: C, 57.3; H, 6.19; N, 8.0); after HBr in TFA[5b] treatment: R_f[6] 0.74, R_f[7] 4.1 × His, prepared by the reaction of N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester[8] with the product obtained by hydrogenolysis of N-carbobenzoxy-L-threonyl-L-serine methyl ester,[9] was deblocked on exposure to HBr in TFA and condensed with N-carbobenzoxy-S-benzyl-L-cysteine *p*-nitrophenyl ester to give N-carbobenzoxy-S-benzyl-L-cysteinyl-S-benzyl-L-cysteinyl-L-threonyl-L-serine methyl ester (I): mp 174–177°; $[\alpha]^{27}D$ −26.0° (*c* 1, DMF) (*Anal.* Calcd for $C_{36}H_{44}N_4O_9S_2$: C, 58.4; H, 6.00; N, 7.6. Found: C, 57.9; H, 6.04; N, 7.3); after HBr in TFA treatment: R_f[6] 0.87, R_f[7] 4.45 × His. Exposure of I to HBr in TFA and reaction of the resulting product with N-carbobenzoxy-L-glutamine *p*-nitrophenyl ester[8] yielded N-carbobenzoxy-L-glutaminyl-S-benzyl-L-cysteinyl-S-benzyl-L-cysteinyl-L-threonyl-L-serine methyl ester (II): mp 220–221°; $[\alpha]^{27}D$ −26.9° (*c* 1, DMF) (*Anal.* Calcd for $C_{41}H_{52}N_6O_{11}S_2$: C, 56.7; H, 6.00; N, 9.7. Found: C, 56.4; H, 6.27; N, 9.2); for the hydrobromide: R_f[6] 0.75, R_f[7] 4.36 × His.

N-Carbobenzoxy-L-valyl-L-glutamic acid γ-*t*-butyl-α-methyl ester [mp 91–93°; $[\alpha]^{27}D$ −5.8° (*c* 1, DMF); lit[10] mp 90°; lit[10] $[\alpha]^{25}D$ −29.5° (*c* 1, methanol); after hydrogenolysis: R_f[6] 0.65, R_f[7] 4.04 × His], prepared by the reaction of N-carbobenzoxy-L-valine *p*-nitrophenyl ester[11] and L-glutamic acid γ-*t*-butyl-α-methyl ester, was hydrogenolyzed and condensed with N-carbobenzoxyglycyl-L-isoleucine azide[12] to give N-carbobenzoxyglycyl-L-isoleucyl-L-valyl-L-glutamic acid γ-*t*-butyl-α-methyl ester (III): mp 171–174°; $[\alpha]^{27}D$ −13.0° (*c* 1, DMF); lit[10] mp 202–203°; lit[10] $[\alpha]^{25}D$ −13.2° (*c* 2, DMF) (*Anal.* Calcd for $C_{31}H_{48}N_4O_9$: C, 60.0; H, 7.74; N, 9.0. Found: C, 59.8; H, 7.86; N, 9.5); after hydrogenolysis: R_f[6] 0.86, R_f[7] 4.81 × His. Treatment of III with hydrazine afforded

(1) (a) Part of this work was reported (P. G. K.) in the National Academy of Sciences Autumn Meeting, Seattle, Wash., Oct 11–13, 1965; (b) presented (P. G. K.) at the Brookhaven National Laboratory Symposium on "Structure and Function of Polypeptide Hormones: Insulin," Upton, N. Y., Nov 8–11, 1965.
(2) P. G. Katsoyannis, A. M. Tometsko, J. Z. Ginos, and M. A. Tilak, *J. Am. Chem. Soc.*, 88, 164 (1966).
(3) Prepared from crystalline zinc insulin by a new procedure: P. G. Katsoyannis and A. Tometsko, unpublished data.
(4) D. S. H. W. Nicol and L. F. Smith, *Nature*, 187, 483 (1960).
(5) (a) DMF stands for N,N-dimethylformamide; (b) TFA stands for trifluoroacetic acid.
(6) The R_f refers to the Partridge system: S. M. Partridge, *Biochem. J.*, 42, 238 (1948).
(7) The R_f refers to the system 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (S. G. Waley and G. Watson, *Biochem. J.*, 55, 328 (1953)), and is expressed as a multiple of the distance traveled by a histidine marker.
(8) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, 81, 5688 (1959).
(9) K. Hofmann, W. Haas, M. J. Smithers, and G. Zanetti, *ibid.*, 87, 631 (1965).
(10) H. Zahn, H. Bremer, W. Sroka, and J. Meienhofer, *Z. Naturforsch.*, 20b, 646 (1965).
(11) B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 40, 373 (1957).
(12) Y. Wang, *et al.*, *Sci. Sinica* (Peking), 13, 2030 (1964).