dicated that the product consisted with three components having the retention times of 22.2, 23.0, and 32.0 min on a 5% OV-17 column, 3 m × 6 mm i.d. The product was fractionated into two portions, fraction A including the 22.2 and 23.0 min peaks and fraction B including the 32.0 min peak.

The structure of fraction B was determined as **14** by the spectra: nmr ($CDCl_3$) δ 0.94 (d, 3, J = 4.8 Hz, CH_3-CH), 1.63 (s, 9, CH_3 on double bond), 2.04 (s, 3, O-Ac), 4.10 (t, 2, J = 6.5 Hz, H_2C-O); ir (liquid film) 1740 cm^{-1} (C=O).

Fraction A in methylene chloride was ozonized for 30 min under cooling with Dry Ice–acetone. After the reaction mixture was treated with Zn and acetic acid, the mixture was filtered. Then, to the filtrate, a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* HCl was added and the mixture was stirred for 10 min at room temperature. The product was extracted with methylene chloride. Thus, 113 mg of a hydrazone mixture was obtained, which was separated by preparative tlc on silica gel G.

From the fraction, R_f 0.8, 13 mg of methyl isopropyl ketone DNP, mp 121°, was obtained. This substance agreed in melting point and ir with an authentic specimen. The DNP showing R_f 0.6, 9 mg, was identical with formaldehyde DNP in all respects.

From the fraction of R_f 0.2, 71 mg of yellow oil was obtained which was purified by alumina chromatography. The *n*-hexane–benzene (7:3) fraction gave 5 mg of oil, the structure of which was determined as **15** DNP by the nmr and mass spectra: nmr ($CDCl_3$) δ 1.05 (d, 3, J = 6.0 Hz, CH_3-CH), 1.25 (s, H_3CCH-C=N), 1.58 (s, 3, H_3CC=N), 2.05 (s, 3, O-Ac); mass spectrum *m/e* 394. By glc analysis, this substance was shown to contain a small amount of **17** DNP by the comparison with authentic sample. The benzene fraction gave 20 mg of 5-acetoxy-3-methylpentanal (**16**) DNP. The structure was confirmed by the nmr and mass spectra: nmr ($CDCl_3$) δ 1.00 (d, 2, J = 4.5 Hz, CH_3-CH), 2.05 (s, 3, O-Ac), 4.15 (t, 2, J = 6.0 Hz, H_2C-OAc); mass spectrum *m/e* 338.

The retention times of DNP derivatives of **16**, **17**, and **15** were 4.4, 7.35, and 8.1 min on a 2% OV-1 at 270°, and 6.4, 9.0, and 11.0 min on a 1.5% OV-17 at 270°.

Registry No.—**5**, 20981-59-3; **6**, 26308-99-6; **7**, 10184-81-3; **10**, 26314-72-7; **11**, 26358-50-9; **14**, 26358-51-0; **15** DNP, 26358-52-1; **16** DNP, 26314-73-8; stigmasta-5,25-dien-3β-ol, 2364-23-0; stigmasta-5,25-dien-3β-ol acetate, 2456-00-0.

Acknowledgment.—The authors are grateful to Dr. W. Sucrow, Organic Chemistry Institute of Technischen Universitat Berlin, for the samples of stigmasta-5,25-dien-3β-ol acetate and 24-nor-5-cholene-3β,23-diol 3-acetate.

Sulfur-Containing Polypeptides. XIII. Bis Cystine Peptide Derivatives[1-3]

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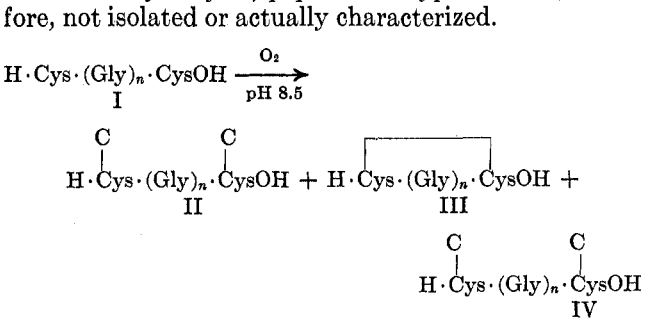
A method of synthesis of parallel cyclic bis cystine peptides has been devised. The cyclic dimers have been found to undergo base-catalyzed rearrangement to the corresponding cyclic monomers.

As the role of the disulfide bridges in protein molecules becomes better established,[5] the need for model peptides containing suitably placed sulfur–sulfur bonds has become more critical. The necessity for evaluating the chemical and conformational properties of cystine-containing peptides was recognized some years ago by Rydon, *et al.*[6] In a classic series of papers which to this date represent the only serious effort to evaluate the effect of the disulfide bond on the chemical and physical properties of a homologous series of peptides, Rydon, *et al.*, obtained the following data. Air oxidation of the L-cysteinyl-polyglycyl-L-cysteines (I), generated by the action of sodium in liquid ammonia on the fully blocked peptides, provided a varying series of products depending on the number of glycine residues separating the two sulhydryls. In no case was the parallel bis cystine peptide, IV, obtained; rather the antiparallel isomer, II, and the cyclic monomer, III, were the major products. Furthermore, Rydon, *et al.*, found that, as the number of glycine residues separating the two cysteine residues was increased to four or greater, the cyclic monomer was essentially the only product of the oxidation. In these investigations, the presence of the antiparallel bis cystine peptides II (n = 1, 2, 3) was established indirectly by careful dinitrophenylation experiments and subsequent controlled hydrolysis; peptides of type II were, therefore, not isolated or actually characterized.



Other investigators have noted, however, that a 20-membered disulfide "loop" (n = 4) may not be the most stable form under all conditions. For example, treatment of oxytocin[7] or lysine vasopressin[8,9] (both n = 4 systems) with weak bases resulted in loss of biological activity and formation of a dimer; oxidation of oxytocein in concentrated solution was observed to provide

* To whom correspondence should be addressed.

(1) The preceding paper of this series: R. G. Hiskey and B. F. Ward, Jr., *J. Org. Chem.*, **35**, 1118 (1970).

(2) Supported by Grants A-3416 and GM-07966 from the Institute of Arthritis and Metabolic Diseases and the Institute of General Medical Science, National Institutes of Health, U. S. Public Health Service.

(3) The following abbreviations have been employed in the text: Bz = benzoyl; Tr = trityl; Z = carbobenzoxy; WRK = 2-ethyl-5-phenylisoxazolium 3′-sulfonate; Phth = phthaloyl; BOC = *tert*-butyloxycarbonyl; Bzh = benzhydryl; DCC = *N,N*-dicyclohexylcarbodiimide; WSC = ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride.

(4) Abstracted in part from the dissertations of G. W. Davis, M. E. Safdy, R. A. Upham, and W. C. Jones, Jr. Submitted to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, 1966–1969.

(5) See, for example, H. Neuman, I. Z. Steinberg, J. R. Brown, R. F. Goldberger, and M. Sela, *Eur. J. Biochem.*, **3**, 171 (1967).

(6) G. S. Heaton, H. N. Rydon, and J. A. Schofield, *J. Amer. Chem. Soc.*, 3157 (1956); D. Jarvis, H. N. Rydon, and J. A. Schofield, *ibid.*, 1752 (1961); D. G. Large, H. N. Rydon, and J. A. Schofield, *ibid.*, 1749 (1961).

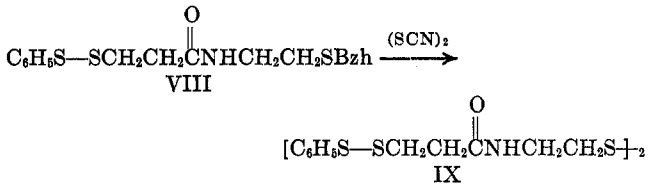
(7) C. Ressler, *Science*, **128**, 1281 (1958).

(8) A. V. Schally and R. Guillemin, *J. Biol. Chem.*, **239**, 1038 (1964).

(9) A. V. Schally and J. F. Barrett, *J. Amer. Chem. Soc.*, **87**, 2497 (1965).

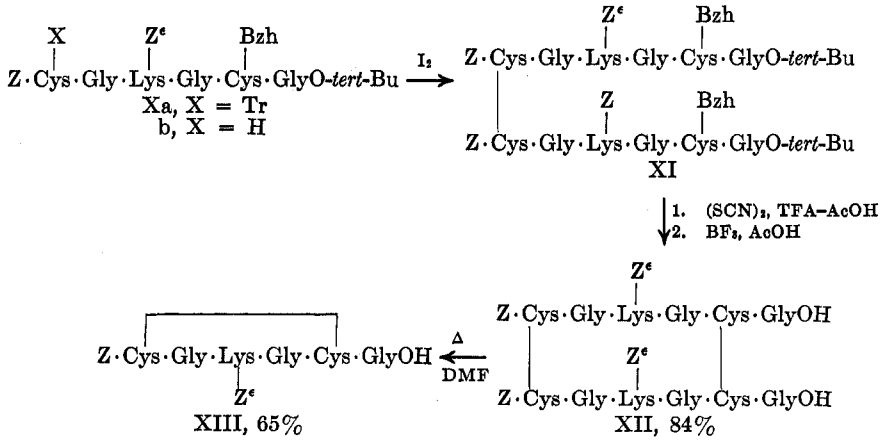
substantial amount of polymer.[10] More recent studies[11] indicated the oxytocein oxidation also yields the parallel and antiparallel oxytocin dimers in addition to the cyclic monomer. The dimers were also produced by the action of triethyl amine on oxytocin. These data together with those of Rydon, *et al.*, suggest, as might be expected, that the relative stability of the cyclic monomer and dimers depends not only on ring size, but also on the amino acid sequence of the peptide. Thus, a study of the relative stabilities of various peptides containing cystine and other amino acid residues might provide a different sort of probe for evaluation of the effect of amino acid sequence on the pairing of cystine residues. However, in order to conduct a program of this type, unambiguous methods for the synthesis of cyclic monomers and the parallel and antiparallel bis cystine derivatives were necessary. The present report concerns the synthesis of some parallel bis cystine peptides and the corresponding cyclic monomers and describes a possible approach to stability studies; a subsequent paper will describe the preparation of antiparallel dimers and a comparison of the properties of the three types of molecules.

The general synthetic approach adopted for the preparation of the cyclic bis cystine peptides is shown schematically. Previous studies have established that (a) cysteine peptides containing both *S*-trityl and *S*-benzhydryl protective groups can be selectively oxidized by a sulfenylthiocyanate or thiocyanogen at the *S*-trityl containing residue[12] and (b) a linear molecule containing a preformed disulfide bond and an *S*-trityl[13] or an *S*-benzhydryl[14] residue can be selectively oxidized in a similar manner to produce a linear bisdisulfide. Thus, the *S*-trityl or *S*-benzoyl group could be utilized as blocking group A and the *S*-benzhydryl group as B in the precursor to the protected disulfide, VI. Cyclization of VI would be formally analogous to the conversion[14] of *N*-(2-benzhydrylthioethyl)-5-phenyl-4,5-dithiapentanamide (VIII) to *N*-(3-thiapropyl)-5-phenyl-4,5-dithiapentanamide disulfide (IX).



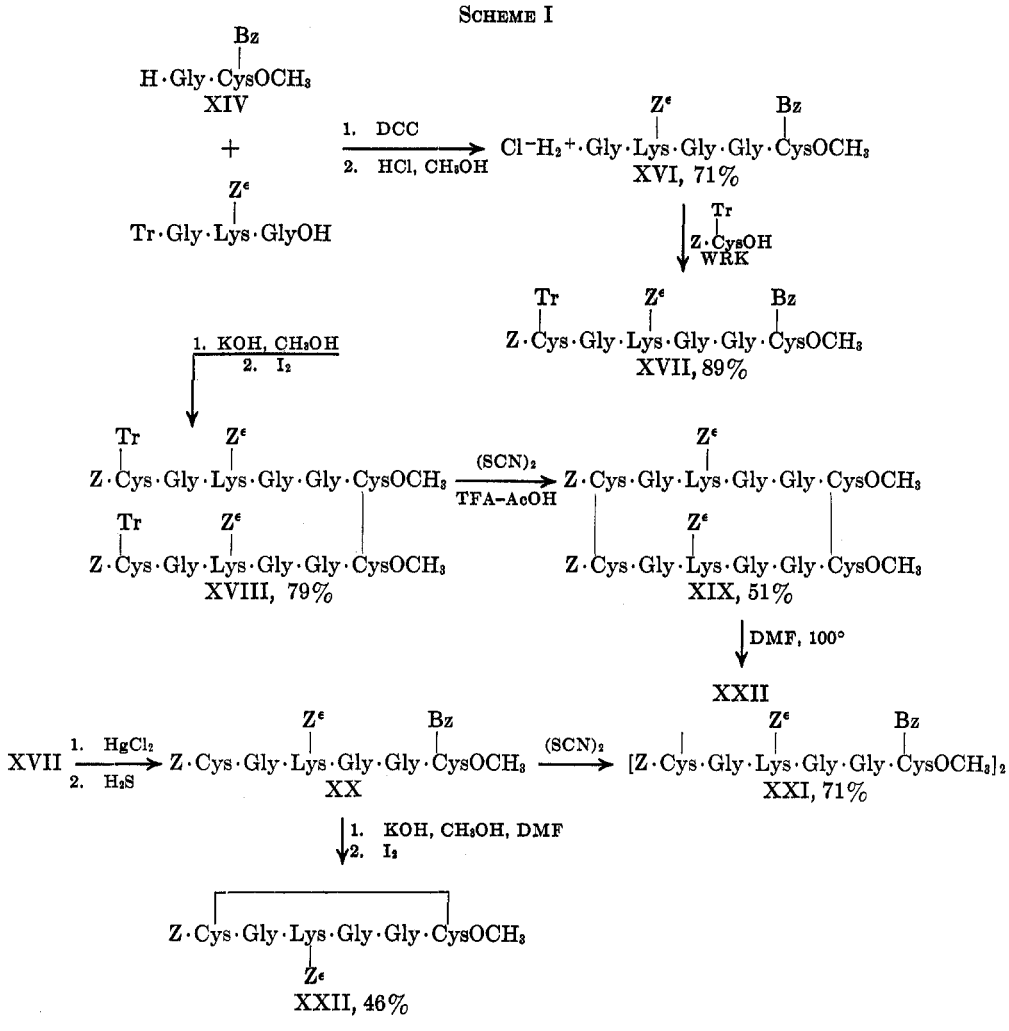
A substance meeting the structural requirements of VI was available from earlier experiments.[15] Thus, the disulfide XI (obtained *via* iodine oxidation of Xb) was allowed to react with thiocyanogen in a trifluoroacetic acid–acetic acid solvent. The tlc of the reaction mixture suggested partial ester hydrolysis had occurred under these conditions and, therefore, the reaction mixture was subsequently treated with boron trifluoride in acetic acid. The resulting product was homogeneous on tlc; elemental and amino acid analysis were consistent with the expected structure of the cyclic bis cystine peptide, XII. In order to differentiate between the desired dimer and the cyclic monomer, an osmometric molecular weight determination was carried out in DMF at 100°. The number average value obtained by this procedure was, however, 714 and suggested that the substance was the cyclic monomer, XIII, rather than XII. A weight-average molecular weight value (1400) supporting the cyclic biscystine structure, XII, was, however, obtained by ultracentrifugation[16] in DMF at 25°.

Since disulfide interchange had not been observed when the thiocyanogen reaction was conducted on the more labile linear unsymmetrical bisdisulfides,[14] another reason for the molecular weight discrepancy was sought. A possible explanation appeared to lie in the conditions used for the two determinations; the cyclic bis cystine peptide, XII, presumably stable at 25° in DMF might be expected to rearrange to the cyclic monomer, XIII, when heated to 100° in that solvent. Preliminary evidence in support of this possibility was obtained by polarimetric measurements. When the optical rotation of the substance obtained by cyclization and hydrolysis of XI, $[\alpha]^{25}D$ 66.5°, was measured at various time intervals in DMF at 100°, a regular de-



(10) M. Bodansky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 2504 (1959).
(11) D. Yamashiro, D. B. Hope, and V. du Vigneaud, *ibid.*, **90**, 3857 (1968).
(12) R. G. Hiskey, T. Mizoguchi, and E. L. Smithwick, Jr., *J. Org. Chem.*, **32**, 97 (1967).
(13) R. G. Hiskey and D. N. Harpp, *J. Amer. Chem. Soc.*, **87**, 3965 (1965).
(14) R. G. Hiskey and M. A. Harpold, *Tetrahedron*, **23**, 3923 (1967).
(15) R. G. Hiskey and J. B. Adams, Jr., *J. Org. Chem.*, **31**, 2178 (1966).
(16) H. K. Schachman, "Ultracentrifugation in Biochemistry," Academic Press, New York, N. Y., 1959.

SCHEME I



crease in rotation was observed. After 2 hr at 100° the rotation had fallen to a constant value, $[\alpha]^{25}D$ −50.7°, and the reaction product was precipitated with ether and examined by tlc. The substance obtained by precipitation was homogeneous and exhibited a slightly higher mobility than the original material; the elemental analysis of the material (subsequently identified as XIII) was consistent with the empirical formula. An approximate molecular weight value, obtained by ultracentrifugation in DMF at 25°, was 850 and thus it appeared that the cyclic bis cystine derivative, XII, had rearranged to the cyclic monomer, XIII, during the osmometric molecular weight determination.

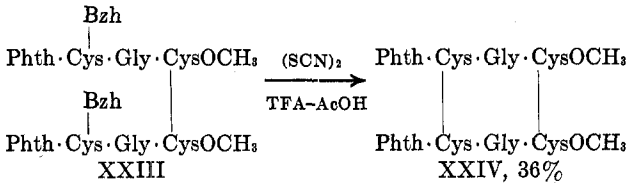
At this point a second peptide derivative of type V was also available for study. The synthetic route leading to methyl *N*-carbobenzoxy-*S*-trityl-L-cysteinylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycylglycyl-*S*-benzoyl-L-cysteinate (XVII) is indicated in Scheme I. The *S*-benzoyl protective group of XVII was removed by the action of methanolic potassium hydroxide; separation and oxidation of the crude thiol provided the symmetrical disulfide, XVIII, in 79% yield. Lower yields of XVIII were obtained when sodium methoxide in methanol was employed or when the crude thiol was oxidized without separation from the reaction mixture. When XVIII was allowed to react with thiocyanogen in trifluoroacetic acid–acetic acid (1:3 v/v) the cyclic bis cystine peptide derivative, XIX, was obtained in 51% yield. The elemental analysis was in agreement with structure XIX; the molecular weight value obtained by ultracentrifugation (DMF, 25°) was 1600. Again the molecular weight value obtained by osmometry (DMF, 100°) was one-half that expected for XIX (808). When XIX was heated in DMF at 100° for 1 hr and examined by tlc, the mobility of the single spot was slightly greater than that of the starting material suggesting the cyclic monomer XXII had been produced.

In order to obtain an authentic sample of the cyclic monomer, XXII, for tlc comparison, XVII was detritylated using mercuric chloride followed by treatment with hydrogen sulfide. For characterization the resulting thiol, XX, was oxidized with thiocyanogen to the symmetrical disulfide, XXI. Treatment of the *S*-benzoylthiol XX with potassium hydroxide in methanol–DMF,[17] followed by oxidation of a dilute solution of the crude dithiol with iodine, provided the cyclic monomer, XXII, in 46% yield. The elemental analysis and molecular weight of the substance were in agreement with the structure XXII. A tlc comparison of this material with the product produced by the action of DMF at 100° on XIX, indicated the two to be identical. Thus, it appeared that rearrangement of a cyclic bis cystine peptide to the corresponding cyclic monomer occurred in both the $n = 3$ and 4 systems.

Following these experiments, the utility of *o*-chlorophenol as a solvent for osmometric molecular weight de-

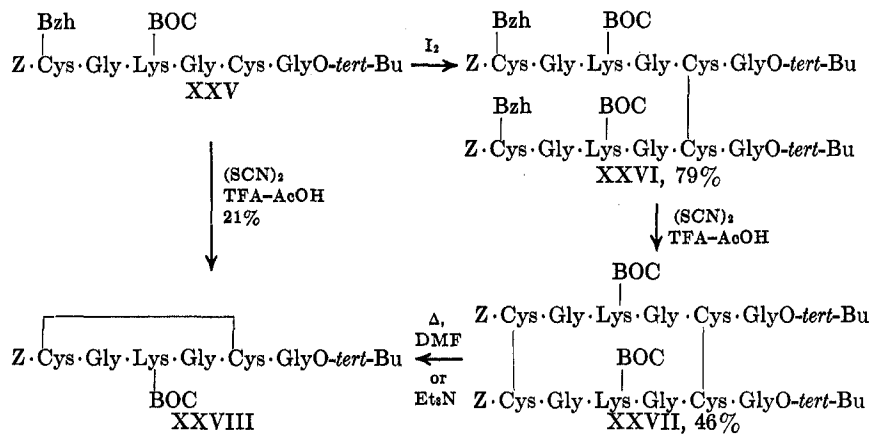
(17) The relatively low yield of crude dithiol probably results from β elimination of mercaptide caused by the use of strong base in a highly polar solvent: R. G. Hiskey, R. A. Upham, G. M. Beverly, and W. C. Jones, Jr., *J. Org. Chem.*, **35**, 513 (1970). This possibility was not considered at the time this reaction sequence was carried out.

terminations was recognized.[18] The use of this solvent allowed molecular weights to be determined at 37° and the use of DMF could be avoided. In order to obtain positive evidence for the dimeric structures, the $n = 1$ parallel dimer, dimethyl *N,N*-diphthaloyl-L-cystinylglycyl-L-cystinate (XXIV), was prepared by oxidation of dimethyl *N,N'*-bis(*N*-phthaloyl-*S*-benzyhdryl-L-cysteinylglycyl)-L-cystinate (XXIII). The elemental analysis and molecular weight value (*o*-chlorophenol, 37°) were consistent with the formulation as a cyclic bis cystine peptide derivative rather than the cyclic monomer. The stability of XXIV toward DMF at 100°



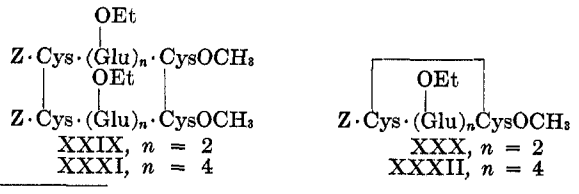
or base was not evaluated although it should be noted that several attempts to obtain the cyclic monomer in the $n = 1$ system have provided the corresponding antiparallel dimer.[19] A similar result was observed in the earlier work of Rydon, *et al.*[6]

A second $n = 3$ parallel dimer and corresponding cyclic monomer were prepared from *tert*-butyl *N*-carbobenzoxy-*S*-benzhydryl-L-cysteinylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglycyl-L-cysteinylglycinate[17] (XXV); the cyclic monomer, XXVIII, could be obtained in low yield by cyclization of a dilute solution of XXV with thiocyanogen. Additional synthetic experiments of a similar nature provided two other sets of parallel dimers and cyclic monomers; these included γ-ethyl-L-glutamyl



derivatives, XXIX and XXX of the $n = 2$ system and the similar homologs of the $n = 4$ system, XXXI and XXXII (Schemes II and III). With these samples in hand the rearrangement of the cyclic biscystine peptides to cyclic monomers was considered in more detail. The earlier data did not distinguish between a thermal rearrangement and a process which would proceed by base catalysis. The presence of trace quantities of dimethylamine in DMF is well established and this route appeared the more likely possibility. When XXVII was heated to 100° in DMF the substance was completely converted to the cyclic monomer, XXVIII, in 0.5 hr; under the same conditions XXVIII was unchanged. However, when the bis cystine derivative, XXVII, was heated to 100° in 2,2,2-trifluoroethanol for periods up to 4 hr no XXVIII could be detected by tlc. This result suggested the conversion was base catalyzed. Support for this view was obtained by treatment of XXVII with triethylamine in a chloroform–methanol solvent. Although no change in the tlc pattern was observed after 3 hr at room temperature, XXVII was almost completely converted to XXVIII after 16.5 hr at 50°. In contrast to the data obtained with oxytocin[11] no rearrangement of XXVIII → XXVII (monomer to dimer) was noted during this period. The addition of triethylamine to a solution of XXVII in DMF at 25° resulted in the formation of a substantial amount of XXVIII (tlc) after 24 hr; the cyclic monomer XXVIII was again not effected by these conditions.

A comparison of the specific rotations of the available cyclic bis cystine peptides and the corresponding cyclic monomers indicate that the parallel dimers exhibit larger negative specific rotations than the monomers. Preliminary studies indicate that the dimer → monomer conversion can be followed polarimetrically; we anticipate that future studies on the kinetics of this conversion will provide information relating the effect of ring size and amino acid composition of other bis cystine peptides to the rate of this process.



(18) The initial observation was made by Dr. R. L. Smith of this laboratory: R. G. Hiskey and A. J. Dennis, *Anal. Lett.*, **1**, 221 (1968).

(19) W. C. Jones, Jr., and K. Tomibe, unpublished observations.

Experimental Section[20]

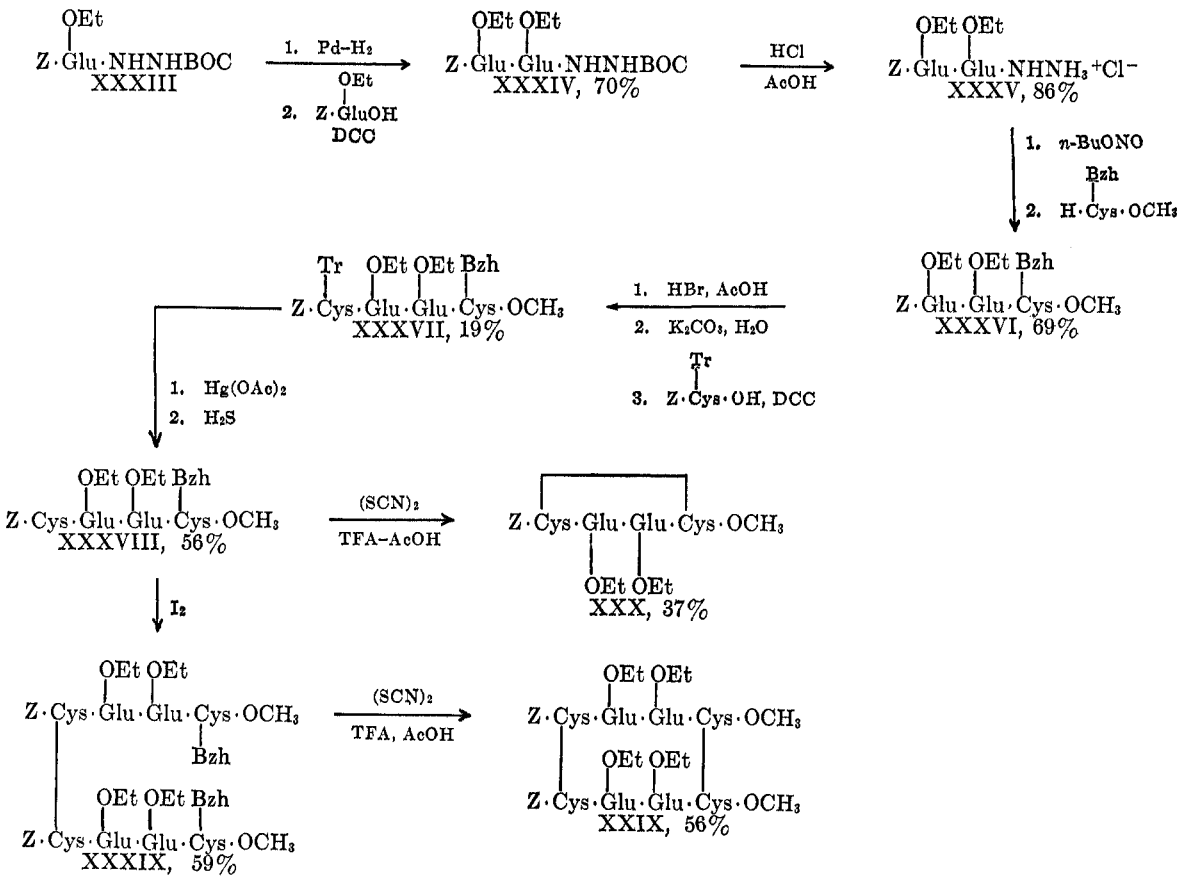
N-Phthaloyl-*S*-benzhydryl-L-cysteinylglycine[21] was prepared in 71% yield as previously described.

(20) Melting points are uncorrected and were taken in capillary tubes or on a Kofler hot stage. Optical rotations were measured on a Perkin–Elmer polarimeter Model 141. Elemental analyses were performed by Micro Tech Laboratories, Skokie, Ill. Molecular weights were determined using a Mechrolab Model 301A vapor pressure osmometer or a Beckman Model E ultracentrifuge. Amino acid analyses were performed on a Beckman Model 116 amino acid analyzer. Thin layer chromatography was conducted on microscope slides coated with silica gel GF_{254} which were developed with iodine vapor, ultraviolet light, or ninhydrin. Solvent systems employed were system A, chloroform–methanol, 9:1; system B, chloroform–methanol, 19:1; system C, chloroform–methanol–acetic acid, 8:1:1; system D, chloroform–methanol–acetic acid, 14:2:1.

(21) R. G. Hiskey, J. T. Staples, and R. L. Smith, *J. Org. Chem.*, **32**, 2772 (1967).

SCHEME II

SYNTHESIS OF XXIX AND XXX



Dimethyl *N,N'*-Bis[(*N*-phthaloyl-*S*-benzhydryl-L-cysteinyl-glycyl)-L-cystinate] (XXIII).—To a suspension of 2.00 g (4.2 mmol) of *N*-phthaloyl-*S*-benzhydryl-L-cysteinylglycine, 0.72 g (4.2 mmol) of L-cysteine methyl ester hydrochloride, and 0.57 ml (4.1 mmol) of triethylamine in 5 ml of methylene chloride, cooled to −10°, was added 0.88 g (4.2 mmol) of DCC. After 2 hr the suspension was filtered, the filtrate evaporated, and the resulting foam was dissolved in ethyl acetate. The solution was washed with 0.1 *N* hydrochloric acid, water, and brine. The ethyl acetate was replaced with methanol and the resulting solution was treated with excess (26.2 ml) 0.1 *N* iodine–potassium iodide solution. The yellow solution was poured into ice water and the resulting solid was filtered, washed with water, dried, and dissolved in ethyl acetate. The solution was washed with 1% sodium thiosulfate solution, water, and brine; the ethyl acetate was evaporated and replaced with 5% methanol in chloroform. The solution was filtered through 20 g of silica gel and the solvent was removed to provide 1.77 g (71%) of solid. The analytical sample was prepared by filtration of an ethyl acetate solution of the substance through silica gel, $[\alpha]^{26}D$ −57.8° (*c* 1.02, $CHCl_3$).

Anal. Calcd for $C_{60}H_{56}N_6O_{12}S_4$: C, 61.00; H, 4.78; N, 7.11; S, 10.86. Found: C, 60.88; H, 4.87; N, 7.25; S, 11.32.

Dimethyl *S,S*-Bis-*N*-phthaloyl-L-hemicystylglycyl-*S',S'*-L-hemicystine (XXIV).—Thiocyanogen was generated by the action of 200 mg (1.25 mmol) of bromine, contained in 10 ml of dry ethyl acetate, on a suspension of lead thiocyanate (487 mg, 1.50 mmol) in 20 ml of dry ethyl acetate. The thiocyanogen solution was filtered into an addition funnel and added over 15 min to a cold solution of the di-*S*-benzhydryl disulfide (XXIII) (1.21 g, 1.02 mmol) in 100 ml of 1:1 v/v acetic acid–trifluoroacetic acid solution. The reaction mixture was stirred 19 hr in the dark and then lyophilized. The residue was treated with 500 ml of hexane and the resulting solid collected and dissolved in chloroform and treated with two portions of decolorizing charcoal. The chloroform solution was filtered and diluted with hexane, and the resulting solid was filtered and applied to a column of silica gel (9 g). Gradient elution with chloroform to neat ethyl acetate provided 312 mg (36%) of a powder homogeneous by tlc (system B), $[\alpha]^{31}D$ −120.6° (*c* 0.50, $CHCl_3$).

Anal. Calcd for $C_{84}H_{84}N_6O_{12}S_4$: C, 48.22; H, 4.05; N, 9.92; S, 15.14; mol wt, 847. Found: C, 48.50; H, 4.30; N, 9.69; S, 14.78; mol wt (osmometry, *o*-chlorophenol, 37°), 851.

S,S-Bis-*N*-carbobenzoxy-L-hemicystylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl-*S',S'*-L-hemicystylglycine (XII).—To a cold solution of 1.22 g (0.60 mol) of di-*tert*-butyl *S,S*-bis(*N*-carbobenzoxy-L-hemicystylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl)-*S*-benzhydryl-L-cysteinylglycinate[15] (XI) in 120 ml of acetic acid–trifluoroacetic acid (1:1 v/v) was added 35 ml of a filtered ethyl acetate solution containing 0.75 mol of thiocyanogen prepared from 0.29 g (0.90 mmol) of lead thiocyanate and 0.12 g (0.75 mmol) of bromine in the usual fashion. The addition required 30 min and the reaction mixture was stirred in the dark at 0–5° for 14 hr. The reaction mixture was filtered into 1600 ml of ice water; the white solid was collected, washed with water and ether, and dried. The solid was suspended in hot ethyl acetate, cooled, and collected, and washed with ethyl acetate, methanol, and ether. Precipitation from DMF with ether yielded 0.94 g of a white solid, mp 212–214°. The substance exhibited two spots in system A, presumably the acid and ester derivatives.

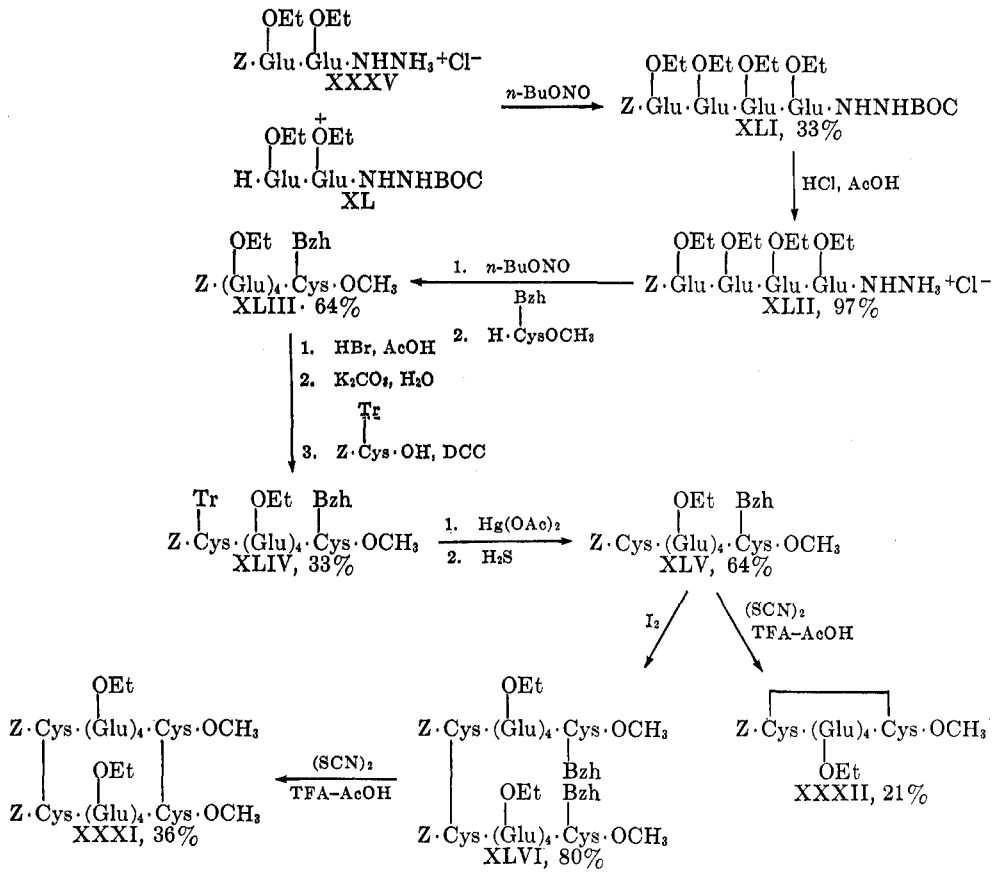
The crude solid was suspended in 20 ml of acetic acid and treated with 1.46 ml (11.2 mmol) of boron trifluoride etherate. The reaction mixture was stirred for 1 hr at room temperature, poured into 250 ml of ice water, and filtered, and the resulting white solid washed with water and ether. The dried product was precipitated from DMF with ether to yield 0.79 g (84%) of the bis cystine peptide, mp 226–229°, $[\alpha]^{25}D$ −66.5° (*c* 0.34, DMF). Amino acid analysis of an acid hydrolysate gave the following composition: $Cys_{1.1}$, $Gly_{2.5}$, $Lys_{1.0}$.

Anal. Calcd for $C_{68}H_{86}N_{14}O_{22}S_4$: C, 51.70; H, 5.49; N, 12.40; S, 8.12; mol wt, 1579. Found: C, 50.86; H, 5.57; N, 11.98; S, 8.27; mol wt, 1445 (ultracentrifuge, DMF, 25°, a partial specific volume of 0.69 cc/g was assumed).

Rearrangement of XII and Isolation of *S,S',N*-Carbobenzoxy-L-hemicystylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl-L-hemicystyl-

SCHEME III

SYNTHESIS OF XXXI AND XXXII



glycine (XIII).—A solution of 0.065 g (41.0 mmol) of XII in 10 ml of DMF was heated at 100° for 2 hr. The white solid precipitated by addition of ether was washed with ether and dried to yield 0.042 g (65%) of the cyclic monomer, mp 215–127°, $[\alpha]^{25}D$ −50.7° (*c* 0.65, DMF). The material was homogeneous on tlc, system C, and exhibited a larger R_f value than the starting bis-disulfide.

Anal. Calcd for $C_{34}H_{43}N_7O_{11}S_2$: C, 51.70; H, 5.49; N, 12.40; S, 8.12; mol wt, 789. Found: C, 50.77; H, 5.68; N, 12.73; S, 7.41; mol wt, 850 (ultracentrifuge, DMF, 25°, a partial specific volume of 0.69 cc/g was assumed).

Methyl Glycyl-*S*-benzoyl-L-cysteinate Hydrochloride (XIV).—A solution of 25.8 g (0.06 mol) of methyl *N*-carbobenzoxyglycyl-*S*-benzoyl-L-cysteinate[22] in 90 ml of trifluoroacetic acid containing 12.0 g of phenol was refluxed for 1 hr and evaporated to dryness *in vacuo*. To a solution of the residue in 80 ml of ether was added 80 ml of ether saturated with hydrogen chloride; IV separated as a syrupy gum and was crystallized by washing with ether and ethyl acetate. The product was obtained as 17.3 g (86.5%), of white solid, mp 182–184° dec. Recrystallization from a methanol–ether mixture raised the melting point to 187.5–188° dec, $[\alpha]^{24.5}D$ +5.7° (*c* 1.04, MeOH).

Anal. Calcd for $C_{13}H_{17}N_2O_4SCl$: C, 46.91; H, 5.14; N, 8.42; S, 9.64. Found: C, 46.97; H, 5.08; N, 8.21; S, 9.34.

Methyl Glycyl-N^{ϵ}-carbobenzoxy-L-lysylglycyl-*S*-benzoyl-L-cysteinate Hydrochloride (XVI).—Ethyl *N*-tritylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycinate[15] (43.2 g, 0.065 mol) was saponified using 78 ml (0.078 mol) of 1 *N* sodium hydroxide in 235 ml of dioxane for 2 hr at room temperature. The reaction mixture was diluted with 650 ml of water and acidified with 90 ml of 1 *N* sulfuric acid providing an oil which was dissolved in ethyl acetate. Evaporation of the dried solution provided a syrup; trituration with ether afforded 41.5 g of the crude acid XV. To a cold solution of 20.0 g (0.060 mol) of methyl glycyl-*S*-benzoyl-L-cysteinate in 100 ml of methylene chloride, containing 8.4 ml (0.060 mol) of triethylamine, was added a solution of 40.0 g (0.063 mol) of crude acid, XV, in 100 ml of methylene chloride followed by a solution of 12.8 g (0.062 mol) of DCC in 30 ml of methylene chloride. After being kept overnight at 5°, the precipitated DCU was removed by filtration, and the filtrate was washed with dilute hydrochloride acid, dilute potassium bicarbonate solution, and water. The dried solution was evaporated to dryness *in vacuo* and the residue was dissolved into ethyl acetate to separate the remaining DCU. The ethyl acetate solution was evaporated *in vacuo* leaving the crude pentapeptide derivative homogeneous by tlc system A. The syrup (47.9 g) was treated with 63 ml (0.063 mol) of 1 *N* hydrogen chloride in methanol. The solution was refluxed on a steam bath for 5 min, the methanol was evaporated *in vacuo*, and the residue was triturated with dry ether to yield a crystalline hydrochloride which was collected and washed with ether. Recrystallization from methanol–ethyl acetate provided 30.3 g (71.3%) of the desired pentapeptide, XVI, mp 198–199° dec, $[\alpha]^{23.5}D$ −26.4° (*c* 1.04 DMF).

Anal. Calcd for $C_{31}H_{40}N_6O_9S\cdot HCl$: C, 52.50; H, 5.83; H, 11.85; S, 4.51. Found: C, 52.24; H, 5.79; N, 11.53; S, 4.30.

***N*-Carbobenzoxy-*S*-trityl-L-cysteine** was obtained by acidification of the diethylammonium salt[23] with dilute sulfuric acid. The resulting oil was crystallized from 30–60° petroleum ether and recrystallized from benzene–*n*-hexane, mp 114–115°, $[\alpha]^{25}D$ +17.8° (*c* 1.41, MeOH).

Anal. Calcd for $C_{30}H_{27}NO_4S$: C, 72.41; H, 5.47; N, 2.82; S, 6.44. Found: C, 72.68; H, 5.56; N, 3.07; S, 6.29.

Methyl *N*-Carbobenzoxy-*S*-trityl-L-cysteinylglycyl-N^{ϵ}-carbobenzoxy-L-lysylglycylglycyl-*S*-benzoyl-L-cysteinate (XVII).—To an ice-cold suspension of 2.533 g (10 mmol) of 2-ethyl-5-phenylisoxazolium 3′-sulfonate[24] in 40 ml of acetonitrile was added a solution of 4.977 g (10 mmol) of *N*-carbobenzoxy-*S*-trityl-L-cysteine in 40 ml of acetonitrile containing 1.40 ml (10 mmol) of triethylamine over 15 min. The cold suspension was treated with a cold solution containing 7.092 g (10 mmol) of XVI in 80 ml of DMF containing 1.40 ml (10 mmol) of triethylamine. The reaction mixture was stirred for 24 hr at room temperature,

(22) R. G. Hiskey, T. Mizoguchi, and T. Inui, *J. Org. Chem.*, **31**, 1192 (1966).

(23) L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, **84**, 3887 (1962).

(24) R. B. Woodward, R. A. Olofson, and H. Mayer, *ibid.*, **83**, 1010 (1961).

poured into 600 ml of water, and filtered. The resulting white solid was washed with water and ether to yield 10.3 g (89.8%) of the hexapeptide derivative XVII, mp 179–181°, $[\alpha]_D^{26.5}$ −5.2° (c 2.58, DMF). The analytical sample was recrystallized from methanol.

Anal. Calcd for $C_{61}H_{65}N_7O_{12}S_2$: C, 63.58; H, 5.69; N, 8.51; S, 5.56. Found: C, 63.57; H, 5.64; N, 8.38; S, 5.67.

When the coupling reaction was carried out (a) using *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride[25] as a coupling reagent in DMF and (b) by mixed anhydride method with isobutyl chloroformate in DMF, the yields of hexapeptide were (a) 26% (mp 176–179°) and (b) 16% (mp 178–180°).

Crude Methyl *N*-Carbobenzoxy-*S*-chloromercuri-L-cysteinylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-*S*-benzoyl-L-cysteinate.—A solution of 1.152 g (1.00 mmol) of hexapeptide XVII in 40 ml of acetic acid was treated with a solution of 544 mg (2.00 mmol) of mercuric chloride in 4.0 ml of methanol, followed by the addition of a solution containing 136 mg (1.00 mmol) of sodium acetate trihydrate in 4.0 ml of methanol. The reaction mixture was kept for 4 hr at room temperature and diluted with 40 ml of water and the white precipitate collected and washed with AcOH–H_2O (2:3), water, methanol, and finally ether to yield 1.07 g of the crude *S*-chloromercury derivative, mp 199–200° dec.

Anal. Calcd for $C_{42}H_{50}N_7O_{12}S_2HgCl$: C, 44.05; H, 4.40; N, 8.56; S, 5.60; Cl, 3.10. Found: C, 42.00; 42.13; H, 4.55, 4.39; N, 8.36; S, 5.72; Cl, 2.96.

Methyl *N*-Carbobenzoxy-L-cysteinylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-*S*-benzoyl-L-cysteinate (XX).—A solution of 1.03 g (0.90 mmol) of *S*-chloromercuripeptide in 20 ml of DMF–methanol (1:1) was treated with hydrogen sulfide for 5 min at room temperature. After removal of the precipitated mercuric sulfide, the filtrate was poured into 180 ml of water. The resulting white precipitate was collected and washed with water and ether to yield 655 mg (80.0%) of the thiol, mp 156–158°. The substance exhibited an intense color with sodium nitroprusside and was homogeneous on tlc, system D. The analytical sample was prepared by recrystallization from methanol–ether, mp 156–158°, $[\alpha]^{24}D$ −16.7° (c 1.17, DMF).

Anal. Calcd for $C_{42}H_{51}N_7O_{12}S_2$: C, 55.43; H, 5.65; N, 10.78; S, 7.05. Found: C, 55.24; H, 5.68; N, 10.97; S, 7.03.

***S,S*-Bis(methyl *N*-carbobenzoxy-L-hemicystylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-*S*-benzoyl-L-cystinate) (XXI). A. With Thiocyanogen.**—Thiocyanogen in ethyl acetate solution was prepared by dropwise addition of 100 mg (0.63 mmol) of bromine in 10 ml of ethyl acetate to a suspension of 244 mg (0.75 mmol) of lead thiocyanate in 15 ml of ethyl acetate over 12-min period at 0–5° in the dark. The cold, filtered thiocyanogen solution was then added dropwise (18 min) to a cold, dark solution of 1.152 g (1.00 mmol) of the *S*-trityl hexapeptide (XVII) in 100 ml of acetic acid–trifluoroacetic acid (3:1 v/v). After being kept overnight at 0–5°, the reaction mixture was poured into 600 ml of water and the white precipitate was collected and washed with water. The crude product, obtained by extraction of the triphenylmethylthiocyanate with ether, was purified by ether precipitation from acetic acid. The resulting peptide, 649 mg (71.4%), mp 144–146°, $[\alpha]^{24}D$ −49.8° (c 1.00, DMF), was homogeneous on tlc.

Anal. Calcd for $C_{84}H_{100}N_{14}O_{24}S_4$: C, 55.49; H, 5.54; N, 10.79; S, 7.05. Found: C, 55.76; H, 5.72; N, 10.69; S, 7.20.

Evaporation of ether extract provided 213 mg of triphenylmethyl thiocyanate, mp 133–136°.

B. With Iodine.—A solution of 136 mg (0.15 mmol) of the thiol (XX) in 3.0 ml of methanol was oxidized with 0.1 *N* iodine–potassium iodide in 80% ethanol solution. The reaction mixture consumed 1.45 ml of this reagent (96.8% of theoretical). The reaction mixture was poured into 50 ml of water containing a small amount of sodium thiosulfate. The white precipitate was collected by filtration and washed with water and finally ether to yield 120 mg (88.3%) of solid, mp 146–147°. A mixture melting point with the material obtained using thiocyanogen was not depressed.

***S',S'*-Bis(methyl *N*-carbobenzoxy-*S*-trityl-L-cysteinylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-L-hemicystinate) (XVIII). A. Using Potassium Hydroxide in Methanol and with Isolation of the Intermediate Thiol.**—A suspension of 2.304 g (2.00 mmol) of hexapeptide XVII in a mixture of 33 ml of methanol and 11 ml of DMF was treated with 4.40 ml of 0.5 *N* potassium hydroxide in methanol solution under nitrogen atmosphere for 30 min at room temperature. After acidification with 3.60 ml of acetic acid, the solution was poured into 350 ml of water and the crude mercaptan derivative was collected and washed with water and ether to yield 1.8 g (87.8%), mp 187–188°, as a white solid. The tlc of this material exhibited minor spots due to starting peptide and the disulfide derivative.

The crude thiol was oxidized with 0.1 *N* iodine–potassium iodide in 80% ethanol solution in 50 ml of methanol. The oxidation required 13.8 ml of this reagent. The disulfide, XVIII, crystallized from the reaction mixture to yield 1.4 g (79.3%) of white solid, mp 215–217°. The analytical sample was recrystallized from DMF and acetonitrile: mp 218–219°; $[\alpha]^{21}D$ −18.3° (c 1.00, DMF); homogeneous, system D.

Anal. Calcd for $C_{108}H_{120}N_{14}O_{22}S_4$: C, 61.93; H, 5.78; N, 9.36; S, 6.12. Found: C, 61.91; H, 5.90; N, 9.37; S, 5.96.

B. With Potassium Hydroxide without Isolation of Thiol.—A suspension of 1.729 g (1.50 mmol) of the *S*-trityl hexapeptide in a mixture of 24 ml of methanol and 8 ml of DMF was treated with 3.30 ml (1.65 mmol) of 0.5 *N* potassium hydroxide in methanol solution as described in A. After acidification with 2.70 ml of acetic acid, the reaction mixture was oxidized with 0.1 *N* iodine–potassium iodide in 80% ethanol solution (11.72 ml of this reagent, 78.3% based on the thiol, was consumed) and poured nito 300 ml of water containing a small amount of sodium thiosulfate. The white precipitate was collected and washed with water and ether to yield 1.46 g of crude disulfide, mp 198–203°. Purification in the manner previously described provided the disulfide, XVII, in 44% yield.

C. With Sodium Methoxide.—A suspension of 1.152 g (1.00 mmol) of the hexapeptide XVII in a mixture of 16 ml of methanol and 8 ml of DMF was treated with 2.30 ml (1.15 mmol) of 0.5 *N* sodium methoxide in methanol solution in the manner described as method A. After acidification with 1.80 ml of acetic acid, the resulting mixture was oxidized with 0.1 *N* iodine–potassium iodide in 80% ethanol solution (7.0 ml of this reagent, 70.0% based on the thiol, was consumed) and poured into 200 ml of water containing a small amount of sodium thiosulfate. The fine powder (0.88 g) which separated upon standing 48 hr melted at 189–195°. Purification provided the pure disulfide (XVIII) in 25% yield.

Methyl *S,S',N*-Carbobenzoxy-L-hemicystylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-L-hemicystinate (XXII).—A suspension of 1.238 g (1.36 mmol) of the *S*-benzoylthiol, XX, in 27.5 ml of methanol–DMF (4:1) was treated with 3.00 ml (1.50 mmol) of 0.5 *N* potassium hydroxide in methanol solution in the same manner previously described. The crude dithiol yield [1.033 g (94.5%), mp 170°, sinters at 172–174°] was contaminated with a minor amount of starting material as indicated by tlc, system D.

To a solution of 344 mg (1.36 mmol) of iodine in 400 ml of methanol was added dropwise a solution of 1.033 g (1.28 mmol) of the crude dithiol in 400 ml of methanol–DMF (4:1) during a 2.5-hr period at room temperature. After evaporation of methanol, the residual solution was poured into 500 ml of water containing 1.0 g of sodium thiosulfate and 0.5 hr later 350 ml of saturated sodium chloride aqueous solution was added. The white product was collected and washed with water and ether. The dried product (810 mg) was dissolved in 8.5 ml of acetic acid and 17.0 ml of ether was added. The material precipitated (190 mg) was removed; the filtrate was diluted with large amounts of ether to yield the cyclic monomer as a white powder 510 mg (49.6% based on crude dithiol, 46.6% based on XX), mp 194°, sinters at 203–206°. The analytical sample was prepared by washing the powder with hot acetone, mp 205–208°, $[\alpha]^{28.5}D$ −44.7° (c 1.10, DMF).

Anal. Calcd for $C_{84}H_{45}N_7O_{11}S_2$: C, 52.29; H, 5.64; N, 12.20; S, 7.98. Found: C, 52.96, 53.14; H, 5.82, 5.65; N, 12.02; S, 7.73.

Dimethyl *S,S,S',S'*-Bis(methyl *N*-carbobenzoxy-L-hemicystylglycyl-N^ϵ-carbobenzoxy-L-lysylglycylglycyl-L-hemicystinate) (XIX).—To a solution of 1.047 g (0.50 mmol) of the *S*-trityl disulfide, XVIII, in a mixture of acetic acid and trifluoroacetic acid, prepared by addition of 25 ml of trifluoroacetic acid to a suspension of *S*-trityl disulfide in 75 ml of acetic acid, was added dropwise a solution of thiocyanogen in ethyl acetate, which was obtained from 100 mg (0.63 mmol) of bromine and 243 mg (0.75 mmol) of lead thiocyanate in 25 ml of ethyl acetate in the manner previously described. The addition required 20 min; the temperature was maintained at 0–5 and the stirring was continued in the dark for 9 hr. The reaction mixture was poured into 600 ml

(25) J. C. Sheeham, P. A. Cruickshank, and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961).

of water and the solid was collected, washed with water and ether, and dried. The substance was dissolved in 8.5 ml of DMF and 100 ml of acetone was added. The precipitate (108 mg) was removed by filtration and the filtrate was poured into 600 ml of water. The gelatinous mass which separated from the aqueous solution was collected and washed with water to yield 413 mg (51.4%) of bisdisulfide, mp 197–201°. The analytical sample was prepared by washing the substance with hot methanol, mp 197°, sinters at 201–213°, $[\alpha]^{23.5}D$ −73.9° (*c* 1.11, DMF). The R_f value on tlc (system D) of the substance was lower than that exhibited by the cyclic monomer.

Anal. Calcd for $C_{70}H_{90}N_{14}O_{22}S_4$: C, 52.29; H, 5.64; N, 12.20; S, 7.98; mol wt, 1607.9. Found: C, 52.02; H, 5.82; N, 12.40; S, 8.26; mol wt, 1600 (ultracentrifuge, 25°, DMF, a partial specific volume of 0.69 cc/g was assumed).

S',S'-Bis(*tert*-butyl *N*-carbobenzoxy-*S*-benzhydryl-L-cysteinylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglycyl-L-hemicystylglycinate) (XXVI).—A solution of 0.196 g (0.20 mmol) of *tert*-butyl *N*-carbobenzoxy-*S*-benzhydryl-L-cysteinylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglcyl-L-cysteinylglycinate[17] (XXV) in 100 ml of absolute methanol was treated with a solution of iodine in methanol until a yellow color persisted. A small amount of sodium thiosulfate solution was added to discharge the color, and the reaction mixture was poured into 600 ml of water. The aqueous solution was saturated with sodium chloride to coagulate the product. The resulting solid was collected, washed with water, and dried *in vacuo* over phosphorus pentoxide at 100° to give 154.7 mg (79%) of the disulfide, mp 183–185°, $[\alpha]^{26}D$ −52.6° (*c* 0.35, DMAC). The compound exhibited one spot on tlc (system B).

Anal. Calcd for $C_{98}H_{128}N_{14}O_{22}S_4$: C, 58.88; H, 6.59; N, 10.01; S, 6.55. Found: C, 59.13; H, 6.62; N, 9.84; S, 6.48.

S,S',*N*-Carbobenzoxy-L-hemicystylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglycyl-L-hemicystylglycine *tert*-Butyl Ester (XXVIII).—A stirred suspension of 0.4 g (1.2 mmol) of lead thiocyanate in 20 ml of dry ethyl acetate was treated in the dark with a solution of 0.160 g (1.0 mmol) of bromine in 20 ml of dry ethyl acetate. Stirring in the dark was continued until the solution was colorless (about 10 min). A solution of 75.0 mg (0.77 mmol) of XXV in 4 ml of dry acetic acid was added dropwise to 2.0 ml (0.082 mmol) of thiocyanogen solution at 0° and then cooled to −10°. To the sulfenylthiocyanate solution was added a mixture of 25 ml of dry trifluoroacetic acid and 50 ml of dry acetic acid at −10°. The reaction mixture was stirred at −10° in the dark for 9 hr and then lyophilized to a pink amorphous solid. The solid was dissolved in 2 ml of DMAC and applied to 3.5 g of silica gel in chloroform on a 1 × 13 cm column. Elution with chloroform removed the DMAC and benzhydryl thiocyanate. Further elution with 19:1 v/v chloroform–methanol produced 18.2 mg (30%) of cyclic disulfide. Recrystallization from chloroform, methanol, ether, and hexane gave 13 mg (21%) of the product, mp 194.5–196° dec, $[\alpha]^{26}D$ −43° (*c* 0.255, DMAC), homogeneous (system B).

Anal. Calcd for $C_{34}H_{53}N_7O_{11}S_2$: C, 51.77; H, 6.58; N, 12.08; S, 7.90; mol wt, 811. Found: C, 51.70; H, 6.57; N, 11.65; S, 8.02; mol wt (osmometry, 37°, *o*-chlorophenol), 1070.

S,S,S',S'-Bis(*N*-carbobenzoxy-L-hemicystylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglycyl-L-hemicystylglycine *tert*-Butyl Ester) (XXVII).—To a rapidly stirred suspension of 0.4 g (1.2 mmol) of lead thiocyanate in 20 ml of dry ethyl acetate was added a solution of 0.160 g (1.0 mmol) of bromine in 20 ml of dry ethyl acetate. The solution was stirred in the dark for 10 min (solution was colorless). A solution of 142 mg (0.0725 mmol) of S',S'-bis(*N*-carbobenzoxy-*S*-benzhydryl-L-cysteinylglycyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysylglycyl-L-hemicystylglycine *tert*-butyl ester)[17] (XXVI) in 30 ml of dry acetic acid at −10° was treated with 3.1 ml (0.0775 mmol) of the thiocyanogen solution and 15 ml of dry trifluoroacetic acid at −10°. The reaction mixture was stirred in the dark at −10° for 9 hr and then lyophilized to a pink amorphous solid. Thin layer chromatography of the mixture indicated a spot at the origin, a spot with a very high R_f (probably benzhydryl thiocyanate), and a spot at R_f 0.27. The pink solid was dissolved in 2 ml of DMAC and applied to 4 g of silica gel in chloroform on a 1 × 13 cm column. Elution with chloroform removed the DMAC and the benzhydryl thiocyanate. Elution with a large volume (*ca.* 2 l.) of 19:1 chloroform–methanol (v/v) gave 55 mg (46.5%) of (XII), mp 202–204° dec, $[\alpha]^{26}D$ −77° (*c* 0.250, DMAC), homogeneous (system B). The amino acid analysis of an acid hydrolysate of performic acid oxidized sample was $CySO_3H_{1.9}Gly_{3.0}Lys_{1.0}$.

Anal. Calcd for $C_{70}H_{106}N_{14}O_{22}S_4$: C, 51.77; H, 6.58; N, 12.08; S, 7.90; mol wt, 1624. Found: C, 51.70; H, 6.60, N, 11.89; S, 7.96; mol wt (osmometry, 37°, *o*-chlorophenol); 1900.

N-Carbobenzoxydi(γ-ethyl-L-glutamyl)-S-benzhydryl-L-cysteine Methyl Ester (XXXVI).—A suspension of 4.0 g (0.012 mol) of *S*-benzhydryl-L-cysteine methyl ester hydrochloride in ether was shaken with 6% potassium carbonate solution. The organic layer was separated, washed with water, dried, and evaporated to yield the free base as an oil.

A stirred suspension of 4.0 g (7.8 mmol) of *N*-carbobenzoxy-γ-ethyl-L-glutamyl-γ-ethyl-L-glutamic acid hydrazide hydrochloride[26] (XXXV) in 25 ml of acetic acid saturated with hydrogen chloride was cooled to a slush and treated with 9 ml (80 mmol) of *n*-butyl nitrite. The reaction mixture was allowed to warm to 15° over 15 min, diluted with 250 ml of cold ethyl acetate, and washed repeatedly with cold 1% sodium bicarbonate solution until the washings were neutral to litmus. The organic layer was dried and added to the free base. After standing at −10° for 48 hr, the reaction mixture was concentrated *in vacuo* and the resulting white solid filtered and recrystallized from ethyl acetate–hexane to afford 4.2 g (69%) of white powder, mp 124–125°, $[\alpha]^{25}D$ −42.2° (*c* 0.54, TFE).

Anal. Cacld for $C_{39}H_{47}N_3SO_{10}$: C, 62.46; H, 6.32; N, 5.60; S, 4.28. Found: C, 62.76; H, 6.31; N, 5.74; S, 4.21.

N-Carbobenzoxytetra(γ-ethyl-L-glutamyl)-S-benzhydryl-L-cysteine Methyl Ester (XLIII).—The substance was prepared in 70% yield from *N*-carbobenzoxy-tri-(γ-ethyl-L-glutamyl)-α,γ-diethyl-L-glutamic acid hydrazide[26] (XLII) and *S*-benzhydryl-L-cysteine methyl ester as described above. The product was recrystallized from chloroform–hexane and was obtained as a powder, $[\alpha]^{25}D$ −31.4° (*c* 0.5, TFE).

Anal. Calcd for $C_{53}H_{69}N_5SO_{16}$: C, 59.81; H, 6.54; N, 6.58; S, 3.01. Found: C, 59.27; H, 6.45; N, 6.99; S, 2.92.

N-Carbobenzoxy-S-trityl-L-cysteinyldi(γ-ethyl-L-glutamyl)-S-benzhydryl-L-cysteine Methyl Ester (XXXVII).—A solution of 0.4 g (0.54 mmol) of XXXVI in 5 ml of saturated hydrogen bromide in acetic acid solution was allowed to stand for 20 min at 25°. The solution was then lypohilized and the residue dissolved in 100 ml of ethyl acetate. The solution was shaken with 6% potassium carbonate solution and the organic layer dried and evaporated *in vacuo* to a clear colorless oil.

A solution of 0.274 g (0.54 mmol) of *N*-carbobenzoxy-*S*-trityl-L-cysteine in 20 ml of methylene chloride was added to the free base obtained above. The resulting solution was cooled to −10° and treated with 0.11 g (0.54 mmol) of DCC. After stirring for 3 hr at −10° and 8 hr at 25°, the solvent was evaporated *in vacuo* and the residue was triturated with hot ethyl acetate and filtered. Evaporation of the filtrate provided a white solid which was crystallized and recrystallized from a chloroform–ether–hexane solvent. The tetrapeptide derivative appeared as 0.11 g (19%) of white solid, mp 113–116°, $[\alpha]^{25}D$ −26.0° (*c* 0.5, TFE).

Anal. Calcd for $C_{61}H_{66}N_4S_2O_{11}$: C, 66.89; H, 6.07; N, 5.12; S, 5.86. Found: C, 66.17; H, 6.48; N, 5.83; S, 5.26.

N-Carbobenzoxy-S-trityl-L-cysteinyltetra(γ-ethyl-L-glutamyl)-S-benzhydryl-L-cysteine Methyl Ester (XLIV).—The substance was prepared in overall 33% yield by the DCC coupling of *N*-carbobenzoxy-*S*-trityl-L-cysteine with the free base obtained from the treatment of XLIII as above. The product was recrystallized from chloroform–hexane to yield a white powder, $[\alpha]^{25}D$ −20.5° (*c* 0.56, TFE).

Anal. Calcd for $C_{75}H_{88}N_6S_2O_{17}$: C, 63.90; H, 6.29; N, 5.96; S, 4.55. Found: C, 63.77; H, 6.29; N, 6.01; S, 4.62.

Generation of Crude N-Carbobenzoxy-L-cysteinyldi(γ-ethyl-L-glutamyl)-S-benzhydryl-L-cysteine Methyl Ester (XXXVIII).—A 1-ml solution (0.050 mmol) of 0.05 *M* mercuric acetate in methanol was treated at 0° with a solution containing 54 mg (0.05 mol) of XXXVII in 2 ml of DMAC. The solution was allowed to stir at 25° for 4 hr and the methanol was removed *in vacuo*. The DMAC solution was treated with 25 ml of ether, the suspension centrifuged, and the supernatant liquid decanted. The resulting solid was dissolved in 4 ml of acetic acid containing two drops of β-mercaptoethanol and treated with hydrogen sulfide. The mercuric sulfide was filtered and the crude thiol precipitated by the addition of water to the filtrate. The white, nitroprusside-positive solid was washed with water to provide 0.24 g (56%) of crude thiol as a white powder, mp 155–160°, homogeneous on tlc (system B).

(26) M. Goodman, I. G. Rosen, and M. Safdy, *Biopolymers*, **2**, 502 (1964).

Generation of Crude *N*-Carbobenzoxy-L-cysteinyltetra(γ-ethyl-L-glutamyl)-*S*-benzhydryl-L-cysteine Methyl Ester (XLV).—The crude hexapeptide thiol was prepared from XXXV in 64% yield as described above. The substance was obtained as a white nitroprusside-positive powder, homogeneous on tlc (system B).

***S,S',N*-Carbobenzoxy-L-hemicystyldi(γ-ethyl-L-glutamyl)-L-hemicysteine Methyl Ester (XXX).**—A stirred suspension of 0.4 g (1.2 mmol) of lead thiocyanate in 20 ml of ethyl acetate was treated in the dark with a solution of 160 mg (1.0 mmol) of bromine in 20 ml of ethyl acetate. The mixture was stirred for 10 min in the dark, at which time the solution was colorless. A solution of 24 mg (0.028 mmol) of XXXVIII in 4 ml of acetic acid was added dropwise over 10 min to 1.3 ml (0.031 mmol) of thiocyanogen solution in the dark. The solution was stirred for 10 min at 0°. To the sulfenyl thiocyanate solution was added a mixture of 30 ml of acetic acid and 15 ml of TFA. The solution was stirred in the dark at 0° for 32 hr and lyophilized to a pink residue which was crystallized from chloroform–methanol–hexane, filtered, and washed with water and ether to yield 7 mg (37%) of white powder, MTLC (system A) homogeneous, $[\alpha]^{22}D$ −20.3 (*c* 0.15, DMF).

Anal. Calcd for $C_{29}H_{40}N_4S_2O_{11}$: C, 50.86; H, 5.89; N, 8.18; S, 9.37. Found: C, 50.91; H, 5.92; N, 7.96; S, 9.46.

***S,S',N*-Carbobenzoxy-L-hemicystyltetra(γ-ethyl-L-glutamyl)-L-hemicystine Methyl Ester (XXXII).**—The title compound was prepared in 21% yield from crude *N*-carbobenzoxy-L-cysteinyltetra(γ-ethyl-L-glutamyl)-*S*-benzhydryl-L-cysteine methyl ester using the procedure described above. The substance was obtained as a white powder, MTLC (system A) homogeneous, $[\alpha]^{22}D$ −45.9 (*c* 0.05 DMF).

Anal. Calcd for $C_{43}H_{62}N_6S_2O_{17}$: C, 51.69; H, 6.26; N, 8.41; S, 6.42. Found: C, 51.27; H, 6.08; N, 8.65; S, 6.26.

***S,S*-Bis[*N*-carbobenzoxy-L-hemicystyldi(γ-ethyl-L-glutamyl)-*S*-benzhydryl-L-cysteine Methyl Ester] (XXXIX).**—A solution of 220 mg (0.26 mmol) of XXXVIII in 2 ml of chloroform–methanol (1:1) was treated dropwise with an ethereal solution of iodine until the color persisted. Ether was added to precipitate the product and the mixture was centrifuged, the supernatant decanted, and the resulting gum was dissolved in acetic acid. Several drops of sodium thiosulfate solution was added to destroy the iodine color, and the product was precipitated by addition of water and filtered. Recrystallization from chloroform–methanol–ether afforded 195 mg (89%) of a white powder, homogeneous (system B), nitroprusside negative.

Anal. Calcd for $C_{84}H_{102}N_8S_4O_{22}$: C, 59.20; H, 6.03; N, 6.58; S, 7.53. Found: C, 59.24; H, 6.28; N, 6.68; S, 7.43.

***S,S*-Bis[*N*-carbobenzoxy-L-hemicystyltetra(γ-ethyl-L-glutamyl)-*S*-benzhydryl-L-cysteine Methyl Ester] (XLVI).**—The compound was prepared in 80% yield from XXXV as described above. The substance was obtained as a white powder homogeneous (system A), nitroprusside negative.

Anal. Calcd for $C_{112}H_{146}N_{12}S_4O_{34}$: C, 57.66; H, 6.31; N, 7.21; S, 5.50. Found: C, 57.63; H, 6.32; N, 7.40; S, 5.63.

***S,S,S',S'*-Bis[*N*-carbobenzoxy-L-hemicystyldi(γ-ethyl-L-glutamyl)-L-hemicystyl Methyl Ester] (XXIX).**—A stirred suspension of 0.4 g (1.2 mmol) of lead thiocyanate in 20 ml of ethyl acetate was treated with a solution of 160 mg (1.0 mmol) of bromine in 20 ml of ethyl acetate. The mixture was stirred for 10 min in the dark, at which time the solution was colorless. A stirred solution of 100 mg (0.0588 mmol) of XXXIX in 40 ml of acetic acid was cooled to a slush and treated in the dark with 2.6 ml (0.065 mol) of thiocyanogen solution, followed by 20 ml of TFA. The mixture was stirred in the dark at 0° for 48 hr and lyopholized to a pink residue which was crystallized from chloroform–methanol–ether. The filtered product was washed with ether and warm hexane. Recrystallization from acetic acid–water afforded 45 mg (56%) of white powder, homogeneous (system A), $[\alpha]^{22}D$ −43.1° (*c* 0.1 DMF).

Anal. Calcd for $C_{58}H_{80}N_8S_4O_{22}$: C, 50.86; H, 5.89; N, 8.18; S, 9.37. Found: C, 50.97; H, 5.91; N, 7.85; S, 9.16.

***S,S,S',S'*-Bis[*N*-carbobenzoxy-L-hemicystyltetra(γ-ethyl-L-glutamyl)-L-hemicystyl Methyl Ester] (XXXI).**—The title compound was prepared in 36% yield from XLVI using the procedure described above, homogeneous (system A), $[\alpha]^{22}D$ −69.4° (*c* 0.1, DMF).

Anal. Calcd for $C_{86}H_{124}N_{12}S_4O_{34}$: C, 51.69; H, 6.26; N, 8.41; S, 6.42. Found: C, 51.60; H, 6.19: N, 8.53; S, 6.33.

Registry No.—XII, 26315-81-1; XIII, 26315-82-2; XIV, 26315-83-3; XVI, 26315-84-4; XVII, 26315-85-5; XVII (*S*-chloromercury derivative), 26311-05-7; XVIII, 26438-52-8; XIX, 26315-86-6; XX, 26315-87-7; XXI, 26315-88-8; XXII, 26315-89-9; XXIII, 26358-53-2; XXIV, 26358-54-3; XXVI, 26315-90-2; XXVII, 26409-05-2; XXVIII, 26315-91-3; XXIX, 26310-96-3; XXX, 26310-97-4; XXXI, 26310-98-5; XXXII, 26310-99-6; XXXVI, 26311-00-2; XXXVII, 26358-55-4; XXXVIII, 26311-01-3; XXXIX, 26311-02-4; XLIII, 26311-03-5; XLIV, 26358-56-5; XLVI; 26358-57-6; *N*-carbobenzoxy-*S*-trityl-L-cysteine, 26311-04-6.