The *trans,anti* isomer **5** was obtained by trituration of the crude product with Skellysolve B as a crystalline solid, mp 142–143°, in 61% yield. Recrystallization from Skellysolve B gave an analytical sample, mp 145–146°.

Anal. Calcd for $C_{20}H_{26}N_2$: C, 81.58; H, 8.90; N, 9.52. Found: C, 81.67; H, 9.10; N, 9.23.

The *trans,syn* isomer **7** was obtained by dissolving the crude product in ether and adding excess hydrogen bromide. The resulting salt was shaken with 10% sodium hydroxide solution and methylene chloride. The methylene chloride layer was washed with water and dried over sodium sulfate, and the solvent was removed. Recrystallization of the residue from methanol gave a solid, mp 125–126°, in 38% yield.

Anal. Calcd for $C_{20}H_{26}N_2$: C, 81.58; H, 8.90; N, 9.52. Found: C, 81.30; H, 8.80; N, 9.27.

The *cis,syn* isomer **10** was obtained by crystallization of the crude product from Skellysolve B as a solid, mp 144–149°, in 55% yield. Recrystallization from methanol gave an analytical sample, mp 150–151°.

Anal. Calcd for $C_{20}H_{26}N_2$: C, 81.58; H, 8.90; N, 9.52. Found: C, 81.52; H, 8.87; N, 9.38.

The *cis,anti* isomer **12** was obtained by crystallization of the crude product from Skellysove B as a solid, mp 110–111°, in 36% yield. Recrystallization from methanol gave an analytical sample, mp 112–113°.

Anal. Calcd for $C_{20}H_{26}N_2$: C, 81.58; H, 8.90; N, 9.52 Found: C, 81.77; H, 9.11; N, 9.30.

Registry No.—**3,** 13388-62-0; **4,** 13388-63-1; **5,** 13388-64-2; **6,** 13388-65-3; **7,** 13428-18-7; **8,** 13388-66-4; **9,** 13388-67-5; **9**-14b-*d*, 13388-68-6; **10,** 13388-69-7; **11,** 13388-70-0; **12,** 13388-71-1.

Acknowledgment.—The authors are indebted to Mr. A. Lewis and his associates and to Mr. R. Puchalski for the spectral data and to Mrs. U. Zeek for analytical determinations. We also wish to thank the Chemical Development Department under the supervision of Dr. A. W. Ruddy for the large-scale preparation of intermediates.

Sulfur-Containing Polypeptides. VII. Synthesis of S-Trityl-L-cysteine Peptides Using Acid-Labile Amino and Carboxy Protective Groups[1-3]

RICHARD G. HISKEY, JON T. STAPLES,[4,5] AND ROBERT L. SMITH

Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina 27515

Received March 24, 1967

The development of a versatile synthetic route to L-cysteine containing polypeptides employing acid-labile protective groups which can be removed cleanly in the presence of the S-trityl thioether moiety has facilitated the synthesis of *t*-butyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (I) in good over-all yield.

Previous reports have considered synthetic routes to suitably protected cysteine derivatives[6] and selective oxidation[7] of S-trityl thioethers of cysteine. These studies have provided a basis for preparation of peptides containing several cysteine residues. The present report describes the synthesis of such a molecule and summarizes our efforts to develop amino and carboxyl protective groups that are compatible with S-trityl thioethers.

The synthetic goal of these experiments was the protected octapeptide derivative, *t*-butyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (I). This substance was desired as the precursor to a molecule containing three disulfide bridges, shown schematically as IV. The projected synthesis of IV (Scheme I) is designed to permit stepwise introduction of the three disulfide bonds and provide a model from which synthesis of more complex related peptides can be achieved. Octapeptide II is the key intermediate in the synthesis of IV; thus the protective groups incorporated into I must permit the selective oxidation of cysteine residues I and VI and removal of the carboxyl protective group in a subsequent step. Chain extension of II, using an ester containing an S-trityl-L-cysteine residue, would lead to the A chain, III. This segment of the molecule contains the intact intrachain disulfide bridge and two cysteine residues with S-protective groups that can be selectively removed. Oxidation of III, using the appropriate B chain, should provide IV.

Our approach to the preparation of I was governed by the need to obtain this substance in quantity and the desire to develop a method of synthesis that could be utilized for other molecules containing intact disulfide bridges. However, an obstacle to the successful preparation of molecules similar to I and II concerns the availability of amino and carboxyl protective groups that can be cleanly removed in the presence of the S-trityl group. The elegant work of Zervas, *et al.*,[8] which resulted in the synthesis of Va–c, provided significant data on the compatibility of various N-protective groups with the S-trityl residue. The approach adopted in this synthesis involved the use of a methyl ester as the carboxyl protective group and allowed elongation of V from the C-terminal serine methyl ester residue. Although utilization of acid-stable esters simplifies the coupling and chain-extension steps of their synthesis, removal of esters with alkali could lead to

(1) Part VI of this series: R. G. Hiskey and E. L. Smithwick, Jr., *J. Am. Chem. Soc.*, **89**, 437 (1967).

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

(3) The following abbreviations have been employed in the text: Z = carbobenzoxy; Tr = trityl; Bzh = benzhydryl; t_{Bu} = *t*-butyl; BOC = *t*-butyloxycarbonyl; Bz = benzoyl; *o*-NPS = *o*-nitrophenylsulfenyl; DCC = N,N-dicyclohexylcarbodiimide; WSC = 1-ethyl-3-(3-N,N-dimethylaminopropyl)carbodiimide hydrochloride; Phth = phthaloyl; EOCP = ethoxycarbonylphthalimide; BHOC = benzhydryloxycarbonyl; DMF = N,N-dimethylformamide; Ox^- = oxalate.

(4) Le Doux Fellow, 1965–1966.

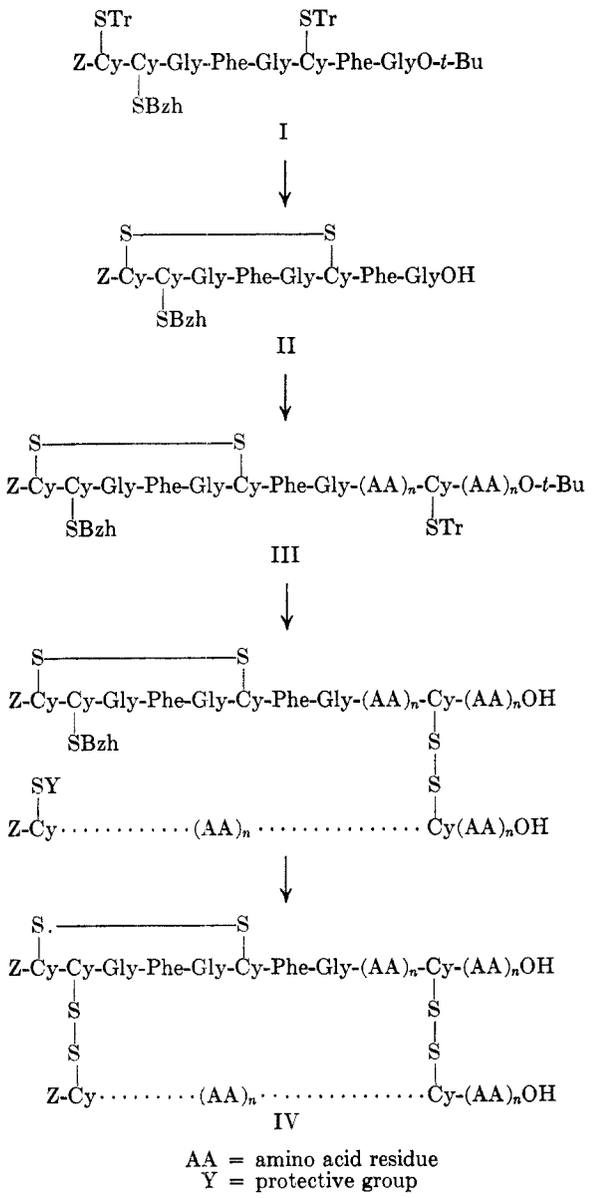
(5) Abstracted in part from a dissertation by J. T. Staples submitted to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, June 1966.

(6) R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **31**, 2178 (1966).

(7) R. G. Hiskey and D. N. Harpp, *J. Am. Chem. Soc.*, **87**, 3965 (1965); R. G. Hiskey, T. Mizoguchi, and E. L. Smithwick, Jr., *J. Org. Chem.*, **32**, 97 (1967).

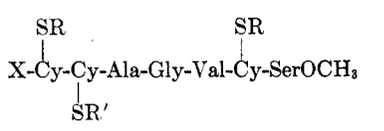
(8) L. Zervas, I. Photaki, A. Cosmatos, and D. Borovas, *J. Am. Chem. Soc.*, **87**, 4922 (1965), and earlier references cited.

SCHEME I



AA = amino acid residue
Y = protective group

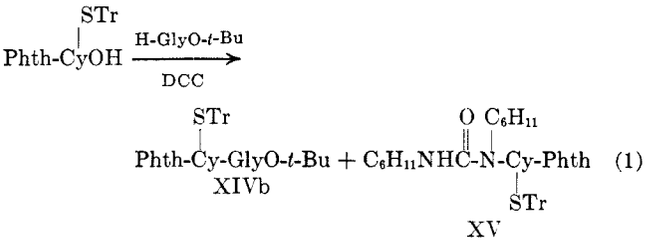
numerous side reactions including alkaline decomposition of disulfide bonds, β elimination in the thioethers,



Va, R = Tr; R' = BzH; X = Z
b, R = Tr; R' = Bzh; X = BOC
c, R = Bz; R' = Tr; X = *o*-NPS

and disulfide interchange in molecules containing two or more disulfide bonds. Hydrazinolysis of the ester group and oxidative conversion of the hydrazide to the azide have been accomplished[8] in the presence of an S-trityl-L-cysteine residue; whether this sequence could be applied to a complex peptide such as V remains to be established.

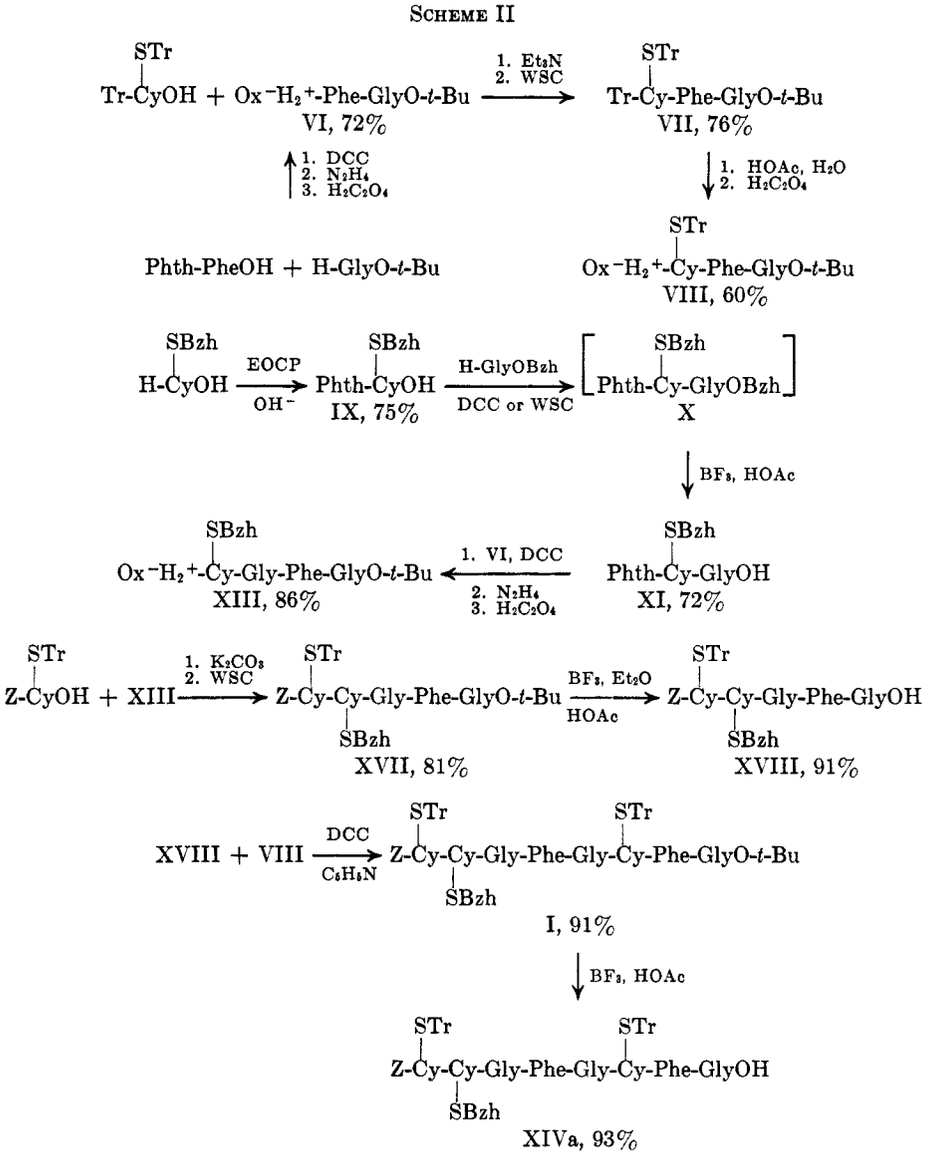
In our approach to the synthesis of I we have attempted to utilize protective groups on the amino and carboxyl portions of the molecule that can be removed under acid conditions in the presence of the S-trityl group. The reactions involved in the synthesis of I are outlined in Scheme II. The compatibility of the N-phthaloyl, S-benzhydryl, and *t*-butyl groups was predictable and facilitated the preparation of *t*-butyl S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XIII) in good over-all yield. Preparation of this subunit was effected as follows. Coupling of benzhydryl glycinate with N-phthaloyl-S-benzhydryl-L-cysteine was accomplished using either DCC or WSC. The benzhydryl ester of glycine gave consistantly higher yields than *t*-butyl glycinate in the synthesis of X. Presumably, the lowered yields of *t*-butyl N-phthaloyl-S-benzhydryl-L-cysteinylglycinate resulted from steric interaction between the carbodiimide adduct of IX and *t*-butyl glycinate. When N-phthaloyl-S-trityl-L-cysteine was coupled with *t*-butyl glycinate, approximately equal quantities of *t*-butyl N-phthaloyl-S-trityl-L-cysteinylglycinate (XIVb) and the N-acylurea (XV) were obtained (eq 1). Removal of the



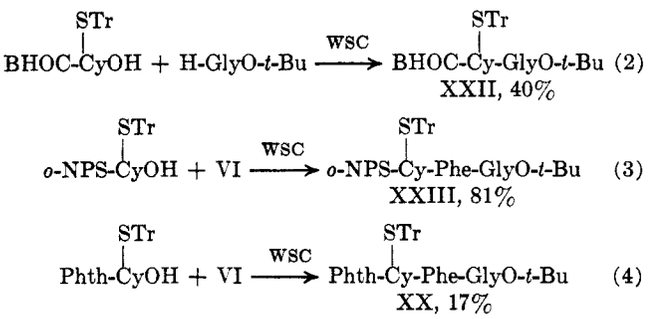
benzhydryl ester was accomplished with boron trifluoride.[6] The resulting acid, N-phthaloyl-S-benzhydryl-L-cysteinylglycine (XI), was coupled with VI to provide *t*-butyl N-phthaloyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XII) in 94% yield. The protected tetrapeptide, XII, could be deblocked with boron trifluoride to provide N-phthaloyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XVI) in good yield or with hydrazine to afford XIII in 86% over-all yield. Although XIII was purified and characterized, the crude preparation was sufficiently pure to be utilized in subsequent coupling experiments. Neutralization of XIII followed by coupling with N-carbobenzoxy-S-trityl-L-cysteine provided *t*-butyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XVII) in 81% yield. Cleavage of ester XVII was accomplished using boron trifluoride; N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XVIII) resulted in 95% yield. Considerable flexibility is available in this sequence; we anticipate that N-benzhydryloxycarbonyl-S-trityl-L-cysteine, N-*t*-butyloxycarbonyl-S-trityl-L-cysteine, and N-phthaloyl-S-trityl-L-cysteine can be substituted for N-carbobenzoxy-S-trityl-L-cysteine with no subsequent complications during ester removal with boron trifluoride.

The key intermediate in this sequence is the tripeptide, *t*-butyl S-trityl-L-cysteinyl-L-phenylalanylglycinate (VIII). Several precursors to this substance were prepared in order to evaluate the selective removal of the N-protective group. These substrates included *t*-butyl N,S-ditrityl-L-cysteinyl-L-phenylalanylglycinate (VII), *t*-butyl N-(*o*-nitrophenylsulfenyl)-S-trityl-L-cysteinyl-L-phenylalanylglycinate (XIX), *t*-butyl N-phthaloyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (XX), and *t*-butyl N-benzhydryloxycarbonyl-S-trityl-L-cysteinylglycinate (XXI). Potentially, these deriva-

SCHEME II



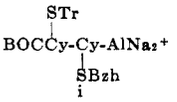
tives are among the first examples[9] of protected cysteine derivatives which contain an S-trityl thioether and labile amino as well as carboxy protecting groups (eq 2–4). Treatment of XXII with dry HCl gave a product



mixture which was resolved into three ninhydrin-positive components by tlc. Similar treatment of XXIII provided the desired hydrochloride derivative of VIII; however, the product was always accompanied by colored impurities which could not be removed. When XX was treated with hydrazine hydrate, only low yields of VIII were obtained. The acid-catalyzed N-detritylation of VII was much more satisfactory; VIII was obtained as a homogeneous (tlc) product using either concentrated hydrochloric acid in acetone (60% yield) or 80% acetic acid (78% yield). Hence, the N-trityl group appears to be the blocking group of choice for this particular series.

Having successfully obtained intermediate VIII, the formation of octapeptide I was studied. Coupling XVIII and VIII using DCC in pyridine provided I in 91% yield. A similar reaction using N-ethyl-5-phenylisoxazolium-3′-sulfonate in acetonitrile–DMF gave 45% of I, whereas WSC in methylene chloride–DMF afforded I in 55% yield. The properties of various preparations, obtained by varying coupling conditions, were identical. Selective cleavage of the *t*-butyl ester group was accomplished by treatment of I with boron trifluoride; under these conditions N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycyl-S-trityl-L-cysteinyl-L-phenylalanylglycine (XXIV) was obtained in 93.4% yield.

(9) The only additional example of which we are aware is the acyl diazonium salt (i) prepared in ref 8 by iodine oxidation of the corresponding acyl hydrazide.



Experimental Section[10]

t-**Butyl N-Phthaloyl-L-phenylalanylglycinate.**—To a rapidly stirred, cold (−10°) solution containing 7.37 g (0.025 mole) of N-phthaloyl-L-phenylalanine[11] and 3.56 g (0.027 mole) of freshly prepared *t*-butyl glycinate[6] in 25 ml of methylene chloride was added 5.16 g (0.025 mole) of DCC. The solution was stirred at −10° for 2 hr and at 20° for 12 hr. The reaction mixture was diluted with 25 ml of methylene chloride, filtered, and evaporated. The residue was dissolved in 300 ml of hot ethyl acetate and washed with 5% aqueous sodium bicarbonate, water, and saturated aqueous sodium chloride. The organic layer was dried and evaporated to a solid; recrystallization from acetone–hexane afforded 8.6 g (84%) of fine needles: mp 167–168.5°, $[\alpha]^{18}D$ −101.7° (*c* 1.01, $CHCl_3$).

Anal. Calcd for $C_{23}H_{24}N_2O_5$: C, 67.63; H, 5.92; N, 6.86. Found: C, 67.41; H, 5.99; N, 7.13.

t-**Butyl L-Phenylalanylglycinate Oxalate Salt (VI).**—A solution containing 8.17 g (0.02 mole) of N-phthaloyl-L-phenylalanylglycine *t*-butyl ester in 50 ml of methanol was treated with 1 ml (0.0206 mole) of hydrazine (99%). The solution was refluxed for 1 hr, cooled, and filtered. The filtrate was evaporated to a yellow oil which was partitioned between 100 ml of 5% potassium carbonate and 200 ml of ether. The aqueous phase was separated, extracted with two 100-ml portions of ether, and the combined extracts were dried. The ether extract was slowly diluted with a solution containing 2.52 g (0.02 mole) of oxalic acid dihydrate in methanol–ether (1:2, 30 ml). The solution was cooled and the precipitated product collected. Recrystallization from methanol–ether provided 6.3 g (85.5%): mp 164° dec, $[\alpha]^{18}D$ +8.0° (*c* 1.0, DMF).

Anal. Calcd for $C_{17}H_{24}N_2O_7$: C, 55.43; H, 6.57; N, 7.60. Found: C, 55.26; H, 6.59; N, 7.74.

t-**Butyl N,S-Dityrityl-L-cysteinyl-L-phenylalanylglycinate (VII).**—A solution of 33.9 g (0.05 mole) of N,S-dityrityl-L-cysteine[12] (prepared by neutralization of the diethylamine salt) and 19.0 g (0.052 mole) of *t*-butyl L-phenylalanylglycinate (prepared by neutralization of VI in 60 ml of methylene chloride cooled to −10°) was treated with 10.0 g (0.051 mole) of WSC. The reaction was allowed to proceed at −10° for 1 hr and at 25° for 15 hr. Evaporation of the solvent provided a foam which was dissolved in 250 ml of ethyl acetate–ether (1:1) and washed with cold 1 *N* hydrochloric acid, water, and saturated sodium chloride solution. The solvent was removed and the resulting solid foam was dissolved in chloroform and filtered through a Florisil column (200 g). The first fraction (35 g, 82%) was homogeneous by tlc (system A, B), $[\alpha]^{18}D$ +55.2° (*c* 0.909, $CHCl_3$); however, repeated combustion analyses of the material provided varying analytical results. Subsequent intermediates derived from this material were found to be analytically pure.

Anal. Calcd for $C_{56}H_{55}N_3O_4S$: C, 77.66; H, 6.40; N, 4.85; S, 3.70. Found: C, 79.37, 78.45; H, 6.57, 7.34; N, 3.36, 4.30; S, 3.62, 3.23.

t-**Butyl S-Trityl-L-cysteinyl-L-phenylalanylglycinate Oxalate Salt (VIII).**—A solution containing 2.86 ml of concentrated hydrochloric acid in 5 ml of acetone was added to a solution of 24.8 g (0.0286 mole) of N,S-dityrityl ester VII in 40 ml of acetone. The reaction mixture was stirred 2 hr at 20°, evaporated, and the resulting residue partitioned between 200 ml of 5% sodium bicarbonate and 500 ml of ethyl acetate. The organic layer was washed with water and saturated sodium chloride solution, dried, and evaporated. The gummy residue was dissolved in ether (500 ml) and treated with a solution containing 3.61 g (0.0286 mole) of oxalic acid dihydrate in 15 ml of methanol. The turbid solution was evaporated and the salt, 12.2 g (60%), $[\alpha]^{18}D$ +7.47° (*c* 0.75, DMF), crystallized from 200 ml of ether.

Anal. Calcd for $C_{39}H_{43}O_6S$: C, 65.61; H, 6.07; N, 5.89; S, 4.50. Found: C, 65.48; H, 6.03; N, 5.88; S, 4.51.

Benzhydryl N-Tritylglycinate.—To a solution of 15.2 g (0.048 mole) of N-tritylglycine in 60 ml of THF was added 250 ml of an ethereal diphenyldiazomethane solution containing 11.0 g (0.057 mole) of diphenyldiazomethane. The solution was refluxed 6 hr, cooled, and acidified with cold 5 *N* hydrochloric acid. The solution was evaporated to a residue which crystallized from acetone–methanol to give 20.0 g (86%) of ester, mp 137–139°.

Anal. Calcd for $C_{34}H_{29}NO_2$: C, 84.44; H, 6.04; N, 2.90. Found: C, 84.25; H, 6.12; N, 3.18.

Benzhydryl Glycinate *p*-Toluenesulfonate Salt.—A solution containing 48.0 g (0.1 mole) of benzhydryl N-tritylglycinate in 275 ml of acetone, 25 ml of 12.1 *N* hydrochloric, and 5 ml of water was kept at 35° for 30 min. The acetone was removed and the slurry dried *in vacuo*. The residue was dissolved in ether, washed with 5% potassium carbonate, and the organic layer was dried. The ether solution was treated with 19.0 g of *p*-toluenesulfonic acid and the product collected: 33.0 g (80%), mp 138–141°. The analytical sample was recrystallized from methylene chloride–ether.

Anal. Calcd for $C_{22}H_{23}NO_5S$: C, 63.90; H, 5.61; N, 3.39; S, 7.75. Found: C, 64.22; H, 5.69; N, 3.22; S, 7.57.

N-Phthaloyl-S-benzhydryl-L-cysteinylglycine (XI).—To a solution of 25.0 g (0.06 mole) of benzhydryl glycinate *p*-toluenesulfonate salt,[13] 8.4 ml (0.06 mole) of triethylamine, and 25.0 g (0.06 mole) of N-phthaloyl-S-benzhydryl-L-cysteine in 90 ml of methylene chloride at −10° was added 12.0 g (0.0615 mole) of WSC. The reaction was kept at −10° for 1 hr and 20° for 20 hr. The solvent was removed and the residue dissolved in 300 ml of ethyl acetate. The organic layer was washed with 1 *N* hydrochloric acid, water, 5% sodium bicarbonate, water, and saturated sodium chloride solution. The dried organic layer was evaporated and the residue (X) was dissolved in glacial acetic acid–chloroform (2:1, 300 ml). The solution was treated with 39 ml (0.3 mole) of boron trifluoride–diethyl ether complex at 20° for 1 hr and poured into ice water–chloroform (5:2, 2.8 l.). The aqueous layer was extracted with chloroform and the combined organic extracts were dried and evaporated. The residue was crystallized from ethyl acetate to provide 20.5 g (72%) of product: mp 188–190°, $[\alpha]^{18}D$ −108.6° (*c* 1.1, acetone).

Anal. Calcd for $C_{26}H_{22}O_5S$: C, 65.81; H, 4.67; N, 5.90; S, 6.76. Found: C, 65.27; H, 4.96; N, 5.89; S, 6.77.

t-**Butyl N-Phthaloyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XII).**—The oxalate salt of *t*-butyl L-phenylalanylglycinate (8.0 g, 0.0218 mole) was neutralized with 5% potassium carbonate and the free base extracted into ether. The dried ether solution was evaporated to an oil which was dissolved in 20 ml of methylene chloride (cooled to −10°) containing 9.48 g (0.02 mole) of XI. The cold solution was treated with 4.12 g (0.02 mole) of DCC and kept at −10° for 1 hr and at 20° for 19 hr. The resulting suspension was diluted with ethyl acetate and filtered. The filtrate was washed with 1 *N* hydrochloric acid, water, 5% potassium bicarbonate, water, and saturated sodium chloride solution. The solvent was removed and the residue crystallized from a chloroform–hexane solution to provide 13.8 g (94%) of solid, mp 162–165°. Recrystallization from acetone–hexane raised the melting point to 165–167°, $[\alpha]^{18}D$ −62.3° (*c* 1.0, DMF).

Anal. Calcd for $C_{41}H_{42}N_4O_7S$: C, 67.01; H, 5.76; N, 7.62; S, 4.36. Found: C, 66.96; H, 5.61; N, 7.92; S, 4.20.

N-Phthaloyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XVI).—A solution of 22.0 g (0.03 mole) of ester XII in 250 ml of acetic acid was treated with 39 ml (0.03 mole) of boron trifluoride–diethyl etherate and allowed to stand at 25° for 1 hr. The reaction mixture was poured into 1.1 l. of ice water and the suspension extracted with two 450-ml portions of ethyl acetate. The extracts were washed with water and saturated salt solution and dried. After effecting solvent removal, the residue was dissolved in diethyl ether–ethyl acetate (1:1) and treated with 1 equiv of diethylamine. The oil which separated was isolated by decantation, suspended in ethyl acetate, and extracted with 1 *N* hydrochloric acid. The ethyl acetate layer was washed with saturated brine, dried, and evaporated. The residue crystallized when treated with chloroform. The yield in three crops was 16.6 g (81%): mp 110–114°, $[\alpha]^{22}D$ −85.90° (*c* 1.46, acetone).

(10) Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and Triangle Laboratories, Chapel Hill, N. C. Optical rotations were determined with a Perkin-Elmer polarimeter, Model 141 (glass cell). Thin layer chromatography (tlc) was used as the criterion of purity. Microscope slides were used as support for the adsorbents, silica gel G and silica gel G_{254}. Iodine vapor, ninhydrin, and ultraviolet light were employed to develop the chromatograms. The following solvent systems were employed: system A, benzene–ethyl acetate (8:2); system B, chloroform–methanol (9:1). Melting points were taken in unsealed capillary tubes and on a Kofler hot stage and are uncorrected. Solvents were dried and distilled prior to use. Solvent removal was effected with rotary evaporators; bath temperatures were maintained at or below 40°.

(11) J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Am. Chem. Soc.*, **74**, 3822 (1952).

(12) L. Zervas and I. Photaki, *ibid.*, **84**, 3887 (1962).

(13) When the substance was prepared by the procedure of A. A. Boderin, G. R. Delpierre, and J. S. Fruton, *J. Am. Chem. Soc.*, **87**, 5469 (1965), the yield was 90%.

Anal. Calcd for $C_{37}H_{34}N_4O_7S$: C, 65.47; H, 5.05; N, 8.25; S, 4.72. Found: C, 64.76; H, 5.32; N, 8.60; S, 4.61.

N-Benzhydryloxycarbonyl-S-trityl-L-cysteine Diethylammonium Salt.—To a solution of 4.24 g of sodium carbonate in 70 ml of water and 120 ml of dioxane was added 14.52 g (0.04 mole) of S-trityl-L-cysteine. The solution was treated with 15.1 g (0.06 mole) of benzhydryloxycarbonyl azide[14] and the suspension stirred 72 hr at 25°. The reaction mixture was poured into 1 l. of ice water and acidified with 5 *N* hydrochloric acid. The separated oil was extracted with ethyl acetate, dried, and evaporated. The residue was dissolved in ether and treated with an equivalent amount of diethylamine. The resulting precipitate was collected and crystallized from chloroform–ether–hexane to yield 20.5 g (76.5%) of product: mp 167.5–169°, $[\alpha]^{26}D$ +19.4° (*c* 1.0, $CHCl_3$).

Anal. Calcd for $C_{40}H_{42}N_2O_4S$: C, 74.50; H, 6.55; N, 4.34; S, 4.96. Found: C, 74.55; H, 6.81; N, 4.17; S, 4.82.

***t*-Butyl N-Benzhydryloxycarbonyl-S-trityl-L-cysteinylglycinate (XXII).**—A suspension of N-benzhydryloxycarbonyl-S-trityl-L-cysteine diethylammonium salt (3.3 g, 0.005 mole) in 100 ml of ethyl acetate was treated with 100 ml of 1% sulfuric acid. The solution was washed with water and the organic layer was dried and evaporated. The residue was dissolved in 12 ml of methylene chloride, treated with 0.67 g (0.005 mole) of *t*-butyl glycinate, cooled to −10°, and treated with 1.03 g (0.0051 mole) of DCC. After 1 hr at −10° and 10 hr at 25°, 200 ml of ethyl acetate containing a few drops of acetic acid was added and the reaction mixture filtered. The filtrate was washed with 10% citric acid, water, 5% potassium bicarbonate solution, and saturated sodium chloride solution. The dried organic extract was evaporated and lyophilized from benzene. Chromatography of the crude solid (3.22 g) on florisil using benzene as the eluent provided 1.37 g (40% of XXII, homogeneous on tlc (system A): mp 81–82°; $[\alpha]^{25}D$ +3.6° (*c* 0.5, C_2H_5OH).

Anal. Calcd for $C_{42}H_{42}N_2O_5S$: C, 73.44; H, 6.16; N, 4.08; S, 4.67. Found: C, 73.62; H, 6.39; N, 4.04; S, 4.41.

N-Phthaloyl-S-trityl-L-cysteine Diethylammonium Salt.—To a solution containing 10.6 g of sodium carbonate in 180 ml of water and 120 ml of dioxane was added 36.3 g (0.10 mole) of S-trityl-L-cysteine and 22.5 g (0.0103 mole) of ethoxycarbonylphthalimide.[15] After 7 hr at 25°, the reaction mixture was diluted with 900 ml of cold water and acidified with 5 *N* hydrochloric acid. The resulting oil was extracted into ether. The ether layer was washed with saturated salt solution and dried. The ether solution was treated with an equivalent amount of diethylamine and evaporated to dryness. The residue was crystallized from ethyl acetate–ether to give 46.5 g (83%) of product: mp 161–164°, $[\alpha]^{21}D$ −22.7° (*c* 1.15, $CHCl_3$).

Anal. Calcd for $C_{34}H_{34}N_2O_4S$: C, 72.06; H, 6.05; N, 4.94; S, 5.66. Found: C, 71.96; H, 6.22; N, 4.96; S, 5.69.

***t*-Butyl N-Phthaloyl-S-trityl-L-cysteinylglycinate (XIVb).**—A suspension of 6.97 g (0.0123 mole) of N-phthaloyl-S-trityl-L-cysteine diethylammonium salt in ethyl acetate was neutralized by addition of 1 *N* hydrochloric acid until solution was complete. The organic layer was dried, evaporated, and the residue dissolved in 15 ml of methylene chloride. The solution was cooled to −10° and treated with 1.61 g (0.0123 mole) of *t*-butyl glycinate followed by 2.53 g (0.0125 mole) of DCC. After 1 hr at −10° and 18 hr at 25° the reaction mixture was filtered and the filtrate washed with 10% citric acid, water, 5% potassium bicarbonate, water, and saturated sodium chloride solution. The dried organic layer was evaporated to a residue which was lyophilized from benzene to yield 7.65 g of crude product. A 1.0-g sample of the crude product was chromatographed on 100 g of Florisil using benzene as the eluent. The slower moving component was identified as the desired dipeptide derivative: 0.46 g (46%), $[\alpha]^{22}D$ −1.3° (*c* 1.1, acetone).

Anal. Calcd for $C_{36}H_{34}N_2O_5S$: C, 71.26; H, 5.65; N, 4.62; S, 5.28. Found: C, 71.34; H, 5.78; N, 4.76; S, 5.28.

The faster moving component of the reaction mixture proved to be the N-acylurea, N-cyclohexyl-N-(N-phthaloyl-S-trityl-L-cysteinyl)-N¹-cyclohexylurea (XV).

Anal. Calcd for $C_{43}H_{45}N_3O_4S$: C, 73.79; H, 6.48; N, 6.00; S, 4.58. Found: C, 74.01; H, 6.69; N, 6.01; S, 4.27.

***t*-Butyl N-*o*-Nitrophenylsulfenyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (XXIII).**—Neutralization of 3.80 g (0.0103 mole) of VI followed by extraction into ether provided the free amine, which was dried and dissolved in 15 ml of methylene chloride. The solution (cooled to −10°) was treated with 5.16 g (0.01 mole) of *o*-nitrophenylsulfenyl-S-trityl-L-cysteine[16] followed by 2.0 g (0.0103 mole) of WSC. After 1 hr at −10° and 19 hr at 25°, the methylene chloride was replaced with ether and extracted with 2 *N* sulfuric acid. The ethereal solution was washed with 5% sodium bicarbonate, water, and saturated salt solution. The dried solution was evaporated and the residue lyophilized from benzene to give 7.2 g (92%) or crude product. A 2-g portion was chromatographed on 150 g of Florisil to give 1.76 g (88% recovery) of material that was homogeneous on tlc (system A), $[\alpha]^{22}D$ −10.7° (*c* 1.04, methanol).

Anal. Calcd for $C_{43}H_{44}N_4O_6S_2$: C, 66.47; H, 5.71; N, 7.21; S, 8.25. Found: C, 68.11; H, 5.84; N, 7.39; S, 7.93.

***t*-Butyl N-Phthaloyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (XX).**—L-Phenylalanylglycine *t*-butyl ester oxalate (1.90 g, 0.0052 mole) was neutralized with 5% potassium carbonate, affording the free amine which was extracted into ether. After drying, the ethereal solution was evaporated to dryness and the residue dissolved in 10 ml of methylene chloride. To this solution was added a methylene chloride solution of N-phthaloyl-S-trityl-L-cysteine (prepared by neutralizing 2.83 g (0.005 mole) of N-phthaloyl-S-trityl-L-cysteine diethylamine salt). The solution was concentrated to 10 ml *in vacuo*, cooled to −10°, and treated with 1.02 g of WSC. After 24 hr the solvent was removed *in vacuo* and the residue dissolved in 200 ml of ethyl acetate. The organic layer was washed with 1 *N* hydrochloric acid, water, 5% sodium bicarbonate, water, and saturated salt solution. The solution was dried and evaporated. The residue was lyophilized from benzene to give 1.1 g (29%) of product. Purification was accomplished by filtering a chloroform solution of the crude product through florisil. The recovery was 655 mg (17%): $[\alpha]^{22}D$ −5.5° (*c* 1.033, DMF).

Anal. Calcd for $C_{45}H_{43}N_3O_6S$: C, 71.64; H, 5.75; N, 5.57; S, 4.25. Found: C, 71.47; H, 5.71; N, 5.53; S, 4.14.

***t*-Butyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XVII).** **A. *t*-Butyl S-Benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XIII).**—To a hot solution of 10.3 g (0.014 mole) of *t*-butyl N-phthaloyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XII) in 40 ml of methanol–*t*-butyl alcohol (1:1) was added 0.75 ml (0.015 mole) or 99% hydrazine hydrate. The solution was refluxed 1 hr, cooled, and evaporated. The residue was suspended in 50 ml of 5% potassium carbonate solution and extracted with two 100-ml portions of ether–ethyl acetate (1:1). The organic extract was dried and treated with 0.014 mole of oxalic acid dihydrate. The resulting solid was collected and dried *in vacuo* to yield 8.66 g (90%) of crude product. Prior to use, the oxalate salt was neutralized with 5% potassium carbonate, extracted into ethyl acetate, dried, and coupled without purification.

B. Coupling of XIII and N-Carbobenzoxy-S-trityl-L-cysteine.—The oxalate salt of S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine *t*-butyl ester (XIII) (14.0 g, 0.0203 mole) was neutralized by suspension in 100 ml of ether and 50 ml of ethyl acetate followed by treatment with 60 ml of 5% potassium carbonate until all solids were dissolved. The aqueous layer was extracted with 90 ml of water and 30 ml of ethyl acetate. The combined organic extracts were washed with water and saturated salt solution, dried, and evaporated *in vacuo* to dryness. The residual oil and 9.96 g (0.02 mole) of N-carbobenzoxy-S-trityl L-cysteine were dissolved in 25 ml of DMF and 10 ml of methylene chloride, cooled to −10°, and treated with 4.0 g of WSC at −10° for 1 hr. After 3 hr the reaction mixture was diluted with 30 ml of methylene chloride and stirred for a total reaction time of 21 hr. The mixture was poured (with the aid of methanol) into 200 ml of 1 *N* hydrochloric acid and 200 ml of ice. The white solid was collected, washed thoroughly with methanol and ether, and dried *in vacuo* to give 17.2 g (81%) of product. The analytical sample was prepared by trituration with hot methanol and precipitation of the residue from chloroform with methanol and ether. The compound was homogeneous by thin layer chromatography (benzene–dioxane–acetic acid, 90:25:4); its nmr spectrum indicated the proper ratio of *t*-butyl to aromatic protons; it had mp 219–221°, $[\alpha]^{18}D$ −51.4° (*c* 1.0, DMF).

(14) R. G. Hiskey and J. B. Adams, Jr., *J. Am. Chem. Soc.*, **87**, 3969 (1965).

(15) G. H. L. Nefkins, G. I. Tesser, and R. J. F. Nivard, *Rec. Trav. Chim.*, **79**, 688 (1960).

(16) L. Zervas, D. Borovas, and E. Gaziz, *J. Am. Chem. Soc.*, **85**, 3660 (1963).

Anal. Calcd for $C_{63}H_{65}N_5O_8S_2$: C, 69.78; H, 6.04; N, 6.46; S, 5.91. Found: C, 69.88; H, 6.12; N, 6.33; S, 6.24.

N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XVIII).—A solution of 10.7 g (0.01 mole) of *t*-butyl N-carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycinate (XVII) in 200 ml of acetic acid and 150 ml of chloroform was treated with 13.0 ml of born trifluoride–diethyl etherate and allowed to stand for 1 hr at 25°. The reaction mixture was poured into 3 l. of ice water containing 500 ml of chloroform. After extracting the aqueous layer with 300 ml of chloroform, the combined chloroform extracts were evaporated to a solid residue which was suspended in 350 ml of methanol, filtered, washed with methanol and ether, and dried to yield 9.35 g (91%) of product, mp 236.5–238.5°. An analytical sample was prepared by dissolving the peptide derivative in DMF, adding a few drops of acetic acid, and reprecipitating with ether: mp 240–242°, $[\alpha]^{18}D$ −14.7° (*c* 1.0, DMF).

Anal. Calcd for $C_{59}H_{57}N_5O_8S_2$: C, 68.92; H, 5.59; N, 6.81; S, 6.24. Found: C, 68.67; H, 5.74; N, 7.03; S, 6.18.

***t*-Butyl N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycyl-S-trityl-L-cysteinyl-L-phenylalanylglycinate (I). Method A.**—N-Ethyl-5-phenylisoxazolium-3′-sulfonate (254 mg, 0.001 mole) was added to a cold (0–5°), stirred solution of N-carbobenzoxy-S-trityl-L-cysteinylglycyl-L-phenylalanylglycine (0.028 g, 0.001 mole) and triethylamine (0.14 ml, 0.001 mole) in acetonitrile–DMF (10:1). After 3 hr, 8 ml of DMF was added and stirring was maintained at 0–5° until solution was complete. The reaction mixture was treated with a solution containing 624 mg (0.001 mole) of *t*-butyl S-trityl-L-cysteinyl-L-phenylalanylglycinate in acetonitrile–DMF (2:1) and was stirred for 44 hr at room temperature. The thick suspension was diluted with 300 ml of chloroform and evaporated *in vacuo*. The oily residue was azeotropically dried with benzene, extracted with hot chloroform, and filtered. The insoluble product was washed with chloroform and dried to yield 846 mg (51.8%) of white solid. Recrystallization from pyridine–water (1:1) afforded 730 mg (44.7%) of the octapeptide derivative: mp 235–236°, $[\alpha]^{18}D$ −16.0° (*c* 1.15, DMF).

Anal. Calcd for $C_{95}H_{96}N_8O_{11}S_3$: C, 70.56; H, 5.92; N, 6.86; S, 5.89. Found: C, 70.50; H, 5.89; N, 7.17; S, 5.69.

Method B.—*t*-Butyl S-trityl-L-cysteinyl-L-phenylalanylglycinate oxalate salt (780 mg, 0.001 mole) was neutralized with 5% potassium carbonate and the free amine extracted into ethyl acetate–ether (1:1). The organic solution was dried, evaporated, and the residue dissolved in 2 ml of DMF. N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (1.08 g, 0.00107 mole) was added; the solution was cooled to −10° and treated with 200 mg (0.00103 mole) of WSC followed by 3 ml of methylene chloride. After 4 hr the reaction mixture was diluted with 20 ml of methylene chloride and stirred for 4 hr. The methylene chloride was removed *in vacuo* and the residual DMF solution was treated with cold 2.5 *N* hydrochloric acid (45 ml) and methanol (45 ml). The resulting precipitate was washed with cold methanol and ether and dried to afford 1.33 g (81.5%) of crude octapeptide derivative, mp 228–231°. The dried compound was boiled in methanol, filtered, and recrystallized from pyridine–water (1:1) to yield 892 mg (54.5%) of product melting at 234–236°, $[\alpha]^{18}D$ −16.0 (*c* 1.15, DMF). Another recrystallization from DMF–pyridine–acetone did not alter the melting point. A mixture melting point determination with an authentic sample from method A showed no depression.

Method C.—A solution containing 8.244 g (8.0 mmoles) of XVIII and 4.992 g (8.0 mmoles) of the free base of VIII in 40 ml of dry pyridine was cooled to −10° and treated with 1.648 g (8.0 mmoles) of DCC. The reaction mixture was maintained at −10° for 2 hr and then at 25° for 13 hr. During the reaction an additional 40 ml of pyridine was added to maintain solution. The reation mixture was diluted with 80 ml of water and the resulting solid collected, washed with four 100-ml portions of water, and dried. The solid was washed with hot methanol–chloroform (2:1, 240 ml) and four 50-ml portions of methanol: yield, 11.89 g (91%); mp 235–236°; $[\alpha]^{18}D$ −16.2° (*c* 1.06, DMF); homogeneous tlc (system B). In separate experiments, yields of 88 and 85% were obtained.

N-Carbobenzoxy-S-trityl-L-cysteinyl-S-benzhydryl-L-cysteinylglycycl-L-phenylalanylglycyl-S-trityl-L-cysteinyl-L-phenylalanylglycine (XIVa).—To a suspension of 327 mg (0.2 mmole) of I in 10 ml of glacial acetic acid was added 0.254 ml (2.0 mmoles) of boron trifluoride–diethyl etherate. The yellow suspension was stirred at 20° for 1.3 hr, diluted with 5 ml of acetic acid, and filtered into 25 ml of ice water. The precipitated white solid (197 mg) was washed with acetic acid (5 ml) and three 5-ml portions of water. Tlc (system B) indicated the material to be mainly unreacted ester. The filtrate was diluted with 20 ml of saturated sodium chloride solution and the resulting solid collected and dried (118 mg); tlc indicated this fraction was mainly the desired acid.

The two fractions (combined weight 315 mg) were suspended in 20 ml of glacial acetic acid and treated with 0.254 ml of boron trifluoride–diethyl etherate for 6 hr at 20°. The resulting suspension was poured into ice water (30 ml), diluted with 20 ml of saturated sodium chloride solution, filtered, and dried. The resulting solid (302 mg) still contained a trace of the starting ester (tlc); thus the solid was treated with boron trifluoride–diethyl etherate (0.254 ml) as previously described. The dried solid, obtained by dilution of the reaction mixture, was homogeneous on tlc (system B) and amounted to 294 mg (93%). The analytical sample was recrystallized from 24 ml of DMF–ether (1:5): mp 228–229°, $[\alpha]^{18}D$ −17.4° (*c* 1.0, DMF).

Anal. Calcd for $C_{92}H_{88}N_8O_{11}S_3$: C, 70.00; H, 5.61; N, 7.10; S, 6.14. Found: C, 70.01; H, 5.33; N, 6.83; S, 6.13.

Registry No.—I, 13342-47-7; VI, 13342-48-8; VII, 13342-49-9; VIII, 13342-50-2; XI, 13342-51-2; XII, 13342-42-2; XIV, 13342-43-3; XIVa, 13342-87-5; XV, 13318-39-3; XVI, 13365-00-9; XVII, 13342-59-1; XVIII, 13396-28-6; XX, 13342-44-4; XXII, 13342-45-5; XXIII, 13342-46-6; Phth-Phe-Gly-O-*t*-Bu, 13342-82-0; Tr-Gly-B_2h, 6479-35-2; H_2N-Gly-B_2h *p*-tosylate, 5042-82-0; B_2hO_2C-Cy(STr)OH-Et_2N, 13342-85-3; PhthCy(STr)OH·Et_2N, 13342-86-4.