Ethyl 2-Acetylaminofluorenyl-7-carbamate.—A solution of 0.15 g. of 2-acetylaminofluorenyl-7-isocyanate in 15 ml. of ethanol was refluxed 10 minutes, diluted, and cooled. On filtration there was obtained 0.10 g. (57% yield) of product. Recrystallization from ethanol gave an analytical sample of m.p. 248.5–250.5°. *Anal.* Calcd. for $C_{18}H_{18}N_2O_3$: N, 9.0. Found: N, 9.2, 9.0.

PHILADELPHIA, PENNA. RECEIVED JULY 25, 1951

[CONTRIBUTION NO. 830 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

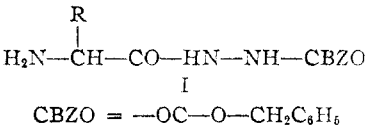
Studies on Polypeptides. III.[1] Novel Routes to α-Amino Acid and Polypeptide Hydrazides

BY KLAUS HOFMANN,[2] ADOLF LINDENMANN,[3] MARGARET Z. MAGEE AND NOORUL HAQ KHAN

A novel procedure for the synthesis of acylated α-amino acid and polypeptide hydrazides is presented. The method involves the synthesis of α-amino acid carbobenzoxyhydrazides, their incorporation into polypeptide derivatives, followed by liberation of the hydrazide group by hydrogenolysis. Three procedures for the preparation of α-amino acid carbobenzoxyhydrazides are described. The transformation of three L-amino acid amides into the respective 4-substituted-2-thio-5-thiazolidones is shown to involve racemization. A method allowing the preparation of phthalyl amino acid and peptide hydrazides is given. A number of acylated tripeptide carbobenzoxyhydrazides were synthesized in good yields by the interaction of acylated dipeptide azides with glycine carbobenzoxyhydrazide. Triglycine hydrazide dihydrochloride was prepared in good yield by the hydrogenation of carbobenzoxytriglycine carbobenzoxyhydrazide.

The conventional method for the preparation of acylated polypeptide hydrazides involves the treatment of acylated peptide esters with hydrazine. Although highly successful with acylated amino acid and dipeptide esters, this method is not generally applicable when the preparation of more highly complex acylated polypeptide hydrazides is desired.

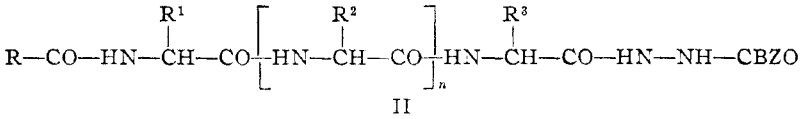
The application to the synthesis of peptide hydrazides of α-amino acid carbobenzoxyhydrazides of the general structure (I) offers distinct advantages.



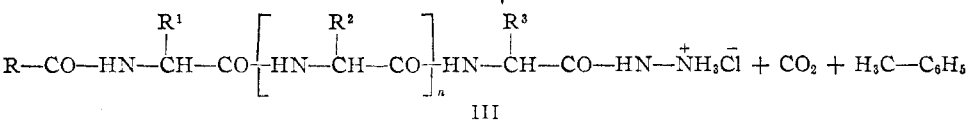
I

CBZO = —OC—O—$CH_2C_6H_5$

These compounds by way of their free amino group may be readily combined with other acylated peptide structures to form polypeptide carbobenzoxyhydrazides of the general formula (II)



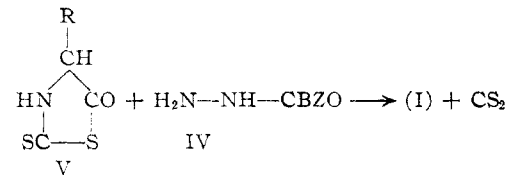
II



III

Hydrogenolysis uncovers the hydrazide function thus producing an acylated polypeptide hydrazide (III), which may be attached to other peptide derivatives through its azide. This novel method for the synthesis of hydrazides allows the introduction of a potential hydrazide group into a peptide moiety at the monoamino acid stage, thus avoiding the exposure of complex sensitive peptides to hydrazine. The present communication describes procedures for the preparation of α-amino acid carbobenzoxyhydrazides and illustrates some uses of these compounds. The key reagent for this purpose was carbobenzoxyhydrazine (IV)[4] which has not been used hitherto in the preparation of hydrazides.

The reaction of 2-thio-5-thiazolidones (V) with amines or amino acid esters leads, under suitable conditions, to the formation of amino acid amides or dipeptide esters.[5] This scheme seemed applicable to the synthesis of α-amino acid carbobenzoxyhydrazides. Heating of an equimolar mixture of 2-thio-5-thiazolidone[6] (V, R = H) and carbobenzoxyhydrazine in glacial acetic acid led to the evolution of carbon disulfide. From the reaction mixture it was possible to isolate glycine carbobenzoxyhydrazide (I, R = H) in the form of its well-crystallized hydrochloride. The elementary analysis and the presence of a free primary amino group were in accord with the expected structure.



IV

V

The amides of DL-alanine and of DL-phenylalanine were then converted into the corresponding 2-thio-

(1) For paper No. II see K. Hofmann, M. Z. Magee and A. Lindenmann, THIS JOURNAL, **72**, 2814 (1950).

(2) This investigation was supported by grants from the U. S. Public Health Service, The Rockefeller Foundation in New York and Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

(3) Postdoctorate Research Fellow from the University of Basel, Switzerland.

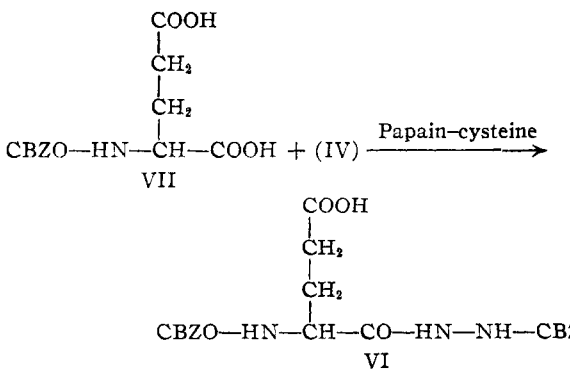
(4) N. Rabjohn, THIS JOURNAL, **70**, 1181 (1948).

(5) I. Heilbron, *J. Chem. Soc.*, 2099 (1949).

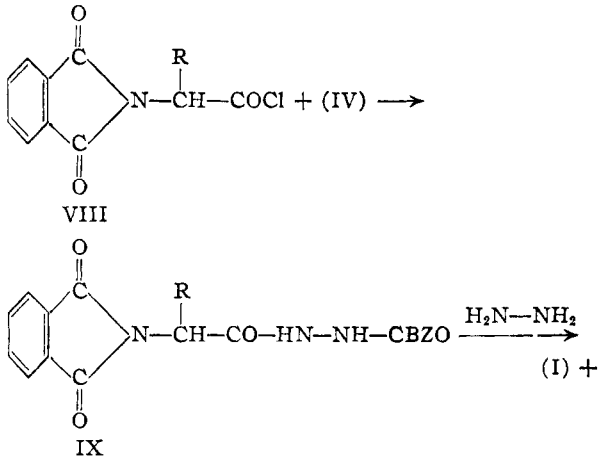
(6) A. H. Cook, I. Heilbron and A. L. Levy, *ibid.*, 201 (1948).

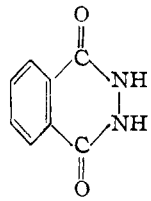
5-thiazolidones[6,7] and the latter, upon reaction with carbobenzoxyhydrazine in glacial acetic acid, afforded the carbobenzoxyhydrazides of DL-alanine and DL-phenylalanine, respectively. The success of these experiments prompted an extension of the procedure to a number of L-α-amino acids. Optically active 4-substituted-2-thio-5-thiazolidones have not as yet been prepared. In order to test the possibility of obtaining such compounds the amides of L-leucine and L-tyrosine were converted into the respective thiazolidones by treatment with an excess of carbon disulfide in the presence of potassium carbonate. In a similar manner L-isoasparagine was converted into 2-thio-4-(carboxymethyl)-5-thiazolidone; in this instance potassium hydroxide was employed since the desired thiazolidone was obtained in extremely poor yield by the use of potassium carbonate. The three thiazolidones exhibited no measurable optical rotation under our experimental conditions. This finding may have two explanations; either the conversion of optically active amino acid amides into their 2-thio-5-thiazolidones is accompanied by racemization or the optically active 2-thio-5-thiazolidones exhibit extremely small optical rotations. The former possibility seems the more likely one since the reaction of 2-thio-4-isobutyl-5-thiazolidone with carbobenzoxyhydrazine afforded the optically inactive carbobenzoxyhydrazide of DL-leucine. Attempts to transform the other two thiazolidones into the carbobenzoxyhydrazides of aspartic acid and tyrosine led to the formation of polymeric materials. The realization that the conversion of α-amino acids into their carbobenzoxyhydrazides by way of the 2-thiazolidones proceeds in low yields and is accompanied by racemization prompted a search for other methods for the preparation of α-amino acid carbobenzoxyhydrazides.

The classical demonstration by Bergmann[8] that the enzyme papain catalyzes the formation of phenylhydrazides from acylated L-α-amino acids provided the basis for such a method. The synthesis of carbobenzoxy-L-glutamic acid-α-carbobenzoxyhydrazide (VI) has been accomplished in this manner. The compound precipitated in good yield when carbobenzoxy-L-glutamic acid (VII) was incubated with carbobenzoxyhydrazine in the presence of papain–cysteine at *p*H 5. This application of carbobenzoxyhydrazine opens the way to the enzymatic synthesis of acylated L-amino acid hydrazides. The direct reaction of carbobenzoxy-L-amino acids with hydrazine in the presence of papain leads to the formation of *sym*-bis-(carbobenzoxy-L-amino acid)-hydrazines[9] and not to hydrazides.



The coupling of phthalylamino acid chlorides (VIII) with carbobenzoxyhydrazine (IV) led in high yields to the formation of phthalylamino acid carbobenzoxyhydrazides of the general structure (IX). The carbobenzoxyhydrazides of phthalylglycine, phthalyl-DL-alanine and phthalyl-DL-phenylalanine were prepared in this manner. These substances represent convenient starting materials for the synthesis of α-amino acid carbobenzoxyhydrazides, since the phthalyl blocking-group is readily removed upon exposure to hydrazine at room temperature.[10] A number of racemic α-amino acid carbobenzoxyhydrazides were prepared and isolated as the hydrochlorides. The presence of a free amino group in these compounds was demonstrated by Van Slyke amino-nitrogen determinations. This method will be

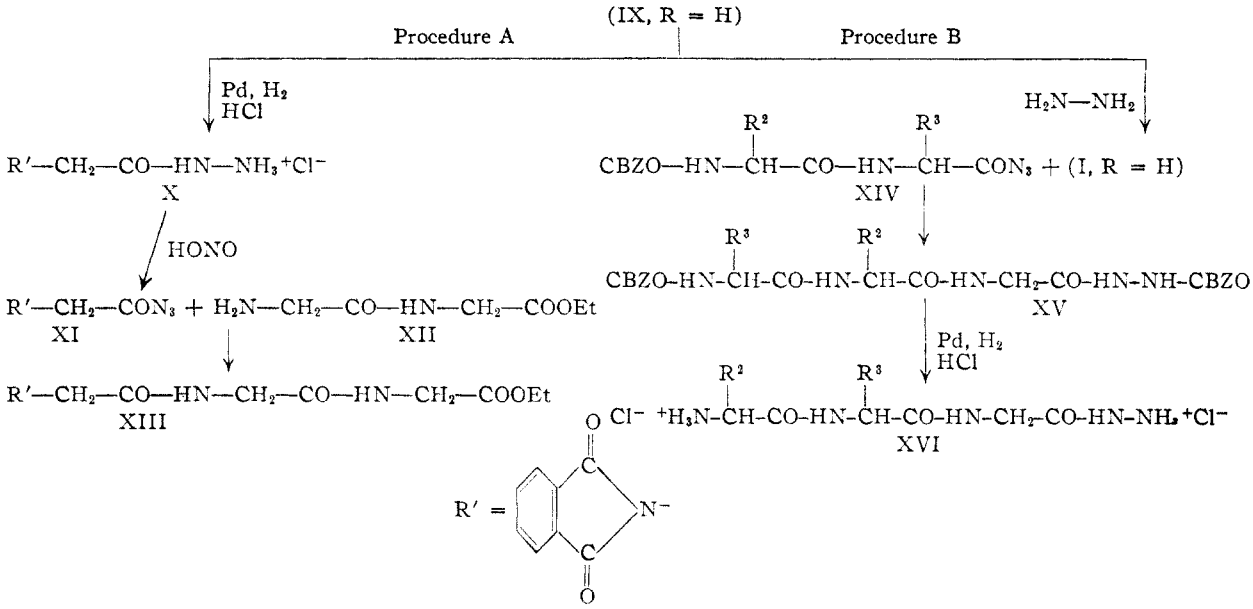




applied to the preparation of optically active α-amino acid carbobenzoxyhydrazides, as it has been demonstrated[10] that under carefully controlled conditions it is possible to remove a blocking phthalyl group from optically active phthalylamino acids without racemization.

In order to demonstrate their value for the preparation of hydrazides, the carbobenzoxyhydrazides of benzoyltriglycine, tosylglycine and phthalylglycine were subjected to hydrogenation in the presence of an equimolar quantity of hydrogen chloride. In all instances the respective hydrazide hydrochlorides were obtained in high yields. The necessary carbobenzoxyhydrazides were prepared by the reaction of glycine carbobenz-

(7) J. D. Billimoria and A. H. Cook, *J. Chem Soc.*, 2323 (1949).
(8) M. Bergmann and H. Fränkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).
(9) F. W. Holly, J. J. Cahill, Jr., and K. Folkers, THIS JOURNAL, **73**, 2944 (1951).
(10) F. A. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).



oxyhydrazide with benzoyldiglycine azide, or *p*-toluenesulfonyl chloride, respectively. The synthesis of phthalyl-glycine carbobenzoxyhydrazide from phthalylglycyl chloride and carbobenzoxyhydrazine has already been mentioned. Phthalylamino acid and phthalylpeptide hydrazides have previously been inaccessible since the reaction of phthalylamino acid esters and phthalylpeptide esters with hydrazine results in the removal of the phthalyl group rather than in the conversion of the ester group into a hydrazide function.[11] In combination with the present hydrazide synthesis, however, the phthalyl procedure is readily adaptable for this purpose. Phthalylglycine carbobenzoxyhydrazide (IX, R = H) may serve to illustrate the transformations which have been accomplished. Depending upon the desired peptide derivative this unit may be incorporated into more complex peptides from the carboxyl side (procedure A) or from the amino end (procedure B). Catalytic hydrogenation of (IX, R = H) in the presence of hydrogen chloride according to procedure A afforded phthalylglycine hydrazide hydrochloride (X) and this material through its azide (XI) was coupled with diglycine ethyl ester (XII) to give phthalyltriglycine ethyl ester (XIII). On the other hand, as has been mentioned above, the treatment of compound (IX, R = H) with hydrazine hydrate in methanol at room temperature (procedure B) removed the phthalyl group with the formation of glycine carbobenzoxyhydrazide (I, R = H). Mention has been made of the reaction between glycine carbobenzoxyhydrazide and benzoyldiglycine azide and *p*-toluenesulfonyl chloride. In addition to these examples glycine carbobenzoxyhydraide has been coupled with a number of carbobenzoxydipeptide azides (XIV) to give a series of carbobenzoxytripeptide carbobenzoxyhydrazides of the general structure (XV). By this method were prepared the carbobenzoxyhydrazides of carbobenzoxytriglycine, carbobenzoxyglycyl-L-leucylglycine, carbobenzoxyglycyl-L-tyrosylglycine, carbobenzoxy-α-L-glutamyl-glycylglycine, carbobenzoxyglycycl-DL-phenylalanylglycine and carbobenzoxy-α-L-glutamyl-L-tyrosylglycine. Also prepared was tosylglycyl-L-tyrosylglycine carbobenzoxyhydrazide. Obviously, the carbobenzoxyhydrazides of other amino acids may serve in a similar manner. For example, the coupling of carbobenzoxydiglycine azide with the carbobenzoxyhydrazide of DL-leucine afforded carbobenzoxydiglycycl-DL-leucine carbobenzoxyhydrazide.

Hydrogenation of carbobenzoxytriglycine carbobenzoxyhydrazide (XV, R^2 and R^3 = H) in the presence of two equivalents of hydrochloric acid afforded triglycine hydrazide dihydrochloride (XVI, R^2 and R^3 = H) which was also obtained from the hydrogenation of carbobenzoxytriglycine hydrazide.

Amino acid and peptide hydrazides which are readily available from their dicarbobenzoxy derivatives by catalytic hydrogenation may represent valuable intermediates for the preparation of complex acylated polypeptide hydrazides. They embody within their structure two basic groups, namely, an amino and a hydrazide group which may be expected to exhibit different dissociation constants. It should be possible to convert them into acylated polypeptide hydrazides by coupling with acylated peptide azides at *p*H values at which the amino end is uncharged while the more basic hydrazide group is still ionized. Experiments to test this possibility are under way.

Experimental[12]

Carbobenzoxyhydrazine Hydrochloride.[13]—A mixture of dibenzyl carbonate[14] (30 g.) and hydrazine hydrate (9.6 ml.) was heated at 100–110° for four hours with stirring. The resulting clear solution was cooled in an ice-bath, acidified

(11) R. Radenhausen, *J. prakt. Chem.*, **52**, 446 (1895). These findings have been confirmed in our laboratory.

(12) The melting points were determined with short-stem Anschütz thermometers and are uncorrected. The microanalyses were determined in our microanalytical laboratory by Mr. George L. Stragand. The Van Slyke determinations were carried out by Miss Anna Bridgwater.

(13) In our experience the method here described is superior to the one given by Rabjohn.[4]

(14) C. A. Bischoff, *Ber.*, **36**, 159 (1903).

to congo red with concentrated hydrochloric acid, and the ensuing mass triturated with ether (200 ml.) and filtered. The resulting mixture of carbobenzoxyhydrazine hydrochloride and hydrazine hydrochloride was washed with ether and dried *in vacuo* at room temperature; yield 17.5 g.

The dry hydrochlorides were then suspended in absolute ethanol (300 ml.) and the suspension heated on a steam-bath, filtered, and the clear filtrate cooled at −20° for 12 hours. The hydrazine dihydrochloride which had crystallized was removed by filtration and the filtrate concentrated to a volume of 100–150 ml., when the hydrochloride of carbobenzoxyhydrazine began to crystallize. The mixture was warmed to give a clear solution which was kept at room temperature for 12 hours and the crystals collected and washed with ice-cold absolute ethanol and dried; yield 12.5 g.; m.p. 167–167.5°. Concentration of the mother liquors afforded an additional amount of crystals (3.3 g.); m.p. 166–168°. The two crops were combined and recrystallized from absolute ethanol; yield 11.5 g. (46%); m.p. 170–170.5°.

Anal. Calcd. for $C_8H_{11}O_2N_2Cl$: C, 47.4; H, 5.5; N, 13.8; Cl, 17.5. Found: C, 47.5; H, 5.2; N, 14.2; Cl, 17.3.

Carbobenzoxyhydrazine.—To an ice-cold suspension of carbobenzoxyhydrazine hydrochloride (10 g.) in chloroform (75 ml.) was added diethylamine (5.2 ml.) to give a clear solution. Ether (200 ml.) was added and the resulting precipitate of diethylamine hydrochloride removed by filtration. The filtrate was concentrated to dryness *in vacuo* and the residue dissolved in boiling ether (60 ml.). Traces of insoluble materials were removed by filtration and the solution concentrated to a small volume and placed in a refrigerator. The resulting crystals were collected and dried; yield 7.9 g. (96%); m.p. 67–69°; literature melting point[4] 69–70°. A sample was recrystallized from ether.

Anal. Calcd. for $C_8H_{10}O_2N_2$: C, 57.8; H, 6.1; N, 16.9. Found: C, 58.0; H, 6.1; N, 17.1.

DL-2-Thio-4-methyl-5-thiazolidone.—To a solution of potassium carbonate (16.2 g.) in water (65 ml.) was added carbon disulfide (7.15 ml.) and DL-alanine amide (9.0 g.)[15] and the mixture shaken at room temperature for nine hours. The resulting yellow suspension was extracted with three 40-ml. portions of ether, the aqueous phase cooled at −2°, and acidified to congo red with concentrated hydrochloric acid. The mixture was placed in a refrigerator for 30 minutes, and the yellow crystals collected and repeatedly washed with ice-water and dried *in vacuo* at room temperature. The compound was recrystallized from aqueous methanol; yield 6.2 g. (41%); m.p. 124–126°. This compound was previously prepared by a different procedure[16] and is reported as melting at 127–128°.

Anal. Calcd. for $C_4H_5ONS_2$: C, 32.6; H, 3.4; N, 9.5; S, 43.6. Found: C, 32.7; H, 3.2; N, 9.5; S, 43.3.

DL-2-Thio-4-isobutyl-5-thiazolidone.[17]—To a solution of potassium carbonate (17.8 g.) in water (95 ml.) was added carbon disulfide (8.2 ml.) and L-leucine amide[15] (16 g.) and the mixture shaken at room temperature for nine hours. The product was isolated in the manner described above and recrystallized from aqueous methanol; yield 10.3 g. (44%); m.p. 96–97°; $\alpha^{26}D$ 0 ± 2° (*c* 1, in methanol).

Anal. Calcd. for $C_7H_{11}ONS_2$: C, 44.4; H, 5.9; N, 7.4; S, 33.9. Found: C, 44.5; H, 5.8; N, 7.2; S, 33.2.

DL-2-Thio-4-(*p*-hydroxybenzyl)-5-thiazolidone.—This compound was prepared from L-tyrosine amide (3.7 g.),[15] potassium carbonate (2.9 g.), carbon disulfide (1.3 ml.) and water (50 ml.) in the manner described above, and was recrystallized from aqueous ethanol; yield 2.5 g. (51%); m.p. 166–169°; $\alpha^{27}D$ 0 ± 1° (*c* 10, in ethanol). This compound was previously prepared by another method and is reported[7] as melting at 163–164°.

Anal. Calcd. for $C_{10}H_9O_2NS_2$: C, 50.2; H, 3.8; N, 5.9; S, 26.8. Found: C, 50.3; H, 3.8; N, 5.7; S, 27.1.

DL-2-Thio-4-(carboxymethyl)-5-thiazolidone.—A solution of L-isoasparagine (5 g.),[18] $\alpha^{26}D$ +8°, in 1 *N* potassium hydroxide (66.6 ml.) was shaken at room temperature for eight hours with carbon disulfide (2 ml.) and the mixture extracted with three 40-ml. portions of ether. The aqueous phase was cooled at −2°, acidified to congo red with concentrated hydrochloric acid and extracted with four 130-ml. portions of ether. The combined ether extracts were washed with saturated sodium chloride solution (60 ml.), dried over sodium sulfate and the ether evaporated; the residue was recrystallized from ether; yield 2.6 g. (36%); m.p. 161–163°; $\alpha^{27}D$ 0 ± 1° (*c* 5, in ethanol).

Anal. Calcd. for $C_5H_5O_3NS_2$: C, 31.4; H, 2.6; N, 7.3; S, 33.5. Found: C, 31.3; H, 2.4; N, 7.5; S, 33.2.

Phthalylglycine Carbobenzoxyhydrazide.—To an ice-cold solution of carbobenzoxyhydrazine (7.6 g.) in ethyl acetate (110 ml.) was added a solution of phthalylglycyl chloride (5.13 g.)[19] in ethyl acetate (40 ml.) and the mixture was kept at room temperature for six hours. The solid precipitate was collected, dried *in vacuo*, ground with water in a mortar, refiltered, and dried. The ethyl acetate filtrates were evaporated to dryness *in vacuo*, the residue combined with the precipitate described above, and the material recrystallized from absolute ethanol; yield 7.2 g. (89%); m.p. 194–195°.

Anal. Calcd. for $C_{18}H_{15}O_5N_3$: C, 61.2; H, 4.3; N, 11.9. Found: C, 61.3; H, 4.5; N, 12.0.

Phthalyl-DL-phenylalanine Carbobenzoxyhydrazide.—To an ice-cold solution of phthalyl-DL-phenylalanyl chloride (2.4 g.)[19] in ether (100 ml.) was added a solution of carbobenzoxyhydrazine (2.5 g.) in ether (100 ml.) and the mixture kept at room temperature for 24 hours. The product was isolated in the manner described above and was recrystallized from a mixture of isoamyl alcohol and petroleum ether; yield 3.2 g. (95%); m.p. 155–157°.

Anal. Calcd. for $C_{25}H_{21}O_5N_3$: C, 67.7; H, 4.8; N, 9.5. Found: C, 67.7; H, 4.9; N, 9.6.

Glycine Carbobenzoxyhydrazide Hydrochloride.—a. From 2-Thio-5-thiazolidone.—A solution of 2-thio-5-thiazolidone (10 g.)[6] and carbobenzoxyhydrazine (12.4 g.) in glacial acetic acid (220 ml.) was heated at 100–120° for 45 minutes. The glacial acetic acid was then removed *in vacuo* and the residue evaporated with three 50-ml. portions of water. The material was then dissolved in 50% aqueous ethanol (50 ml.) and the solution passed through a column containing sodium hydroxide-activated Amberlite IRA-400 (140 g.). The column was washed with 50% aqueous ethanol until the eluates were neutral to litmus paper. The combined eluates were then evaporated to a small volume *in vacuo* and acidified to congo red with ethanolic hydrogen chloride. The mixture was kept at room temperature for 20 hours and the resulting crystals collected. The mother liquors were concentrated and an additional amount of the hydrochloride precipitated by the addition of ether. The combined crops of the hydrochloride were recrystallized from ethanol; yield 10.5 g. (54%); m.p. 176–178°.

Anal. Calcd. for $C_{10}H_{14}O_3N_3Cl$: C, 46.2; H, 5.4; N, 16.2; Cl, 13.7; NH_2-N, 5.4. Found: C, 46.5; H, 5.3; N, 15.8; Cl, 13.7; NH_2-N, 5.3.

b. From Phthalylglycine Carbobenzoxyhydrazide.—To a warm suspension of phthalylglycine carbobenzoxyhydrazide (900 mg.) in methanol (30 ml.) was added hydrazine hydrate (1.5 ml.) and the resulting clear solution kept at room temperature for 24 hours, when the methanol was removed *in vacuo*. Water (30 ml.) and 2 *N* hydrochloric acid (2 ml.) were added to the residue and the precipitate of 1,2,3,4-tetrahydrophthalazine-1,4-dione removed by filtration; yield 405 mg. (98%); m.p. 330° with decomposition. The filtrate was evaporated to dryness *in vacuo* and the resulting crystalline mass dried. Absolute ethanol (20 ml.) was added to the residue, the mixture was warmed, filtered and the clear filtrate concentrated to a small volume. The mixture was kept at room temperature for 20 hours and the resulting hydrochloride twice recrystallized from absolute ethanol; yield 360 mg. (55%); m.p. 177–178°. No depression of the melting point was observed when this material was admixed with the substance prepared according to a above.

Anal. Calcd. for $C_{10}H_{14}O_3N_3Cl$: NH_2-N, 5.4. Found: NH_2-N, 6.0.

DL-Alanine Carbobenzoxyhydrazide Hydrochloride. a. From DL-2-Thio-4-methyl-5-thiazolidone.—A solution of

(15) P. S. Yang and M. M. Rising, THIS JOURNAL, **53**, 3183 (1931).

(16) A. H. Cook and A. L. Levy, *J. Chem. Soc.*, 642 (1950).

(17) In our preliminary communication[1] this compound was erroneously designated as the L-form.

(18) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(19) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

carbobenzoxyhydrazine (1.9 g.) in glacial acetic acid (15 ml.) was added to a solution of DL-2-thio-4-methyl-5-thiazolidone (1.7 g.) in glacial acetic acid (30 ml.) and the mixture heated at 110–125° for 30 minutes. The glacial acetic acid was removed *in vacuo* and the residue evaporated with three 50-ml. portions of water. The resulting oily material was dissolved in 50 per cent. aqueous ethanol (40 ml.) and the solution passed through a column containing sodium hydroxide-activated Amberlite IRA-400 (35 g.). The column was washed with 50 per cent. aqueous ethanol until the eluates were neutral to litmus paper, and the combined eluates evaporated to dryness *in vacuo*. The residue was dissolved in ethanol (20 ml.) and the solution acidified by the addition of ethanolic hydrogen chloride (2.8 ml.) (1 ml. contained 0.147 g. of hydrogen chloride). The resulting hydrochloride was collected and recrystallized from a mixture of ethanol and ether; yield 1.1 g. (43%); m.p. 192–193°.

Anal. Calcd. for $C_{11}H_{16}O_3N_3Cl$: C, 48.3; H, 5.9; N, 15.3; Cl, 13.0; NH_2-N, 5.1. Found: C, 48.5; H, 6.0; N, 15.6; Cl, 13.1; NH_2-N, 5.5.

b. From Phthalyl-DL-alanine Carbobenzoxyhydrazide.—To an ice-cold solution of carbobenzoxyhydrazine (1.4 g.) in ether (25 ml.) was added a solution of phthalyl-DL-alanyl chloride (1 g.)[20] in ether (20 ml.) and the mixture kept at room temperature for 20 hours. The suspension was washed with water, dried over sodium sulfate and the ether removed. The resulting oily product was used for the next step without further purification; yield 1.3 g. (84%).

To a solution of the above phthalyl-DL-alanine carbobenzoxyhydrazide (1.3 g.) in methanol (20 ml.) was added hydrazine hydrate (0.17 ml.) and the mixture kept at room temperature for 20 hours. The methanol was removed *in vacuo*, the residue triturated with water (30 ml.) and 2 *N* hydrochloric acid (2 ml.) and the insoluble 1,2,3,4-tetrahydrophthalazine-1,4-dione removed by filtration. The clear filtrate was evaporated to drynesss and the residue crystallized from a mixture of ethanol and ether; yield 0.9 g. (92%); m.p. 187–189°. No depression of the melting point was observed when this material was admixed with the compound prepared according to a above.

Anal. Calcd. for $C_{11}H_{16}O_3N_3Cl$: NH_2-N, 5.1. Found: NH_2-H, 5.1.

DL-Leucine Carbobenzoxyhydrazide Hydrochloride.[17]—A solution of DL-2-thio-4-isobutyl-5-thiazolidone (4.66 g.) and carbobenzoxyhydrazine (4.1 g.) in glacial acetic acid (85 ml.) was heated at 115–120° for 20 minutes. The glacial acetic acid was removed *in vacuo* and the residue evaporated with three 30-ml. portions of water. The product was then dissolved in 50% aqueous ethanol (50 ml.) and the solution passed through a column containing sodium hydroxide-activated Amberlite IRA-400 (110 g.). The column was eluted with 50% aqueous ethanol until the eluates were neutral to litmus paper. The combined eluates were acidified to congo red with ethanolic hydrogen chloride and evaporated to dryness *in vacuo*. The residue was dissolved in a small quantity of ethyl acetate, and petroleum ether added until the solution became turbid; the mixture was placed in a refrigerator for 56 hours. The resulting crystals were collected and recrystallized from a mixture of ethanol and ether; yield 3.2 g. (41%); m.p. 171–174°; $\alpha^{27}D$ 0 ± 1° (*c* 10, in water).

Anal. Calcd. for $C_{14}H_{22}O_3N_3Cl$: C, 53.2; H, 7.0; N, 13.3; Cl, 11.2; NH_2-N, 4.4. Found: C, 53.0; H, 6.9; N, 13.4; Cl, 10.9; NH_2-N, 4.5.

DL-Phenylalanine Carbobenzoxyhydrazide Hydrochloride. a. From DL-2-Thio-4-benzyl-5-thiazolidone.—A solution of DL-2-thio-4-benzyl-5-thiazolidone (920 mg.)[7] and carbobenzoxyhydrazine (680 mg.) in glacial acetic acid (40 ml.) was heated at 110–125° for 40 minutes and then evaporated to dryness *in vacuo*. The crystalline residue was evaporated with one 25-ml. portion of water, dissolved in warm ethanol (40 ml.) and acidified to congo red with ethanolic hydrogen chloride. The mixture was filtered and the clear filtrate concentrated to a small volume *in vacuo*. The resulting crystals were collected and recrystallized from ethanol; yield 600 mg. (41%); m.p. 196–198°.

Anal. Calcd. for $C_{17}H_{20}O_3N_3Cl$: C, 58.4; H, 5.7; N, 12.0; Cl, 10.1; NH_2-N, 4.0. Found: C, 58.1; H, 5.8; N, 12.0; Cl, 9.9; NH_2-N, 4.4.

(20) S. Gabriel, *Ber.*, **41**, 242 (1908).

b. From Phthalyl-DL-phenylalanine Carbobenzoxyhydrazide.—To a solution of phthalyl-DL-phenylalanine carbobenzoxyhydrazide (1 g.) in methanol (20 ml.) was added hydrazine hydrate (0.11 ml.) and the mixture kept at room temperature for 20 hours. The methanol was removed *in vacuo*, and water (30 ml.) and 2 *N* hydrochloric acid (2 ml.) added to the residue. The resulting suspension was filtered, and the clear filtrate concentrated to a small volume when crystallization occurred. The crystals were collected and recrystallized from ethanol; yield 0.52 g. (66%); m.p. 191–193°. No depression of the melting point was observed when this material was admixed with the substance prepared according to a above.

Anal. Calcd. for $C_{17}H_{20}O_3N_3Cl$: NH_2-N, 4.0. Found: NH_2-N, 4.1.

Carbobenzoxy-L-glutamic Acid α-Carbobenzoxyhydrazide.[21]—To a solution of carbobenzoxy-L-glutamic acid[18] (4 g.) in 0.85 *N* sodium hydroxide (16.8 ml.) was added a solution of carbobenzoxyhydrazine (2.37 g.) in 0.1 *M* citrate buffer of *p*H 5 (80 ml.) and water (20 ml.). Solutions of cysteine hydrochloride (0.14 g.) in water (5 ml.) and of papain (1.5 g.) in water (10 ml.) were added and the mixture incubated at 37° for 24 hours. After this time the contents of the flask had completely solidified. The mixture was filtered, the solid dried and crystallized from ethyl acetate; yield 3 g. (49%); m.p. 131–132°; $\alpha^{26}D$ −26 ± 2° (*c* 1, in ethanol).

Anal. Calcd. for $C_{21}H_{23}O_7N_3$: C, 58.7; H, 5.4; N, 9.8. Found: C, 58.5; H, 5.3; N, 9.7.

Phthalylglycine Hydrazide Hydrochloride.—A suspension of phthalylglycine carbobenzoxyhydrazide (3.5 g.) in methanol (180 ml.) and ethanolic hydrogen chloride (2.5 ml.) (1 ml. contained 0.147 g. of hydrogen chloride) was shaken with hydrogen in the presence of spongy palladium until the evolution of carbon dioxide ceased. The mixture was warmed, filtered, evaporated to dryness *in vacuo* and the residue recrystallized from a mixture of methanol and ether; yield 2.2 g. (87%); m.p. 300° with decomposition.

Anal. Calcd. for $C_{10}H_{10}O_3N_3Cl$: N, 16.4. Found: N, 16.6.

Phthalyltriglycine Ethyl Ester.—Phthalylglycine hydrazide hydrochloride (2 g.) was dissolved in water (80 ml.) and 2 *N* hydrochloric acid (4 ml.), and the solution cooled at −2°. An ice-cold solution of sodium nitrite (0.55 g.) in water (4 ml.) was added with stirring, and the mixture kept at 0° for five minutes. The azide was collected, washed with ice-water and dried *in vacuo* at 5° for 24 hours; yield 1.69 g. The azide was dissolved in ice-cold ethyl acetate (70 ml.) and the solution added to an ethanol solution (35 ml.) of diglycine ethyl ester (prepared from 1.55 g. of the hydrochloride). The mixture was kept at room temperature for 18 hours and the crystalline reaction product collected, ground with 1 *N* hydrochloric acid (50 ml.), washed with water and dried; yield 1.35 g. (50%); m.p. 228–230°.

Anal. Calcd. for $C_{16}H_{17}O_6N_3$: C, 55.4; H, 4.9; N, 12.1. Found: C, 55.6; H, 5.0; N, 12.1.

Benzoyltriglycine Carbobenzoxyhydrazide.—An ice-cold ethyl acetate solution (320 ml.) of benzoyldiglycine azide (prepared from 3.66 g. of the hydrazide[22]) was added to a methanol solution (40 ml.) of glycine carbobenzoxyhydrazide (prepared from 3.53 g. of the hydrochloride with sodium ethoxide) and the mixture kept at room temperature for 24 hours. The resulting precipitate was collected and recrystallized from water; yield 3.2 g. (46%); m.p. 223–225° with decomposition.

Anal. Calcd. for $C_{21}H_{23}O_6N_5$: C, 57.2; H, 5.3; N, 15.9. Found: C, 57.1; H, 5.1; N, 15.6.

Benzoyltriglycine Hydrazide Hydrochloride.—A suspension of benzoyltriglycine carbobenzoxyhydrazide (250 mg.) in methanol (15 ml.), water (5 ml.) and 0.77 *N* hydrochloric acid (0.74 ml.) was hydrogenated in the presence of spongy palladium in the usual manner until the evolution of carbon dioxide ceased. The mixture was heated, filtered, the filtrate evaporated to dryness *in vacuo* and the residue recrystallized from a mixture of ethanol and ether; yield 180 mg. (93%); m.p. 235–242° with decomposition.

Anal. Calcd. for $C_{13}H_{18}O_4N_5Cl$: C, 45.4; H, 5.3; N,

(21) This experiment was performed by Dr. I. Gordon.

(22) T. Curtius, *J. prakt. Chem.*, **70**, **78** (1904).

20.4; Cl, 10.3. Found: C, 45.7; H, 5.1; N, 20.4; Cl, 10.0.

Tosylglycine Carbobenzoxyhydrazide.—To a solution of glycine carbobenzoxyhydrazide (prepared from 500 mg. of the hydrochloride) in dry pyridine (15 ml.) was added *p*-toluenesulfonyl chloride (383 mg.) and the mixture kept at room temperature for 24 hours. The pyridine was removed *in vacuo* and the residue triturated with water (20 ml.), when crystallization occurred. The crystals were collected and recrystallized from aqueous ethanol; yield 420 mg. (58%); m.p. 132–134°.

Anal. Calcd. for $C_{17}H_{19}O_5N_3S$: C, 54.1; H, 5.1; N, 11.1; S, 8.5. Found: C, 54.2; H, 4.9; N, 10.9; S, 8.7.

Tosylglycine Hydrazide Hydrochloride.—Tosylglycine carbobenzoxyhydrazide (250 mg.) was dissolved in a mixture of water (25 ml.) and ethanolic hydrogen chloride (25 ml.) (1 ml. contained 0.147 g. of hydrogen chloride) and hydrogenated in the presence of spongy palladium until the evolution of carbon dioxide ceased. The catalyst was removed by filtration and the clear filtrate evaporated to dryness *in vacuo*. The compound was recrystallized from a mixture of ethanol and ether; yield 180 mg. (97%); m.p. 174–177°.

Anal. Calcd. for $C_9H_{14}O_3N_3SCl$: N, 15.0. Found: N, 15.2.

Carbobenzoxytriglycine Methyl Ester.—To a methanol solution of diglycine methyl ester (prepared from 6.3 g. of the hydrochloride) was added an ice-cold ethyl acetate solution of carbobenzoxyglycine azide (7 g.) and the mixture allowed to stand at room temperature for 20 hours. The solution was evaporated to a small volume *in vacuo*, water (100 ml.) was added and the carbobenzoxytriglycine methyl ester collected and recrystallized from aqueous methanol; yield 6.8 g. (58%); m.p. 151–155°.

Anal. Calcd. for $C_{16}H_{19}O_6N_3$: N, 12.5. Found: N, 12.7.

Carbobenzoxytriglycine Hydrazide.—Carbobenzoxytriglycine methyl ester (5 g.) was dissolved in dry methanol (200 ml.); hydrazine hydrate (1.0 ml.) was added and the mixture allowed to stand at room temperature for 20 hours. The resulting precipitate was collected and recrystallized from water; yield 3.5 g. (70%); m.p. 225° with decomposition.

Anal. Calcd. for $C_{14}H_{19}O_5N_5$: N, 20.8. Found: N, 20.9.

Carbobenzoxytriglycine Carbobenzoxyhydrazide.—An ice-cold solution of carbobenzoxydiglycine azide (prepared from 0.7 g. of the hydrazide) was added to an ethanol solution of carbobenzoxyhydrazine (prepared from 0.8 g. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The resulting copious precipitate was collected, ground with water in a mortar, dried and recrystallized from ethanol; yield 0.9 g. (92%); m.p. 212–215°.

Anal. Calcd. for $C_{22}H_{25}O_7N_5$: C, 56.1; H, 5.3; N, 14.9. Found: C, 56.1; H, 5.2; N, 14.6.

For the purpose of identification carbobenzoxytriglycine carbobenzoxyhydrazide was prepared also by carbobenzoxylation of triglycine hydrazide dihydrochloride. The resulting compound melted at 213–215° and did not depress the melting point of a sample prepared according to the method described above.

Anal. Calcd. for $C_{22}H_{25}O_7N_5$: C, 56.1; H, 5.3; N, 14.9. Found: C, 55.9; H, 5.0; N, 14.8.

Carbobenzoxyglycyl-L-leucine Hydrazide.—L-leucine methyl ester (prepared from 6.4 g. of the hydrochloride) was coupled with carbobenzoxyglycyl chloride (4 g.)[18] and the resulting oily ester dissolved in methanol (10 ml.). The solution was heated, hydrazine hydrate (1.1 ml.) added and the mixture kept at room temperature for 48 hours. The methanol was removed *in vacuo* and the oily residue triturated with ether, when crystallization occurred. The crystals were collected and recrystallized from a mixture of methanol and ether; yield 3.8 g. (32%); m.p. 130–131°.

Anal. Calcd. for $C_{16}H_{24}O_4N_4$: N, 16.7. Found: N, 17.3.

Carbobenzoxyglycyl-L-leucylglycine Carbobenzoxyhydrazide.—An ice-cold ethyl acetate solution of carbobenzoxyglycyl-L-leucine azide (prepared from 7.3 g. of the hydrazide) was added to a methanol solution (250 ml.) of glycine carbobenzoxyhydrazide (prepared from 5.6 g. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The solvent was evaporated to dryness *in vacuo* and the residue dissolved in ethyl acetate. The ethyl acetate solution was washed with 1 *N* hydrochloric acid, water and 10% sodium bicarbonate and dried over sodium sulfate. Evaporation of the ethyl acetate gave crystals which were recrystallized from a mixture of ethanol and ether; yield 5.7 g. (49%); m.p. 142–144°; $\alpha^{26}D$ −18 ± 2° (*c* 1, in methanol).

Anal. Calcd. for $C_{26}H_{33}O_7N_5$: C, 59.2; H, 6.3; N, 13.3. Found: C, 59.1; H, 6.4; N, 13.1.

Carbobenzoxyglycyl-DL-phenylalanylglycine Carbobenzoxyhydrazide.—Coupling, in the usual manner, of carbobenzoxyglycyl-DL-phenylalanine azide (prepared from 5 g. of the hydrazide) with glycine carbobenzoxyhydrazide (prepared from 3.5 g. of the hydrochloride) gave carbobenzoxyglycyl-DL-phenylalanylglycine carbobenzoxyhydrazide which was recrystallized from a mixture of ethyl acetate and ethanol; yield 4.6 g. (60%); m.p. 162–163°.

Anal. Calcd. for $C_{29}H_{31}O_7N_5$: C, 62.0; H, 5.6; N, 12.5. Found: C, 62.4; H, 5.4; N, 12.6.

Carbobenzoxyglycyl-L-tyrosine Hydrazide.—Carbobenzoxyglycyl chloride (12 g.)[18] was coupled with L-tyrosine methyl ester (prepared from 36 g. of the hydrochloride) and the resulting oily ester converted into the hydrazide; yield 6.2 g. (30%); m.p. 205–206°.

Anal. Calcd. for $C_{19}H_{22}O_5N_4$: N, 14.5. Found: N, 14.2.

Carbobenzoxyglycyl-L-tyrosylglycine Carbobenzoxyhydrazide.—Carbobenzoxyglycyl-L-tyrosine azide (prepared from 5 g. of the hydrazide) was coupled in the usual manner with glycine carbobenzoxyhydrazide (prepared from 3.4 g. of the hydrochloride) and the product recrystallized from a mixture of methanol and ether; yield 4.0 g. (53%); m.p. 175–177°; $\alpha^{26}D$ −3 ± 2° (*c* 1, in methanol).

Anal. Calcd. for $C_{29}H_{31}O_8N_5$: C, 60.3; H, 5.4; N, 12.1. Found: C, 60.4; H, 5.1; N, 11.8.

Carbobenzoxy-α-L-glutamylglycylglycine Carbobenzoxyhydrazide.—To a solution of carbobenzoxy-α-L-glutamylglycine methyl ester (4.36 g.)[23] in methanol (20 ml.) was added hydrazine hydrate (1.2 ml.) and the mixture kept at room temperature for 20 hours. The methanol was removed *in vacuo*, the oily residue dissolved in 1 *N* hydrochloric acid (62 ml.) and the solution cooled at 0°. A solution of sodium nitrite (1.7 g.) in water (8 ml.) was added and the resulting azide extracted with three 40-ml. portions of ice-cold ethyl acetate. The combined ethyl acetate extracts were washed in the usual manner and dried over sodium sulfate. The solution of the azide was then added to an ethanol solution (35 ml.) of glycine carbobenzoxyhydrazide (prepared from 3.2 g. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The suspension was filtered, the clear filtrate evaporated to dryness *in vacuo* and the residue triturated with 1 *N* hydrochloric acid (30 ml.), when crystallization occurred. The crystals were collected, washed with water, dried, and recrystallized from a mixture of ethanol and ether; yield 3.9 g. (58%); m.p. 162–164°; $\alpha^{26}D$ −7 ± 2° (*c* 1, in methanol).

Anal. Calcd. for $C_{25}H_{29}O_9N_5$: C, 55.3; H, 5.4; N, 12.9. Found: C, 55.5; H, 5.5; N, 12.7.

Carbobenzoxy-α-L-glutamyl-L-tyrosylglycine Carbobenzoxyhydrazide.—An ice-cold ethyl acetate solution (70 ml.) of carbobenzoxy-α-L-glutamyl-L-tyrosine azide (prepared from 1.15 g. of the hydrazide)[24] was added to a methanol solution (20 ml.) of glycine carbobenzoxyhydrazide (prepared from 650 mg. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The solvents were removed *in vacuo* and the residue washed with 2 *N* hydrochloric acid and water, and dried. The substance was recrystallized from aqueous methanol; yield 0.84 g. (48%); m.p. 200–202° with decomposition; $\alpha^{27}D$ −6.5 ± 1° (*c* 2, in glacial acetic acid).

Anal. Calcd. for $C_{32}H_{35}O_{10}N_5$: C, 59.2; H, 5.4; N, 10.8. Found: C, 59.0; H, 5.7; N, 10.8.

Tosylglycyl-L-tyrosine Hydrazide.—To an ice-cold suspension of tosylglycine (2.5 g.) (prepared according to Harrington and Moggridge)[25] in ether (30 ml.) was added phos-

(23) W. Grassmann and F. Schneider, *Biochem. Z.*, **273**, 452 (1934).

(24) J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **127**, 627 (1939).

(25) C. R. Harrington and R. C. G. Moggridge, *J. Chem. Soc.*, 706 (1940).

phorus pentachloride (2.9 g.) in small portions with shaking. The mixture was shaken until a clear solution resulted and the ether evaporated *in vacuo* at a bath temperature of 10–15°. The resulting crystalline acid chloride was washed with petroleum ether and dried *in vacuo;* yield 2.2 g. (81%).

A solution of the above acid chloride in ether (40 ml.) was added to an ice-cold ethyl acetate solution (210 ml.) of L-tyrosine methyl ester (prepared from 5 g. of the hydrochloride) and the mixture kept at room temperature for 24 hours. The suspension was filtered, the filtrate evaporated to dryness *in vacuo* and the resulting oily compound dissolved in methanol (30 ml.). Hydrazine hydrate (800 mg.) was added, the mixture refluxed for five minutes and then kept at room temperature for 24 hours. The hydrazide which had crystallized was collected and recrystallized from aqueous methanol; yield 3.5 g. (97%); m.p. 204–207° with decomposition.

Anal. Calcd. for $C_{18}H_{22}O_5N_4S$: C, 53.2; H, 5.5; N, 13.8; S, 7.9. Found: C, 53.4; H, 5.4; N, 13.8; S, 7.6.

Tosylglycyl-L-tyrosylglycine Carbobenzoxyhydrazide.—Tosylglycyl-L-tyrosine hydrazide (2.5 g.) was dissolved in 2 *N* hydrochloric acid (13.4 ml.) and the solution cooled at 0°. A solution of sodium nitrite (460 mg.) in water (2 ml.) was added, and the azide which precipitated was extracted with three 100-ml. portions of ice-cold ethyl acetate. The ethyl acetate extracts were combined, washed with water and dried over sodium sulfate. The solution was then added to a methanol solution (40 ml.) of glycine carbobenzoxyhydrazide (prepared from 1.73 g. of the hydrochloride) and the mixture kept at room temperature for 20 hours. The suspension was filtered, the solvents removed *in vacuo* and the residue triturated with 1 *N* hydrochloric acid (20 ml.) when crystallization occurred. The compound was collected, washed with water, dried and recrystallized from aqueous methanol; yield 2.35 g. (64%); m.p. 185–187°; $\alpha^{26}D$ −3 ± 2°(*c* 1, in methanol).

Anal. Calcd. for $C_{28}H_{31}O_8N_5S$: C, 56.3; H, 5.2; N, 11.7; S, 5.4. Found: C, 56.3; H, 5.2; N, 12.0; S, 5.5.

Carbobenzoxyglycylglycyl-DL-leucine Carbobenzoxyhydrazide.[17]—An ice-cold ethyl acetate solution (120 ml.) of carbobenzoxydiglycine azide (prepared from 1.57 g. of the hydrazide) was added to an ethanol solution (10 ml.) of DL-leucine carbobenzoxyhydrazide (prepared from 1.39 g. of the hydrochloride) and the mixture kept at room temperature for 19 hours. The suspension was filtered, and the filtrate evaporated to dryness *in vacuo* and the residue dissolved in ethyl acetate (25 ml.). The solution was washed with 1 *N* hydrochloric acid (30 ml.), 10% sodium bicarbonate (30 ml.) and water (30 ml.), dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was recrystallized from ethanol; yield 1.4 g. (47%); m.p. 196–199°; $\alpha^{26}D$ 0 ± 1° (*c* 10, in glacial acetic acid).

Anal. Calcd. for $C_{26}H_{33}O_7N_5$: C, 59.2; H, 6.3; N, 13.3. Found: C, 59.1; H, 6.2; N, 13.4.

Triglycine Hydrazide Dihydrochloride. a. From Carbobenzoxytriglycine Hydrazide.—A suspension of carbobenzoxytriglycine hydrazide (1.0 g.) in water (5 ml.) and 1 *N* hydrochloric acid (6.3 ml.) was hydrogenated over spongy palladium until the evolution of carbon dioxide ceased. The catalyst was removed by filtration, the filtrate concentrated to a small volume and placed in a refrigerator. The resulting crystals were collected and dried; yield 0.7 g. (84%); m.p. 195–205° with decomposition.

Anal. Calcd. for $C_6H_{15}O_3N_5Cl_2$: N, 25.4; Cl, 25.7. Found: N, 25.7; Cl, 25.4.

b. **From Carbobenzoxytriglycine Carbobenzoxyhydrazide.**—A suspension of carbobenzoxytriglycine carbobenzoxyhydrazide (0.3 g.) in 50% aqueous methanol (14 ml.) and 3 *N* hydrochloric acid (4.4 ml.) was hydrogenated over spongy palladium until the evolution of carbon dioxide ceased. The catalyst was removed by filtration, the filtrate evaporated to dryness *in vacuo* and the resulting oil placed in a refrigerator where crystallization occurred. The crystals were washed with absolute ethanol and dried; yield 0.16 g. (91%); m.p. 195–205° with decomposition. A sample for analysis was recrystallized from water.

Anal. Calcd. for $C_6H_{15}O_3N_5Cl_2$: N, 25.4. Found: N, 25.8.

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Studies on Polypeptides. IV.[1] Remarks Regarding the Use of the Phenylthiocarbonyl Protecting-group in Peptide Synthesis

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A number of phenylthiocarbonyl dipeptide esters were prepared and their reaction with lead acetate in 70% ethanol was investigated. Contrary to previous claims this reaction led to the formation of hydantoin derivatives and not to dipeptide esters. This observation limits the practical applicability of the phenylthiocarbonyl group as a tool in peptide synthesis. The method may provide the basis for a generally applicable procedure for the preparation of hydantoins. The reaction of phenylthiocarbonylglycine carbobenzoxyhydrazide with lead acetate in ethanol afforded 2-carbobenzoxy-3,6-dioxohexahydro-1,2,4-triazine and not glycine carbobenzoxyhydrazide.

Phenylthiocarbonyl chloride (I) has been suggested as a group-protecting reagent in peptide synthesis.[4] This acid chloride reacts with α-amino acid esters (II) to form phenylthiocarbonyl-α-amino acid esters (III) which are readily converted into phenylthiocarbonyl-α-amino acids (IV) by acid hydrolysis. These latter compounds in the form of their chlorides (V) may be coupled with other α-amino acid esters (VI) to form phenylthiocarbonyldipeptide esters (VII). Short heating with lead acetate in 70% ethanol, it is claimed, removes the phenylthiocarbonyl blocking-group from such dipeptide derivatives with the formation of lead phenylmercaptide and dipeptide esters. Since this method seemed to offer considerable promise as a new, generally applicable procedure in peptide chemistry, we have undertaken the present investigation.

Phenylthiocarbonylglycine ethyl ester (III, R^1 = H) and phenylthiocarbonyl-DL-alanine methyl ester were prepared and were converted into the respective phenylthiocarbonyl-α-amino acids by acid hydrolysis. By coupling of the appropriate phenylthiocarbonyl-α-amino acid chloride with the desired α-amino acid ester, phenylthiocarbonyldiglycine ethyl ester (VII, R^1 and R^2 = H), phenylthiocarbonylglycyl-DL-alanine methyl ester and phenylthiocarbonyl-DL-alanyl-DL-alanine ethyl ester (VII)

(1) For paper III see K. Hofmann, A. Lindenmann, M. Z. Magee and N. H. Khan, THIS JOURNAL, **74**, 470 (1952).

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(4) G. C. H. Ehrensvärd, *Nature*, **159**, 500 (1947).