N, 6.36; S, 29.11. Found: C, 49.33; H, 4.69; N, 6.46; S, 29.38.

Fractions 13–17 gave 0.12 g. of an uncharacterized oily solid. Fractions 18 and 19 provided 0.12 g. (16.2%) of the trisulfide VIIIa, m.p. 108–111°. A mixture melting point with an authentic sample was not depressed.

Preparation of Methyl N-Carbobenzoxy-L-cysteinate. A solution of 2.78 g. (0.01 mole) of N-carbobenzoxy-L-cysteine[17] in 15 ml. of dry methanol was treated with 5 drops of concentrated sulfuric acid and allowed to stand at room temperature for 20 hr. The solution was concentrated *in vacuo* and the residue was extracted with ether. The extract was washed with 3% sodium bicarbonate solution and water. The dried extract was concentrated *in vacuo* to provide 2.52 g. (86%) of ester as a clear, viscous oil. The substance exhibited a single spot on the thin layer chromatogram.

Preparation of IIId by Esterification of IIIc. Treatment of an ether suspension of 0.150 g. (0.29 mmole) of IIIc with excess diazomethane solution provided 0.06 g. (39.6%) of IIId, m.p. 67–69°. One recrystallization from methanol–hexane raised the melting point to 71–74°. A mixture melting point with the material obtained from the thiocyanogen reaction melted at 71–74°.

Preparation of IIIe. A solution of 0.091 g. (0.44 mmole) of DCC, 0.165 g. (0.40 mmole) of glycine benzhydryl ester *p*-toluenesulfonic acid salt,[18] and 0.041 g. (0.40 mmole) of triethylamine in 8 ml. of methylene chloride was treated with a solution containing 0.211 g. (0.4 mmole) of IIIc in 7 ml. of methylene chloride. The mixture was stirred in the cold for 1 hr. and at room temperature overnight. The filtrate was washed with successive 30-ml. portions of 1% sodium bicarbonate solution and 0.5 *N* hydrochloric acid. The solution was dried and concentrated *in vacuo* to yield 0.3 g. of white solid. Recrystallization from an ethanol–hexane mixture provided 0.205 g. (68.3%) of IIIe, m.p. 87–88°. The analytical sample was prepared by filtering a solution of IIIe through a short Florisil column followed by recrystallization of the resulting solid from an ethanol–hexane mixture: m.p. 87.5–88.5°, $[\alpha]^{24}D$ −17.8° (*c* 1.0, dioxane); ν_{max}^{KBr} 3414, 1748, 1718, and 1664 cm.$^{-1}$.

Anal. Calcd. for $C_{37}H_{39}N_3O_6S_4$: C, 59.25; H, 5.24; N, 5.60; S, 17.10. Found: C, 59.11; H, 5.54; N, 5.39; S, 17.12.

Cyanide Ion Cleavage of IIIf. Treatment of a solution of 0.486 g. (0.001 mole) of IIIf in 40 ml. of acetonitrile with 0.060 g. (0.001 mole) of sodium cyanide provided a black solution. Thin layer chromatography (benzene–ethyl acetate, 1:1) of the reaction mixture after 3 hr. showed the presence of at least four compounds. After 11 hr. the reaction mixture was worked up as previously described.[19] The only pure component of the reaction mixture which could be isolated by chromatography on Florisil was identified as 2,4-dinitrophenyl benzyl sulfide, 0.130 g. (44.7%), m.p. 124–126°. A mixture melting point with an authentic sample was 127–129°. The infrared spectrum of the substance was identical with that of the authentic sulfide and different from the infrared spectrum of 2,4-dinitrophenyl benzyl disulfide.

Treatment of Va with Sodium Acetate. Anhydrous sodium acetate, 0.082 g. (0.001 mole), was added to a solution of 0.516 g. (0.001 mole) of Va in 10 ml. of methylene chloride. The mixture was stirred at 5° for 1 hr. and at room temperature for 1 hr. Work-up in the usual manner provided 0.470 g. (91.2%) of Va, m.p. 99.5–101°. A mixture melting point with authentic Va was not depressed. A similar experiment using sodium thiocyanate provided 0.470 g. (91.2%) of recovered Va.

(17) C. A. Bunton, J. N. E. Day, R. H. Flowers, P. Sheel, and J. L Wood, *J. Chem. Soc.*, 963 (1957).

(18) Prepared by Mr. J. T. Staples of this laboratory.

(19) R. G. Hiskey, W. H. Bowers, and D. N. Harpp, *J. Am. Chem. Soc.*, 86, 2010 (1964).

Sulfur-Containing Polypeptides. I. Use of the N-Benzhydryloxycarbonyl Group and the Benzhydryl Ester[1,2]

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Contribution from the Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina. *Received May 8, 1965*

The N-benzhydryloxycarbonyl (BhOC) group has been utilized in the synthesis of several peptides. The group is introduced via the stable, solid reagent, benzhydryl azidoformate. The group is removed by mild acid hydrolysis and is stable to many conditions encountered in peptide synthesis. The benzhydryl ester has been found useful in conjunction with the BhOC group.

Introduction

Since the introduction of the carbobenzoxy fragment as an amino-protecting group in peptide synthesis a number of analogous groups have been utilized.[4] While several reagents have found limited application, none surpasses the general applicability of carbobenzoxy chloride. There are, however, circumstances in which the N-carbobenzoxy group is

(1) Supported in part by Grant A-3416 from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

(2) Abstracted in part from a dissertation submitted by J. B. Adams, Jr., to the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the Ph.D. degree, June 1965.

(3) Shell Chemical Corporation Fellow, 1963–1964.

(4) Several excellent reviews on amino-protecting groups have appeared, including: (a) R. A. Boissonnas in "Advances in Organic Chemistry: Methods and Results," Vol. 3, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y. 1963, p. 183; (b) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 885.

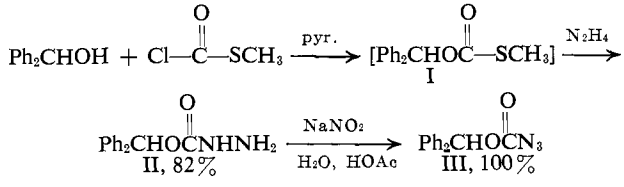
Table I. N-Benzhydryloxycarbonyl-L-amino Acids

Compound[a]	M.p., °C.	Yield, %	$[\alpha]^{24}D$, deg.	Base
N-BhOC-L-phenylalanine[b]	194–195	60	+54.8 (*c* 0.33, $CHCl_3$)	$NaHCO_3$
N-BhOC-L-alanine[c]	188–189	83	+9.5 (*c* 0.4, $CHCl_3$)	MgO
N-BhOC-L-valine[d]	156–157	54	+6.1 (*c* 0.39, $CHCl_3$)	$NaHCO_3$
N-BhOC-S-benzhydryl-L-cysteine[e]	140	61	−3.05 (*c* 1.36, C_2H_5OH)	$NaHCO_3$

[a] The BhOC amino acids were prepared by the general method described for N-BhOC-glycine and were isolated as the N,N-dicyclohexylammonium salts. [b] *Anal.* Calcd. for $C_{35}H_{44}N_2O_4$: C, 75.50; H, 7.97; N, 5.03. Found: C, 75.29; H, 7.90; N, 5.05. Recrystallized from acetone–chloroform–ether. [c] *Anal.* Calcd. for $C_{23}H_{40}N_2O_4$: C, 72.47; H, 8.39; N, 5.83. Found: C, 72.39; H, 8.23; N, 5.85. Recrystallized from acetone–chloroform–ether. [d] *Anal.* Calcd. for $C_{31}H_{44}N_2O_4$: C, 73.19; H, 8.72; N, 5.51. Found: C, 73.00; H, 8.45; N, 5.52. Recrystallized from acetone–chloroform–ether. [e] *Anal.* Calcd. for $C_{42}H_{50}N_2O_4S$: C, 74.30; H, 7.42; N, 4.13; S, 4.72. Found: C, 74.14; H, 7.33; N, 4.16; S, 4.75. Recrystallized from acetone–ether–petroleum ether.

clearly not the blocking group of choice. For example, in connection with our program involving the synthesis of unsymmetrical cystine peptides, carboxylic acid-protecting groups which could be removed under mild acidic conditions were required. In these molecules conditions forceful enough to remove the N-carbobenzoxy group invariably caused simultaneous removal of the ester carboxy-protecting group. Further attempts to remove the N-carbobenzoxy group from simple unsymmetrical cystine peptides using either liquid hydrogen bromide or trifluoroacetic acid have given sizable quantities of the corresponding symmetrical cystine derivatives.[5] In view of these difficulties the development of amino- and carboxy-protecting groups which could be utilized for the synthesis of various deblocked, unsymmetrical cystine peptides was essential. The present report concerns the use of the benzhydryloxycarbonyl (BhOC) group as a amino-protecting group and the benzhydryl ester as a carboxy-protecting function.

Amino Group Protection. Despite the wide assortment of amino-protecting groups, only about five can be incorporated directly into amino acids, used under the conditions of peptide formation and purification, and subsequently removed under milder acidic conditions[6–8] than those required for removal of the N-carbobenzoxy group. These include the formyl,[4] trityl,[4] *p*-methoxybenzyl,[9] *o*-nitrophenylsulfenyl,[10] and *t*-butyloxycarbonyl[11] groups. Of these groups the *t*-butyloxycarboxyl has been most widely used; in the present discussion the N-benzhydryloxycarbonyl (BhOC) fragment will be compared with this group. Benzhydryloxycarbonylation of amino acids can be achieved, under mildly basic conditions, by the use of benzhydryl azidoformate (III). This reagent is a stable solid, easily prepared in quantity and excellent over-all yield from benzhydryl methylthiolcarbonate (I) *via* benzhydryloxycarbonylhydrazide (II). The intermediates in this sequence are solids and, in our opinion, more easily accessible than *t*-butyl azidoformate[12] and its corresponding precursors.[13] Several N-benzhydryloxycarbonylamino acids were prepared from III with either magnesium oxide or sodium bicarbonate as the added base. The results of these preparations are given in Table I.



In order to investigate the stability of the N-benzhydryloxycarbonyl group, benzhydryl carbanilate (IV) and N-benzhydryloxycarbonylglycine (V) were subjected to a variety of reaction conditions. The results of these experiments are described in Tables II and III.

Table II. Cleavage Studies on Benzhydryl Carbanilate[a]

Cleavage conditions	Temp., °C.	Time, min.	% recovery of BhOC-NHC_6H_5 or % yield (Y) of $C_6H_5NH_3^+ Cl^-$
1 *N* HCl–THF	*b*	30	8.5 (Y)[f]
4 *N* HCl–THF	*b*	15	73 (Y)[f]
4 *N* HCl–THF	*b*	30	83 (Y)[f]
4 *N* HCl–THF	*b*	60	94 (Y)[f]
Satd. HCl–ether	*b*	60	98 (Y)[f]
Satd. HCl–HOAc	*b*	5	97 (Y)[f]
CF_3CO_2H	0	5	79 (Y)[f]
HOAc–EtOAc (2:1)	40	30	100 (R)[g]
N_2H_4–CH_3OH	*c*	60	100 (R)[h]
HOAc	*d*	5	100 (R)[h]
4 *N* NaOH–dioxane	*e*	60	100 (R)[h]

[a] Conducted on 1 *M* solutions. [b] Room temperature. [c] Reflux with 2 equiv. of 85% hydrazine. [d] Warm on steam bath. [e] 7.5 ml. of dioxane, 5.0 ml. of 4 *N* NaOH at room temperature. [f] Product precipitated by addition of excess ether. [g] Product precipitated by addition of excess water after evaporation of the ethyl acetate. [h] Product precipitated by addition of excess water.

Table III. Cleavage Studies on N-Benzhydryloxycarbonylglycine

Cleavage conditions	Time, min.	% cleavage, m.t.l.c. (system A)
CF_3CO_2H, 0°	1	100[a]
4 *N* HCl–CH_3OH, 25°	60	100[b]
c	10	*d*

[a] Glycine trifluoroacetate obtained in 92% yield. [b] Glycine methyl ester hydrochloride obtained in 99% yield. [c] Reaction conditions: 50 ml. of chloroform, 2 drops of concentrated sulfuric acid, 0.1 mole of isobutylene, room temperature. [d] Approximately 80% glycine precipitated.

From these cleavage experiments it is evident that the BhOC group is smoothly cleaved under mild acidic conditions. Of particular importance was the ob-

(5) (a) Mr. E. L. Smithwick, unpublished observation. (b) H. N. Rydon and F. O. dos S. P. Serrão, *J. Chem. Soc.*, 3638 (1964), have also observed this type of acid-catalyzed disulfide interchange.
(6) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).
(7) M. Brenner and H. C. Curtius, *Helv. Chim. Acta*, **46**, 2126 (1963).
(8) F. Weygand and W. Steglich, *Z. Naturforsch.*, **14b**, 472 (1959).
(9) F. Weygand and E. Csendes, *Angew. Chem.*, **64**, 136 (1952).
(10) (a) L. Zervas, D. Borovas, and E. Gazis, *J. Am. Chem. Soc.*, **85**, 3660 (1963); (b) L. Zervas, and C. Hamalidis, *ibid.*, 87, 99 (1965).
(11) (a) G. W. Anderson and A. C. McGregor, *ibid.*, **79**, 6180 (1957); (b) F. C. McKay and N. F. Albertson, *ibid.*, **79**, 4686 (1957).
(12) L. A. Carpino, C. A. Giza, and B. A. Carpino, *ibid.*, **81**, 955 (1959).
(13) L. A. Carpino, *J. Org. Chem.*, **28**, 1909 (1963).

servation that the BhOC group was cleanly removed with 1.7 *N* hydrogen chloride in tetrahydrofuran (Table V). Subsequent experiments[5a] have established that unsymmetrical cystine derivatives are stable under these conditions. Thus the BhOC group is compatible with unsymmetrical disulfides and can probably be used in the synthesis of deblocked cystine derivatives. The BhOC group resists the action of several reagents known to remove certain other amino- or carboxy-protecting groups, *e.g.*, hydrazine in refluxing methanol, sodium hydroxide in aqueous dioxane, and aqueous hydrochloric acid in acetone. The stability of the BhOC group in reactions involving peptide bond formation is indicated by the preparation of N-benzhydryloxycarbonyltriglycine (VI) and N-benzhydryloxycarbonyl-L-alanylglycine ethyl ester (VII). N-Benzhydryloxycarbonylglycine could also be converted to the

$$\underset{\text{V}}{\text{BhOC}\cdot\text{GlyOH}} \xrightarrow[\text{HCl}\cdot\text{HGly}_2\text{OEt}]{\text{DCC, Et}_3\text{N}} [\text{BhOC}\cdot\text{Gly}_3\text{OEt}] \xrightarrow{-\text{OH}} \underset{\text{VI}}{\text{BhOC}\cdot\text{Gly}_3\text{OH}}$$

$$\text{BhOC}\cdot\text{AlaO}^{-}{}^{+}\text{DCHA} \xrightarrow[\text{HCl}\cdot\text{H}\cdot\text{GlyOEt}]{\text{DCC}} \underset{\text{VII}}{\text{BhOC}\cdot\text{Ala}\cdot\text{GlyOEt}}$$

DCHA = N,N-dicyclohexylammonium

corresponding *p*-nitrophenyl ester in 85% yield.

Carboxy Group Protection. The introduction of a carboxy-deblocked C-terminal cysteine residue into a peptide presents a rather difficult synthetic problem if other blocking groups in the peptide molecule (*e.g.*, N-carbobenzoxy, S-trityl, S-benzhydryl) are to be kept intact. This situation arises from the alkali lability of the sulfur-containing amino acids and their "poisoning" effect on hydrogenation catalysts; thus both saponification and hydrogenolysis of the appropriate esters is largely precluded. Therefore a carboxy-protecting group which can be introduced into an S,N-protected cysteine residue, kept intact while the amino-protecting group is removed and the coupling accomplished, and ultimately removed by mild acid hydrolysis, would be very desirable.

Several types of esters of intermediate acid lability have been utilized in peptide synthesis. These include the *t*-butyl,[14] phthalimidomethyl,[15] and *p*-methoxybenzyl.[16] The *t*-butyl ester has been widely used in a number of circumstances; however, in the particular case of cysteine the use of the isobutylene–sulfuric acid method or silver salt method[17] for *t*-butyl esterification does not appear to be compatible with the S-trityl protective group. The *p*-methoxybenzyl ester has received relatively little attention; hydrolysis of the ester occurs with trifluoroacetic acid at 0°.[16,18]

Another potentially useful acid-labile ester is the benzhydryl ester. Although these esters have been known for some time and have been removed from various carboxylic acids by catalytic hydrogenation[19] or other methods,[20] their potential use in peptide synthesis had not been exploited. An appealing feature of benzhydryl esters was the possibility that the ester could be introduced into N-protected amino acids by diphenyldiazomethane[21] (under neutral conditions) or by the azeotropic distillation method, previously utilized for the preparation of benzyl[22] esters. This possibility has been confirmed; in the present investigation both methods have been utilized, although the latter procedure provides good yields of the ester only when the N-protected amino acid is appreciably soluble in benzene. The diphenyldiazomethane method provided an 87% yield of S,N-ditrityl-L-cysteine benzhydryl ester, whereas the azeotropic method would be inapplicable, owing to the acid lability of the N-trityl group. Attempts to prepare a benzhydryl ester by the reaction of an N-carboxy-α-amino acid anhydride with benzhydrol were unsuccessful. Similar results were obtained when direct esterification of several amino acids with benzhydrol by the azeotropic method were attempted. Likewise diphenyldiazomethane failed to react with either glycine or S-benzoyl-L-cysteine; however, an N,N-dimethylformamide solution of glycine hydrochloride afforded a detectable amount of glycine benzhydryl ester when treated with diphenyldiazomethane.

Cleavage studies on the benzhydryl ester were carried out with benzhydryl hippurate. The susceptibility of the ester to both acid and alkaline conditions are indicated in Table IV. The compatibility of the N-benzhydryloxycarbonyl and benzhydryl ester groups is indicated by selective removal of the former group from N-benzhydryloxycarbonylglycine benzhydryl ester (Table V). The optimum conditions in this particular situation appear to be 1.7 *N* hydrogen chloride in tetrahydrofuran at room temperature.

Table IV. Cleavage Studies on Benzhydryl Hippurate

Cleavage conditions	Time, min.	% cleavage, m.t.l.c. (system B)
4 *N* HCl–THF, 25°	5	*a*
$BF_3\cdot Et_2O$–HOAc (1:6), 25°	1	*b*
HCl–HOAc satd., 25°	1	*b*
CF_3CO_2H, 0°	1	*b*
HOAc, 70°	5	*c*
N_2H_4–CH_3OH[d]	60	*e*
NaOH–dioxane,[f] 25°	60	*g*

[a] No cleavage; when reaction time increased to 30 min. some cleavage observed. [b] Complete ester cleavage. [c] Quantitative recovery of starting ester obtained by dilution with water. [d] Two equivalents of 85% hydrazine hydrate in refluxing methanol. [e] Quantitative yield of hippuryl hydrazide obtained by evaporation and trituration with ether. [f] Two equivalents of sodium hydroxide in aqueous dioxane. [g] Quantitative yield of benzhydrol obtained by dilution with water and ethyl acetate extraction.

The acid labilities of the benzhydryl and *t*-butyl esters appear to be quite similar. The sensitivity of the former ester to the action of hydrazine does not pre-

(14) R. Roeske, *J. Org. Chem.*, 28, 1251 (1963), and earlier references cited.

(15) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, 82, 941 (1963).

(16) F. Weygand and K. Hunger, *Chem. Ber.*, 95, 1 (1962).

(17) G. W. Anderson and F. M. Callahan, British Patent 878,732; German Patent 1,118,214; *Chem. Abstr.*, 56, 15606*b* (1962).

(18) Although ref. 16 reports a 32% yield of N-carbobenzoxyglycine *p*-methoxybenzyl ester *via* DCC, Dr. J. A. Maclaren (unpublished observation) obtained a 76% yield of the same ester by this procedure and a 94% yield of the ester by refluxing an ethyl acetate solution of *p*-methoxybenzyl chloride, N-carbobenzoxyglycine, and triethylamine.

(19) E. Hardegger, Z. E. Heweihi, and F. G. Robinet, *Helv. Chim. Acta*, 31, 439 (1948).

(20) (a) C. A. Bunton, J. N. E. Day, R. H. Flowers, P. Sheel, and J. L. Wood, *J. Chem. Soc.*, 963 (1957); (b) A. C. Cope, and W. R. Lyman, *J. Am. Chem. Soc.*, 75, 3312 (1953); (c) G. J. Harvey and V. R. Stimson, *J. Chem. Soc.*, 3629 (1956); (d) S. Sarel, L. A. Pohoryles, and R. Ben Shoshan, *J. Org. Chem.*, 24, 2067 (1959).

(21) J. B. Miller, *ibid.*, 24, 560 (1959), describes a preparation of diphenyldiazomethane.

(22) J. E. Shields, W. H. McGregor, and F. H. Carpenter, *ibid.*, 26, 1491 (1961).

Table V. Cleavage Studies on N-Benzhydryloxycarbonylglycine Benzhydryl Ester

Cleavage conditions	Time, min.	Result, m.t.l.c. (system B)
Aq. HCl–acetone,[a] 25°	1–60	No cleavage[b]
c	1	*d*
c	5–60	*e*
c	5	*f*
c	15	*g*
1.7 *N* HCl–THF, 65°	10	*h*
HCl–Et_2O, 25°	4	*e*
Aq. CH_3OH–H_2SO_4, 65°	2	*h*
H_2–Pd black, CH_3OH	19 hr.	*i*

[a] 5 ml. of 5 *N* hydrochloric acid, and 20 ml. of acetone. [b] Benzhydryl benzoate also gave a quantitative recovery of starting material. [c] 1.7 *N* HCl in THF. [d] Partial N-cleavage; no C-cleavage. [e] Complete N-cleavage; no C-cleavage. [f] 43% of glycine benzhydryl ester hydrochloride obtained by quenching with sodium bicarbonate, extraction into ether, and precipitation with dry hydrogen chloride. [g] 72% yield of glycine benzhydryl ester hydrochoride obtained as in *f*. [h] Complete N- and C-cleavage. [i] 60% yield of glycine obtained.

clude its successful combination with the N-phthaloyl group, although more care must be taken than in the case of removal of an N-phthaloyl group in the presence of a *t*-butyl ester. The benzhydryl ester appears to be more acid labile than the phthalimidomethyl ester and of similar alkali lability.

Experimental[23]

Preparation of Benzhydryloxycarbonylhydrazide (II). A mixture containing 184.2 g. (1 mole) of benzhydrol, 81.3 ml. (1.01 moles) of pyridine, and 300 ml. of chloroform was cooled in an ice bath and treated with 84.4 ml. (106 g., 0.95 mole) of methyl chlorothiolcarbonate during 20–30 min. Stirring was continued for 1.5 hr. while the mixture was allowed to warm to room temperature. Most of the chloroform was removed *in vacuo*,[24] 500 ml. of ether was added, and the pyridine hydrochloride removed by filtration. The ether solution was washed with water and saturated sodium chloride solution and dried. Evaporation of the ether provided a yellow liquid (I), which solidified when dried *in vacuo* over calcium chloride.

The crude benzhydryl methylthiolcarbonate (I) was dissolved in 900 ml. of ether, cooled, and slowly treated with a solution of 79 ml. (0.9 mole) of 85% hydrazine hydrate in 854 ml. of methanol. The mixture was allowed to stand at room temperature for 6 hr., and any aqueous layer was separated and discarded. The organic layer was evaporated to dryness and dried *in vacuo* over sulfuric acid. The solid was recrystallized from ether to give 179 g. (82%) of II as a white solid, m.p. 101–102°.

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.40; H, 5.82; N, 11.57. Found: C, 69.59; H, 5.96; N, 11.44.

(23) Melting points are uncorrected and were taken in capillary tubes. Elemental analyses were performed by the Triangle Chemical Laboratories, Chapel Hill, N. C. Optical rotations were taken with a Rudolph Model 80 polarimeter equipped with a Model 200 photoelectric attachment. Chromatographic procedures were carried out either in 1-butanol–acetic acid–water (4:1:5) (system A) or in benzene–chloroform–ethanol (12:12:1) (system B) usless otherwise indicated. Thin layer chromatography using silica gel G was performed on microscope slide plates and is referred to as micro thin layer chromatography (m.t.l.c.). Ascending paper chromatography was performed with Whatman No. 1 paper.

(24) Excessive warming caused loss of carbon dioxide.

Preparation of Benzhydryl Azidoformate (III). A mixture of 24.2 g. (0.1 mole) of II, 33 ml. of acetic acid, and 18 ml. of water was stirred until solution was complete and then cooled to 0–5°. The solution was treated with 8.3 g. (0.12 mole) of powdered sodium nitrite, added in portions. Solid azide began precipitating during the sodium nitrite addition. The mixture was poured into 400 ml. of water and extracted with ether; the extract was washed with 1 *M* sodium bicarbonate solution until the washes were alkaline. The extract was then washed with saturated sodium chloride solution, dried, and evaporated to a yellow oil. Cooling the oil provided 25.3 g. (100%) of III as a pale yellow solid, m.p. 37–39°.

Anal. Calcd. for $C_{14}H_{11}N_3O_2$: C, 66.39; H, 4.38; N, 16.59. Found: C, 66.45; H, 4.29; N, 16.56.

Preparation of N-Benzhydryloxycarbonylglycine (V). A mixture of 7.51 g. (0.1 mole) of glycine, 8.06 g. of magnesium oxide, 50.7 g. (0.2 mole) of III, 130 ml. of water, and 310 ml. of dioxane was shaken for 48 hr. The filtered solution was diluted with 1500 ml. of water and extracted with ethyl acetate. The aqueous layer was cooled to 0°, acidified to pH 1–2 with 5 *N* hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water and saturated sodium chloride solution, dried, and evaporated to a pale yellow oil. The oil was crystallized from ether–petroleum ether to yield 25.3 g. (89%) of V as a white granular solid, m.p. 114.5–115°.

Anal. Calcd. for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.30; H, 5.34; N, 4.93.

Preparation of Benzhydryl Carbanilate (IV). A benzene solution of 18.4 g. (0.1 mole) of benzhydrol and 10.5 ml. (0.096 mole) of phenyl isocyanate was refluxed for 24 hr. After the addition of 10 ml. of triethylamine, refluxing was continued for 18 hr. Removal of the solvent provided a white solid which was recrystallized from acetone–hexane to give 25.7 g. (88%) of product, m.p. 140–141, lit.[25] m.p. 140°.

Preparation of N-Benzhydryloxycarbonylglycylglycylglycine (VI). The ethyl ester of the tripeptide was generated from V and glycylglycine ethyl ester hydrochloride using DCC. The reaction was carried out in N,N′-dimethylformamide and the product was crystallized from acetone–water. The ester was saponified by treatment with 1.5 equiv. of sodium hydroxide in 50% aqueous dioxane for 3 hr. Dilution with water and acidification precipitated the tripeptide. The product was washed with acetone and petroleum ether to provide the analytical sample, m.p. 189–189.5° dec.

Anal. Calcd. for $C_{20}H_{21}N_3O_6$: C, 60.14; H, 5.30; N, 10.54. Found: C, 59.83; H, 5.56; N, 10.60.

Preparation of N-Benzhydryloxycarbonyl-L-alanylglycine Ethyl Ester (VII). The dipeptide was prepared in chloroform from N-benzhydryloxycarbonyl-L-alanine N,N′-dicyclohexylamine salt and glycine ethyl ester hydrochloride by the DCC method. Two recrystallizations from acetone–petroleum ether provided VII in 76% yield, m.p. 114–114.5°, $[\alpha]^{20}D$ −22.4° (*c* 0.720, EtOH).

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.71; H, 6.36; N, 7.39.

(25) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th Ed., John Wiley and Sons, Inc., New York, N. Y., 1960, p. 281.

Preparation of N-Benzhydryloxycarbonylglycine p-Nitrophenyl Ester. A mixture of 2.85 g. (0.01 mole) of V and 1.39 g. (0.01 mole) of *p*-nitrophenol in ethyl acetate was cooled and treated with 2.06 g. (0.01 mole) of DCC. After 1.5 hr. the mixture was filtered and the filtrate washed with 0.5 *N* hydrochloric acid, dilute sodium chloride solution, saturated sodium bicarbonate solution, and saturated sodium chloride solution. The organic layer was dried and evaporated to a yellow oil. The oil was crystallized from ether and recrystallized from ethanol–petroleum ether to give 3.46 g. (85%) of active ester, m.p. 96–97°.

Anal. Calcd. for $C_{22}H_{18}N_2O_6$: C, 65.02; H, 4.46; N, 6.90. Found: C, 64.63; H, 4.47; N, 6.92.

Preparation of Diphenyldiazomethane. The procedure of Miller[21] was employed. A yield of 89% diphenyldiazomethane from benzophenone hydrazone was assumed. All esterifications were conducted using a ratio of 0.12 mole of diphenyldiazomethane to 0.1 mole of monocarboxylic acid.

Preparation of S,N-Dityrityl-L-cysteine Benzhydryl Ester. S,N-Dityrityl-L-cysteine diethylamine salt (106 g., 0.156 mole) was converted to the free acid by Dowex 50 W X-8 (acid form) in aqueous tetrahydrofuran (1:2). The amorphous acid was dissolved in 500 ml. of toluene and treated with excess diphenyldiazomethane solution. The reaction mixture was kept 12 hr. at room temperature and 6 hr. on the steam bath. The orange solution was treated with 50 g. of Florisil and the mixture allowed to stand 2 hr. The filtered solution was evaporated to a yellow oil, redissolved in ether, and crystallized by the addition of methanol. Recrystallization from ether–methanol provided 104 g. (87%) of the ester, m.p. 124–124.5°, $[\alpha]^{25}D$ 51.0° (*c* 1.93, acetone).

Anal. Calcd. for $C_{54}H_{45}NO_2S$: C, 84.02; H, 5.88; N, 1.82; S, 4.15. Found: C, 84.51; H, 5.76; N, 1.85; S, 4.01.

Preparation of N-Benzhydryloxycarbonylglycine Benzhydryl Ester. An ethyl acetate solution of 7.13 g. (0.025 mole) of V was added to an ethyl acetate solution of excess diphenyldiazomethane. After 2 days at 25° the purple color was discharged with concentrated hydrochloric acid and the organic layer washed with water, saturated sodium bicarbonate solution, water, and saturated sodium chloride solution. The dried organic layer was evaporated to an oil which was crystallized from ether–petroleum ether. Recrystallization from the same solvent provided 10.3 g. (91%) of white solid, m.p. 119.5–121°. The sample was homogeneous on m.t.l.c. in systems A and B.

Anal. Calcd. for $C_{29}H_{25}NO_4$: C, 77.14; H, 5.58; N, 3.10. Found: C, 77.09; H, 5.67; N, 3.02.

Preparation of Benzhydryl Benzoate. Azeotropic Distillation Procedure. A solution of 13.2 g. (0.11 mole) of benzoic acid, 18.4 g. (0.1 mole) of benzhydrol, and a catalytic amount of *p*-toluenesulfonic acid monohydrate in 225 ml. of benzene was refluxed for 6 hr. under a Dean–Stark trap. The cooled solution was washed with cold 1 *N* sodium hydroxide solution, water, and saturated sodium chloride solution. The dried organic layer was evaporated to an oil which solidified on cooling. Recrystallization from acetone–petroleum ether gave 22.1 g. (77%) of ester, m.p. 87.5–88°; lit. m.p. 88.5,[26a] 87,[19] 91.5°.[26b]

Preparation of N-Phthaloylglycine Benzhydryl Ester. This ester was prepared by the azeotropic distillation procedure. The yield was 63% after two recrystallizations from acetone–petroleum ether, m.p. 131–131.5°.

Anal. Calcd. for $C_{23}H_{17}NO_4$: C, 74.38; H, 4.61; N, 3.77. Found: C, 74.08; H, 4.57; N, 3.97.

Preparation of Benzhydryl Hippurate. The ester was prepared by azeotropic distillation in 18% yield, m.p. 123–124°.

Anal. Calcd. for $C_{22}H_{19}NO_3$: C, 76.50; H, 5.54; N, 4.06. Found: C, 76.49; H, 5.33; N, 3.97.

When the diphenyldiazomethane method of esterification was employed in acetone (1 day at room temperature) the yield of ester was 83%.

Preparation of Glycine Benzhydryl Ester Oxalate. To a solution of 37.1 g. (0.1 mole) of N-phthaloylglycine benzhydryl ester in 250 ml. of hot methanol was added 8.76 ml. (0.1 mole as hydrazine) of 85% hydrazine hydrate. The solution was refluxed for 10 min., evaporated, and treated with 150 ml. of 6% potassium carbonate solution and 250 ml. of ether. The ether extract was dried, filtered, and treated with 9.0 g. (0.1 mole) of anhydrous oxalic acid. The cold solution was filtered and provided 19 g. (58%) of product, m.p. 155–155.5° after recrystallization from methanol. Paper chromatography exhibited one spot using system A, R_f 0.92, and one spot using system B, R_f 0.84.

Anal. Calcd. for $C_{17}H_{17}NO_6$: C, 61.62; H, 5.17; N, 4.23. Found: C, 61.65; H, 5.17; N, 4.41.

Preparation of S-Trityl-L-cysteine Benzhydryl Ester Oxalate. To a solution of 7.72 g. (0.01 mole) of S,N-dityrityl-L-cysteine benzhydryl ester in 25 ml. of tetrahydrofuran was added 2.46 ml. (0.01 mole as HCl) of 4.06 *N* hydrogen chloride in tetrahydrofuran and 2 ml. of water. The solution was refluxed for 5 min. and made basic with sodium bicarbonate solution; the solvent was removed *in vacuo*. The aqueous portion was decanted and the yellow gum dissolved in ether. The ether solution was washed with saturated sodium chloride solution, dried, and treated with a solution of 0.9 g. (0.01 mole) of anhydrous oxalic acid. The white precipitate was recrystallized from acetone–petroleum ether–ether to give 2.1 g. (33%) of the oxalate salt, melting point ill-defined (<80°); paper chromatography (system B), one spot, R_f 0.88; m.t.l.c. (system A), homogeneous (iodine, ninhydrin, ultraviolet); $[\alpha]^{20}D$ 60.7° (*c* 1.6, EtOH).

Anal. Calcd. for $C_{37}H_{33}NO_6S$: C, 71.71; H, 5.37; N, 2.26; S, 5.17. Found: C, 72.29; H, 5.50; N, 2.19; S, 5.41.

The hydrochloride derivative of S-trityl-L-cysteine benzhydryl ester (melting point ill-defined) was also obtained in yields of 85% by N-detritylation with 5 *N* hydrochloric acid–acetone (1:4) and purification by trituration with ether–hexane. Paper chromatography (system A) showed one spot, R_f 0.97.

Anal. Calcd. for $C_{35}H_{31}ClNO_2S$: C, 74.38; H, 5.53. Found: C, 74.51; H, 5.83.

(26) (a) E. Linnemann, *Ann.*, 133, 22 (1865); (b) G. J. Harvey and V. R. Stimson, *J. Chem. Soc.*, 3629 (1956).