

186 mg (87%) of a white solid, mp 219–221°,  $[\alpha]^{25}_D -5.16^\circ$  (*c* 0.62, DMF); homogeneous (system D).

*Amino acid anal.* Found: CysSO<sub>3</sub>H, 2.4; Ser, 0.84; Gly, 1.00; Ala, 1.02; Val, 0.98; Leu, 0.95.

*Anal.* Calcd for C<sub>30</sub>H<sub>74</sub>N<sub>8</sub>S<sub>3</sub>O<sub>12</sub>: 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-alanyl-glycyl-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°;  $[\alpha]^{25}_D -12.06^\circ$  (*c* 0.92, DMF); homogeneous (system D); ninhydrin positive.

*Amino acid anal.* Found: CysSO<sub>3</sub>H, 2.4; Ser, 0.88; Gly, 1.00; Ala, 1.04; Val, 1.00; Leu, 1.00.

*Anal.* Calcd for C<sub>46</sub>H<sub>86</sub>N<sub>8</sub>S<sub>3</sub>O<sub>10</sub>·H<sub>2</sub>O: 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-alanyl-glycyl-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°,  $[\alpha]^{25}_D -13.33^\circ$  (*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<sub>3</sub>H, 2.2; Ser, 0.81; Gly, 0.99; Ala, 1.02; Val, 1.00; Leu, 0.96.

*Anal.* Calcd for C<sub>41</sub>H<sub>83</sub>N<sub>8</sub>O<sub>10</sub>S<sub>3</sub>·CF<sub>3</sub>CO<sub>2</sub>H·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 diketo-piperazine, 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*-ditrityl-L-cysteine *N*-hydroxysuccinimide ester, 27560-18-5.

## Sulfur-Containing Polypeptides. XVI. Synthesis of the A<sub>14–21</sub> Fragment of Ovine Insulin<sup>1–3</sup>

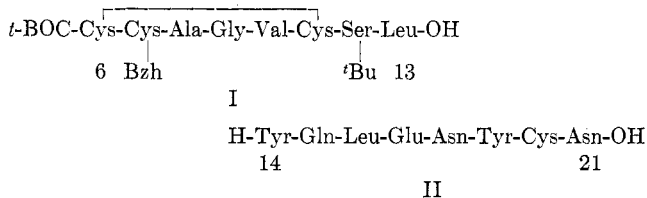
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The protected octapeptide *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl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

In the accompanying report<sup>1</sup> a synthetic route to a suitably blocked peptide containing the A<sub>6–13</sub> sequence of ovine insulin (I) was described. The present report concerns the development of a synthesis leading to the A<sub>14–21</sub> sequence (II) and describes our experience with the *N*-2-(*p*-diphenyl)isopropylloxycarbonyl protective group developed by Sieber and Iselin<sup>5</sup> for the elegant synthesis of thyrocalcitonin.<sup>6</sup>



(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*-diphenyl)isopropylloxycarbonyl; *o*-NPS = *o*-nitrophenylsulfenyl; <sup>t</sup>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.

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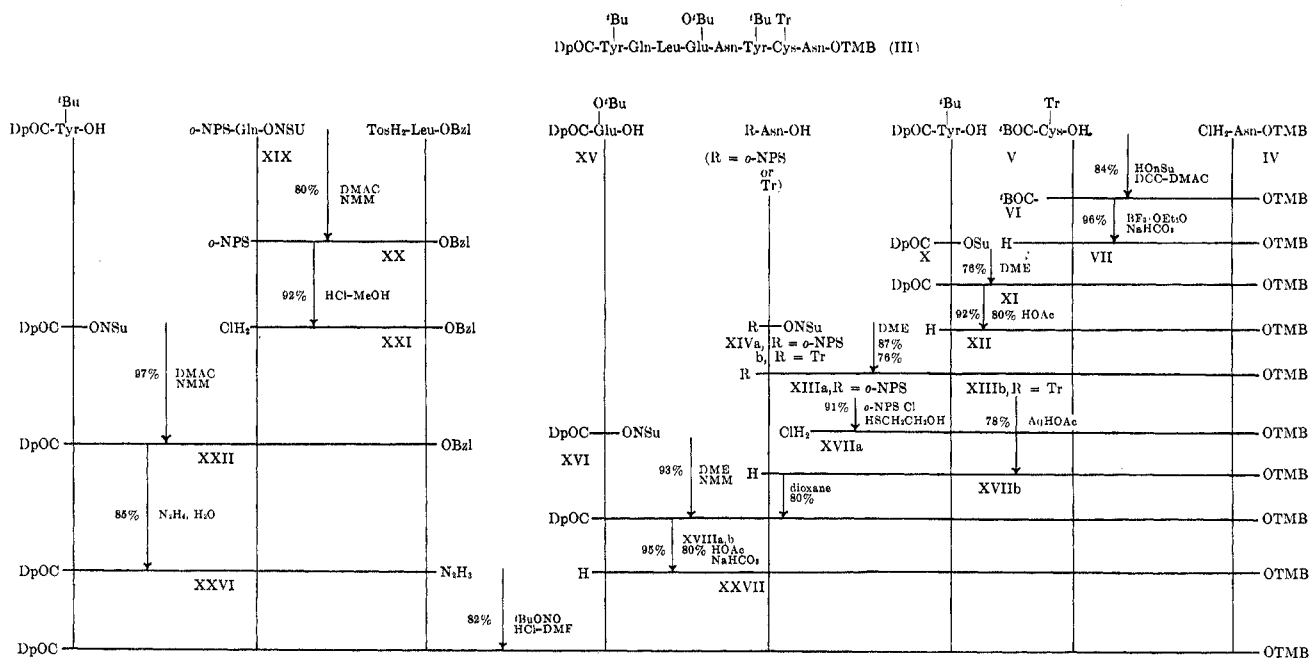
(5) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 622 (1968).

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

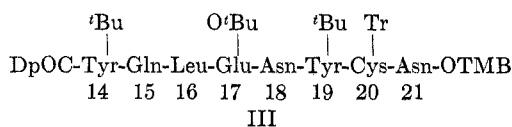
The preparation of the A<sub>14–21</sub> sequence was complicated by the presence of seven functional side chains in the octapeptide; four of these required protection. Since acid-labile protective groups were required and the presence of a cysteine residue ruled out the possibility of removal of groups 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<sub>14,19</sub> was the *tert*-butyl ether; the *S*-trityl group was required for the A<sub>20</sub> cysteine residue to permit selective formation of the two interchain disulfide bonds at A<sub>7</sub>B<sub>7</sub> and A<sub>20</sub>B<sub>19</sub>. The *tert*-butyl ester seemed to be suitable for the A<sub>17</sub> 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<sup>7</sup> 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<sup>1</sup> 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).

## SCHEME I

SYNTHESIS OF THE PROTECTED A<sub>14-21</sub> PEPTIDE DERIVATIVE

nitrophenylsulfenyl<sup>8</sup> (*o*-NPS), and the *N*-2-(*p*-diphenyl)isopropylloxycarbonyl<sup>5,6</sup> (DpOC) groups. The synthetic goal was then the fully protected octapeptide derivative III; the route finally adopted is shown in Scheme I.



*L*-Asparagine 2,4,6-trimethylbenzyl ester hydrochloride (IV) was coupled *via* the *N*-hydroxysuccinimide method to *tert*-butyloxycarbonyl-*S*-trityl-*L*-cysteine dicyclohexylamine salt (V). The *t*-BOC group of the dipeptide VI was subsequently removed by the action of boron trifluoride in acetic acid, and *S*-trityl-*L*-cysteinyll-*L*-asparagine 2,4,6-trimethylbenzyl ester (VII) was obtained in 82% overall yield. The DpOC group was not employed at this point since a group of this lability was not required and since the preparation of this particular cysteine derivative has provided low-melting solids that are difficult to purify. In our early experiments VII was converted to the oxalate salt VIII for characterization purposes; subsequently VII was used directly in the following coupling step.

Since the acid-labile *tert*-butyl ether was required for the protection of the phenolic hydroxyl of Tyr<sub>19</sub>, clearly either the *o*-NPS, the DpOC, or the Tr group was necessary for amino protection. Despite the fact that a number of separate steps are required for the preparation of *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-*L*-tyrosine dicyclohexylamine salt (IX), this group was preferable to the *o*-NPS group since *S*-trityl cleavage can sometimes occur when the *o*-NPS group of an *S*-trityl-*L*-cysteine peptide is removed from the amino terminus<sup>9,10</sup> or to the *N*-trityl group which is

known to give lowered yields in the coupling steps because of steric hindrance. In the preparation of IX, *O*-*tert*-butyl-*L*-tyrosine was cleanly acylated by the action of [2-(*p*-diphenyl)isopropyl]phenyl carbonate; IX was obtained in 61% yield and could readily be converted into the crystalline *N*-hydroxysuccinimide ester derivative (X) in 66% yield. The coupling reaction between X and the crude free base VII proceeded smoothly and afforded the tripeptide derivative, *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-*L*-tyrosyl-*S*-trityl-*L*-cysteinyl-*L*-asparagine 2,4,6-trimethylbenzyl ester (XI) in 76% yield. Alternatively, XI could be prepared from the crystalline oxalate salt VIII and the active ester X by using 2 equiv of *N*-methylmorpholine. Although both preparations exhibited identical behavior on tlc and essentially the same melting point, the product obtained from 2 equiv of base showed a slightly lower specific rotation and hence subsequent preparations were conducted using VII. Removal of the *N*-DpOC group was accomplished using the conditions described by Sieber and Iselin.<sup>5</sup> The free base XII was obtained as a ninhydrin-positive solid, homogeneous on tlc; cleavage over a 17-hr period gave better results than when shorter times were employed.

The choice of an amino protective group for asparagine-18 was complicated by the earlier observations of Sieber and Iselin concerning the DpOC derivative of *L*-asparagine. This derivative was obtained in rather low yield and exhibited low solubility in common solvents employed for coupling. Thus it appeared that *o*-nitrophenylsulfenyl-*L*-asparagine would provide better results despite the anticipated deblocking problems. *N*-*o*-Nitrophenyl-*L*-asparagine *N*-hydroxysuccinimide ester (XIVa) was prepared by the procedure of Walter, *et al.*,<sup>11</sup> the coupling reaction between XII and XIVa

(8) L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, **85**, 3660 (1963).

(9) W. C. Jones, Jr., Ph.D. Dissertation, University of North Carolina, June 1969.

(10) D. Brandenburg, *Tetrahedron Lett.*, 6201 (1966).

(11) C. Meyers, R. T. Havran, I. L. Schwartz, and R. Walter, *Chem. Ind. (London)*, 136 (1969).

proceeded readily in DME to provide *N*-*o*-nitrophenylsulfenyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl ester (XIIIa) in 88% yield.

Removal of the *o*-NPS group from XIIIa was studied rather carefully. Cleavage experiments in acetic acid, methanol-pyridine, or acetic acid-pyridine-DMF gave incomplete reaction. Treatment of XIIIa with thio-glycolic acid in DMF gave no reaction; similar results were also obtained using *o*-nitrothiophenol.<sup>10</sup> Complete cleavage was finally observed using exactly 1 equiv of *o*-nitrophenylsulfenyl chloride in the presence of  $\beta$ -mercaptoethanol.<sup>10</sup> This reagent generated 1 equiv of hydrogen chloride and produced the hydrochloride salt of the tetrapeptide XVIIa, in 91% crude yield. The product was homogeneous on tlc, and colored impurities due to *S*-trityl cleavage<sup>9,10</sup> were not observed. The salt XVIIa was converted to the free base XVIIb and coupled with *N*-2-(*p*-diphenyl)isopropylloxycarbonyl- $\gamma$ -*tert*-butyl-L-glutamic acid *N*-hydroxysuccinimide ester (XVI), obtained in 83% yield from the corresponding acid XV. When the reaction was carried out on a small scale, a good yield of the pentapeptide *N*-2-(*p*-diphenyl)isopropylloxycarbonyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl ester (XVIII) was obtained. However, when the conversion of the *o*-NPS peptide XIIIa to the hydrochloride salt XVIIa was performed on a large scale, the resulting salt XVIIa was not homogeneous and mobile colored impurities were detected on tlc. Since purification of XVIIa from this preparation was difficult, the use of the *o*-NPS group was abandoned in favor of the *N*-trityl group. *N*-Trityl-L-asparagine *N*-hydroxysuccinimide ester (XIVb) was prepared in 52% yield and was allowed to react with XII in dioxane solution. The coupling reaction appeared to proceed smoothly and *N*-trityl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl ester (XIIIb) was obtained in 76% yield. Treatment of XIIIb with aqueous acetic acid at room temperature provided the free base XVIIb in 78% crude yield. The product was homogeneous on tlc and was coupled directly with XVI without further purification. The pentapeptide XVIII was obtained in reasonable yield (80%) and high purity as indicated by tlc, elemental, and amino acid analysis. Thus in subsequent experiments using larger quantities the route involving *N*-trityl-L-asparagine has been the method of choice.

At this point a second fragment corresponding to the A<sub>14-16</sub> portion of III was prepared and coupled to the free base XXVII by the azide method. Initially *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine methyl ester (XXIII) was prepared from the *N*-hydroxysuccinimide ester (X) and *N*-*o*-nitrophenylsulfenyl-L-glutamyl-L-leucine methyl ester (XXIV). Although the resulting tripeptide was obtained in fair yield and pure condition, the procedure was complicated by the hygroscopic nature of the dipeptide hydrochloride XXV resulting from the removal of the *o*-NPS group from XXIV with hydrogen chloride. Attempts to obtain a crystalline free base invariably led to diketopiperazine formation. More satisfactory results were obtained using the corresponding benzyl ester. Treatment of L-leucine benzyl ester

*p*-toluenesulfonate salt with *N*-*o*-nitrophenylsulfenyl-L-glutamine *N*-hydroxysuccinimide ester<sup>11</sup> (XIX) provided *N*-*o*-nitrophenylsulfenyl-L-glutamyl-L-leucine benzyl ester (XX) in 80% yield. Removal of the amino protective group proceeded smoothly and provided the crystalline hydrochloride of L-glutamyl-L-leucine benzyl ester (XXI) in 92% yield. The dipeptide was then coupled with X to provide *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine benzyl ester (XXII) in 97% yield. Treatment of either the methyl ester XXIII or the benzyl ester XXII with hydrazine provided the same hydrazide derivative, *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-L-tyrosyl-L-glutamyl-L-leucine hydrazide (XXVI). The substance was obtained as a gel which could be solidified by crystallization from alcohol and was homogeneous on tlc.

Formation of the azide from the hydrazide XXVI was now considered. In order to avoid any cleavage of the DpOC group, our initial diazotization experiments utilized 1 equiv of hydrogen chloride in DMF. Treatment of the azide, generated by this method, with the free base of the pentapeptide XXVII, obtained by acetic acid cleavage of the DpOC group XVIIIa, gave low yields of the desired octapeptide derivative, *N*-2-(*p*-diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-*S*-trityl-L-cysteinyl-L-asparagine 2,4,6-trimethylbenzyl ester (III). However, subsequent control experiments with XXVI established that the DpOC group was stable to excess hydrogen chloride in THF-DMF mixtures at low temperatures ( $-20$  to  $-40^\circ$ ). The coupling between the azide, prepared by the Rudinger method,<sup>12</sup> and the free base XXVII proceeded smoothly and provided good yields (75-85%) of the desired octapeptide III. The product was homogeneous on tlc and gave the expected elemental and amino acid analyses. Future experiments will deal with the formation of the fully blocked A chain and the combination of this material with an appropriate B chain.

### Experimental Section<sup>13</sup>

***N*-*o*-Nitrophenylsulfenyl-L-asparagine.**—L-Asparagine (79.2 g, 0.6 mol) was dissolved in 750 ml of dioxane, cooled to  $5^\circ$ , and treated with 300 ml of 2 *N* sodium hydroxide solution. The clear solution was treated simultaneously with 126 g (10% excess) of nitrophenylsulfenyl chloride and 360 ml of cold 2 *N* sodium hydroxide. The pH of the solution was maintained at 9-10. Vigorous stirring was continued for 2 hr at room temperature and 600 ml of water was added, and the reaction mixture filtered. The filtrate was acidified with cold 2 *N* sulfuric acid and the product washed with water to yield 154 g (92%) of yellow solid, mp 161-162°, homogeneous (system B) (lit.<sup>5</sup> mp 165-166°).

***N*-*o*-Nitrophenylsulfenyl-L-asparagine 2,4,6-Trimethylbenzyl Ester.**—A solution of 46 g (0.16 mol) of *N*-*o*-nitrophenylsulfenyl-L-asparagine in 80 ml of DMF was treated with 22.5 ml of tri-

(12) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).

(13) Melting points are uncorrected. Combustion analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Amino acid analyses were determined on a Beckman Model 116 amino acid analyzer and have not been corrected for destruction during hydrolysis. Thin layer chromatography (tlc) was conducted on silica gel GF<sub>254</sub> with the following solvent systems: (A) chloroform-methanol (9:1); (B) chloroform-methanol-acetic acid (8:1:1); (C) 1-butanol-acetic acid-water (10:1:3); (D) *n*-heptane-*tert*-butyl alcohol-acetic acid-water-pyridine (25:70:6:24:20); (E) *sec*-butyl alcohol-3% ammonium hydroxide (7:3); (F) 1-butanol-acetic acid-water-pyridine (60:6:24:20). Unless otherwise stated products were dried *in vacuo* over phosphorus pentoxide and sodium hydroxide pellets.

ethylamine and 27.6 g (0.16 mol) of molten 2,4,6-trimethylbenzyl chloride. The clear solution was stirred 4 days at room temperature and diluted with cold 10% sodium bicarbonate solution. The crude product was filtered and washed with water; recrystallization from a cyclohexane-chloroform mixture provided 45.2 g (67.6%) of yellow solid, homogeneous (system D), mp 173–174° (lit.<sup>14</sup> mp 173–174°).

**L-Asparagine 2,4,6-trimethylbenzyl ester hydrochloride (IV)** was prepared by the procedure of Stewart in 69% yield, homogeneous (system B), mp 198–199° (lit.<sup>14</sup> mp 194.5–195.5°).

***N*-tert-Butyloxycarbonyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (VI)**.—A suspension of 12.86 g (0.02 mol) of *N*-tert-butyloxycarbonyl-S-trityl-L-cysteine dicyclohexylamine salt (V) and 6.02 g (0.02 mol) of L-asparagine 2,4,6-trimethylbenzyl ester hydrochloride (IV) in 140 ml of DMAc was cooled to –10° and treated with 2.3 g (0.02 mol) of *N*-hydroxysuccinimide and 4.2 g (0.02 mol) of DCC. The stirred suspension was allowed to warm to room temperature overnight and was stirred an additional 10 hr. The suspension was filtered into a cold brine solution and the filtrate extracted with ethyl acetate. The organic layer was washed with cold brine, cold 10% citric acid solution, cold 1 M sodium bicarbonate solution, and cold brine. The dried solid (13.6 g) was recrystallized from a petroleum ether-ether mixture to yield 12.0 g (84.6%) of white solid, mp 179–180°,  $[\alpha]^{25D} + 20.6^\circ$  (c 1.4, methanol).

*Anal.* Calcd for C<sub>41</sub>H<sub>47</sub>N<sub>3</sub>O<sub>6</sub>S: C, 69.36; H, 6.67; N, 5.92; S, 4.51. Found: C, 69.18; H, 6.71; N, 6.01; S, 4.32.

**S-Trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (VII)**.—The protected dipeptide VI was dissolved in 100 ml of glacial acetic acid and treated dropwise with 13 ml (0.1 mol) of boron trifluoride diethyl etherate at room temperature. After 2 hr the solution was diluted with cold water, treated with 34 g (0.3 mol) of sodium acetate, and precipitated by saturating the solution with sodium chloride. The resulting precipitate was filtered, washed with brine, partitioned between ethyl acetate and 10% sodium bicarbonate solution, and washed with water and brine. Removal of the solvent provided 14.6 g (85.2%) of white solid, homogeneous (system A). The material was used directly in the subsequent coupling reaction.

**S-Trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester Oxalate Salt (VIII)**.—An oxalate salt of VII was prepared in the following manner. A 10.79-g (0.0179 mol) sample of VII was dissolved in 10 ml of methanol and treated with 1.6 g (0.0177 mol) of anhydrous oxalic acid. The salt was precipitated with ether and recrystallized from methanol-ether to yield 7.8 g (56%) of white solid,  $[\alpha]^{25D} + 48.9^\circ$  (c 1.65, methanol).

*Anal.* Calcd for C<sub>33</sub>H<sub>41</sub>N<sub>3</sub>O<sub>8</sub>S: C, 65.22; H, 5.91; N, 6.00; S, 4.58. Found: C, 64.68; H, 5.97; N, 6.06; S, 4.78.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-tert-butyl-L-tyrosine Dicyclohexylamine Salt (IX)**.—A solution containing 4.7 g (0.02 mol) of *O*-tert-butyl-L-tyrosine in 9.1 ml of Triton B (40% in methanol) was maintained at 50° and the methanol was evaporated. The remaining oil was carefully dried *in vacuo*, dissolved in 15 ml of DMF, and treated with 6.6 g (0.02 mol) of [2-(*p*-diphenyl)isopropyl] phenyl carbonate.<sup>5</sup> The solution was stirred for 3 hr at 50° and then partitioned between water and ether. The aqueous layer was acidified at 0° with 10% citric acid solution and extracted with ether. The ether extract was washed, dried, and evaporated to an oil which was dissolved in ether and treated with 4 ml of dicyclohexylamine. The salt was recrystallized from isopropyl alcohol to yield 8.0 g (61.5%) of salt, mp 160–161°,  $[\alpha]^{25D} + 42.5^\circ$  (c 1.2, methanol).

*Anal.* Calcd for C<sub>41</sub>H<sub>56</sub>O<sub>2</sub>N<sub>2</sub>: C, 74.96; H, 8.59; N, 4.26. Found: C, 74.74; H, 8.71; N, 4.22.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-tert-butyl-L-tyrosine *N*-Hydroxysuccinimide Ester (X)**.—A 18.38-g (0.028 mol) sample of the dicyclohexylamine salt IX was dissolved in ethyl acetate and extracted with 15% citric acid solution. The citric acid layer was reextracted with ethyl acetate, and the combined organic extracts were washed with citric acid solution, water, and brine. The dried organic layer was evaporated to an oil which was dissolved in 40 ml of 1,2-dimethoxyethane, cooled to 0°, and treated with 3.22 g (0.028 mol) of *N*-hydroxysuccinimide and 5.80 g (0.028 mol) of DCC. The reaction mixture was stirred for 3 hr at 0° and allowed to stand at 0° overnight. Evaporation of the filtrate provided a solid which was crystallized twice from isopropyl alcohol to yield 10.51 g (66%) of the active ester, mp 136–138°,  $[\alpha]^{25D} - 18.6^\circ$  (c 1.7, methanol).

*Anal.* Calcd for C<sub>33</sub>H<sub>36</sub>O<sub>7</sub>N<sub>2</sub>: C, 69.21; H, 6.34; N, 4.89. Found: C, 69.09; H, 6.30; N, 4.82.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-tert-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XI)**.—A solution containing 4.52 g (0.0074 mol) of the *S*-trityl dipeptide in 30 ml of 1,2-dimethoxyethane was treated with 4.26 g (0.0074 mol) of the *N*-hydroxysuccinimide ester. Stirring was continued for 3 hr at room temperature. The solution was poured into ice water and filtered. The solid was washed with 10% sodium bicarbonate solution and water. Recrystallization from a methanol-water mixture provided 6.0 g (76.0%) of the protected tripeptide, mp 189–190° homogeneous (system A),  $[\alpha]^{25D} - 2.08$  (c 1.25, DMF).

*Anal.* Calcd for C<sub>65</sub>H<sub>70</sub>N<sub>4</sub>O<sub>8</sub>S: C, 73.07; H, 6.69; N, 5.24; S, 3.00. Found: C, 72.93; H, 6.52; N, 5.24; S, 3.00.

The tripeptide XI could also be obtained from the oxalate salt VIII. To a solution of 6.99 g (0.01 mol) of VIII in 20 ml of DME at 0° was added 1.1 ml (0.02 mol) of *N*-methylmorpholine and 5.72 g (0.01 mol) of X. After 3 hr of stirring at 20° the oxalate salt of *N*-methylmorpholine was precipitated with water and the aqueous layer was extracted with chloroform. Evaporation of the solvent provided an oil which could be crystallized from a methanol-water-2-propanol mixture to yield 7.7 g (73%) of XI, mp 187–188°,  $[\alpha]^{25D} - 1.53$  (c 1.25, DMF), homogeneous (system A).

*Anal.* Calcd for C<sub>65</sub>H<sub>70</sub>N<sub>4</sub>O<sub>8</sub>S: C, 73.07; H, 6.69; N, 5.24; S, 3.00. Found: C, 73.01; H, 6.74; N, 5.19; S, 2.86.

***O*-tert-Butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XII)**.—A suspension of 3.6 g (3.34 mmol) of the fully blocked tripeptide in 75 ml of 80% acetic acid was vigorously stirred at room temperature for 17 hr. The solution was poured into 200 ml of cold brine and the product partitioned between 100 ml of ethyl acetate and 10% sodium bicarbonate, washed with water and brine, and dried. Removal of the solvent and recrystallization from isopropyl alcohol provided 2.69 g (97.9%) of white solid, mp 182–183°, homogeneous (system A),  $[\alpha]^{25D} + 6.9^\circ$  (c 1.1, methanol).

*Anal.* Calcd for C<sub>49</sub>H<sub>56</sub>O<sub>6</sub>N<sub>4</sub>S: C, 70.90; H, 6.92; N, 6.75; S, 3.86. Found: C, 70.99; H, 6.92; N, 6.84; S, 3.71.

***N*-*o*-Nitrophenylsulfenyl-L-asparagine *N*-Hydroxysuccinimide Ester (XIVa)**.—A solution of 11.41 g (0.04 mol) of *N*-*o*-nitrophenylsulfenyl-L-asparagine in 40 ml of DMAc was cooled to 0° and treated with 4.61 g (0.04 mol) of *N*-hydroxysuccinimide and 8.7 g (0.04 mol) of DCC. The solution was stirred for 2 hr at 0° and stored in the cold overnight. The solution was filtered and washed with 2 ml of DMAc; the filtrate was poured into 600 ml of cold isopropyl alcohol. The yellow solid was filtered, washed with cold isopropyl alcohol, and dried to yield 8.9 g (59%) of the active ester, mp 150–151°,  $[\alpha]^{25D} - 51.1^\circ$  (c 1, dioxane) [lit. mp 150–151°,  $[\alpha]^{25D} - 52.7^\circ$  (c 1, dioxane)].

*Anal.* Calcd for C<sub>14</sub>H<sub>14</sub>O<sub>6</sub>N<sub>2</sub>S: C, 43.97; H, 3.69; N, 14.65; S, 8.38. Found: C, 43.88; H, 3.77; N, 14.49; S, 8.27.

***N*-*o*-Nitrophenylsulfenyl-L-asparaginyl-*O*-tert-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XIIIa)**.—A solution containing 5.13 g (0.0062 mol) of the free base XII in 60 ml of 1,2-dimethoxyethane was treated, at room temperature, with 2.46 g (0.0062 mol) of the *N*-hydroxysuccinimide ester XIVa. The slurry was stirred overnight and filtered and the product washed with cold chloroform. Recrystallization from a chloroform-methanol solvent provided 6.0 g (88.5%) of tetrapeptide, mp 204–206°, homogeneous (system A),  $[\alpha]^{25D} - 22.8^\circ$  (c 0.90, DMF).

*Anal.* Calcd for C<sub>59</sub>H<sub>66</sub>O<sub>10</sub>N<sub>7</sub>S<sub>2</sub>: C, 64.57; H, 6.06; N, 8.93; S, 5.84. Found: C, 64.31; H, 5.86; N, 8.98; S, 5.92.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl- $\gamma$ -tert-butyl-L-glutamic Acid Dicyclohexylamine Salt (XV)**.—A solution containing 6 g (0.03 mol) of  $\gamma$ -tert-butyl-L-glutamic acid<sup>15</sup> in 13.6 ml of Triton B (40% in methanol) was maintained at 50° and the methanol was evaporated. The remaining oil was carefully dried *in vacuo*, dissolved in 20 ml of DMAc, and treated with 9.99 g (0.03 mol) of [2-(*p*-diphenyl)isopropyl] phenyl carbonate. The solution was stirred for 3 hr at 50° and then partitioned between water and ether. The aqueous layer was acidified at 0° with 10% citric acid solution and extracted with ether. The ether extract was washed, dried, and evaporated to an oil which was dissolved in ethyl acetate and treated with 6 ml of dicyclohexylamine. The salt was recrystallized from an isopropyl alcohol-ether-petroleum

(15) E. Schroder and E. Klieger, *Justus Liebigs Ann. Chem.*, **673**, 196 (1964).

(14) F. H. C. Stewart, *Aust. J. Chem.*, **20**, 365 (1967).

ether mixture to yield 12.0 g (65%) of the salt, mp 136–138°,  $[\alpha]^{20D} +12.9^\circ$  (*c* 1.7, methanol).

*Anal.* Calcd for  $C_{37}H_{54}O_6N_2$ : C, 71.35; H, 8.74; N, 4.50. Found: C, 71.17; H, 8.51; N, 4.37.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl- $\gamma$ -*tert*-butyl-L-glutamic Acid *N*-Hydroxysuccinimide Ester (XVI).**—A 6.22-g (0.01 mol) sample of the salt XV was partitioned between a 10% aqueous citric acid solution and ethyl acetate at 0°. The layers were separated and the aqueous phase extracted again with ethyl acetate. The organic layers were washed with 10% citric acid solution, water, and brine. The dried extract was concentrated to a clear oil which was dissolved in 20 ml of 1,2-dimethoxyethane and treated with 1.30 g (0.012 mol) of *N*-hydroxysuccinimide and 2.5 g (0.012 mol) of DCC at 0°. The mixture was stirred for 12 hr in an ice bath and stored overnight at 0°. Filtration and evaporation of the filtrate yielded an oil which crystallized on trituration with isopropyl alcohol to yield 4.5 g (83%) of the active ester, mp 117–118°,  $[\alpha]^{20D} -24.9^\circ$  (*c* 1.55, dioxane).

*Anal.* Calcd for  $C_{29}H_{34}O_5N_2$ : C, 64.67; H, 6.36; N, 5.20. Found: C, 64.75; H, 6.28; N, 5.12.

***N*-Trityl-L-asparagine *N*-Hydroxysuccinimide Ester (XIVb).**—A solution of *N*-tritylasparagine<sup>16</sup> (2.6 g, 0.007 mol) in 30 ml of dioxane was cooled to 10° and treated with 0.88 g (0.0077 mol) of *N*-hydroxysuccinimide and 1.6 g (0.0077 mol) of DCC. The solution was stirred for 4 hr at 10° and was allowed to stand at 4° overnight. The dicyclohexylurea was filtered and washed with cold dioxane. The filtrate was concentrated *in vacuo* and the solid on crystallization from ethyl acetate-*n*-hexane provided 1.7 g (52%) of the active ester, mp 152–153°,  $[\alpha]^{25D} -73.1^\circ$  (*c* 1, dioxane).

*Anal.* Calcd for  $C_{27}H_{36}O_5N_3$ : C, 68.78; H, 5.30; N, 8.91. Found: C, 68.90; H, 5.47; N, 9.07.

***N*-Trityl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XIIIb).**—A solution containing 2.49 g (0.003 mol) of the free base XII in 25 ml of dioxane was treated at room temperature with 1.9 g (0.004 mol) of the *N*-hydroxysuccinimide ester XIVb and stirred overnight. The dioxane was evaporated and the residue dissolved in chloroform, washed with 1 *N* sodium bicarbonate and water, and dried over sodium sulfate. Evaporation of the chloroform, trituration of the residue with ether, and crystallization from ethyl acetate-*n*-hexane provided 2.7 g (76%) of the tetrapeptide, mp 202–205°, homogeneous (system A),  $[\alpha]^{25D} -13.3^\circ$  (*c* 2, dioxane).

*Anal.* Calcd for  $C_{72}H_{76}O_8N_8S$ : C, 72.91; H, 6.47; N, 7.08; S, 2.76. Found: C, 72.87; H, 6.76; N, 7.04; S, 3.15.

***L*-Asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparaginyl 2,4,6-Trimethylbenzyl Ester (XVIIb).**—A suspension of 1.0 g of the protected tetrapeptide XIIIb in a mixture of 10 ml of acetic acid and 2 ml of water was stirred at room temperature for 6 hr. The reaction mixture was diluted with brine and triturated (three 10-ml portions), and the resulting gum was partitioned between ethyl acetate and 1 *N* sodium bicarbonate. The ethyl acetate solution was washed with water, dried over sodium sulfate, and evaporated. The residue was triturated with ether to provide 0.62 g (78%) of the crude product, homogeneous (system B). The crude solid was used directly in the next step.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XVIIIb).**—A solution of 0.471 g (0.0005 mol) of the crude tetrapeptide XVIIb and 0.54 g (0.001 mol) of the *N*-hydroxysuccinimide ester in dioxane was stirred for 24 hr at room temperature. The mixture was diluted with ice and 1 *N* sodium bicarbonate solution, followed by brine. The solid was filtered, washed with water, and triturated with ether to yield, after recrystallization from chloroform-hexane, 0.55 g (80%) of the product, mp 160–161°, homogeneous (system A),  $[\alpha]^{25D} -8.6^\circ$  (*c* 1, methanol).

*Anal.* Calcd for  $C_{78}H_{91}O_{13}N_7S$ : C, 68.54; H, 6.71; N, 7.17; S, 2.34. Found: C, 68.53; H, 6.66; N, 7.08; S, 2.40.

Amino acid analysis after performic acid oxidation and acid hydrolysis showed  $Asp_{1.9}CySO_3H_{1.0}Glu_{1.0}$ . Amino acid analysis of an acid hydrolysate in the presence of phenol showed  $Asp_{2.0}Glu_{1.0}Tyr_{1.0}$ .

***L*-Asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester Hydrochloride Salt (XVIIIa).**—A suspension of 2.2 g (2.0 mmol) of the protected

tetrapeptide in 80 ml of chloroform, containing 1 ml of  $\beta$ -mercaptoethanol, was stirred vigorously and treated with 0.568 g (3 mmol) of *o*-nitrophenylsulfenyl chloride in 40 ml of chloroform, containing 0.5 ml of  $\beta$ -mercaptoethanol. The suspension was stirred 30 min at room temperature, filtered, and evaporated. The residue was triturated with ether and the resulting hydrochloride salt (1.8 g), homogeneous in system A, was used directly in the following coupling reaction.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (XVIIIa).**—A solution of 1.77 g (1.8 mmol) of the crude tetrapeptide hydrochloride salt XVIIa and 1.08 g (2.0 mmol) of the *N*-hydroxysuccinimide ester was dissolved in 20 ml of 1,2-dimethoxyethane and at 0° treated with 0.25 ml of *N*-methylmorpholine. The mixture was stirred 24 hr at room temperature and diluted with water and the product filtered. The solid was washed with water and then triturated with ether to yield a gelatinous solid, 2.5 g (93%), mp 160° (recrystallized from chloroform-*n*-hexane), homogeneous (system A),  $[\alpha]^{20D} -7.3^\circ$  (*c* 1, methanol).

*Anal.* Calcd for  $C_{78}H_{91}O_{13}N_7S$ : C, 68.54; H, 6.71; N, 7.17; S, 2.34. Found: C, 68.64; H, 6.92; N, 7.05; S, 2.09.

Amino acid analysis after performic acid oxidation and acid hydrolysis showed  $Asp_{1.9}CySO_3H_{1.0}Glu_{1.0}$ . Amino acid analysis of an acid hydrolysate in the presence of phenol showed  $Asp_{2.0}Glu_{1.0}Tyr_{1.0}$ .

**$\gamma$ -*tert*-Butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparaginyl 2,4,6-Trimethylbenzyl Ester (XXVII).**—The blocked pentapeptide XVIIIa (2.3 g, 1.68 mmol) was dissolved in 20 ml of glacial acetic acid and after 24 hr diluted with 3 ml of water. The reaction mixture was stirred 36 hr at room temperature, and diluted with 200 ml of brine; the resulting semisolid was isolated by decantation. The product was dissolved in chloroform, washed with 2 *N* sodium bicarbonate and water, and dried. Evaporation and trituration of the solid with ether provided 1.8 g (95%) of the crude deblocked pentapeptide. The crude solid was used directly in the subsequent coupling reaction with the azide generated from XXVI.

***N*-*o*-Nitrophenylsulfenyl-L-glutamine *N*-Hydroxysuccinimide Ester (XIX).**—A solution of 2.99 g (0.01 mol) of *N*-*o*-nitrophenylsulfenyl-L-glutamine in 20 ml of DMAc was treated at 0° with 1.15 g (0.01 mol) of *N*-hydroxysuccinimide and 2.06 g (0.01 mol) of DCC. After stirring 3 hr at 0° the reaction was stored at 0° overnight, filtered and diluted with 200 ml of isopropyl alcohol. Recrystallization of the solid from isopropyl alcohol provided 3.2 g (79%) of the active ester, mp 146–148°,  $[\alpha]^{25D} -55.6^\circ$  (*c* 2.0, DMF) (lit.<sup>10</sup> mp 142–146°).

***N*-*o*-Nitrophenylsulfenyl-L-glutamyl-L-leucine Benzyl Ester (XX).**—A solution containing 7.92 g (0.02 mol) of the active ester and 7.86 g (0.02 mol) of *L*-leucine benzyl ester *p*-toluenesulfonate salt in 120 ml of 1,2-dimethoxyethane was cooled to 0° and treated with 2.2 ml of *N*-methylmorpholine. The reaction mixture was stored overnight at room temperature, the solvent removed, and the residue dissolved in ethyl acetate. The solution was washed with water, 10% sodium bicarbonate, and water. Removal of the solvent and trituration of the residue with cold ether provided 8.1 g (80.5%) of product, mp 116–120°,  $[\alpha]^{25D} -54.6^\circ$  (*c* 1, methanol).

*Anal.* Calcd for  $C_{24}H_{30}O_5N_4S$ : C, 57.82; H, 6.01; N, 11.16; S, 6.38. Found: C, 57.82; H, 6.07; N, 10.78; S, 6.34.

***L*-Glutamyl-L-leucine Benzyl Ester Hydrochloride Salt (XXI).**—A solution of 5.5 g (0.011 mol) of the dipeptide derivative in 15 ml of methanol was treated with 5 ml of 4 *N* hydrogen chloride in methanol solution. The reaction mixture was stirred for 5 min and evaporated; the resulting oil was triturated with ether. The solid was recrystallized from a chloroform-ether mixture to yield 3.9 g (92%) of product, mp 151–152°,  $[\alpha]^{25D} -10.6^\circ$  (*c* 1, methanol).

*Anal.* Calcd for  $C_{15}H_{20}ClO_4N_2$ : C, 55.93; H, 7.04; N, 10.92. Found: C, 55.90; H, 7.25; N, 11.01.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine Benzyl Ester (XXII).**—A solution of the hydrochloride (3.1 g, 8 mmol) was dissolved in 15 ml of DMAc and cooled to 0°. The cold solution was treated with 4.6 g (8 mmol) of the active ester and 0.9 ml of *N*-methylmorpholine and allowed to stir overnight at room temperature. The reaction mixture was diluted with cold water and filtered and the product washed with water. The product appeared as 6.3 g (97%) of white solid, mp 150–151°,  $[\alpha]^{25D} -15.4^\circ$  (*c* 1, methanol), homogeneous (system A).

(16) L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, **78**, 1359 (1956).

*Anal.* Calcd for  $C_{47}H_{88}O_8N_4$ : C, 69.90; H, 7.24; N, 6.94. Found: C, 70.17; H, 7.26; N, 7.03.

***N*-o-Nitrophenylsulfenyl-L-glutamyl-L-leucine Methyl Ester (XXIV).**—A mixture containing 3.2 g (0.008 mol) of XIX and 1.5 g (0.008 mol) of leucine methyl ester hydrochloride salt in 25 ml of DME was cooled to 0°, treated with 1.2 ml (0.008 mol) of triethylamine, and stirred for 2 hr at room temperature. The reaction mixture was diluted with water and the product was extracted with chloroform and washed with 10%  $NaHCO_3$ , 0.2 *N* sulfuric acid, and  $H_2O$ . Evaporation of the solvent gave a crude product which on recrystallization from methanol-ether yielded 2.5 g (73%) of product, mp 124–125°,  $[\alpha]^{25}_D +6.35$  (*c* 2, DMF), homogeneous (system A).

*Anal.* Calcd for  $C_{15}H_{25}O_3N_3S$ : C, 50.70; H, 6.10; N, 13.14; S, 7.51. Found: C, 51.24; H, 6.26; N, 13.30; S, 7.47.

**Diketopiperazine of XXIV.**—A solution of 2.13 g (0.005 mol) of XXIV in 5 ml of MeOH was treated with 2.5 ml of 4 *N* HCl in absolute methanol. The reaction mixture was stirred for 3 min and diluted with ether. The clear solution was decanted, and the remaining oil on trituration with ether provided a white hygroscopic solid. The semisolid was dissolved in 5 ml of  $H_2O$ , neutralized with saturated  $NaHCO_3$  solution, and extracted with chloroform. The chloroform was removed and the bicarbonate solution cooled to yield the diketopiperazine derivative, 0.73 g (44%), mp 239–240° (recrystallized from methanol-chloroform).

*Anal.* Calcd for  $C_{11}H_{19}O_3N_3$ : C, 54.77; H, 7.88; N, 17.49. Found: C, 54.43; H, 7.87; N, 17.45.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine Hydrazide (XXVI).**—The tripeptide benzyl ester XXII (0.8 g, 1.0 mmol) was dissolved in 10 ml of dry methanol and treated with 1.3 ml of hydrazine monohydrate (90%). The solution was stirred for 5 days at room temperature and diluted with ether and the resulting solid collected. The product was washed with ether and recrystallized from a methanol-ether mixture to yield 0.62 g (85%) of solid, mp 183–184°, homogeneous (system A),  $[\alpha]^{25}_D -11^\circ$  (*c* 1.05, MeOH).

*Anal.* Calcd for  $C_{40}H_{54}O_7N_6$ : C, 65.73; H, 7.45; N, 11.50. Found: C, 65.27; H, 7.39; N, 11.31.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucyl- $\gamma$ -*tert*-butyl-L-glutamyl-L-asparaginyl-*O*-*tert*-butyl-L-tyrosyl-S-trityl-L-cysteinyl-L-asparagine 2,4,6-Trimethylbenzyl Ester (III).**—A solution of the hydrazide (0.6 g, 0.81 mmol) in 30 ml of DMF was cooled to –20° and treated with 2.2 ml of 3 *N* hydrogen chloride in tetrahydrofuran solution. The temperature was lowered to –40° and 0.11 ml of *n*-butyl nitrite was added dropwise. The reaction mixture was stirred at –20 to –25° for 40 min, cooled to –60°, and treated with 0.8 ml of *N*-methylmorpholine. The solution of the azide at –40° was treated with a precooled (–40°) solution of the penta-

peptide (0.9 g, 0.8 mmol) in 10 ml of DMF. The stirring was continued for 1 hr at –30 to –20° and in an ice bath (0–2°) for 3.5 days.

The reaction mixture was diluted with ice water and saturated with sodium chloride. The separated product was washed with water, dried, and triturated with ether. A chloroform solution of the octapeptide derivative was applied to a silica gel column and eluted with chloroform-methanol (98:2). The product was collected and recrystallized from chloroform-petroleum ether to yield 1.2 g (82%) of white solid,  $[\alpha]^{25}_D -13.2^\circ$  (*c* 0.5, DMF).

*Anal.* Calcd for  $C_{102}H_{123}O_{18}N_{11}S \cdot 2H_2O$ : C, 65.71; H, 7.13; N, 8.26; S, 1.72. Found: C, 65.80; H, 7.05; N, 7.98; S, 1.85.

The amino acid analysis of a performic acid oxidized, acid hydrolysate was  $Asp_{2.1}CysSO_3H_{1.1}Glu_{2.2}Leu_{1.0}$ . The amino acid analysis of an acid hydrolysate in the presence of phenol was  $Asp_{2.0}Glu_{2.0}Leu_{1.0}Tyr_{2.0}$ .

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine Methyl Ester (XXIII).**—A solution of the crude hydrochloride XXV (2.07 g, 6.7 mmol) in 40 ml of DME and 4.13 g (6.7 mmol) of the active ester X was treated at 0° with 0.75 ml (6.7 mmol) of *N*-methylmorpholine, stirred overnight at room temperature, and diluted with water. The product was extracted with chloroform and washed with cold 1 *N* sodium hydroxide solution, water, 0.2 *N* sulfuric acid, and water. Evaporation of the chloroform and crystallization from a mixture of methanol-ethyl acetate gave 1.6 g (33.2%) of the tripeptide, mp 161–163°,  $[\alpha]^{25}_D -7.33^\circ$  (*c* 1.65, DMF).

*Anal.* Calcd for  $C_{41}H_{54}O_8N_4$ : C, 67.37; H, 7.45; N, 7.67. Found: C, 67.33; H, 7.30; N, 7.58.

***N*-2-(*p*-Diphenyl)isopropylloxycarbonyl-*O*-*tert*-butyl-L-tyrosyl-L-glutamyl-L-leucine Hydrazide (XXVI).**—A solution of the methyl ester XXIII (0.82 g 1.12 mmol) in 20 ml of methanol was stirred with 1.3 ml of hydrazine monohydrate at 50° for 1 hr and an additional 2 hr at 30°. Dilution with ether afforded 0.67 g (87%) of the product which on recrystallization from ethanol melted at 180–182°.

**Registry No.**—III, 33608-46-7; VI, 30806-18-9; VIII, 30806-19-0; IX, 33532-10-4; X, 33527-03-6; XI, 33527-04-7; XII, 33527-05-8; XIIIa, 33608-48-9; XIIIb, 33527-06-9; XIVa, 21753-83-3; XIVb, 33527-08-1; XV, 25461-15-8; XVI, 33527-10-5; XVIII, 33527-11-6; XX, 33527-12-7; XXI, 33527-13-8; XXII, 33527-14-9; XXIII, 33527-15-0; XXIV, 33527-16-1; XXIV diketopiperazine, 33527-17-2; XXVI, 33527-18-3.