2-Amino-3-cyano-5-methyl-6-methoxypyrazine 4-Oxide (11). A solution of 0.50 g of 2-amino-3-cyano-5-methylpyrazine 1,4-dioxide in 20 ml of dry methanol containing 0.43 g of sodium methoxide was heated under reflux overnight. The resulting brownish-red solution was evaporated to about half its volume under reduced pressure, cooled, and filtered. The collected solid was crystallized from acetic acid (charcoal) to give 0.25 g (46%) of colorless crystals: mp >235° dec; nmr (CF_3COOH) 2.07 (3, s, C_5-CH_3), 3.71 (3, s, OCH_3).

2-Amino-3-cyano-5-methyl-6-guanidinopyrazine 4-Oxide (12). A mixture of 0.20 g of 2-amino-3-cyano-5-methyl-6-methoxypyrazine 4-oxide, 0.32 g of guanidine hydrochloride, and 0.23 g of sodium methoxide in 12 ml of dry methanol was heated under reflux for 14 hr. The yellow solid which had separated was collected by filtration and crystallized from glacial acetic acid. The resulting acetate salt of **12** was stirred with aqueous sodium bicarbonate to give a colorless solid which was crystallized from aqueous DMF: yield 0.13 g (57%); mp >280° dec; ir 2210 cm^{-1} (CN).

Photoalkylation of Peptides. Visible Light-Induced Conversion of Glycine Residues into Branched α-Amino Acids

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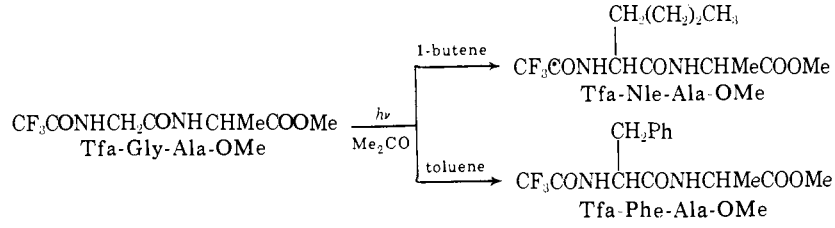
Abstract: Glycine residues in dipeptides and polypeptides have been modified by a photoalkylation procedure which results in their preferential conversion into branched α-amino acids. The reactions were induced with visible light using a combination of an α-diketone and a peroxide as the photoinitiator. The degree of selectivity and that of the asymmetric induction were found to increase with the increase in molecular weight of the peptide. Results indicate that the reactions proceed through energy transfer from the photoexcited triplet α-diketone to the peroxide leading subsequently to fragmentation of the latter and thereby to a free-radical reaction.

Photochemical modifications of glycine-containing peptides and proteins have been described by us in a series of publications.[2] These modifications involve an alkylation process by which glycine residues are converted into residues of a variety of branched α-amino acids through the substitution of a preselected alkyl or aralkyl group for a hydrogen atom at the α position of the glycine. The reactions were induced photochemically with ultraviolet light (λ >260 nm or >290 nm) using acetone as a photoinitiator, and were found to be selective for glycine residues in peptides and proteins. While employing 1-butene or toluene as reagents, glycine residues were converted into norleucine and phenylalanine, respectively. A mechanism



involving free-radical intermediates has been proposed for these reactions.[2a] In the initiation step an excited acetone molecule abstracts a hydrogen atom from the α carbon of glycine, thus leading to a free radical which subsequently reacts with an olefin (*e.g.*, 1-butene) or an aralkyl radical (*e.g.*, benzyl) to yield the new branched α-amino acid.

The extension of these photoalkylation reactions to peptides and proteins containing ultraviolet-sensitive amino acids, as well as the incorporation of sensitive amino acid residues (*e.g.*, tyrosine) into a protein molecule, may require the use of light of longer wavelengths and employment of suitable photoinitiators. The use of a variety of photoinitiators might also lead to a broader scope of these modification reactions and to the possible incorporation of new side chains into glycine (*e.g.*, the production of aspartic acid or serine derived from acetic acid[3] or methanol,[4] respectively). α-Diketones could be considered as suitable photoinitiators for these reactions, since they absorb ultraviolet light of long wavelengths, and their absorption extends into the visible region. However, the hydrogen atom abstraction ability of diketones in the excited state is rather weak as compared to that of the corresponding monoketones.[5] Indeed, we found that α-diketones failed to initiate the photoalkylation reactions. Peroxides, such as di-*tert*-butyl peroxide (DBP), afford free radicals which are powerful hydrogen atom abstracting agents and may be considered

(1) In partial fulfillment of the requirements for a Ph.D. Thesis submitted to the Feinberg Graduate School, 1972.

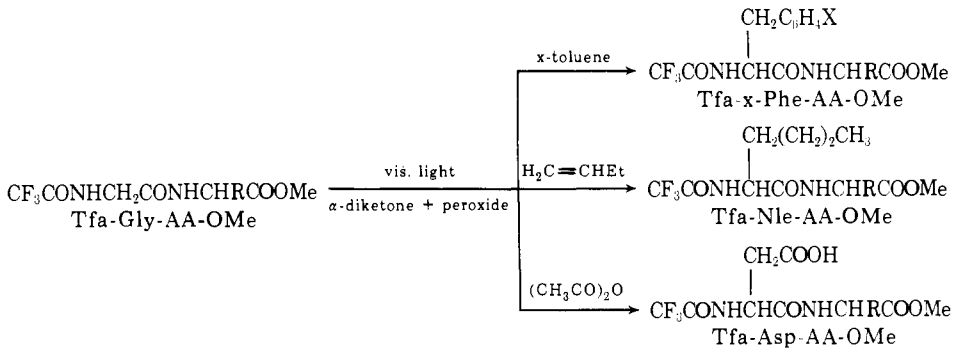
(2) (a) D. Elad and J. Sperling, *J. Chem. Soc. C*, 1579 (1969); (b) J. Sperling and D. Elad, *J. Amer. Chem. Soc.*, **93**, 967 (1971); (c) *ibid.*, **93**, 3839 (1971), and references cited therein.

(3) J. C. Allen, J. I. G. Cadogan, and D. H. Hey. *J. Chem. Soc.*, 1918 (1965).

(4) W. H. Urry, F. W. Stacey, E. S. Huyser, and O. O. Juveland, *J. Amer. Chem. Soc.*, **76**, 450 (1954).

(5) O. W. Jolly and P. de Mayo, *Can. J. Chem.*, **42**, 170 (1964); W. G. Bentrude and K. R. Darnall, *Chem. Commun.*, 810 (1968); M. B. Rubin, *Fortschr. Chem. Forsch.*, **13**, 251 (1969), and references cited therein; N. J. Turro and T. J. Lee, *J. Amer. Chem. Soc.*, **91**, 5651 (1969); B. Monroe, *Advan. Photochem.*, **8**, 77 (1971).

Scheme I



x = *p*-H, *p*-F, or *p*-OMe
R = H, CH_3, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, CH_2Ph, or CH_2Ph-*p*-OMe
AA = Gly, Ala, Val, Leu, Phe, or Tyr(OMe)

as suitable photoinitiators; however, the absorption of such peroxides does not extend to long enough wavelengths.[6] It was assumed that a combination of an α-diketone and a peroxide might function as a suitable photoinitiator, the former serving as the light absorbing system, and the latter acting as the hydrogen atom abstraction agent. Such an assumption seemed plausible by analogy to the previously reported photosensitized decomposition of peroxides by ketones.[7] The present publication includes a detailed description of the photoalkylation of glycine residues in di-, tri-, and polypeptides induced with visible light using a variety of α-diketone–peroxide combinations.[8]

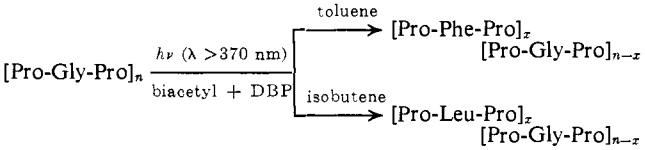
Results and Discussion

Visible light irradiation of glycine-containing peptides with 1-butene, substituted toluenes, or acetic acid derivatives, in the presence of an α-diketone and a peroxide, led to the conversion of glycine residues in the peptides into norleucine, substituted phenylalanines, and aspartic acid, respectively. The reactions can be represented as in Scheme I (for dipeptides).

Reactions of Dipeptides. The dipeptides employed were protected by esterification and *N*-trifluoroacetylation in order to minimize side reactions of the free amino or carboxyl groups.[2] The most common combination of an α-diketone and a peroxide employed for initiation was that of biacetyl (BA) and di-*tert*-butyl peroxide (DBP); however, other combinations of α-diketones and peroxides, such as camphorquinone (CQ) and dicumyl peroxide (DCP), proved to be effective. The reactions studied and the major products obtained are summarized in Table I.

Progress of the reactions was followed by direct glpc analysis on silicon–oil columns.[9] The amino acid composition of the reaction mixtures was determined by periodic analysis of hydrolyzed samples with an amino acid analyzer.[10] The modified peptides, as well as the nonpeptidic products, were isolated by column chromatography on silica gel and characterized by their physical properties and comparison with authentic samples. The major peptides obtained were 1:1 adducts resulting from preferential substitution at the glycine residue. In reactions with 1-butene, low molecular weight telomers, mainly 2:1 adducts, were obtained in low yields. These telomers result from the addition of two olefin moieties to a single glycine residue, thus giving rise to a C_{10}-α-amino acid. Yields of the products were found to depend upon the ratio of peroxide–alkylating reagent (*e.g.*, toluene). In the absence of toluene, quantitative destruction of the dipeptide derivative occurred, probably due to the decomposition of the glycine radicals formed, whereas the use of an excess of toluene led to the supression of the desired alkylation process. Optimal yields of alkylation products of glycine were obtained when the toluene–peroxide ratio was maintained between 2:1 and 3:1. The predominant nonpeptidic products isolated from the reactions of the toluenes were the corresponding bibenzyls and 1,2,3-triarylpropanes. These products result from the combination of benzyl radicals formed in the reaction mixtures.[11] The nonpeptidic by-products of the butene reactions were predominantly mixtures of higher hydrocarbons which result from the polymerization of the olefin.

Reactions of Polypeptides. The photoalkylation of glycine-containing polypeptides led to the preferential conversion of glycine residues into the appropriate branched α-amino acids, as described in the following scheme illustrating, for example, photoalkylation of $[Pro\text{-}Gly\text{-}Pro]_n$ copolymer. Sequential as well as



ramdom copolymers[12] containing glycine, alanine, and proline were employed for these studies. The polypeptides were fractionated on Sephadex G-25 columns and fractions of molecular weights ranging from 2000 to 6000 were lyophilized and used for the photochemical reactions. The polypeptides varied in

(6) E. R. Bell, F. F. Rust, and W. Vaughan, *J. Amer. Chem. Soc.*, 72, 337 (1950); J. G. Calvert and J. N. Pitts, Jr., "Photochemistry," Wiley, New York, N. Y., 1967, p 443.
(7) C. Walling and M. J. Gibian, *J. Amer. Chem. Soc.*, 87, 3413 (1965).
(8) Preliminary communication: D. Elad, M. Schwarzberg, and J. Sperling, *Chem. Commun.*, 617 (1970).
(9) F. Weygand, B. Kolb, A. Prox, M. A. Tilak, and I. Tomida, *Hoppe-Seyler's Z. Physiol. Chem.*, 322, 38 (1960).
(10) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).
(11) *Cf.* E. H. Farmer and C. G. Moore, *J. Chem. Soc.*, 131 (1957).
(12) N. S. Andreeva, N. G. Esipora, M. I. Millinova, N. V. Rogulenkova, and C. A. Shibner, "Conformation of Biopolymers," Vol. 2, G. N. Ramachandran, Ed. Academic Press, New York, N. Y., 1967, pp 469–481; E. Katchalski and M. Sela, *Advan. Protein Chem.*, 13, 243 (1958).

Table I. Alkylation of Glycine-Containing Dipeptides (Initiated by visible light with α-diketones and peroxides)

Dipeptide	Initiation system[a]	Alkylating reagent	Product (yield, %)[b]
Tfa-Gly-Gly-OMe	BA + DBP[c]	Toluene	Tfa-Phe-Gly-OMe (30) Tfa-Gly-Phe-OMe (29) Tfa-Phe-Phe-OMe (10)
Tfa-Gly-Gly-OMe	DBP[d]	Toluene	Tfa-Phe-Gly-OMe (30) Tfa-Gly-Phe-OMe (30) Tfa-Phe-Phe-OMe (6)
Tfa-Gly-L-Ala-OMe	BA + DBP[c]	Toluene	Tfa-Phe-L-Ala-OMe (47) Tfa-Gly-α-MePhe-OMe (12)
Tfa-Gly-L-Ala-OMe	BA + DCP[e]	Toluene	Tfa-Phe-L-Ala-OMe (40) Tfa-Gly-α-MePhe-OMe (15)
Tfa-Gly-L-Ala-OMe	DCP[f]	Toluene	Tfa-Phe-L-Ala-OMe (35) Tfa-Gly-α-MePhe-OMe (15)
Tfa-Gly-L-Val-OMe	BA + DBP[c]	Toluene	Tfa-Phe-L-Val-OMe (50)
Tfa-Gly-L-Val-OMe	DBP[f]	Toluene	Tfa-Phe-L-Val-OMe (36)
Tfa-Gly-L-Val-OMe	DCP[f]	Toluene	Tfa-Phe-L-Val-OMe (32)
Tfa-Gly-L-Leu-OMe	BA + DBP[c]	Toluene	Tfa-Phe-L-Leu-OMe (54)
Tfa-Gly-L-Leu-OMe	CQ + DBP[e]	Toluene	Tfa-Phe-L-Leu-OMe (30)
Tfa-Gly-L-(γ-OMe)Asp-OMe	CQ + DCP[c]	Toluene	Tfa-Phe-(γ-OMe)Asp-OMe (30)
Tfa-Gly-L-Phe-OMe	BA + DBP[c]	Toluene	Tfa-Phe-L-Phe-OMe (45) Tfa-Gly-α-BzPhe-OMe (10)
Tya-Gly-L-Tyr(OMe)-OMe	BA + DBP[c]	Toluene	Tfa-Phe-L-Tyr(OMe)-OMe (43)
Tfa-Gly-Gly-OMe	BA + DBP[c]	*p*-Fluorotoluene	Tfa-Gly-(*p*-F)Phe-OMe (20) Tfa-(*p*-F)Phe-Gly-OMe (20) Tfa-(*p*-F)Phe-(*p*-F)Phe-OMe (5)
Tfa-Gly-Gly-OMe	BA + DBP[c]	*p*-Methoxytoluene	Tfa-Tyr(OMe)-Gly-OMe (21) Tfa-Gly-Tyr(OMe)-OMe (20)
Tfa-L-Phe-Gly-OMe	BA + DBP[c]	*p*-Methoxytoluene	Tfa-L-Phe-Tyr(OMe)-OMe (35)
Tfa-Gly-Gly-OMe	BA + DBP[c]	Acetic anhydride	Tfa-Asp-Gly-OMe (30) Tfa-Gly-Asp-OMe (30)
Tfa-Gly-Gly-OMe	BA + DBP[c]	1-Butene	Tfa-Gly-Nle-OMe (20) Tfa-Nle-Gly-OMe (20) Tfa-Gly-C_{10}AA-OMe (10) Tfa-C_{10}AA-Gly-OMe (10)
Tfa-Gly-Gly-OMe	DBP[d]	1-Butene	Tfa-Gly-Nle-OMe (20) Tfe-Nle-Gly-OMe (20) Tfa-Gly-C_{10}AA-OMe (10) Tfa-C_{10}AA-Gly-OMe (10)
Tfa-Gly-L-Ala-OMe	BA + DBP[c]	1-Butene	Tfa-Nle-L-Ala-OMe (16) Tfa-C_{10}AA-L-Ala-OMe (8)

[a] Biacetyl (BA); camphorquinone (CQ); di-*tert*-butyl peroxide (DBP); dicumyl peroxide (DCP). [b] Yields are based on consumed starting dipeptide; conversions ranged from 25 to 60%. [c] GEC fluorescent lamps (cool white 20 W) were used as source of visible light (irradiation time *ca.* 50 hr). [d] Hanovia 200-W lamp with a Pyrex filter. [e] Sunlight with GWV filter (λ >370 nm). [f] Sunlight.

their glycine content (10–66%) and were used without carboxyl or amino group protection. The weight average molecular weights of the water soluble polypeptides were determined by the Yphantis midpoint method.[13] The number-average molecular weights of the water insoluble polypeptides were determined by the Van Slyke method.[14] Reactions were carried out in aqueous solutions of *tert*-butyl alcohol or in nonaqueous suspensions. Toluene was generally employed as the alkylating reagent but comparable results were obtained with olefins, *e.g.*, isobutene. Results are summarized in Table II.

Isolation of the irradiated polypeptide was achieved by precipitation and washing with an organic solvent. The resulting residue was dissolved in water and centrifuged whenever an insoluble fraction was present. The crude water-soluble polypeptide was fractionated on Sephadex G-25 and the high molecular weight fractions were dialyzed and lyophilized. Amino acid analysis of the water-soluble polypeptides indicated that the modification of glycine residues occurred to the extent of 2–10% while essentially no destruction of other amino acids took place. Molecular weight measurements showed that the irradiated polypeptide had nearly the same molecular weight as that of the starting polypeptide. These results indicate that the modification took place while preserving the original chain lengths of the polypeptides. The water-insoluble fractions, whenever occurred, were analyzed separately and were shown to contain the new amino acid, as did the water-soluble fractions.

High conversion of glycine residues was obtained in polypeptides with a low content of glycine. Thus, in the reaction of [L-Pro-Gly-L-Pro]$_n$ *ca.* 8% of the glycine residues were converted into phenylalanine, as compared with only 2% of the glycine residues which were modified in [Gly-L-Pro-Gly]$_n$. A decrease in the extent of alkylation with the increase in the content of glycine was also observed in (DL-Ala)$_m$-(Gly)$_n$ copolymers. Thus, in a $m:n$ = 9:1 copolmyer, 10% of the glycine residues were alkylated under conditions which led to only 4–5% alkylation with a $m:n$ = 4:1 copolymer. Furthermore, in copolymers containing 40% glycine, alkylation could hardly be detected. It is noteworthy that a similar phenomenon has been observed previously in reactions where acetone was used as a photoinitiator.[2b] This implies that it is the nature of the polypeptide and its glycine con-

(13) D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, 88, 586 (1960).
(14) D. D. Van Slyke, *J. Biol. Chem.*, 83, 425 (1929).

Table II. Photoalkylation of Glycine-Containing Polypeptides (Initiated with visible light, biacetyl, and di-*tert*-butyl peroxide)

Polypeptide	Amino acid[a] composition			Mol wt	Alkylating reagent	Product[b] amino acid[a] composition				
	Ala	Gly	Pro			Ala	Gly	Pro	Phe	Leu
(DL-Ala)$_m$(Gly)$_n$	90	10		5100[c]	Toluene	89.5	9.5		1	
(DL-Ala)$_m$(Gly)$_n$	77	23		6000[d]	Toluene	76.5	22.5		1	
(DL-Ala)$_m$(Gly)$_n$	81	19		3800[c]	Toluene	82.8	16.4		0.8	
[Gly-L-Pro-Gly]$_n$		66.3	33.7	2100[d]	Toluene		66.7	32	1.2	
[L-Ala-L-Pro-Gly]$_n$	33.3	33.3	33.3	3000[c]	Toluene	33.4	32.3	33.4	1.0	
[L-Pro-Gly-L-Pro]$_n$		33.4	66.6	2800[c]	Toluene		32.7	64.8	2.5	
[L-Pro-L-Pro-Gly]$_n$		33.4	66.6	3700[c]	Toluene		33.1	65.9	1.0	
[L-Pro-Gly-L-Pro]$_n$		33.4	66.6	2200[c]	Isobutene		31.6	67.7		0.7

[a] Residues per 100 total residues, determined on a Beckman-Spinco 120c amino acid analyzer. [b] In the reactions reported 2–10% of the glycine content in the polypeptides was converted to the appropriate branched α-amino acid residues. [c] Determined by the Yphantis method. [d] Determined by the Van Slyke method.

tent which affect the extent of alkylation. It seems probable that conformational restrictions imposed by the "polyglycine-character" of the polypeptide are responsible for the decrease in the reactivity of these residues in glycine-rich polypeptides.

Selectivity and Asymmetric Induction. Peptides containing glycine and the representative amino acids alanine, valine, and phenylalanine were used for these studies. All these amino acids underwent photoalkylation at the α position leading to the corresponding α,α-disubstituted derivatives which were isolated and characterized by their elemental analyses and physical properties. The extent of photoalkylation of these amino acids was determined by glpc using either authentic peptide derivative of glycine and the corresponding α,α-disubstituted amino acids, or through hydrolysis of the peptides followed by esterification and trifluoroacetylation of the resulting mixture of amino acids.[15] The degree of selectivity was determined for mixtures of the individual amino acids and compared to that obtained in di-, tri-, and polypeptides. The results of this study are summarized in Table III.

Our results show that as individual amino acids, glycine, alanine, and phenylalanine reacted to nearly the same extent, whereas valine was far less reactive. The preferential reactivity of glycine was observed only in peptides and found to increase with increasing molecular weight of the peptide. In the case of glycine–valine systems, selectivity showed up already in the amino acid mixtures, while in peptides glycine residues reacted exclusively. These results indicate that the type of side chain of the amino acid affects the alkylation process, and that the selectivity toward glycine increases in peptides. It is noteworthy that similar results were obtained while initiating the reactions with acetone,[2] and the difference in the degree of selectivity observed in the two photoinitiation methods is merely quantitative, with acetone exhibiting the higher selectivity.[16]

The conversion of a glycine residue in a peptide into a branched α-amino acid involves asymmetric induction by the chiral center which is four atoms distant from the α carbon of the glycine residue.[2] We have undertaken a study of the asymmetric induction occurring in the photoalkylation reactions initiated with the biacetyl–DBP–visible light system.

(15) C. W. Gehrke and D. L. Stalling, *Separ. Sci.*, 2, 101 (1967).
(16) *Cf.* C. Walling and M. J. Gibian, *J. Amer. Chem. Soc.*, 87, 3361 (1965).

Table III. Selectivity[a] in the Photoalkylation of Glycine-Containing Systems (Initiated with visible light, biacetyl, and di-*tert*-butyl peroxide)

Starting system Peptide	Gly:Ala	Product Phe:MePhe[b]
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	1:8	1:1
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	1:1	1:1
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	8:1	1:1
Tfa-Gly-L-Ala-OMe	1:1	4:1
Tfa-L-Ala-Gly-OMe	1:1	5:1
Tfa-L-Ala-Gly-L-Ala-OMe	1:2	6:1
(DL-Ala)$_m$(Gly)$_n$	1:9	18:1
		Nle:MeNle[c]
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	1:5	1:1
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	1:1	1:1
Tfa-Gly-OMe + Tfa-DL-Ala-OMe	5:1	1:1
Tfa-Gly-L-Ala-OMe	1:1	4:1
Tfa-L-Ala-Gly-OMe	1:1	4:1
Tfa-L-Ala-Gly-L-Ala-OMe	1:2	5:1
	Gly:Phe	F-Phe:F-BzPhe[d]
Tfa-Gly-OMe + Tfa-DL-Phe-OMe	1:5	1:1
Tfa-Gly-OMe + Tfa-DL-Phe-OMe	1:1	1:1
Tfa-Gly-OMe + Tfa-DL-Phe-OMe	5:1	1:1
Tfa-Gly-L-Phe-OMe	1:1	4:1
	Gly:Val	Phe:BzVal[e]
Tfa-Gly-OMe + Tfa-DL-Val-OMe	1:3	14:1
Tfa-Gly-OMe + Tfa-DL-Val-OMe	1:1	11:1
Tfa-Gly-OMe + Tfa-DL-Val-OMe	3:1	11:1
Tfa-Gly-L-Val-OMe	1:1	35:1
Tfa-L-Val-Gly-L-Pro-OMe	1:1	43:1

[a] Obtained by dividing the absolute ratio of the products by the ratio of the respective amino acids in the starting peptide. [b] 2-Methylphenylalanine, resulting from alkylation of alanine residues with toluene; per residue of the respective starting amino acid. [c] 2-Methylnorleucine, resulting from alkylation of alanine residues with 1-butene; per residue of the respective starting amino acid. [d] 2-(*p*-Fluorobenzyl)phenylalanine, resulting from alkylation of phenylalanine residues with *p*-fluorotoluene; per residue of the respective starting amino acid. [e] 2-Benzylvaline, resulting from alkylation of valine residues with toluene; per residue of the respective starting amino acid.

Di-, tri-, and polypeptides containing glycine were used in these studies. The peptides were photoalkylated by the usual procedure using toluene as the alkylating reagent, except for phenylalanine-containing peptides where *p*-xylene (leading to *p*-methylphenylalanine) was used. The ratio of the two enantiomers of the newly formed amino acid was determined both by using L-amino acid oxidase digestion[17] and by glpc. In the former case, digestion of the L-amino acids by L-amino acid oxidase and determination of the intact

(17) A. Meister and D. Wellner, *Enzymes*, 7, 609 (1963).

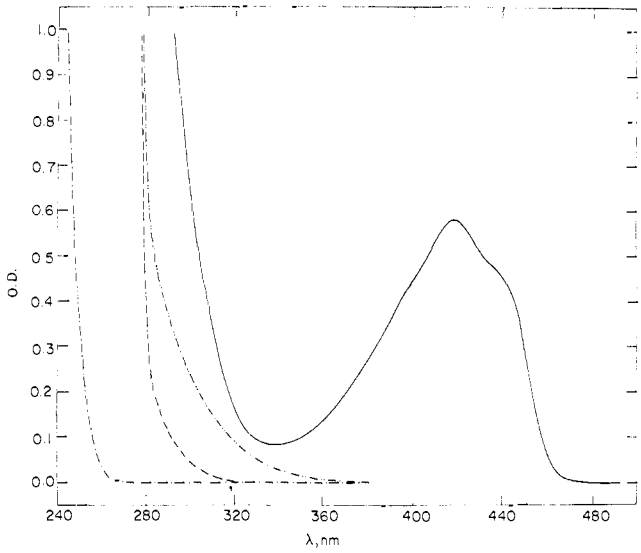


Figure 1. Absorption spectrum of the photoalkylation reaction mixture in *tert*-butyl alcohol. (Light employed for initiation, λ ≃420–460 nm, is absorbed by biacetyl.) (——) Tfa-Gly-Val-OMe (2.5×10^{-2} *M*), toluene (7.1×10^{-1} *M*), di-*tert*-butyl peroxide (1.4×10^{-1} *M*), and biacetyl (3.0×10^{-2} *M*); (·–·–·–·) Tfa-Gly-Val-OMe; (– – –) Tfa-Gly-Val-OMe and toluene; (···–···–) Tfa,Gly-Val-OMe, toluene, and di-*tert*-butyl peroxide.

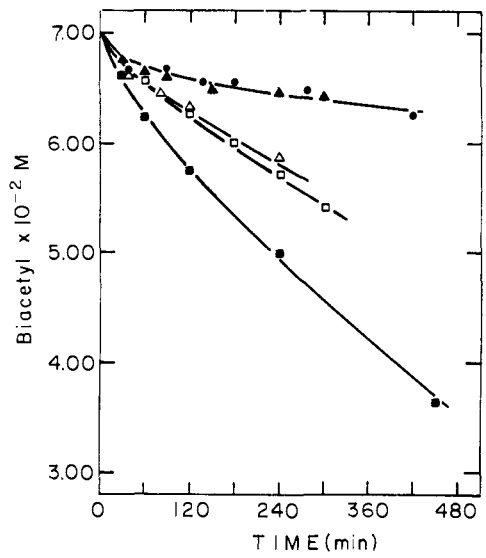


Figure 2. Photolysis of biacetyl (7.0×10^{-2} *M*) with light of λ ≃ 420–460 nm: (▲) in *tert*-butyl alcohol; (■) in the presence of di-*tert*-butyl peroxide (2.74×10^{-1} *M*); (●) in the presence of toluene (1.42 *M*); (△) in the presence of toluene and di-*tert*-butyl peroxide; (□) in the presence of toluene, di-*tert*-butyl peroxide, and Tfa-Gly-OMe (4.82×10^{-2} *M*).

D enantiomer amino acid on an amino acid analyzer allowed the enantiomer ratio to be established. In the glpc method, dipeptide derivatives were determined directly by separation of the diastereoisomers on silicon–oil columns.[18] In addition, the hydrolyzed mixture of the amino acids was analyzed on columns containing optically active stationary phases,[19] which allowed the separation of the D and L enantiomers. The same procedures were used for the determination of the degree of asymmetric induction in the reactions of tri- and polypeptides. The results from the enzymatic method were nearly identical with those obtained by the glpc measurements; the experimental error in these determinations is ±3%. Results of these studies are summarized in Table IV. Our studies indicate that while asymmetric induction in the photoalkylation of di- and tripeptides occurs to a small extent, higher degrees of asymmetric induction are obtained in reactions of sequential polypeptides. This is demonstrated, for example, by the *ca.* 30% asymmetric induction observed for [L-Pro-Gly-L-Pro]$_n$, as compared to 6% asymmetric induction for the corresponding tripeptide unit Tfa-L-Pro-Gly-L-Pro-OMe. In addition, a trend toward the preferential formation of the unnatural D enantiomer is observed in glycine–proline copolymers. Thus, it is indicated that the degree of asymmetric induction depends on the molecular weight and the conformation of the peptide. A similar conclusion has been reached in photoalkylation reactions where acetone served as a photoinitiator. This suggests that the degree of asymmetric induction depends primarily on the nature of the peptide and its constituents.

Table IV. Asymmetric Induction in the Photoalkylation of Glycine-Containing Di-, Tri-, and Polypeptides

Peptide	Alkylating reagent	Formed amino acids[a] L, %	D, %
Tfa-Gly-L-Val-OMe	Toluene	54	46
Tfa-Gly-L-(γ-OMe)Asp-OMe	Toluene	53	47
Tfa-Gly-L-Phe-OMe	*p*-Xylene	54	46
Tfa-L-Phe-Gly-OMe	*p*-Xylene	48.5	51.5
Tfa-Gly-L-Pro-OMe	Toluene	45	55
Tfa-L-Ala-Gly-L-Ala-OMe	Toluene	47	53
Tfa-L-Ala-Gly-L-Phe-OMe	*p*-Xylene	47	53
Tfa-L-Val-Gly-L-Phe-OMe	*p*-Xylene	48	52
Tfa-L-Val-Gly-L-Val-OMe	Toluene	47	53
Tfa-L-Pro-Gly-L-Pro-OMe	Toluene	47	53
[Gly-L-Pro-L-Pro]$_n$	Toluene	39	61
[L-Ala-L-Pro-Gly]$_n$	Toluene	35	65
[L-Pro-Gly-L-Pro]$_n$	Toluene	35	65
[Gly-L-Pro-Gly]$_n$	Toluene	28	72

[a] Phenylalanine or *p*-methylphenylalanine.

Mechanism. In the previous publications of this series we have shown that the role of the photoinitiator in these alkylation reactions is twofold: (i) to absorb the incident light, and (ii) to subsequently abstract a hydrogen atom from the amino acid. Acetone fulfilled these roles, and the interaction of the excited molecule with the amino acid led to the formation of an amino acid free radical, located at the α-carbon atom, which was trapped by the appropriate scavenger (*e.g.*, an olefin) to yield the branched α-amino acid. α-Diketones, which absorb in the visible region, could be considered as suitable substitutes for acetone in these reactions; however, these failed to initiate the photoalkylation reactions, most probably due to their inability to abstract a hydrogen atom from amino acids.[5] The combination of an α-diketone and a peroxide was found suitable for this purpose, the diketone being the light-absorbing system (see Figure 1), and the peroxide serving as the source for reactive species for hydrogen atom abstraction. In an attempt to understand the pathway from the light-absorption

(18) F. Weygand, A. Prox, L. Schmidhamer, and W. Koenig, *Angew. Chem., Int. Ed. Engl.*, 2, 183 (1963).
(19) B. Feibush and E. Gil-Av, *Tetrahedron*, 26, 1361 (1970); B. Feibush, *Chem. Commun.*, 544 (1971); W. Parr, C. Yang, E. Bayer, and E. Gil-Av, *J. Chromatogr. Sci.*, 8, 591 (1970).

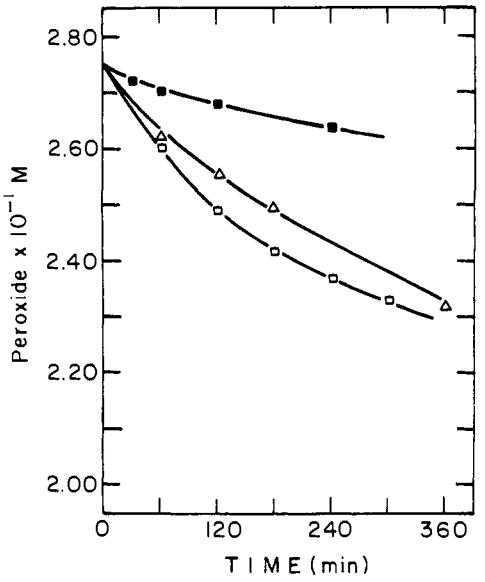


Figure 3. Photodecomposition of di-*tert*-butyl peroxide (2.74 × 10^{-1} *M*) in *tert*-butyl alcohol (λ ≃420–460 nm): (■) in the presence of biacetyl (7.0 × 10^{-2} *M*); (△) in the presence of biacetyl and toluene (1.42 *M*); (□) in the presence of toluene, biacetyl, and Tfa-Gly-Phe-OMe (4.82 × 10^{-2} *M*).

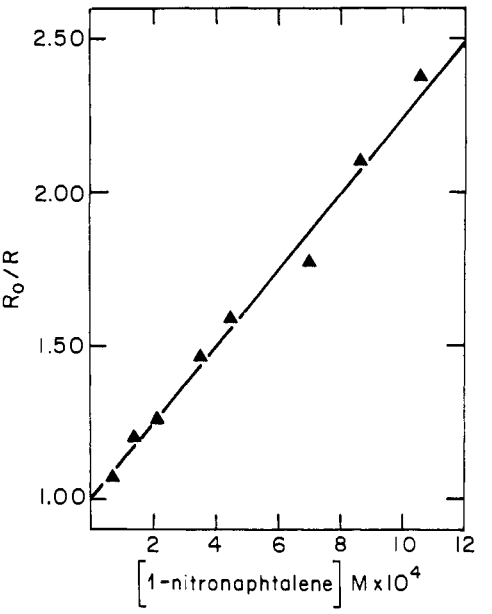


Figure 4. Stern–Volmer plot showing the ratio of the rate of bibenzyl formation in the absence (R_0) to the rate in the presence (R) of 1-nitronaphthalene *vs.* molar concentrations of 1-nitronaphthalene.

step by the diketone to the formation of the appropriate oxy radicals from the peroxide, we investigated the mode of interaction of these compounds under visible light irradiation. We have found that biacetyl is stable in *tert*-butyl alcohol when irradiated with visible light; however, in the presence of di-*tert*-butyl peroxide it is destroyed to a considerable extent (see Figure 2). The addition of toluene to the reaction mixture decreased the rate of decomposition of biacetyl (see Figure 2) and simultaneously increased the rate of disappearance of DBP (see Figure 3). The system behaved similarly when 1-octene replaced toluene and also in the presence of the dipeptide derivative. This behavior can be explained by the toluene (or 1-octene) acting as a scavenger for the free radicals (*e.g.*, *tert*-butoxy radicals) produced, thus avoiding the interaction of the latter with the diketone photosensitizer.[20] Since the light-absorbing system is thus largely protected, the photosensitized decomposition of the peroxide to yield *tert*-butoxy radicals can proceed further.

It seems plausible that the formation of oxy radicals results primarily from interaction of the peroxide with excited diketone, since the decomposition of the peroxide induced by free radicals is quenched in the presence of toluene or an olefin.[21] Furthermore, since intersystem crossing in biacetyl is nearly unity,[22] the triplet state of biacetyl is, most likely, the species involved in this process. In order to clarify this point we used a physical triplet quencher, 1-nitronaphthalene,[23] for quenching the biacetyl triplet (E_T = 55 kcal/mol).[24] Mixtures of biacetyl, DBP, and toluene were irradiated in the presence of varying amounts of 1-nitronaphthalene; the amount of bibenzyl formed during irradiation was the measure of peroxide decomposition to oxy radicals. Quenching of bibenzyl formation was observed upon the progressive addition of 1-nitronaphthalene, and a plot of the reciprocal rate of bibenzyl formation against quencher concentration (Stern–Volmer plot) gave a straight line (Figure 4). Furthermore, total quenching of bibenzyl formation could be achieved by using the appropriate concentration of 1-nitronaphthalene. These results indicate that one excited state is involved in these reactions and that it is the triplet state.

The quantum yield for biacetyl disappearance during the photoalkylation with toluene or an olefin was found to be 0.17, while the decomposition of di-*tert*-butyl peroxide proceeded with a quantum yield of 0.67 (see Experimental Section). These results present a further support to our assumption that the major process leading to oxy radicals is energy transfer from the excited biacetyl, while decomposition of the peroxides by radicals resulting from fragmentation of biacetyl is a minor process. Since excited benzophenone fails to initiate the decomposition of DBP,[7] it seems possible that other mechanisms which eventuate in the regeneration of the diketone, *e.g.*, those involving electron transfer processes, operate in the interaction of the excited α-diketone with peroxides.

The experimental data described in this publication lead us to suggest a pathway from the light-absorption step by the diketone to the formation of the appropriate *tert*-butoxy radicals, and the subsequent generation of amino acid free radicals, which is shown in Scheme II.

Scheme II

$$CH_3COCOCH_3 \xrightarrow[\text{and intersystem crossing}]{h\nu} [CH_3COCOCH_3]^3 \xrightarrow{[(CH_3)_3CO]_2}$$

$$(CH_3)_3C\dot{O} \xrightarrow{-NHCH_2CO^-} -NH\dot{C}HCO- \longrightarrow \text{etc.}$$

(20) *Cf.* W. G. Bentrude and K. R. Darnall, *J. Amer. Chem. Soc.*, 90, 3588 (1968), and references cited therein.
(21) *Cf.* E. S. Huyser and C. J. Bredeweg, *J. Amer. Chem. Soc.*, 86, 2401 (1964).
(22) M. Almgran, *Photochem. Photobiol.*, 6, 829 (1967).
(23) K. Sandros and H. L. J. Backstrom, *Acta Chem. Scand.*, 16, 958 (1962).
(24) T. R. Evans and P. A. Leermakers, *J. Amer. Chem. Soc.*, 89, 4380 (1967).

Finally, the work presented in this publication indicates that the photoalkylation of amino acids, peptides, and proteins can be achieved by the use of a variety of initiation systems, including those operated by visible light, which is not absorbed by any of the common amino acids. The work further demonstrates the selectivity of these reactions for glycine residues, independent of the initiation system, and makes glycine residues in peptides and proteins as potential sites for chemical reactions and cross-linking of proteins with other substrates.

Experimental Section

Chromatography of the dipeptide derivatives was carried out on Kieselgel (0.05–0.2 mm, Merck), while Kieselgel G (Merck) was used for ascending tlc. Mixtures of ethyl acetate–petroleum ether (bp 60–80°) were used as eluents. Spots of dipeptide derivatives were revealed by the *tert*-butyl hypochlorite–potassium iodide–starch reagent. Mixtures of 1-butanol–acetic acid–water (3:1:1, v/v) or 2-butanol:acetic acid:water (5:1:4, v/v, upper phase) were used as eluents for the free amino acids in tlc or paper chromatography. Spots of amino acids were revealed with ninhydrin (0.2% in 1-butanol–ethanol–acetic acid–collidine, 60:60:5:1, v/v). Chromatography of the polypeptides was carried out on columns (100 cm long, 2.5 cm in diameter) of Sephadex (G-25 medium, Pharmacia, Upsala). The eluent was aqueous acetic acid (0.01 *M*) and the flow rate was 1–1.5 ml/min. The void volume of 200 ml was determined with Blue Dextran 2000 (Pharmacia, Upsala). Fractions of 20 ml were collected, and the optical density at 220 or 254 nm was recorded. Amino acid compositions were determined by hydrolysis of peptides in 6 *N* hydrochloric acid at 110° for 22 hr and analysis on a Beckman amino acid analyzer. Values are uncorrected for destruction during hydrolysis. Glpc was performed with a Varian Aerograph 1200-2 instrument using 3% SE-30, 10% Silicon oil DC-710, or 5% QF-1 columns (5 ft × 1/8 in). Nmr spectra were determined with a Varian A-60 instrument in $CDCl_3$ solutions with TMS as internal reference; absorptions are reported in τ values. Mass spectra were recorded with a MAT-Atlas CH4 instrument. Molecular weights of polymer fractions were determined with a Spinco Model E ultracentrifuge using the midpoint method of Yphantis.[13] All measurements were carried out at 22° in 0.2 *N* NaCl solution and at a polymer concentration of 0.8%, except for [L-Pro-L-Pro-Gly]$_n$ which was determined in 12% acetic acid solution.

Experiments were carried out at room temperature in Pyrex tubes (40 cm long, 1 cm in diameter) surrounded with four fluorescent lamps (G. E. C. 20 W) at a distance of 3–5 cm. Oxygen was excluded from the reaction mixture by bubbling nitrogen through the solution followed by evacuation at low pressure. GWV filter tubes (40 cm long, 3 cm in diameter) transmitting light of $\lambda > 370$ nm were frequently used. Experiments with ultraviolet light were carried out in an immersion apparatus with 200-W Hanovia high-pressure mercury vapor lamps which were cooled internally with running water. Pyrex filters were used for these experiments.

Reaction of *N*-Trifluoroacetylglycylglycine Methyl Ester and Toluene. A mixture of *N*-trifluoroacetylglycylglycine methyl ester (400 mg, 1.65 mmol), toluene (5 ml), di-*tert*-butyl peroxide (1 ml), and biacetyl (0.2 ml) in *tert*-butyl alcohol (35 ml) was irradiated for 48 hr. Di-*tert*-butyl peroxide (1 ml) was added twice (after 17 and 40 hr) to the irradiated mixture. The dipeptide composition in the reaction mixture was followed by glpc with the following results.

Time, hr	Tfa-Gly-Gly-OMe, mg	Tfa-Phe-Gly-OMe, mg	Tfa-Gly-Phe-OMe, mg	Tfa-Phe-Phe-OMe, mg
0	400			
17	310	47	41	17
40	250	72	61	25

Amino acid analysis of a hydrolyzed sample after 48 hr showed the presence of glycine (1.81 mmol) and phenylalanine (0.39 mmol). Excess of reagents were removed under reduced pressure, and the oily residue (1.27 g) was chromatographed on silica gel (60 g). Ethyl acetate–petroleum ether (1:20) eluted bibenzyl (190 mg) and 1,2,3-triphenylpropane (80 mg) followed by an unidentified nonpeptide oil (100 mg). The same solvent (1:3) then eluted crude *N*-trifluoroacetylphenylalanylphenylalanine methyl ester which was purified to yield 25 mg of pure product: nmr τ 2.5 (1 H, m, NH), 2.77 (10 H, m, aromatic), 3.58 (1 H, m, NH), 5.23 (2 H, m, NH-C*H*ArCO), 6.29, 7.32 (3 H, two diasteriomeric CO_2CH_3), and 6.97 (4 H, m, CH_2Ar). Ethyl acetate–petroleum ether (3:5) eluted a mixture of *N*-trifluoroacetylphenylalanylglycine methyl ester (62 mg) and *N*-trifluoroacetylglycylphenylalanine methyl ester (58 mg) (determined by glpc). Further elution [ethyl acetate–petroleum ether (1:1)] afforded starting protected dipeptide (205 mg). A mixture of acetone–methanol (1:1) eluted a solid (57 mg) which contained glycine as the sole amino acid (acid hydrolysis and paper chromatography).

Two parallel experiments, one without the peroxide and the other without biacetyl, carried out under similar conditions, did not show any product formation (glpc) after 60 hr of irradiation.

Reaction of *N*-Trifluoroacetylglycylglycine Methyl Ester and 1-Butene. A solution of *N*-trifluoroacetylglycylglycine methyl ester (400 mg, 1.65 mmol), di-*tert*-butyl peroxide (1 ml), and biacetyl (0.2 ml) in *tert*-butyl alcohol (40 ml) was irradiated for 60 hr while 1-butene was bubbled through the solution. Additional peroxide (1 ml) and biacetyl (0.2 ml) were added after 17 and 40 hr. Excess of reagents were removed under reduced pressure and the oily residue was dissolved in ethanol (40 ml) and shaken overnight with 5% palladium–charcoal (400 mg) under hydrogen at atmospheric pressure to remove any unsaturation in the new amino acid. The amino acid composition of the reaction mixture was then determined with an amino acid analyzer with the following results: glycine (2.5 mmol) and norleucine (0.11 mmol). The solvent was removed under reduced pressure and the residue (1 g) was chromatographed on silica gel (50 g). Ethyl acetate–petroleum ether (1:9) eluted an oil (440 mg) which contained mostly polymers of 1-butene. Further elution [ethyl acetate–petroleum ether (1:4)] gave an unidentified peptide oil (55 mg) which upon hydrolysis yielded glycine as the sole amino acid. This was followed by a telomeric mixture [75 mg, eluted with ethyl acetate–petroleum ether (1:3)] which on hydrolysis afforded glycine (0.034 mmol) and a C_{10}-α-amino acid. *N*-Trifluoroacetylnorleucylglycine methyl ester (15 mg) was eluted with ethyl acetate–petroleum ether (1:2) followed by *N*-trifluoroacetylglycylnorleucine methyl ester (17 mg). Ethyl acetate–petroleum ether (3:7) then eluted an unidentified oil (41 mg) which upon hydrolysis yielded glycine as the sole amino acid (paper chromatography). Finally, the same solvent mixture (3:5) eluted starting material, *N*-trifluoroacetylglycylglycine methyl ester (205 mg).

Reaction of *N*-Trifluoroacetylglycyl-L-valine Methyl Ester and Toluene. The reaction was carried out as described above using *N*-trifluoroacetylglycyl-L-valine methyl ester (568 mg, 2 mmol). Amino acid analysis of the reaction mixture after 48 hr irradiation showed glycine (1.2 mmol), valine (1.4 mmol), and phenylanaine (0.2 mmol). The usual work-up and chromatography on silica gel gave bibenzyl (180 mg), 1,2,3-triphenylpropane (60 mg), an unidentified nonpeptide oil (40 mg), and *N*-trifluoroacetylphenylalanyl-L-valine methyl ester (85 mg): mp 120–138° (from acetone–petroleum ether); nmr τ 2.72 (1 H, m, NH), (5 H, apparent s, aromatic), 3.45 (1 H, m, NH), 5.15 (1 H, m, NHC*H*ArCO), 5.4, 5.6 (1 H, dd, J = 5 Hz, NHC*H*RCO), 6.26 (3 H, s, CO_2CH_3), 6.86 (2 H, d, J = 7 Hz, CH_2Ph), 8.02 [1 H, m, RC*H*$(CH_3)_2$], and 9.10, 9.18 [6 H, dd, J = 2.5 Hz, RCH(CH_3)$_2$].

Anal. Calcd for $C_{17}H_{21}N_2O_4F_3$: C, 54.54; H, 5.66; N, 7.48; mol wt, 374. Found: C, 54.56; H, 5.90; N, 7.51; mol wt, 374 (mass spectrum).

This was followed by starting protected dipeptide (450 mg) and polar fractions (47 mg), which contained glycine and valine as the only amino acids (acid hydrolysis and paper chromatography).

Reaction of *N*-Trifluoroacetylglycyl-L-phenylalanine Methyl Ester and Toluene. The reaction was carried out as already described using *N*-trifluoroacetylglycyl-L-phenylalanine methyl ester (664 mg, 2 mmol). The usual work-up and chromatography on silica gel led to bibenzyl (300 mg), 1,2,3-triphenylpropane (40 mg), and *N*-trifluoroacetylphenylalanyl-L-phenylalanine methyl ester (110 mg), mp 154–158° (from acetone–petroleum ether). These were followed by *N*-trifluoroacetylglycyl-α-benzylphenylalanine methyl ester (35 mg): mp 143–144° (from acetone–pentane, at −18°); nmr τ 2.82 (11 H, m, aromatic and NH), 3.67 (1 H, broad s, NH), 6.19 (3 H, s, CO_2CH_3), and 6.42 (4 H, dd, J = 22 Hz, JJ = 54 Hz, CH_2Ph).

Anal. Calcd for $C_{21}H_{21}N_2O_4F_3$: C, 59.71; H, 5.01; N, 6.63; mol wt, 422. Found: C, 59.95; H, 5.01; N, 6.99; mol wt, 422 (mass spectrum).

Further elution gave starting protected dipeptide (455 mg), and a polar fraction (15 mg), which contained glycine and phenylalanine (acid hydrolysis and paper chromatography).

Reaction of *N*-Trifluoroacetylphenylalanylglycine Methyl Ester and *p*-Methoxytoluene. A mixture of *N*-trifluoroacetylphenylalanylglycine methyl ester (664 mg, 2 mmol), *p*-methoxytoluene (2.5 ml), di-*tert*-butyl peroxide (2 ml), and biacetyl (0.3 ml) in *tert*-butyl alcohol (35 ml) was irradiated for 30 hr. *p*-Methoxytoluene (1 ml) and peroxide (2 ml) were added and the irradiation was continued for an additional 30 hr. Excess of reagents was removed under reduced pressure and the oily residue (2.88 g) was chromatographed on silica gel (150 g). Elution with ethyl acetate–petroleum ether afforded *p,p'*-dimethoxybibenzyl (130 mg), unidentified nonpeptidic oils (600 mg), and *N*-trifluoroacetylphenylalanyltyrosine methyl ether methyl ester (170 mg): mp 142–145° (from ethyl acetate–petroleum ether); nmr τ 2.48 (1 H, m, NH), 2.75 (5 H, m, C_6H_5 aromatic), 3.2 (4 H, m, MeO-C_6H_4 aromatic), 3.63 (1 H, m, NH), 5.30 (2 H, m, NHC*H*ArCO), 6.25, 6.30 (6 H, s, s, Ar-OCH_3 and CO_2CH_3), and 7.0 (4 H, m, CH_2Ar).

Anal. Calcd for $C_{22}H_{23}N_2O_5F_3$: C, 58.36; H, 5.12; N, 6.19; mol wt, 452. Found: C, 58.04; H, 5.26; N, 6.31; mol wt, 452 (mass spectrum).

Starting protected dipeptide (500 mg) was eluted next.

Reaction of *N*-Trifluoroacetylglycylglycine Methyl Ester and Acetic Acid Anhydride. A mixture of *N*-trifluoroacetylglycylglycine methyl ester (420 mg, 1.74 mmol), acetic acid anhydride (8 ml), di-*tert*-butyl peroxide (1.5 ml), and biacetyl (0.6 ml) in *tert*-butyl alcohol (30 ml) was irradiated for 40 hr. Amino acid analysis of the reaction mixture showed glycine (2.3 mmol) and aspartic acid (0.25 mmol). Excess of reagents was removed under reduced pressure and the residue was dissolved in absolute methanol (50 ml) and treated with dry hydrogen chloride gas for 1 hr. The solvent was removed and the residue (500 mg) was chromatographed on silica gel in the usual way. Ethyl acetate–petroleum ether (1:3) eluted an unidentified nonpeptidic oil (150 mg). The same solvent mixture (1:2) eluted crude *N*-trifluoroacetylglycylaspartic acid dimethyl ester (32 mg): mp 90–91° (from acetone–hexane); nmr τ 2.07 (1 H, m, NH), 2.56 (1 H, m, NH), 5.08 (1 H, m, NHC*H*RCO), 5.84 (2 H, d, J = 5 Hz, NHCH_2CO), 6.23, 6.32 (6 H, s, s, CO_2CH_3), and 7.03 (2 H, apparent dd, RC$H_2CO_2CH_3$).

Anal. Calcd for $C_{10}H_{13}N_2O_6F_3$: C, 38.22; H, 4.17; N, 8.92; mol wt, 314. Found: C, 38.46; H, 4.24; N, 8.67; mol wt, 314 (mass spectrum).

This was followed by *N*-trifluoroacetylaspartylglycine dimethyl ester (38 mg): mp 138–139° (from acetone–hexane); nmr (*d*-MeOH), τ 5.08 (1 H, m, NHC*H*RCO), 5.98 (2 H, d, J = 3 Hz, NHCH_2CO), 6.27, 6.29 (6 H, s, s, CO_2CH_3), and 7.12 (2 H, apparent dd, RC$H_2CO_2CH_3$).

Anal. Calcd for $C_{10}H_{13}N_2O_6F_3$: C, 38.22; H, 4.17; N, 8.92; mol wt, 314. Found: C, 38.54; H, 4.49; N, 8.27; mol wt, 314 (mass spectrum).

Finally were eluted starting protected dipeptide (250 mg) and a polar residue (50 mg) which contained glycine as the only amino acid.

Reaction of *N*-Trifluoroacetylglycyl-L-aspartic Acid Dimethyl Ester and Toluene (with D-Camphorquinone). A mixture of *N*-trifluoroacetylglycyl-L-aspartic acid dimethyl ester (628 mg, 2 mmol), toluene (5 ml), di-*tert*-butyl peroxide (2 ml), and D-camphorquinone (300 mg) in *tert*-butyl alcohol (35 ml) was irradiated for 48 hr. Additional toluene (1 ml), peroxide (2 ml), and D-camphorquinone (100 mg) were added during irradiation. Amino acid analysis of the reaction mixture showed glycine (1.4 mmol), aspartic acid (1.6 mmol), and phenylalanine (0.2 mmol). The usual work-up and chromatography on silica gel led to bibenzyl (90 mg), 1,2,3-triphenylpropane (20 mg), unidentified nonpeptidic oil (140 mg), D-camphorquinone (80 mg), and *N*-trifluoroacetylphenylalanyl-L-aspartic acid dimethyl ester (80 mg): mp 115–118° (from acetone–hexane); nmr τ 2.72 (5 H, apparent d, aromatic protons superimposed on 1 H, m, NH), 5.18 (2 H, m, NHC*H*RCO, NHC*H*CH_2PhCO), 6.30, 6.33 (6 H, two d, J = 2.5 Hz and J = 1.5 Hz, diasteriomeric CO_2CH_3), 6.83 (2 H, m, CH_2Ph), and 7.25 (2 H, apparent dd, RC$H_2CO_2CH_3$).

Anal. Calcd for $C_{17}H_{19}N_2O_6F_3$: C, 50.49; H, 4.73; N, 6.93; mol wt, 404. Found: C, 50.21; H, 4.50; N, 6.93; mol wt, 404 (mass spectrum).

Starting protected dipeptide (400 mg) was eluted next. Acetone–ethyl acetate (1:1) eluted a polar oil (60 mg) which contained glycine and aspartic acid as the only amino acids.

Reaction of [L-Pro-Gly-L-Pro]$_n$ and Toluene. A mixture of the polypeptide (25 mg), di-*tert*-butyl peroxide (0.4 ml), biacetyl (0.06 ml), toluene (0.7 ml), water (1 ml), and *tert*-butyl alcohol (5 ml) was irradiated for 30 hr. Toluene (0.4 ml), peroxide (0.2 ml), and biacetyl (0.03 ml) were added to the reaction mixture during the irradiation. The solvent was removed under reduced pressure and the residue was washed with acetone and methylene chloride. The solid (22 mg) was chromatographed on Sephadex G-25 and the fractions of high molecular weight were combined and were filtered through a Diaflo UM-2 membrane. Lyophilization gave a polypeptide (18 mg; mol wt, 2800) which showed the following amino acid composition (residues/100 total residues): Pro (64.8), Gly (32.7), and Phe (2.5).

Reaction of [L-Ala-L-Pro-Gly]$_n$ and Toluene. A mixture of the polypeptide (20 mg; mol wt, 3020), toluene (0.8 ml), di-*tert*-butyl peroxide (0.3 ml), biacetyl (0.05 ml), water (1.7 ml), and *tert*-butyl alcohol (5 ml) was irradiated for 14 hr. The solvent was removed under reduced pressure and the remaining polypeptide was precipitated from a small volume of methanol with acetone. The solid was filtered on a Sepheadex G-25 column and the high molecular weight fractions were combined and dialyzed. Lyophilization gave the pure polypeptide (16 mg; mol wt, 3200) which contained Ala (33.4), Pro (33.4), Gly (32.1), and Phe (1.0) (residues/100 total residues).

Reaction of [L-Pro-Gly-L-Pro]$_n$ with Isobutylene. A mixture of the polypeptide (125 mg; mol wt, 2200), di-*tert*-butyl peroxide (2 ml), biacetyl (0.5 ml), water (4 ml), and *tert*-butyl alcohol (30 ml) was irradiated for 70 hr while isobutylene was bubbled through. The usual work-up gave a polypeptide (100 mg; mol wt, 2050) with the amino acid composition of Gly (30.6), Pro (68.7), and Leu (0.7).

Reaction of (DL-Ala)$_m$(Gly)$_n$ (m:n = 4:1) and Toluene. (a) In Solution. The reaction was carried out as described above with 40 mg of the polypeptide (mol wt, 3750). The usual work-up led to an insoluble peptide (5 mg) containing (residue/100) Ala (79.0) and Gly (21.0) and a soluble fraction (29 mg; mol wt, 3800) containing Ala (76.0), Gly (23.0), and Phe (1.0).

(b) In Suspension. A suspension of the polypeptide (40 mg; mol wt, 6350) in a mixture of di-*tert*-butyl peroxide (0.8 ml), biacetyl (0.2 ml), toluene (2 ml), and *tert*-butyl alcohol (25 ml) was irradiated for 16 hr. Toluene (1 ml) and di-*tert*-butyl peroxide (0.4 ml) were added during the irradiation. Solvents were removed under reduced pressure and the residue was washed with ether and *tert*-butyl alcohol. The residue (35 mg) was suspended in water (2 ml) and centrifuged to separate the insoluble fraction (8 mg), which showed the following amino acid composition: Ala (77.8), Gly (17.8), and Phe (4.4). The soluble fraction was chromatographed on Sephadex and the high molecular weight fractions were dialyzed and lyophilized giving a peptide (25 mg; mol wt, 6000) with an amino acid composition of Ala (82.8), Gly (16.4), and Phe (0.8).

Preparation of α,α-Disubstituted Amino Acid Derivatives. *N*-Trifluoroacetyl-α-benzylphenylalanine Methyl Ester. A mixture of *N*-trifluoroacetylphenylalanine methyl ester (11 g, 0.04 mol), toluene (50 ml), di-*tert*-butyl peroxide (15 ml), and *tert*-butyl alcohol (150 ml) was irradiated (Hanovia 200-W high-pressure mercury vapor lamp; Pyrex filter) for 40 hr. The usual work-up and chromatography on silica gel in petroleum ether–ethyl acetate led to bibenzyl (8 g), 1,2,3-triphenylpropane (2.5 g), *N*-trifluoroacetyl-α-benzylphenylalanine methyl ester (2.5 g), and starting material (6.5 g). *N*-Trifluoroacetyl-α-benzylphenylalanine methyl ester exhibited: mp 98–100° (from pentane, at −18°); nmr τ 2.86 (11 H, m, aromatic and NH), 6.03, 6.17 (4 H, dd, J = 14 Hz, CH_2Ar), and 6.17 (3 H, s, CO_2CH_3).

Anal. Calcd for $C_{19}H_{18}NO_3F_3$: C, 62.46; H, 4.97; N, 3.83; mol wt, 365. Found: C, 62.57; H, 4.98; N, 3.70; mol wt, 365 (mass spectrum).

Other α,α-disubstituted amino acids were prepared similarly. *N*-Trifluoroacetyl-α-(*p*-fluorobenzyl)phenylalanine methyl ester showed: mp 68–69° (from pentane, at −18°); nmr τ 2.72–3.12 (10 H, m, aromatic and NH), 6.10, 6.74 (4 H, dd, J = 14 Hz, CH_2Ar), and 6.22 (3 H, s, CO_2CH_3); mass spectrum m/e 383 (M^+), 364 (M^+ − F), 352 (M^+ − OCH_3), 324 (M^+ − $COOCH_3$), 292 (M^+ − CH_2Ph), 274 (M^+ − CH_2Ph − F), 270 (M^+ − CF_3CONH_2), 260 (292 − CH_3OH), 242 (274 − CH_3OH), 211 (270 − $COOCH_3$), 109 (CH_2Ph^+ − F), 91 ($C_7H_7^+$).

N-Trifluoroacetyl-α-benzylvaline methyl ester exhibited: bp 136–140° (0.5 mm); nmr τ 2.72–3.08 (6 H, m, aromatic and NH), 6.12 (3 H, s, CO_2CH_3), 6.17, 6.41 (2 H, dd, J = 14 Hz, CH_2Ar), 7.10 [1 H, m, RC*H*$(CH_3)_2$], and 8.81, 9.07 (6 H, dd, J = 7 Hz, RCH$(CH_3)_2$); mass spectrum m/e 317 (M^+), 298 (M^+ − F), 286 (M^+ − OCH_3), 274 (M^+ − C_3H_7), 258 (M^+ − $COOCH_3$), 226 (M^+ − CH_2Ph), 214 (242 − CO), 204 (PhCH=C$(C_3H_7)$$COOCH_3$), 194 (226 − CH_3OH), 189 (M^+ − CF_3), 172 (204 − CH_3OH), 166 (194 − CO), 145 (204 − $COOCH_3$), 91 ($C_7H_7^+$).

N-Trifluoroacetylglycyl-α-benzylvaline methyl ester exhibited: mp 152–153° (from acetone–pentane); nmr τ 2.72–3.08 (6 H, m,

aromatic and NH), 3.38 (1 H, m, NH), 6.14 (3 H, s, CO_2CH_3 superimposed on 2 H, d, $NHCH_2CO$), 6.20, 6.65 (2 H, dd, J = 14 Hz, CH_2Ph), 7.17 (1 H, m, $RCH(CH_3)_2$), and 8.81, 9.07 (6 H, dd, J = 7 Hz, $RCH(CH_3)_2$); mass spectrum m/e 374 (M^+), 355 ($M^+ - F$), 331 ($M^+ - C_3H_7$), 327 (359 $- CH_3OH$), 315 ($M^+ - COOCH_3$), 305 ($M^+ - CF_3$), 283 ($M^+ - CH_2 - Ph$), 251 (283 $- CH_3OH$), 204 ($PhCH{=}C(C_3H_7)COOCH_3$), 162 ($^+NH_2{=}C(C_3H_7)$-CH_2Ph), 145 (204 $- COOCH_3$), 91 ($C_7H_7^+$).

Determination of Selectivity in Reactions of Tripeptides. A mixture of the peptide (100 mg), di-*tert*-butyl peroxide (0.5 ml), biacetyl (0.05 ml), and the alkylating reagent (toluene or 1-butene, 1.2 g) in *tert*-butyl alcohol (10 ml) was irradiated for 30 hr (10–20% conversion). The solvent was removed under reduced pressure and the residue was hydrolyzed in the usual way. The hydrolyzate was washed with chloroform and evaporated to dryness, and the residue was heated to reflux in 1 N HCl in methanol (40 ml) for 30 min. The solvent was evaporated and the resulting mixture of ester hydrochlorides was dissolved in methylene chloride (20 ml), treated with trifluoroacetic anhydride (3 ml) for 2 hr at room temperature, and evaporated to dryness. The mixture of N-trifluoroacetyl methyl ester derivatives thus obtained was dissolved in methylene chloride and analyzed by glpc. Qualitative and quantitative determinations were achieved by using authentic samples of the appropriate derivatives of the individual amino acids.

Determinations of selectivity in reactions of other peptides were run in a similar manner. In reactions of Tfa-Gly-OMe and other amino acid derivatives, samples from the reaction mixtures were injected and determined directly by glpc, using authentic samples as standards.

Determination of Asymmetric Induction in the Reaction of [L-Pro-Gly-L-Pro]$_n$ and Toluene. (a) With L-Amino Acid Oxidase. The reaction was carried out as described above. A sample of the irradiated polypeptide (10 mg) was hydrolyzed in 6 N hydrochloric acid in the usual way. The solvent was evaporated and the residue was dissolved in 0.15 M Tris–HCl buffer, pH 7.8 (2 ml); 0.37×10^{-3} M solution of D-valine (0.5 ml) and a 0.6×10^{-3} M solution of DL-norleucine (0.5 ml) in the same buffer were added as internal standards. D-Valine served as an internal standard for the undigestable D enantiomer, while DL-norleucine served as a standard for the digestable L enantiomer. An aliquot of the resulting mixture (0.5 ml) was added to a citrate buffer, pH 2.2 (0.5 ml), and the amino acid composition was determined on an amino acid analyzer at 0 hr. A solution of L-amino acid oxidase (Sigma) 10 λ; 6 mg/ml; 6.5 units/mg protein) was then added to the mixture which was left at room temperature. Aliquots (0.5 ml) were analyzed periodically on the amino acid analyzer. A typical example is given in Table V. The phenylalanine residues in the products were found to consist of 35% of the L enantiomer and 65% of the D enantiomer.

Table V

Time, hr	Amino acid, mmol		
	D-Val	DL-Nle	Phe
0	0.185	0.300	0.287
20	0.188	0.156	0.193
40	0.185	0.142	0.193
70	0.184	0.145	0.187

All other reactions and determinations of asymmetric induction were carried out similarly.

(b) By Glpc. A sample of the polypeptide (10 mg) was hydrolyzed with 6 N hydrochloric acid in the usual way. The acid was removed under reduced pressure and the residue was esterified with 1 N HCl in methanol and trifluoroacetylated as described above. The mixture of enantiomers of phenylalanine was resolved on a capillary column (100 ft × 0.02 in.) coated with Tfa-L-Phe-L-Leu cyclohexyl ester.[19] The mixture was shown to consist of 65.5% D-Phe and 34.5% L-Phe.

Quantum Yield Determination. A reaction mixture consisting of N-trifluoroacetylglycylphenylalanine methyl ester (4.8×10^{-2} M), toluene (1.42 M), di-*tert*-butyl peroxide (2.74×10^{-1} M), biacetyl (7×10^{-2} M) in *tert*-butyl alcohol was irradiated (fluorescent lamps with a Corning 5850 filter, transmitting light of $\lambda \simeq 420$–460 nm) in a Pyrex spectroscopic cell (1-cm path) at room temperature. Light intensity and absorption were measured by ferric oxalate actinometry[25] with a similar cell placed in front and behind the reaction cell. Nearly 90% of the light emitted at this region was absorbed by the reaction mixture under these conditions. The disappearance of biacetyl was followed spectrophotometrically as well as by glpc. The disappearance of the peroxide and the starting peptide, as well as the formation of the new peptide, were followed by glpc. (A 10% DC-710 column was used for the peptides and a 20% hallocomide column at 52° for the peroxide and biacetyl.) The rate of decomposition of the peroxide was found to be 13.33×10^{-5} mol $l.^{-1}$ min^{-1}, while 19.86×10^{-5} einstein $l.^{-1}$ min^{-1} was absorbed. The quantum yield for the decomposition of the peroxide under the conditions of the reactions was found to be $\phi_{dec} = 0.67$, that for the decomposition of biacetyl was $\phi_{dec} = 0.17$, while that for the formation of the alkylated peptide was $\phi = 0.084$.

Determination of Decomposition of Biacetyl and Di-*tert*-butyl Peroxide. Solutions of biacetyl with the appropriate mixtures of the reagents in the photoalkylation reactions were irradiated in sealed Pyrex cells (7 × 35 mm) surrounded by fluorescent lamps and a Corning 5850 filter. The initial concentrations of the reactants were: biacetyl (7×10^{-2} M), di-*tert*-butyl peroxide (2.74×10^{-1} M), toluene (1.42 M), and 1-octene (9×10^{-1} M). *tert*-Butyl alcohol served as solvent. The decomposition of biacetyl and di-*tert*-butyl peroxide was followed by glpc with *p*-xylene as standard. Biacetyl was also determined spectrophotometrically. Results of these experiments are described in Figures 2 and 3.

Quenching Experiments. Solutions of biacetyl (5.2×10^{-2} M), di-*tert*-butyl peroxide (2.36×10^{-1} M), toluene (1.0 M), and 1-nitronaphthalene (0–10.5×10^{-4} M) in benzene were irradiated (fluorescent lamps and a Corning GG 435 filter) in a Pyrex cell (1-cm path) at room temperature for 30 min. The formation of bibenzyl, as well as the amount of 1-nitronaphthalene, were determined by glpc (5% QF-1 column; *p,p'*-difluorobibenzyl was used as the internal standard for these measurements, and precision was up to 2–3%). No substantial change in biacetyl absorption or 1-nitronaphthalene concentration could be observed during the irradiation. A plot of the reciprocal rate of formation of bibenzyl against quencher concentration (Stern-Volmer plot) gave a straight line (Figure 4).

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(25) C. A. Parker, *Proc. Roy. Soc., Ser. A*, 220, 104 (1953).