Sulfur-Containing Polypeptides. XV. Synthetic Routes to the A_{6-13} Segment of Ovine Insulin[1-3]

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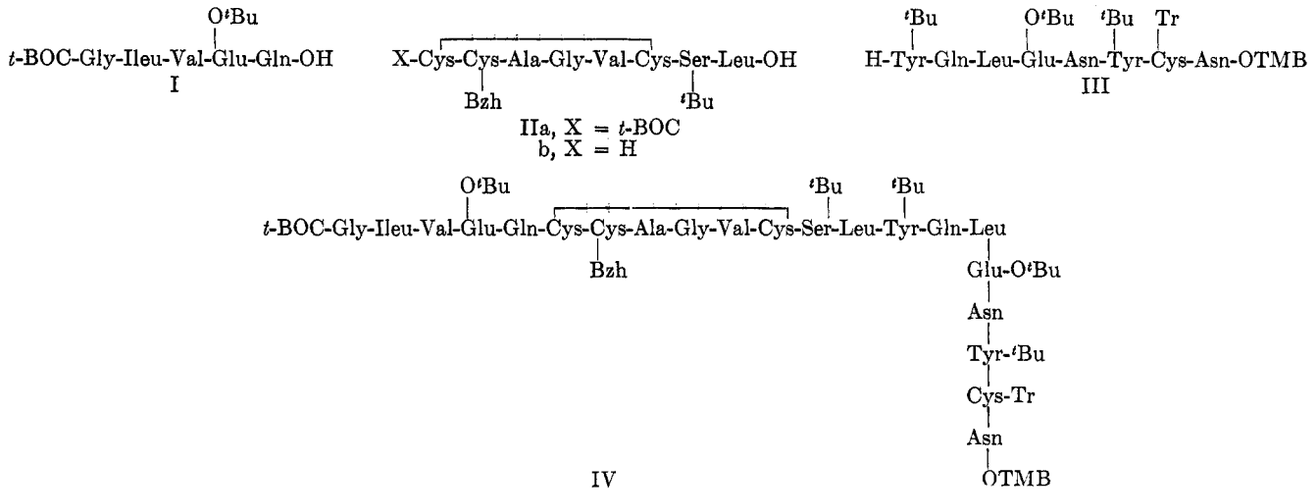
The synthesis of the protected A_{6-13} segment of ovine insulin (IIa) was accomplished by a route utilizing acid-labile protective groups and *N*-hydroxysuccinimide coupling procedures. The resulting octapeptide derivative, *N*-*tert*-butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-*S*-trityl-L-cysteinyl-*O*-*tert*-butyl-L-seryl-L-leucine (XXV) was cyclized to IIa with thiocyanogen. The action of a trifluoroacetic acid–chloroform mixture on IIa selectively removed the *N*-*tert*-butyloxycarbonyl group and provided *S*,*S*-L-hemicystyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-*O*-*tert*-butyl-L-seryl-L-leucine (IIb).

In order to more fully evaluate the general accessibility of complex cystine-containing peptides *via* the sulfenylthiocyanate route[5] and also to develop a synthetic procedure adaptable to the unambiguous synthesis of structural variants of insulin, we have initiated studies directed toward the synthesis of ovine insulin. (a) the proposed route provides S-protective groups of differing acid lability at cysteine residues A_7 and A_{21} (this should allow the A_{21}–B_{19} interchain disulfide bond to be selectively introduced); (b) the synthesis allows the formation of an A chain with a preformed A_{6-11} intrachain sulfur–sulfur bond; and (c) it involves the protection of all side-chain functional groups with acid-labile *tert*-butyl esters or ethers.



The projected plan is similar to that developed from studies with a model system[6] and is designed to allow the stepwise introduction of the three sulfur–sulfur bonds in the molecule.

The most complex aspect of the synthesis, from a protective group point of view, is the preparation of the A-chain derivative IV. The projected route to this substance differs substantially from the elegant and successful syntheses previously described[7] in that

Although several alternative choices of A-chain fragments were available, those finally adopted were the A_{1-5} pentapeptide I, the A_{6-13} octapeptide II, and the A_{14-21} octapeptide III. While the synthesis of I is straightforward and essentially follows a known route, fragments II and III were more formidable because of the restrictions introduced by the synthetic features of the route a–c. The scheme that was adopted for the preparation of the A_{6-13} fragment is described in the present report; an accompanying paper describes the synthesis of III.

A peptide related to II was described by Zervas, *et al.*,[8] in an earlier investigation. Although the route

(1) The preceding paper of this series: R. G. Hiskey, L. M. Beacham, III, V. G. Matl, J. N. Smith, E. B. Williams, Jr., A. M. Thomas, and E. T. Wolters, *J. Org. Chem.*, **36**, 488 (1971).

(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: Z = carbobenzoxy; ^{t}Bu = *tert*-butyl; *t*-BOC = *tert*-butyloxycarbonyl; Su = *N*-hydroxysuccinimide; *o*-NPS = *o*-nitrophenylsulfenyl; Tr = trityl; Bzh = benzyhydryl; DCC = *N*,*N*′-dicyclohexylcarbodiimide; WSC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; TMB = 2,4,6-trimethylbenzyl.

(4) Abstracted in part from the dissertation of L. M. Beacham, III, and the thesis of V. G. Matl submitted to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. and the M.S. degrees, respectively, Jan 1970 and Aug 1968.

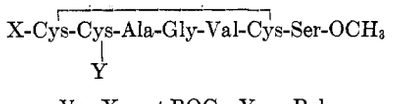
(5) R. G. Hiskey and B. F. Ward, Jr., *J. Org. Chem.*, **35**, 1118 (1970).

(6) R. G. Hiskey, A. M. Thomas, R. L. Smith, and W. C. Jones, Jr., *J. Amer. Chem. Soc.*, **91**, 7525 (1969).

(7) J. Meienhofer, E. Schnabel, H. Bremer, O. Brinkoff, R. Zabel, W. Sroka, H. Klostermeyer, D. Brandenburg, T. Okuda, and H. Zahn, *Z. Naturforsch., B*, **18**, 1120 (1963); Y. Wang, J. Z. Hsu, W. C. Chang, L. L. Cheng, C. Y. Hsing, A. H. Chi, T. P. Loh, C. H. Li, P. T. Shi, and Y. H. Yieh, *Sci. Sinica (Peking)*, **13**, 2030 (1964); P. G. Katsoyannis, A. M. Tometsko, C. Zalut, and K. Fukuda, *J. Amer. Chem. Soc.*, **88**, 5625 (1966); A. Marglin and R. B. Merrifield, *ibid.*, **88**, 5051 (1966); H. Zahn, W. Kanho, H. Klostermeyer, H. G. Gattner, and J. Repin, *Z. Naturforsch., B*, **24**, 1127 (1969).

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

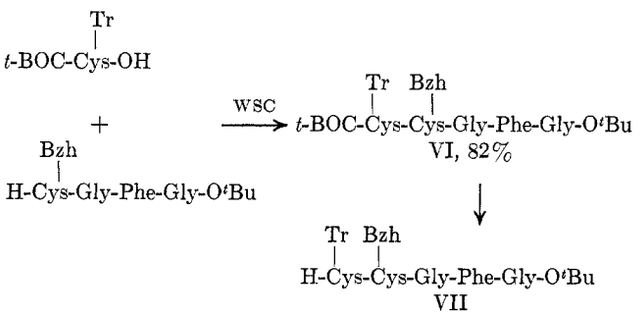
employed for the preparation of Va,b was remarkable in that many new protective groups were used in con-



Va, X = *t*-BOC; Y = Bzh
b, X = *o*-NPS; Y = Tr

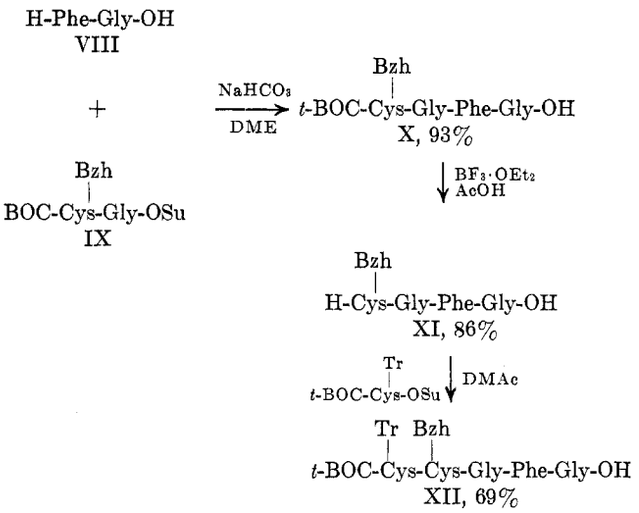
junction on a complicated synthetic problem, several features of the synthesis were relatively unattractive for the purposes of the present studies. For example, the presence of the methyl ester, while simplifying the overall synthesis, limited the choice of coupling methods to be employed for the condensation of A_{6-12} and A_{13-21} to the azide method, since the methyl ester could probably not be removed without serious side reactions elsewhere in the molecule. Furthermore, treatment of V with hydrazine to obtain the hydrazide, and subsequent oxidation to the azide, raised the possibility of side reactions involving the sulfur–sulfur bond. In addition, the presence of a free hydroxyl group on the A_{12} serine residue was to be avoided in order to reduce possible side reactions in the fragment condensations leading to fully protected A chain. Hence a route to II that (a) employed only acid-labile protective groups and (b) allowed the use of blocking groups on the N terminus (A_6) and C terminus (A_{13}) that could be removed without disturbing the A_{6-11} disulfide or protective groups at A_7 and A_{12} was the most attractive.

Initially the combination of the *N-tert*-butyloxycarbonyl group and a *tert*-butyl ester was tested as potential blocking groups for A_6 and A_{13}. Although these groups are quite similar, it appeared in principle that the former could be selectively removed in the presence of the latter. In order to test this possibility, a model peptide, *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine *tert*-butyl ester (VI), was studied. Treatment of VI with hydrogen chloride in dioxane or trifluoroacetic acid invariably led to mixtures of the desired ester VII, unreacted VI, and *S*-trityl-L-cysteinyl-*S*-



benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine, the doubly deblocked substance. Boron trifluoride etherate in acetic acid at 25° produced the same mixture; at 0° only VI and VII resulted. Although VII could be obtained by this route, the separation of VI and VII was not practical on a large scale, and hence alternatives were considered.

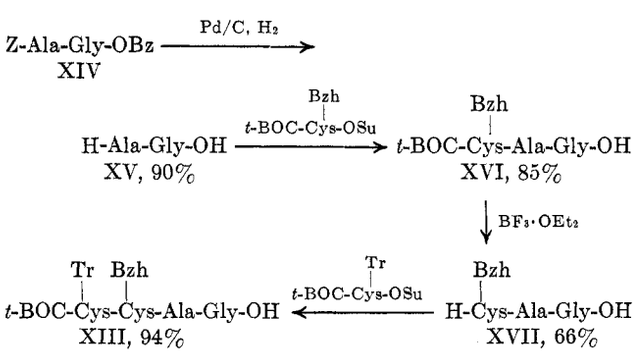
The most desirable solution would be to completely avoid the use of an ester protective group (or other protective groups for that matter). This approach was evaluated by the use of a model system. L-Phenylalanylglycine (VIII) was coupled, in a mixture of dimethoxyethane (DME) and aqueous bicarbonate, to the *N*-hydroxysuccinimide ester[9] of *tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycine (IX). The reaction proceeded smoothly and provided *N-tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (X) in excellent yield. Treatment of X



with boron trifluoride etherate in acetic acid produced *S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XI) which was allowed to react with the *N*-hydroxysuccinimide ester of *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteine. When the coupling was performed in aqueous bicarbonate–DME–DMAc mixtures, low yields (40–50%) resulted because of the low solubility of XI. However, the use of *N*-methylmorpholine, as the base, in DMAc avoided the solubility problem and provided *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XII) in reasonable yield and high purity.

These experiments demonstrated that the *t*-BOC group could be successfully employed without the necessity of an ester protective group, and thus attention was given to the actual synthesis of II. The synthetic plan for the octapeptide involved the preparation of two tetrapeptide fragments. The synthesis of the fully protected N-terminal portion, *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinylalanylglycine (XIII), proceeded smoothly by the procedure utilized for the model compound XII (Scheme I).

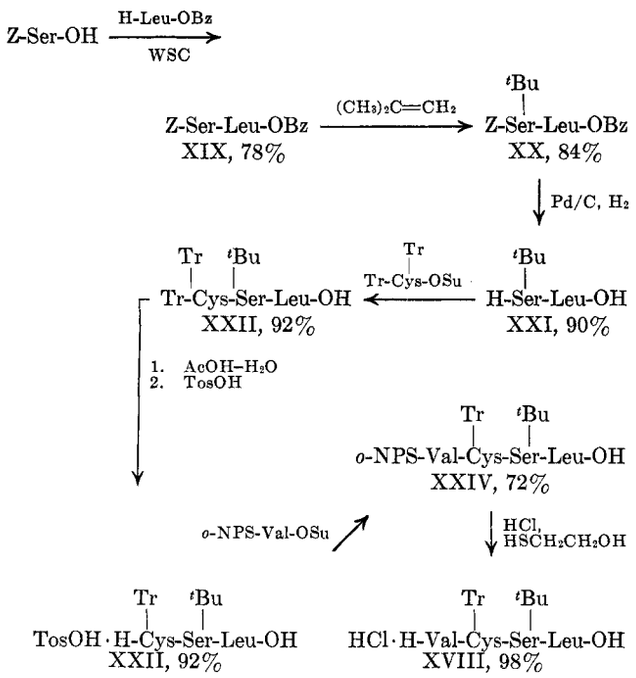
SCHEME I



(9) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, **86**, 1839 (1964); G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *ibid.*, **89**, 178 (1967).

The second tetrapeptide fragment, L-valyl-*S*-trityl-L-cysteinyl-*O*-*tert*-butyl-L-seryl-L-leucine (XVIII), was prepared by the route outlined in Scheme II. Although

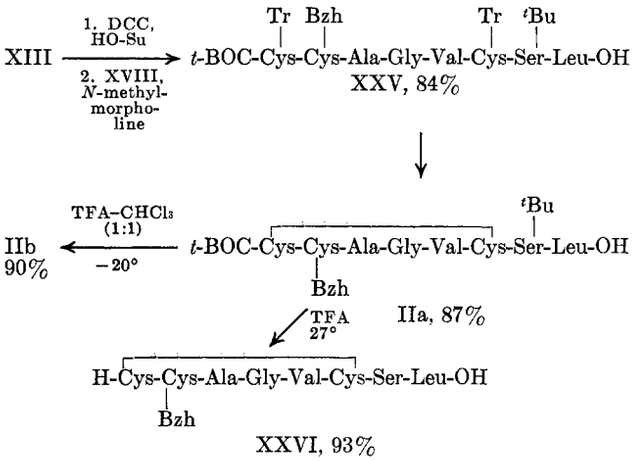
SCHEME II



benzyl *N*-carbobenzoxy-L-seryl-L-leucinate (XIX) could be obtained *via* the *N*-hydroxysuccinimide ester method (57% yield), an improved yield of XIX resulted by the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC); the use of the latter reagent allowed any *N*-acylurea formed to be removed by extraction with dilute acid. Introduction of the *O*-*tert*-butyl protective group followed by hydrogenolysis provided a good overall yield of *O*-*tert*-butyl-L-seryl-L-leucine (XXI). Since the *t*-BOC group had previously been shown to cleave at a rate only slightly greater than the *tert*-butyl ester, the more acid-labile *N*-trityl group was used as the amino protective group for the *S*-tritylcysteine residue, A_{11}. *N*,*S*-Ditrityl-L-cysteine *N*-hydroxysuccinimide ester was readily prepared and coupled with XXI in chloroform to provide *N*,*S*-ditrityl-L-cysteinyl-*O*-*tert*-butyl-L-seryl-L-leucine (XXII). Selective removal of the *N*-trityl group in the presence of O and S ethers was accomplished by the action of 80% acetic acid on XXII; the ether-soluble free base was isolated as the salt XXIII. Formation of XXIV followed by selective removal of the *N*-*o*-nitrophenylsulfenyl group using hydrochloric acid provided L-valyl-*S*-trityl-L-cysteinyl-*O*-*tert*-butyl-L-seryl-L-leucine as the hydrochloride salt (XVIII).

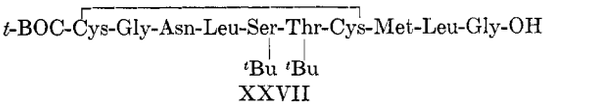
Coupling of the two fragments XIII and XVIII was accomplished *via* the *N*-hydroxysuccinimide ester method (Scheme III). The purification of the active ester of XIII was facilitated by the fact that both unreacted DCC and the urea by-product were reasonably soluble in isopropyl alcohol whereas the active ester was not. Treatment of XVIII with the *N*-hydroxysuccinimide derivative provided the octapeptide, *N*-*tert*-butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-*S*-trityl-L-cysteinyl-*O*-*tert*-butyl-L-seryl-L-leucine (XXV) in 84% yield.

SCHEME III



Previous experience had shown that the cyclization of a di-*S*-trityl-L-cysteine peptide similar to XXV could be accomplished[10] by the action of thiocyanogen in acetic acid without prior liberation of the dithiol form. Although the low solubility of XXV in acetic acid or acetic acid–ethyl acetate mixtures did not permit these solvent systems to be employed, a chloroform–acetic acid system was satisfactory. In contrast to the previous experiments,[10] the cyclization proved to be quite slow but could be driven to completion by the use of excess thiocyanogen. The thiocyanogen polymer could be separated from the peptide by solution of the latter in hot methanol; *S*,*S*′,*N*-*tert*-butyloxycarbonyl-L-hemicystyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-*O*-*tert*-butyl-L-seryl-L-leucine (IIa) was obtained in 84% yield. The results of the combustion and amino acid analyses of a performic acid–oxidized acid hydrolysate of IIa were consistent with the expected structure. An osmometric molecular weight determination indicated the substance was the cyclic monomer as opposed to possible dimeric or polymeric forms.

At this point the selective cleavage of a *N*-*t*-BOC group in the presence of an *O*-*tert*-butyl ether was reinvestigated. Gray and Khoujah[11] reported the selective cleavage of the *N*-*tert*-butyloxycarbonyl group in the presence of a *tert*-butyl ester using an acidic ion-exchange resin. These conditions were not generally adaptable to IIa for solubility reasons, and an attempt to employ this method with a model system gave no reaction. In the elegant synthesis of thyrocalcitonin, Riniker, *et al.*,[12] noted that complete removal of the *O*-*tert*-butyl ethers from the intermediate, XXVII, with



XXVII

90% trifluoroacetic acid required more than 2 hr although a few minutes appeared sufficient for the cleavage of the *N*-*tert*-butyloxycarbonyl group. They suggested that the proximity of the protonated free amine to the *O*-*tert*-butyl ethers retarded the cleavage of the latter groups. For reference purposes the doubly

(10) R. G. Hiskey and R. L. Smith, *J. Amer. Chem. Soc.*, **90**, 2677 (1968).
(11) C. J. Gray and A. M. Khoujah, *Tetrahedron Lett.*, **31**, 2647 (1969).
(12) B. Riniker, M. Brugger, B. Kamber, P. Sieber, and W. Rittel, *Helv. Chim. Acta*, **52**, 1058 (1969).

deblocked peptide XXVI was initially prepared from IIa. Treatment of IIa with boron trifluoride etherate in acetic acid or with neat trifluoroacetic acid at room temperature provided XXVI in good yield, and, using XXVI as a marker, a variety of conditions were investigated by tlc. At room temperature with excess boron trifluoride etherate a second ninhydrin positive spot of slightly greater mobility than XXVI appeared; longer reaction times (up to 12 hr) led to the disappearance of this spot and the enhancement of the spot due to XXVI. The rate of formation of the more mobile product, IIb, was dependent on the amount of acid used; at low temperature with boron trifluoride etherate the formation of IIb was too slow to observe. Hydrogen chloride in dioxane was less effective than the boron trifluoride reagent. Neat trifluoroacetic acid at 27° removed both of the N- and O-blocking groups; however, when the temperature was lowered to −20° the rate of *O-tert*-butyl cleavage was drastically reduced. Using a 1:1 (v/v) mixture of TFA–chloroform, the *O-tert*-butyl group was stable for at least 10 hr at −20°; treatment of IIa with this mixture provided only a trace of XXVI, and a good yield of *S,S'*-L-hemicystyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-*O-tert*-butyl-L-seryl-L-leucine (IIb) was obtained by recrystallization. The material exhibited a single ninhydrin positive spot on tlc and gave combustion and amino acid analyses that were consistent with IIb. Experiments leading to the preparation of the A_{1-13} fragment from the azide of the pentapeptide I and IIb are in progress.

Experimental Section[13]

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteine Dicyclohexylammonium Salt.**—The compound was prepared by the procedure of Schnabel.[14] *tert*-Butyloxycarbonylazide, (10.7 ml, 55 mmol, 10% excess) was added to a suspension of 18.2 g (50 mmol) of *S*-trityl-L-cysteine in 200 ml of dioxane–water (1:1). The pH of the reaction mixture was brought to 9.6 with 4 *N* sodium hydroxide using a pH meter. The stirred suspension was titrated with 4 *N* sodium hydroxide to maintain pH 9.6 (±0.2) for 14 hr. The solution was extracted with 300 ml of ether. The aqueous layer was acidified with citric acid to pH 4.5 and extracted twice with 250 ml of ether and twice with 200 ml of ethyl acetate. The combined organic extract was washed with ten 200-ml portions of water (final washing neutral to Congo Red) and 200 ml of saturated sodium chloride, dried, and evaporated to a foam. A solution of the foam in chloroform was absorbed on a silica gel column (250 g) and eluted with chloroform and 1–6% methanol in chloroform (v,v). The product was dissolved in 150 ml of ether and treated with 9.8 ml (50 mmol) of DCHA to yield an off-white precipitate which was washed with ether. The crude product was twice recrystallized from methanol–ether to afford the salt: 19.5 g (60.6%); mp 210–211° dec; $[\alpha]^{20}D$ +23.8° (*c* 1.0, methanol).

Anal. Calcd for $C_{39}H_{52}N_2O_4S$: C, 72.63; H, 8.13; N, 4.34; S, 4.971 Found: C, 71.96; H, 8.10; N, 4.19; S, 4.97.

(13) Melting points are uncorrected. Combustion analyses were performed by Micro-Tech Laboratories, Skokie, Ill., and Galbraith Laboratories, Knoxville, Tenn. Molecular weights were determined on a Mechrolab Model 301A osmometer, using *o*-chlorophenol at 37°. Amino acid analyses were performed with a Beckman Model 116 amino acid analyzer on samples hydrolyzed for 24 hr in evacuated sealed tubes with 6 *N* hydrochloric acid. The results have not been corrected for cysteic acid or serine destruction. Thin layer chromatograms were run on 3-in. microscope slides or on 5 × 20 cm plates, coated with silica gel GF_{254}. Solvent systems employed were chloroform–methanol, 9:1 (system A); chloroform–acetone–acetic acid, 8:1:1 (system B); chloroform–methanol–17% ammonia, 6:6:1 (system C); chloroform–methanol–17% ammonia, 24:6:1 (system D). Unless otherwise stated, products were dried *in vacuo* over phosphorus pentoxide.

(14) E. Schnabel, *Justus Liebig's Ann. Chem.*, **702**, 188 (1967).

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine *tert*-Butyl Ester (VI).**—The oxalate salt of *S*-benzhydryl-L-cysteinylgycyl-L-phenylalanylglycine *tert*-butyl ester[15] (1.4 g, 2.03 mmol) was partitioned between 10 ml of 5% potassium carbonate and 15 ml of ether–ethyl acetate (2:1). The aqueous phase was extracted twice with 10 ml of ether and twice with 5 ml of ethyl acetate. The combined organic extract was washed with three 30-ml portions of water and a 25-ml portion of saturated sodium chloride, dried, and evaporated to 1.15 g (94%) of the free base as a white foam. A suspension of 1.35 g (2.9 mmol, 10% excess) of *N-tert*-butoxycarbonyl-*S*-trityl-L-cysteine dicyclohexylammonium salt in 25 ml of ethyl acetate was shaken with 15 ml of 0.5 *N* sulfuric acid until complete solution was achieved. The organic extract was washed five times with 25 ml of water (final washing was neutral to Congo Red), washed with 20 ml of saturated sodium chloride, dried, and evaporated to a colorless foam, 0.94 g (70%). WSC (0.372 g, 1.95 mmol) was added to a cold (−10°) solution of 0.884 g (1.95 mmol) of *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteine and 1.15 g (1.95 mmol) of *S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine *tert*-butyl ester in 7 ml of DMF–methylene chloride (2.5:1). The reaction mixture was stirred at −10° for 1 hr and at 20° for 24 hr. An additional 9 ml of methylene chloride was added to effect stirring. The reaction mixture was evaporated to a slurry and transferred to 40 ml of 1 *N* sulfuric acid–ice by means of 20 ml of methanol. The resulting white suspension was stirred for 15 min and filtered. The collected solid was washed with cold methanol–ether and dried. The crude pentapeptide was suspended first in hot methanol, and then in warm ether, and cooled to room temperature. The yield was 1.69 g (82.5%) (including 0.21 g and 0.06 g of the second and third crops), mp 220–221°, homogeneous by tlc (system A). The analytical sample was obtained by recrystallization from methanol–chloroform, mp 217–218°, $[\alpha]D$ −22.3° (*c* 1.0, DMF).

Anal. Calcd for $C_{60}H_{67}N_5O_8S_2$: C, 68.61; H, 6.43; N, 6.67; S, 6.10. Found: C, 68.65; H, 6.49; N, 6.63; S, 6.03.

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycine.**—A solution of *N-tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteine dicyclohexylammonium salt (13.05 g, 23 mmol) in 100 ml of ethyl acetate was washed with 2 *N* sulfuric acid (100 ml) and then with water until the wash was neutral to Congo Red, dried over magnesium sulfate, filtered, and concentrated to an oil. This oil was dissolved in 25 ml of DME, 2.9 g (25 mmol) of *N*-hydroxysuccinimide added, the solution cooled to 0°, and 5.0 g (24 mmol) of DCC added. The reaction was allowed to warm to room temperature overnight, the DCU was filtered and washed with 25 ml of DME, and the combined filtrate and washings were added to a stirred solution of glycine (1.73 g, 23 mmol) and potassium bicarbonate (4.6 g, 46 mmol) in 50 ml of water. This produced considerable gas evolution and a turbid solution which slowly cleared. After 2 hr the solution was cooled to 0° and slowly acidified with 2 *N* sulfuric acid, producing an oil, which was extracted into ether (two 50-ml portions). The ether solution was washed with water until the wash was neutral to Congo Red, dried over magnesium sulfate, and evaporated to a foam (8.75 g, 86%), which tlc (system B) revealed to be slightly contaminated with *N-tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteine. Several attempts at recrystallization or salt formation failed to remove this impurity, and so the product was used without further purification.

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycine *N*-Hydroxysuccinimide Ester (IX).**—To a cold (0°), stirred solution of *N-tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycine (3.4 g, 7.85 mmol) and *N*-hydroxysuccinimide (0.91 g, 7.91 mmol) in 10 ml of DME, DCC (1.62 g, 7.85 mmol) was added. After warming to room temperature overnight, the DCU was filtered and washed with ethyl acetate, and the combined filtrate and washings were concentrated to an oil, which was crystallized from 30 ml of 2-propanol to give a white solid (3.04 g, 72%), mp 154–156°, $[\alpha]^{22}D$ +6.34° (*c* 0.93, chloroform),

Anal. Calcd for $C_{27}H_{31}N_3O_7S$: C, 59.87; H, 5.77; N, 7.76; S, 5.92. Found: C, 59.89; H, 5.73; N, 8.27; S, 5.78.

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (X).**—A solution of IX (7.62 g, 14.1 mmol) in 30 ml of DME was added to a stirred solution of VIII[16] (3.50

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

(16) J. R. Vaughn, Jr., and J. A. Eichler, *J. Amer. Chem. Soc.*, **75**, 5556 (1953).

g, 14.5 mmol) and 2.9 g (29 mmol) of potassium bicarbonate in 30 ml of water, producing considerable evolution of gas. After 1 hr the reaction mixture was diluted with 200 ml of 1 *N* sulfuric acid, producing a gummy precipitate, which was extracted into ethyl acetate (two 100-ml portions). This extract was washed with water (three 200-ml portions) and saturated brine (two 100-ml portions), dried over magnesium sulfate, filtered, and concentrated to 30 ml. Dilution with ether produced a precipitate, which was collected and dried to yield 8.6 g (93%) of a white solid, mp 108–111°, $[\alpha]^{22}D$ +0.01° (*c* 0.89, $CHCl_3$), homogeneous (system B).

Anal. Calcd for $C_{34}H_{40}N_4O_7S$: C, 62.94; H, 6.22; N, 8.64; S, 4.94. Found: C, 62.96; H, 6.20; N, 8.48; S, 4.78.

S-Benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XI).—Boron trifluoride etherate (3.3 ml, 25 mmol) was added to a stirred solution of X (6.5 g, 10 mmol) in 30 ml of acetic acid, producing a faint yellow color and considerable bubbling. After 30 min the solution was poured into 200 ml of 0.1 *N* potassium hydroxide, producing a fine white precipitate, which was collected, washed with water, dried, and recrystallized from DMAc to yield 4.7 g (86%), mp 191–193° dec. Drying at 100° raised the decomposition point to above 230°, $[\alpha]^{22}D$ −18.34° (*c* 1.03, DMF), homogeneous (system C).

Anal. Calcd for $C_{29}H_{32}N_4O_5S \cdot 1.15H_2O$: C, 60.50; H, 6.13; N, 9.73; S, 5.57. Found: C, 60.71; H, 5.91; N, 9.84; S, 5.46.

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinylglycyl-L-phenylalanylglycine (XII).**—DCC (2.27 g, 11 mmol) was added to a stirred solution of *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteine (4.64 g, 10 mmol) and *N*-hydroxysuccinimide (1.27 g, 11 mmol) in 10 ml of DME at −10°. The reaction was allowed to warm to room temperature for 6 hr; the DC was filtered and washed with ethyl acetate (three 10-ml portions). The combined filtrate and washings were concentrated to a foam which was dissolved in 40 ml of DMAc. *N*-Methylmorpholine (1.1 ml, 10 mmol) and XI (5.0 g, 9.1 mmol) were added and the reaction mixture was stirred overnight. *p*-Toluenesulfonic acid (2 g, 10.4 mmol) was added to neutralize the base, and the solution was poured into 100 ml of ice water, producing an immediate fine white precipitate. This was collected and washed with water (three 30-ml portions), methanol (20 ml), and ethyl acetate (two 20-ml portions) and then dried to yield 6.83 g (69%) of a white solid, mp 217–220°, $[\alpha]^{22}D$ −18.8° (*c* 1.0, DMF), homogeneous (system B).

Anal. Calcd for $C_{56}H_{59}N_5O_8S$: C, 67.64; H, 5.98; N, 7.04; S, 6.45. Found: C, 67.69; H, 6.04; N, 7.05; S, 6.78.

***N*-Carbobenzoxy-L-alanylglycine Benzyl Ester (XIV).**—The compound was prepared in 97% yield by the method of Stelakatos,[17] mp 109–111° (lit.[17] mp 111–112°).

L-Alanylglycine (XV).—A solution of XIV (23 g, 0.062 mol) in 350 ml of ethanol and 6 ml of acetic acid was treated with 2.3 g of 10% palladium on charcoal; hydrogen was bubbled through this suspension for 4 hr. Water (150 ml) was added in 50-ml portions as the reaction proceeded to maintain solubility of the product. The catalyst was filtered on a Celite bed; the filtrate was concentrated to 300 ml and diluted with 3.5 l of acetone, producing a white precipitate, which was collected to yield 8.19 g (90%) of a white powder, mp 234–235°, $[\alpha]^{22}D$ +49.36° (*c* 2.04, H_2O) [lit.[18] mp 232–235°, $[\alpha]^{25}D$ +49.1° (*c* 2.0, H_2O)].

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteine Dicyclohexylammonium Salt.**—A stirred suspension of *S*-benzhydryl-L-cysteine[19] (17.5 g, 61 mmol) in dioxane–water, 1:1 (320 ml), was maintained at pH 10.2 by dropwise addition of 4 *N* sodium hydroxide. After 5 hr the clear solution was extracted with ether (50 ml), and the extract was discarded. The solution was acidified with 2 *N* sulfuric acid to pH 3.1 and extracted twice with ethyl acetate; the extracts were washed with water (three 200-ml portions), dried over magnesium sulfate, filtered, and evaporated to an oil. This was dissolved in ether and treated with dicyclohexylamine (15.6 ml, 80 mmol) producing an immediate white precipitate, which was collected and washed with ether. Recrystallization from chloroform–hexane gave 27.4 g (79%) of a white crystalline solid, mp 157–158°, $[\alpha]^{22}D$ +6.38° (*c* 0.93, chloroform).

Anal. Calcd for $C_{33}H_{48}N_2O_4S$: C, 69.68; H, 8.51; N, 4.93; S, 5.64. Found: C, 69.64; H, 8.62; N, 4.82; S, 5.31.

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteine *N*-Hydroxysuccinimde Ester.**—*tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteine was prepared as above, on a 0.1 *M* scale, and the free acid dissolved in 100 ml of DME. *N*-Hydroxysuccinimide (11.5 g, 0.1 mol) was added, the solution cooled to 0°, and DCC (20.6 g, 0.1 mol) added. After stirring 2 hr at 0° and 10 hr at 27°, the solution was filtered and evaporated. The resulting oil was dissolved in 200 ml of ethyl acetate, washed with 200 ml of cold 5% potassium bicarbonate, water (three 200-ml portions), and saturated brine (two 200-ml portions), dried over calcium sulfate, filtered, and evaporated. Crystallization was effected from ethyl acetate–hexane to give a white solid (38.9 g, 76%), which was used without further purification.

***N-tert*-Butyloxycarbonyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycine (XVI).**—A solution of the *N*-hydroxysuccinimide ester (0.653 g, 1.35 mmol) in 5 ml of DME was added to a stirred solution of XV (0.220 g, 1.50 mmol) and potassium bicarbonate (0.30 g, 3.0 mmol) in 5 ml of water. After 2.5 hr the mixture was poured into 50 ml of ice–2 *N* sulfuric acid. The resulting gum was dissolved in 20 ml of ethyl acetate, washed with water (two 20-ml portion) and saturated brine (20 ml), dried over calcium sulfate, filtered, and concentrated to 5 ml. Dilution with 80 ml of ether and cooling to 0° gave a crystalline precipitate, 0.59 g (85%), mp 124–125°, $[\alpha]^{24}D$ +71.06° (*c* 1.13, DMF), homogeneous (system B).

Anal. Calcd for $C_{26}H_{33}N_3O_6S$: C, 60.56; H, 6.45; N, 8.15; S, 6.22. Found: C, 60.96; H, 6.10; N, 8.17; S, 6.34.

S-Benzhydryl-L-cysteinyl-L-alanylglycine (XVII).—Boron trifluoride etherate (2.6 ml, 18 mmol) was added to a stirred solution of XVI (3.17 g, 6.15 mmol) in 25 ml of dry acetic acid. After 30 min the solution was poured into 6% aqueous sodium acetate (150 ml), producing a fine precipitate. After cooling to 0° overnight the solid was collected and washed with water and then recrystallized from DMAc–ethyl acetate, yielding 1.70 g (66%) of a white solid, mp 178–180° dec, $[\alpha]^{24}D$ −5.18 (*c* 0.56, DMF), homogeneous (system C).

Anal. Calcd for $C_{21}H_{25}N_3O_4S \cdot 0.5H_2O$: C, 59.08; H, 5.98; N, 9.95; S, 7.49. Found: C, 59.41; H, 6.17; N, 9.90; S, 7.55.

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycine (XIII).**—A stirred solution of *N-tert*-butyloxycarbonyl-*S*-trityl-L-cysteine (0.92 g, 2.0 mmol) and *N*-hydroxysuccinimide (0.25 g, 2.2 mmol) in 2 ml of DME was cooled to 0° and DCC (0.45 g, 2.2 mmol) added. After 2 hr at 0° and overnight at room temperature the reaction was filtered, the DCU was washed with DME (three 2-ml portions), and the combined filtrate and washings were added to a stirred solution of XVII (0.79 g, 1.9 mmol) and potassium carbonate (0.265 g, 1.9 mmol) in 5 ml of water. This solution was stirred for 6 hr and poured into ice–2 *N* sulfuric acid (60 ml), producing an immediate white precipitate, which was collected and washed with water (three 25-ml portions) and ether (four 25-ml portions) to yield 1.62 g (94%) of a white solid, mp 170–175° dec, $[\alpha]^{28}D$ −6.42° (*c* 1.03, DMF), homogeneous (system B).

Anal. Calcd for $C_{48}H_{52}N_4O_7S_2$: C, 66.95; H, 6.09; N, 6.51; S, 7.45. Found: C, 66.85; H, 6.04; N, 6.50; S, 7.34.

***N*-Carbobenzoxy-L-serine** was prepared in 89% yield by the procedure of Guttman and Boissonnas,[20] mp 115–116° (lit. mp 119.5°).

L-Leucine Benzyl Ester *p*-Toluenesulfonate.—The compound was prepared by the method of Zervas, *et al.*,[21] in 90% yield, mp 154–154.5° (lit. mp 158.5–160°).

***N*-Carbobenzoxy-L-seryl-L-leucine Benzyl Ester (XIX).**—To a stirred solution of *N*-carbobenzoxy-L-serine (35 g, 0.146 mol) in 300 ml of ethyl acetate and 20 ml of DMAc were added L-leucine benzyl ester *p*-toluenesulfonate (57.5 g, 0.146 mol) and *N*-methylmorpholine (16.2 ml, 0.146 mol). The resulting solution was cooled to 0° and WSC (31 g, 0.161 mol, 10% excess) was added. After stirring for 2 hr at 0° and overnight at room temperature, the solution was partitioned between ether (200 ml) and 2 *N* sulfuric acid (400 ml). The organic phase was washed with 2 *N* sulfuric acid (three 400-ml portions), 10% potassium bicarbonate (three 400-ml portions) and saturated brine (300 ml), dried over sodium sulfate, filtered, and evaporated to a clear oil. Crystallization from ethyl acetate–hexane and washing with ether gave a white solid (46.7 g, 73%), mp 81–83°, $[\alpha]^{20}D$ −22.6° (*c* 1.0, chloroform). A sample of the protected dipeptide pre-

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pared *via* the *N*-hydroxysuccinimide ester method was obtained in 57% yield, mp 81–83°, $[\alpha]^{24}D$ −22.5° (*c* 1.0, chloroform) (lit.[22] mp 83–84°).

***N*-Carbobenzoxy-*O-tert*-butyl-L-seryl-L-leucine Benzyl Ester (XX).**—A solution of XIX (26 g, 0.059 mol) in 125 ml of chloroform in a 500-ml pressure flask was treated with 1.3 ml of concentrated sulfuric acid and 60 ml of isobutylene, capped, and stirred at 27° for 12 hr. The solution was washed with 5% potassium bicarbonate (300 ml) and saturated brine (three 200-ml portions) and evaporated to a clear oil, which was purified from traces of XIX by chromatography on 150 g of silica gel, eluting with chloroform. The resulting oil was crystallized from hexane to give a white solid (24.7 g, 84%), mp 69–70°, $[\alpha]^{25}D$ +4.20° (*c* 1.85, chloroform), homogeneous (system A).

Anal. Calcd for $C_{28}H_{38}N_2O_6$: C, 67.44; H, 7.68; N, 5.65. Found: C, 67.92; H, 7.72; N, 5.62.

***O-tert*-Butyl-L-seryl-L-leucine (XXI).**—A solution of XX (10 g, 20 mmol) in ethanol (70 ml) and water (30 ml) was treated with 2 g of 10% palladium on charcoal and hydrogen was bubbled through the solution for 2.3 hr. The catalyst was filtered on a Celite bed and the colorless filtrate evaporated to a white solid, which was collected and washed with ether to yield 5.17 g (90%), mp 96–98°, $[\alpha]^{28}D$ −14.37° (*c* 1.03, methanol), homogeneous (system C).

Anal. Calcd for $C_{18}H_{26}N_2O_4 \cdot 0.8H_2O$: C, 54.06; H, 9.63; N, 9.70. Found: C, 54.16; H, 9.55; N, 9.66.

Diketopiperazine of *O-tert*-Butyl-L-seryl-L-leucine.—A solution of XX (18 g 36 mmol) in acetic acid (200 ml) was treated with 2 g of 10% palladium on charcoal and hydrogen was bubbled through the solution for 2.5 hr. The catalyst was filtered on a Celite bed and the filtrate lyophilized. The material was revealed by tlc to be mostly XX, and was therefore hydrogenated again, this time in neat ethanol. A product (1.7 g, 18.5%) was isolated after filtration and removal of most of the solvent, which was insoluble in ethanol and ninhydrin negative. The nmr was very similar to that of XXI, which was obtained from the filtrate (6.2 g, 63%); the diketopiperazine had mp 267–270°, $[\alpha]^{22}D$ −5.73° (*c* 0.75, hexamethylphosphoramide), homogeneous (system A).

Anal. Calcd for $C_{13}H_{24}N_2O_3$: C, 60.91; H, 9.44; N, 10.93. Found: C, 60.88; H, 9.54; N, 10.89.

***N,S*-Ditrityl-L-cysteine *N*-Hydroxysuccinimide Ester.**—A solution of *N,S*-ditrityl-L-cysteine diethylammonium salt[23] (48 g, 0.071 mol) in ethyl acetate (400 ml) was partitioned with 400 ml of 0.5 *N* sulfuric acid. The organic phase was washed with water (two 400-ml portions), dried over sodium sulfate, and evaporated to a foam, which was dissolved in 70 ml of DME and treated with 9.2 g (0.080 mol) of *N*-hydroxysuccinimide. The solution was cooled to 0° and DCC (16.5 g, 0.080 mol) added. After stirring for 2 hr at 0° and 11 hr at room temperature, the solution was filtered and evaporated to an oil, which was crystallized from 2-propanol to yield a white powder (48.3 g, 97%), mp 109–113°, $[\alpha]^{28}D$ +70.0 (*c* 1.37, chloroform).

Anal. Calcd for $C_{45}H_{38}N_2O_4S$: C, 76.90; H, 5.45; N, 3.99; S, 4.56. Found: C, 76.46; H, 5.63; N, 3.81; S, 4.68.

***N,S*-Ditrityl-L-cysteinyl-*O-tert*-butyl-L-seryl-L-leucine (XXII).**—A solution of XXI (4.65 g, 17 mmol) and the *N*-hydroxysuccinimide ester (10.5 g, 15 mmol) in 30 ml of chloroform was treated with *N*-methylmorpholine (4.0 ml, 37 mmol) and stirred at room temperature for 12 hr. After partitioning between ether (300 ml) and 2 *N* sulfuric acid (300 ml), the organic phase was washed with 2 *N* sulfuric acid (two 300-ml portions), water (three 300-ml portions), and saturated brine (300 ml), dried over sodium sulfate, and evaporated to a white foam (11.86 g, 92%). A 0.5-g sample was chromatographed on a 10-g silica gel column, eluting with chloroform, and crystallized from ether–hexane. The remainder was used without further purification, mp 110–114°, $[\alpha]^{28}D$ +40.52° (*c* 0.96, chloroform).

Anal. Calcd for $C_{54}H_{59}N_3O_5S \cdot 0.5H_2O$: C, 74.45; H, 6.94; N, 4.82; S, 3.68. Found: C, 74.79; H, 6.93; N, 4.75; S, 4.01.

***S*-Trityl-L-cysteinyl-*O-tert*-butyl-L-seryl-L-leucine *p*-Toluenesulfonate Salt (XXIII).**—A solution of XXII (6.7 g, 7.8 mmol) in 15 ml of acetic acid and 3 ml of water was warmed for 10 min on the steam bath, cooled, and lyophilized to a white powder. The powder was dissolved in 100 ml of ether, filtered, and treated with a solution of *p*-toluenesulfonic acid (1.46 g, 7.8 mmol) in ether. The resulting white precipitate was stored at 0° overnight, collected, and washed with ether to yield a white solid (3.84 g, 62%), mp 146–147°, $[\alpha]^{28}D$ +25.09° (*c* 1.08, ethanol).

Anal. Calcd for $C_{42}H_{53}N_3O_8S_2$: C, 63.69; H, 6.75; N, 5.31; S, 8.10. Found: C, 63.59; H, 6.78; N, 5.30; S, 8.30.

***o*-Nitrophenylsulfenyl-L-valine *N*-Hydroxysuccinimide Ester.**—The compound was prepared in 77% yield by the method of Meienhofer,[24] mp 138–139° (lit.[24] mp 138–139°).

***o*-Nitrophenylsulfenyl-L-valyl-*S*-trityl-L-cysteinyl-*O-tert*-butyl-L-seryl-L-leucine (XXIV).**—*N*-Methylmorpholine (0.63 ml, 5.8 mmol) was added to a solution of the *N*-hydroxysuccinimide ester (1.05 g, 2.9 mmol) and XXIII (2.29 g, 2.9 mmol) in 6 ml of DME. After stirring for 48 hr the orange solution was partitioned between ethyl acetate (100 ml) and 1 *N* sulfuric acid. The organic phase was washed with water (two 100-ml portions) and saturated brine (100 ml), dried over sodium sulfate, filtered, and stripped to a yellow foam, which was chromatographed on 60 g of silica gel, eluting with chloroform–acetone–acetic acid, 45:25:1. Crystallization from ether–hexane yielded 1.82 g (72%) of a bright yellow crystalline compound, mp 184–186°, $[\alpha]^{27}D$ −27.2° (*c* 0.99, DMF).

Anal. Calcd for $C_{46}H_{57}N_5O_8S_2$: C, 63.35; H, 6.59; N, 8.03; S, 7.35. Found: C, 63.16; H, 6.67; N, 7.97; S, 7.20.

L-Valyl-*S*-trityl-L-cysteinyl-*O-tert*-butyl-L-seryl-L-leucine Hydrochloride (XVIII).—To a stirred solution of XXIV (1.59 g, 1.82 mmol) and 0.7 ml (10 mmol) 2-mercaptoethanol in 6 ml of chloroform was added 0.31 ml (2.0 mmol) of 6.5 *N* hydrogen chloride in dioxane, dissolved in 30 ml of ether. Precipitation began in a few seconds; after 5 min the precipitate was poured into 200 ml of ether, cooled to −10°, collected, and washed with ether to yield a white solid (1.35 g, 98%), mp 137–138°, $[\alpha]^{28}D$ +11.2° (*c* 1.09, ethanol).

Anal. Calcd for $C_{40}H_{55}O_6SCl \cdot 0.5H_2O$: C, 62.85; H, 7.38; N, 7.32; S, 4.19. Found: C, 62.56; H, 7.31; N, 7.39; S, 4.10.

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycine *N*-Hydroxysuccinimide Ester.**—A solution of XIII (215 mg, 0.25 mmol), *N*-hydroxysuccinimide (58 mg, 0.50 mmol), and DCC (103 mg, 0.50 mmol) in 0.6 ml of DMAc was stirred at 27° for 24 hr and then poured into 20 ml of 2-propanol, producing an immediate precipitate. The suspension was warmed on the steam bath, cooled to 27°, collected, washed with 2-propanol (three 10-ml portions), and dried *in vacuo* to yield 235 mg (97.5%) of product. The compound was used without further purification.

***N-tert*-Butyloxycarbonyl-*S*-trityl-L-cysteinyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-*S*-trityl-L-cysteinyl-*O-tert*-butyl-L-seryl-L-leucine (XXV).**—To a solution of the *N*-hydroxysuccinimide ester of XIII (235 mg, 0.246 mmol) and XVIII (196 mg, 0.26 mmol) in 0.6 ml of DMAc was added *N*-methylmorpholine (0.082 ml, 0.75 mmol). The solution gradually solidified as stirring was continued for 48 hr. The solid was transferred into 0.5 *N* sulfuric acid, stirred well, collected, washed with water, and dried. Tlc (system D) revealed traces of the active ester and XVIII; so the product was suspended in 20 ml of hot ethyl acetate, cooled to room temperature, collected, and washed with ethyl acetate, (three 10-ml portions), yielding 331 mg (84%), mp 222° dec, $[\alpha]^{29}D$ −12.75° (*c* 0.8, DMF), homogeneous (system D).

Amino acid analysis. Found: $CysSO_3H$, 2.5; Ser, 0.92; Gly, 1.0; Ala, 1.1; Val, 1.1; Leu, 0.84.

Anal. Calcd for $C_{88}H_{104}N_8O_{12}S_2$: C, 67.66; H, 6.71; N, 7.17; S, 6.16. Found: C, 67.50; H, 6.87; N, 7.27; S, 5.79.

***S,S',N-tert*-Butyloxycarbonyl-L-hemicystyl-*S*-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-*O-tert*-butyl-L-seryl-L-leucine (IIa).**—Thiocyanogen was generated by the addition of bromine (100 mg, 0.625 mmol) in 15.0 ml of ethyl acetate to a rapidly stirred, dark suspension of lead thiocyanate (243 mg, 0.75 mmol) in 15.0 ml of ethyl acetate. All color had disappeared within 5 min and 10.6 ml (0.22 mmol) of this solution was added to a cold (0°), dark, rapidly stirred solution of XXV (312.4 mg, 0.2 mmol) in chloroform (100 ml) and acetic acid (20 ml). After 24 hr tlc (system D) showed a considerable amount of XXV had not reacted, so thiocyanogen was prepared as above and 0.1 mmol was added to the reaction mixture. After 48 hr the solution was filtered into 400 ml of ice water, and 21 ml of ammonium hydroxide was added to the rapidly stirred solution, producing a very heavy emulsion. After storing at 0° overnight the solution was filtered, and the resulting off-white solid washed with water and chloroform and recrystallized from methanol–water to yield

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186 mg (87%) of a white solid, mp 219–221°, $[\alpha]^{31}D$ −5.16° (*c* 0.62, DMF); homogeneous (system D).

Amino acid anal. Found: $CysSO_3H$, 2.4; Ser, 0.84; Gly, 1.00; Ala, 1.02; Val, 0.98; Leu, 0.95.

Anal. Calcd for $C_{50}H_{74}N_8S_3O_{12}$: C, 55.84; H, 6.94; N, 10.42; S, 8.94; mol wt, 1075.35. Found: C, 56.34; H, 7.05; N, 10.46; S, 8.88; mol wt, 1050 (osmometric, *o*-chlorophenol).

S,S'-L-Hemicystyl-S-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-O-*tert*-butyl-L-seryl-L-leucine (IIb).—Solid IIa (100 mg, 0.093 mmol) was added to 2 ml of trifluoroacetic acid–chloroform, (1:1 v/v) at −20°, producing a bright yellow solution. After 10 hr at −20° the solution was poured into 100 ml of ether at −10° producing an immediate fine white precipitate (82 mg, 90%). After collecting and drying *in vacuo* at 83° overnight, the compound was recrystallized for analysis from DMAc–water, and washed with 2-propanol: mp 218–220°; $[\alpha]^{22}D$ −12.06° (*c* 0.92, DMF); homogeneous (system D); ninhydrin positive.

Amino acid anal. Found: $CysSO_3H$, 2.4; Ser, 0.88; Gly, 1.00; Ala, 1.04; Val, 1.00; Leu, 1.00.

Anal. Calcd for $C_{45}H_{66}N_8S_3O_{10}\cdot H_2O$: C, 54.41; H, 6.90; N, 11.28; S, 9.68. Found: C, 54.28; H, 6.60; N, 11.27; S, 9.67.

S,S'-L-Hemicystyl-S-benzhydryl-L-cysteinyl-L-alanylglycyl-L-valyl-L-hemicystyl-L-seryl-L-leucine (XXVI).—A solution of IIa (50 mg, 0.046 mmol) in 1 ml of TFA was stirred for 50 min at room temperature and then poured into 100 ml of ether at −10°. The resulting fine precipitate was collected and dried to give 40 mg (93%) of a white solid, mp 219–221°, $[\alpha]^{22}D$ −13.33° (*c* 0.51, DMF), homogeneous (system D), ninhydrin positive. The product was recrystallized from DMAc–2-propanol–ether for analysis.

Amino acid anal. Found: $CysSO_3H$, 2.2; Ser, 0.81; Gly, 0.99; Ala, 1.02; Val, 1.00; Leu, 0.96.

Anal. Calcd for $C_{41}H_{58}N_8O_{10}S_3\cdot CF_3CO_2H\cdot DMAC$: C, 50.39; H, 6.12; N, 11.25; S, 8.59. Found: C, 50.64; H, 6.32; N, 11.68; S, 8.90.

Registry No.—IIa, 33578-92-6; IIb, 33515-71-8; VI, 27039-89-0; IX, 33515-73-0; X, 33515-74-1; XI, 33515-75-2; XII, 33515-76-3; XIII, 33511-31-8; XVI, 33511-32-9; XVII, 33511-33-0; XVIII, 33511-34-1; XX, 33511-35-2; XXI, 33511-36-3; XXI diketopiperazine, 33511-37-4; XXII, 33511-38-5; XXIII, 33511-39-6; XXIV, 33511-40-9; XXV, 33511-41-0; XXVI, 33649-61-5; *N*-*tert*-butyloxycarbonyl-*S*-trityl-L-cysteine dicyclohexylammonium salt, 26988-59-0; *N*-*tert*-butyloxycarbonyl-*S*-benzhydryl-L-cysteine dicyclohexylammonium salt, 26988-51-2; *N*,*S*-dityrityl-L-cysteine *N*-hydroxysuccinimide ester, 27560-18-5.

Sulfur-Containing Polypeptides. XVI. Synthesis of the A_{14-21} Fragment of Ovine Insulin[1-3]

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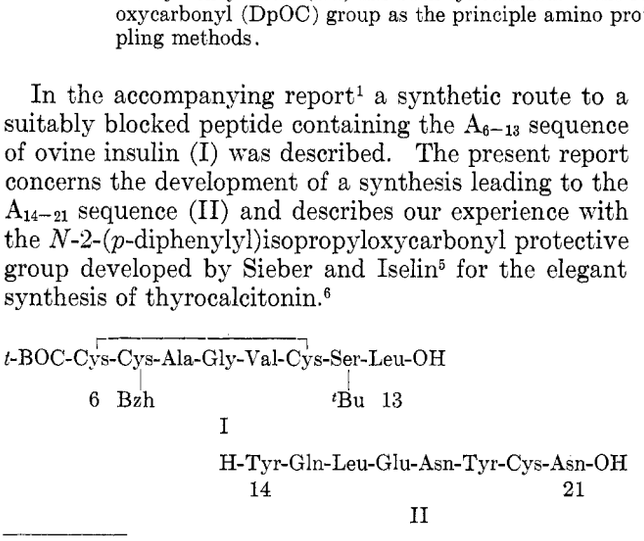
The protected octapeptide *N*-2-(*p*-diphenylyl)isopropyloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutaminyl-L-leucyly-γ-*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl ester (III) has been synthesized. The route involves the use of the *N*-2-(*p*-diphenylyl)isopropyloxycarbonyl (DpOC) group as the principle amino protective group and *N*-hydroxysuccinimide and azide coupling methods.

In the accompanying report[1] a synthetic route to a suitably blocked peptide containing the A_{6-13} sequence of ovine insulin (I) was described. The present report concerns the development of a synthesis leading to the A_{14-21} sequence (II) and describes our experience with the *N*-2-(*p*-diphenylyl)isopropyloxycarbonyl protective group developed by Sieber and Iselin[5] for the elegant synthesis of thyrocalcitonin.[6]



(1) The preceding paper of this series: R. G. Hiskey, L. M. Beacham, III, and V. G. Matl, *J. Org. Chem.*, **37**, 2472 (1972).

(2) Supported by Grant AM-03416 from the Institutes of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service.

(3) The following abbreviations have been employed in the text: *t*-BOC = *tert*-butyloxycarbonyl; DpOC = 2-(*p*-diphenylyl)isopropyloxycarbonyl; *o*-NPS = *o*-nitrophenylsulfenyl; ^{t}Bu = *tert*-butyl; TMB = 2,4,6-trimethylbenzyl; Tr = trityl; Bzl = benzyl, Su = *N*-hydroxysuccinimide; DCC = *N*,*N*'-dicyclohexylcarbodiimide; DME = 1,2-dimethoxyethane; NMM = *N*-methylmorpholine; DMF = *N*,*N*-dimethylformamide; DMAc = *N*,*N*-dimethylacetamide.

(4) Laboratory of Organic Chemistry, Roman Catholic University, Nymegen, The Netherlands.

(5) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 622 (1968).

(6) B. Riniker, M. Brugger, B. Kamber, P. Sieber, and W. Rittel, *ibid.*, **52**, 1058 (1969).

The preparation of the A_{14-21} sequence was complicated by the presence of seven functional side chains in the octapeptide; four of these required protection. Since acid-labile protective groups were required and the presence of a cysteine residue ruled out the possibility of removal of groups by hydrogenolysis, it was clear that only protective groups of very specific acid lability could be utilized. The protective group of choice for the phenolic hydroxyl groups at $A_{14,19}$ was the *tert*-butyl ether; the *S*-trityl group was required for the A_{20} cysteine residue to permit selective formation of the two interchain disulfide bonds at A_7B_7 and $A_{20}B_{19}$. The *tert*-butyl ester seemed to be suitable for the A_{17} carboxyl group. The choice of the 2,4,6-trimethylbenzyl ester as the blocking group for the asparagine-21 residue was governed by the overall stability of this ester and the earlier use by Stewart[7] in a synthesis of a modified sequence of the C-terminal portion of the A chain. Given these choices of ether and ester protective groups, relatively few possibilities were available for amino protective groups. The *N*-*tert*-butyloxycarbonyl group could not be used since *O*-*tert*-butyl ethers and esters generally cleave at comparable rates[1] and the presence of the cysteine residue prevented removal of the *N*-carbobenzyloxy group by hydrogenolysis. Thus the choices of amino protective groups were essentially limited to the *N*-trityl (Tr), the *N*-*o*-

(7) F. H. C. Stewart, *Aust. J. Chem.*, **20**, 1991 (1967).