Butyl).—A solution of acetylacetone (0.10 mole) and 2,4-nonanedione (0.10 mole) in 30 ml. of tetrahydrofuran was added to a suspension of 0.40 mole of sodamide in 600 ml. of ammonia to form disodio salts IX and VI (R = butyl), respectively. The solution was stirred 30 min. and 100 ml. of tetrahydrofuran was added. After 5 min., 9.2 g. (0.09 mole) of ethyl bromide in 10 ml. of tetrahydrofuran was added over 10 min. After 30 min., 6 g. of ammonium chloride was added and the reaction was worked up as above. V.p.c. of the product showed that the ratio of VII to VIII was 50:50.

Peptide Synthesis *via* Oxidation of N-Acetyl-α-amino Acid Phenylhydrazides[1]

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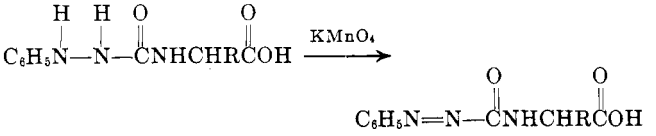
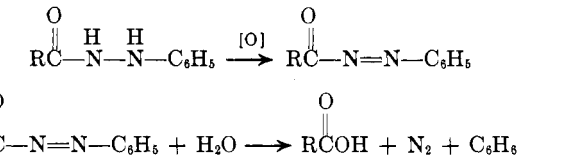
Several N-acyl-α-amino acid phenyldiimides have been prepared by an N-bromosuccinimide oxidation of N-acyl-α-amino acid phenylhydrazides. These amino acid phenyldiimides are strong acylating agents, and may be used in peptide synthesis. A new method for the synthesis of peptides based on the oxidative activation of N-acyl-α-amino acid phenylhydrazides has been developed. The method causes little racemization. Several dipeptides have been prepared by this method.

N-Acyl-α-amino acid phenylhydrazides have been prepared in the papain-catalyzed resolution of amino acids[2] and as intermediates in the enzymatic synthesis of dipeptides.[3] We have recently shown that carbobenzoxy (Cbzo) amino acid phenylhydrazides react with alcoholic potassium hydroxide to give N-carboxyphenylhydrazido amino acids.[4] These N-carboxyphenylhydrazido amino acids were oxidized to N-phenylazocarbonyl amino acids (I) by the method Pieroni used to prepare phenylazoformamide.[5]



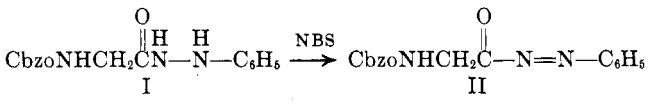
The N-phenylazocarbonyl amino acids were stable under conditions that promote free-radical reactions. The ultraviolet spectra of their ethanol solutions remained unchanged after 24 hr. of refluxing in the presence of oxygen and after being irradiated 24 hr. with ultraviolet light. On the other hand, they did react with dilute sodium hydroxide solutions giving the amino acids, benzene, and a small amount (5%) of *trans*-azobenzene. The above evidence indicated that the N-carboxyphenylazido amino acids reacted by a heterolytic reaction rather than by a free-radical reaction and suggested the possibility that amino acid phenylhydrazides may be oxidized by the same mechanism.

There have been several procedures reported for the removal of the phenylhydrazide group from N-acyl-α-amino acid phenylhydrazides. These all consist of oxidizing the phenylhydrazides in aqueous solutions to give essentially quantitative yields of nitrogen, benzene, and the acylated amino acids or dipeptides.[3,6] The high yields obtained in these oxidations have led to the suggestion[6] that the phenylhydrazides are oxidized first to the azo compounds and that this is followed by the heterolytic elimination of nitrogen and the addition of water to give the carboxylic acids and benzene.



We now wish to report the isolation of N-acyl-α-amino acid phenyldiimides from the oxidation of N-acyl-α-amino acid phenylhydrazides, by a modification of the method Carpino used to prepare *t*-butyl *p*-bromophenylazoformate.[7] These diimides are acylating agents which can be used in a number of reactions including the synthesis of peptides.

Carbobenzoxyglycylphenylhydrazide (I) was oxidized in dichloromethane with N-bromosuccinimide (NBS) to give a red solution. From this solution was isolated a 83% yield of carbobenzoxyglycylphenyldiimide (I).



That compound II was in fact carbobenzoxyglycylphenyldiimide was indicated by the ultraviolet spectrum: $\lambda_{max}^{CH_2Cl_2}$ 450.0 mμ (ε 112) and 302.5 mμ (ε 12,800). Also, the infrared spectrum of II was similar to that of the starting carbobenzoxyglycylphenylhydrazide (I): the N–H absorption at 3.08 μ was reduced, there was a shift in the amide carbonyl peak from 5.43 to 5.85 μ, and the peak at 6.23 μ (which has been attributed to an anilino group) disappeared.[6]

Carbobenzoxyglycylphenyldiimide was very unstable. The infrared spectrum, the ultraviolet spectrum, and the melting point changed after standing a few hours at room temperature. Analysis indicated a loss of nitrogen during the spontaneous decomposition. However, for 3 days carbobenzoxyglycylphenyldiimide was stored at *ca.* −80° without noticeable changes in the spectra or the melting point; a satisfactory analysis was obtained for this sample. The ultraviolet spectra of dilute solutions of carbobenzoxyglycylphenyldiimide in dichloromethane remained unchanged

(1) This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171. Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963.

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(3) E. Waldschmidt-Leitz and K. Kuhn, *Ber.*, **84**, 381 (1951); H. B. Milne, J. E. Halver, D. S. Ho, and M. S. Mason, *J. Am. Chem. Soc.*, **79**, 637 (1957).

(4) (a) H. B. Milne and D. W. Fish, *J. Org. Chem.*, **27**, 3177 (1962); (b) H. B. Milne and W. Kilday, *ibid.*, **30**, 67 (1965).

(5) A. Pieroni, *Gazz. chim. ital.*, **52**, 32 (1922).

(6) R. B. Kelley, *J. Org. Chem.*, **28**, 453 (1963).

(7) L. A. Carpino, P. H. Terry, and P. J. Crowley, *ibid.*, **26**, 4336 (1961).

TABLE I

SYNTHESIS OF PEPTIDES *via* OXIDATION OF ACYL AMINO ACID PHENYLHYDRAZIDES[a]

Acylated peptide ester[b]	Yield,[c] %	Recrystn. solvent	M.p., °C.	$[\alpha]^{25}D$, deg. (concn. of abs. EtOH)	—Carbon, %— Calcd.	Found	—Hydrogen, %— Calcd.	Found	—Nitrogen, %— Calcd.	Found
Cbzo·gly·gly·OEt	61.0[d]	50% ethanol	80–81	...	57.13	57.36	6.16	6.29	9.52	9.81
Cbzo·L-leu·gly·OEt	40.5[e]	50% ethanol	102–103	−25.5 (3.0)	61.60	61.95	7.43	7.55	8.00	8.26
Cbzo·L-leu·L-leu·OMe	65.2[f]	Isopropyl ether	97–98	−35.0 (10)	64.28	64.39	8.19	8.24	7.17	6.94
Cbzo·L-leu·L-phe·OEt	71.4	Isopropyl ether	95–96	−22.2 (1.0)	68.17	68.36	7.27	7.33	6.36	6.46
Cbzo·L-phe·L-gly·OEt	60.7	Isopropyl ether	108–109	−16.5 (5.0)	65.54	65.47	6.25	6.40	7.29	7.21
$BzSO_2$·L-leu·L-leu·OEt	52.5	Ether–ligroin	122–123	−47.8 (3.33)	...	...	...	...	...	...

[a] All of the compounds listed in this table have been reported previously, and numerous references appear in the literature; see J. T. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 1112. [b] The following abbreviations are used: Cbzo = carbobenzoxy, $BzSO_2$ = benzylsulfonyl, peptide linkage = (·), Et = C_2H_5, Me = CH_3, amino acids are denoted by first three letters of name. [c] The yields indicated were obtained by method A. [d] Method C gave 44% yield. [e] Method C gave 35% yield. [f] Method B gave 45% yield.

for several days when they were stored in the dark; however, when the solutions were exposed to fluorescent light, they turned from orange to yellow, and there were changes in the ultraviolet spectra. For these reasons, the preparations and reactions of the N-acyl-α-amino acid phenyldiimides were carried out in the dark.

That carbobenzoxyglycinephenyldiimide reacts with nucleophiles to give the products expected from heterolytic reactions is shown by the following reactions: it reacted with water to give carbobenzoxyglycine, with ethanol to give carbobenzoxyglycine ethyl ester, and with aniline to give benzyloxycarbonylglycylanilide.

$$\mathrm{CbzoNHCH_2\overset{O}{\overset{\|}{C}}{-}N{=}N{-}C_6H_5 + H_2O \longrightarrow CbzoNHCH_2\overset{O}{\overset{\|}{C}}OH + N_2 + C_6H_6}$$

$$\mathrm{CbzoNHCH_2\overset{O}{\overset{\|}{C}}{-}N{=}N{-}C_6H_5 + HOC_2H_5 \longrightarrow CbzoNHCH_2\overset{O}{\overset{\|}{C}}OC_2H_5 + N_2 + C_6H_6}$$

$$\mathrm{CbzoNHCH_2\overset{O}{\overset{\|}{C}}{-}N{=}N{-}C_6H_5 + H_2NC_6H_5 \longrightarrow CbzoNHCH_2\overset{O}{\overset{\|}{C}}NHC_6H_5 + N_2 + C_6H_6}$$

The N-acyl-α-amino acid phenyldiimides may be used also in the synthesis of peptides. When a solution of carbobenzoxyglycinephenyldiimide in dichloromethane was added slowly to a solution of glycine ethyl ester and triethylamine in dichloromethane, a good yield (61%) of carbobenzoxyglycylglycine ethyl ester was isolated from the reaction mixture.

$$\mathrm{CbzoNHCH_2\overset{O}{\overset{\|}{C}}{-}N{=}N{-}C_6H_5 + H_2NCH_2\overset{O}{\overset{\|}{C}}OC_2H_5 \longrightarrow CbzoNHCH_2\overset{O}{\overset{\|}{C}}NHCH_2\overset{O}{\overset{\|}{C}}OC_2H_5 + N_2 + C_6H_6}$$

Because of the instability of the N-acyl-α-amino acid phenyldiimides, a procedure was developed in which the N-acyl-α-amino acid phenylhydrazides were oxidized with N-bromosuccinimide in dichloromethane, and the solutions were used immediately, without isolating the diimides, for the coupling reactions.

The usefulness of this procedure in peptide synthesis was demonstrated by the preparation of six dipeptides, including five which are optically active (Table I). In method A, the acyl α-amino acid phenylhydrazide was oxidized with N-bromosuccinimide in dichloromethane. The resulting solution was added slowly to a solution of an amino acid ethyl or methyl ester and triethylamine in dichloromethane. From the reaction mixture the dipeptides were isolated in from 40 to 71.4% yields.

The carbobenzoxy dipeptide esters were characterized by elemental analyses, by comparing their melting points and optical rotations with the reported values, and by infrared spectra. In each case there were three carbonyl peaks (at *ca.* 5.73, 5.88, and 5.98 μ) corresponding to the ester, urethan, and amide groups.[8]

The infrared spectra, melting point, and mixture melting point of benzylsulfonyl-L-leucyl-L-leucine ethyl ester were identical with those of an authentic sample of benzylsulfonyl-L-leucyl-L-leucine ethyl ester previously prepared in this laboratory.[2]

The progress of these reactions was followed by observing the disappearance of the red color; the relative rates of reaction were carbobenzoxyglycylphenyldiimide, carbobenzoxy-L-leucylphenyldiimide, benzylsulfonyl-L-leucylphenyldiimide. These differences in reaction rates suggest that the acyl groups are participating in the reactions.

Techniques for peptide synthesis similar to those developed by Wolman[9] for the synthesis of peptides *via* the oxidation of N-acyl amino acid hydrazides were also used with moderate success. The N-acyl α-amino acid phenylhydrazide was oxidized in the presence of the amino acid ester and triethylamine. In method B, N-bromosuccinimide was the oxidizing agent and dichloromethane was the solvent. In method C, iodine was the oxidizing agent and dioxane was used as the solvent. Both of these methods resulted in lower yields of dipeptides and larger amounts of colored impurities than were formed when method A was used.

There appear to be several advantages for the synthesis of peptides *via* the oxidation of N-acyl-α-amino acid phenylhydrazides over the synthesis of peptides *via* the oxidation of N-acyl-α-amino acid hydrazides.[9] First, a diimide can be coupled with amino acid esters which would be oxidized by N-bromosuccinimide. Second, a combination of the papain-catalyzed resolution of amino acids *via* the formation of N-acyl-α-

(8) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 180, 222, and 224.

(9) Y. Wolman, P. N. Gallop, A. Patchnarick, and A. Berger, *J. Am. Chem. Soc.*, **84**, 1889 (1962).

amino acid phenylhydrazides[2] with this method provides a procedure for the preparation of optically pure L-peptides from DL-amino acids. Third, the progress of the reaction may be followed spectrophotometrically.

Experimental[10]

Starting Materials.—The N-acyl-α-amino acid phenylhydrazides were prepared by the method of Bergmann and Fraenkel-Conrat.[11]

Carbobenzoxyglycinephenyldiimide.—To a solution of carbobenzoxyglycinephenylhydrazide (0.001 mole) and 0.1 ml. of pyridine in 100 ml. of dichloromethane was added slowly 0.2 g. of N-bromosuccinimide. The oxidations and purifications were carried out in a dark room. The red solution was allowed to stand for 20 min. and then washed three times with 100-ml. portions of water. The solution was dried with anhydrous sodium sulfate, then 20 ml. of methylcyclohexane was added, and the solution was evaporated with the aid of an aspirator at room temperature. When the volume of the solution had been reduced to 25 ml., the solution was filtered. The resulting orange crystals were washed with ligroin (b.p. 30–70°) and dried; yield, 0.25 g. (83%) of product; m.p. 61–62°.

Anal. Calcd. for $C_{16}H_{15}N_3O_3$: C, 64.65; H, 4.94; N, 14.14. Found: C, 64.42; H, 4.93; N, 13.81.

Infrared spectrum of solid film (deposited from dioxane) was N–H stretch, 3.08 μ; C=O stretch, 5.85 μ; and no aniline peak at 6.23 μ. Ultraviolet spectrum was $\lambda_{max}^{CH_2Cl_2}$ 302.5 mμ (ε 12,800) and 450 mμ (ε 112).

Hippurylphenyldiimide.—Hippurylphenyldiimide was prepared by the same method as above in 90% yield, m.p. 124–125°. Ultraviolet spectrum was $\lambda_{max}^{CH_2Cl_2}$ 302.5 mμ (ε 12,800) and 450.0 mμ (ε 112).

Reactions of Carbobenzoxyglycinephenyldiimide. A. With Aniline.—To a solution of 1 g. (3.36 mmoles) of carbobenzoxyglycinephenyldiimide in 100 ml. of dichloromethane was added 2 ml. of aniline. The mixture was stored for 12 hr. in the dark at room temperature. After this time, the solution was washed with 100 ml. of 5% hydrochloric acid, 100 ml. of 5% sodium hydroxide solution, and 100 ml. of water. The dichloromethane solution was evaporated with the aid of an aspirator. The resulting solid was recrystallized from ethanol–water; 0.57 g. (60% yield) of crystals, m.p. 144–145°. The crystals were identified as carbobenzoxyglycine anilide by a mixture melting point determination (m.p. 144–145°) and by comparing the infrared spectrum with the spectrum of an authentic sample of carbobenzoxyglycine anilide (m.p. 144–145°) prepared by the method of Niemann.[12]

B. With Ethanol.—To a solution of 1 g. (3.36 mmoles) of carbobenzoxyglycinephenyldiimide in 100 ml. of dichloromethane was added 10 ml. of absolute ethanol. After standing for 12 hr. in the dark, the solution was washed with 100 ml. of 5% hydrochloric acid, 100 ml. of 5% sodium hydroxide solution, and 100 ml. of water. The resulting solution was dried with anhydrous sodium sulfate and the solvent was evaporated, with the aid of an aspirator, to an oil. This oil was distilled; b.p. 145–150° (0.25 mm.); yield, 0.53 g. (64%) of oil, which solidified to a crystalline solid, m.p. 35.5°. This was identified as carbobenzoxyglycine ethyl ester by a mixture melting point determination (m.p. 35.5°) and comparison of its infrared spectrum with that of an authentic sample of carbobenzoxyglycine ethyl ester.

C. With Water.—A solution of 1.0 g. (3.36 mmoles) of carbobenzoxyglycinephenyldiimide in 100 ml. of dichloromethane was added slowly in the dark to 100 ml. of hot water. The volatile solvent was distilled as the dichloromethane solution was added to the hot water. The resulting aqueous solution was made basic with 5% sodium bicarbonate solution, cooled, and extracted with 50 ml. of dichloromethane. The aqueous layer was acidified with hydrochloric acid, and this was extracted with two 50-ml. portions of dichloromethane. This last dichloromethane extract was dried with anhydrous sodium sulfate and the solvent was evaporated; yield, 0.51 g. of crystals; m.p. 117.5–118.5°. The product was shown to be carbobenzoxyglycine by a melting point determination (m.p. 118–119°) and by comparing the infrared spectrum with that of an authentic sample of carbobenzoxyglycine prepared by the method of Bergmann.[13]

D. With Glycine Ethyl Ester.—A solution of 1 g. (3.36 mmoles) of carbobenzoxyglycinephenyldiimide in 100 ml. of dichloromethane was added slowly (30 min.) in the dark with stirring to a solution of 1.0 g. of glycine ethyl ester hydrochloride and 1.0 ml. of triethylamine in 100 ml. of dichloromethane. The resulting solution was washed with 100 ml. of 6% hydrochloric acid, 100 ml. of 6% sodium hydroxide solution, and 100 ml. of water. The dichloromethane solution was dried with anhydrous sodium sulfate. Evaporation of the solvent gave an oil which was dissolved in absolute ethanol; the solution was decolorized with charcoal, and water was added. This was allowed to stand overnight in the refrigerator, and 0.6 g. of crystals formed (m.p. 80–81°). The product was shown to be carbobenzoxyglycylglycine ester by a mixture melting point determination (m.p. 80–81°) and by comparing the infrared spectrum with that of an authentic sample of carbobenzoxyglycylglycine ethyl ester (m.p. 80–81°) prepared from glycylglycine.

Acyl Peptide Esters. Method A.—N-Bromosuccinimide (3.56 g.) was slowly added to a solution of 10 mmoles of the acyl amino acid phenylhydrazide and 1.6 ml. of pyridine in 100 ml. of dichloromethane. After 20 min. the solution was washed with 100 ml. of water and dried with anhydrous sodium sulfate. The resulting solution was added slowly to a solution of 10 mmoles of the amino acid ester hydrochloride and 4 ml. of triethyl amine in 100 ml. of dichloromethane. The operation was carried out in the dark. After the red color had disappeared, the yellow solution was washed with 100 ml. of 6% hydrochloric acid, 100 ml. of 1 *N* sodium hydroxide solution, and 100 ml. of water. The solution was evaporated with the aid of an aspirator. The resulting oil crystallized on standing in a refrigerator. It was recrystallized from the solvents indicated (Table I). The yields, melting points, optical rotations, and analyses are reported in Table I. The infrared spectra (potassium bromide pellets) of the carbobenzoxy dipeptide esters showed peaks at 3.02, 5.73, 5.88, 5.98, and 6.49 μ.

Method B.—The procedure in method A was modified by adding 3.56 g. of N-bromosuccinimide to a solution of 10 mmoles of the acyl amino acid phenylhydrazide, 10 mmoles of the amino acid ester hydrochloride, and 4 ml. of triethyl amine in 100 ml. of α-dichloromethane. The resulting solution was worked up as indicated in method A.

Method C.—This is a modification of the method Wolman used to synthesize peptides *via* oxidation activation of amino acid hydrazides using iodine. Powdered iodine was slowly added to a cold solution of 5 mmoles of acyl amino acid phenylhydrazide, 5 mmoles of amino acid ester hydrochloride, and 3 ml. of triethylamine in 20 ml. of dioxane and 5 ml. of water. The mixture was stirred for 2 hr. The excess iodine was then removed with solid sodium thiosulfate and the solvent was removed *in vacuo*. The residue was extracted with 100 ml. of dichloromethane which was then washed with 100 ml. of water, 100 ml. of 1 *N* hydrochloric acid, 100 ml. of 1 *N* sodium hydroxide solution, and 100 ml. of saturated sodium chloride solution. The dichloromethane solution was dried over anhydrous sodium sulfate and the solvent was evaporated *in vacuo*. The resulting peptides were recrystallized from the solvents indicated in Table I.

(10) All melting points are corrected. The microanalytical work was performed by the Galbraith Laboratories, Knoxville, Tenn. The infrared spectra were determined on a Beckman IR-5 spectrophotometer; the spectra were obtained from films deposited on rock salt plates. The ultraviolet spectra were determined with a Cary Model 14 recording spectrophotometer.

(11) M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937).

(12) C. Niemann and D. L. Nechols, Jr., *ibid.*, **143**, 191 (1942).

(13) M. Bergmann and L. Zervos, *Ber.*, **65**, 1192 (1932).