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

Richard G. Hiskey,* Erik T. Wolters,4 Gungör Ülkü, and V. Ranga Rao

Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina 27514

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The protected octapeptide H-Tyr-Gln-Leu-Glu-Asn-Tyr-Cys-Asn-OH (II) has been synthesized. The route involves the use of the N-2-(p-diphenylyl)isopropylloxycarbonyl (DpOC) group as the principle amino protective group and O-carboxy-

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 acidity could be utilized. The protective group of choice for the phenolic hydroxyl groups at A<sub>14</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>20</sub>. The tert-butyl ester seemed to be suitable for the A<sub>19</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-butylxycarbonyl 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-0-

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3. The following abbreviations have been employed in the text: t-BOC = tert-butyloxycarbonyl; DpOC = 2-(p-diphenylyl)isopropylxycarbonyl; o-NPS = o-nitrophenylsulphenyl; Bu = tert-butyl; TMB = 2,4,6-trimethylbenzyl; Tr = trityl; Bu = benzyl; Bu = N-hydroxysucinimidic; 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.
Sulfur-Containing Polypeptides. XVI


Scheme I

Synthesis of the Protected Asn-Asp Peptide Derivative

<table>
<thead>
<tr>
<th>Bu</th>
<th>O'Bu</th>
<th>O'Bu</th>
<th>Tr</th>
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<tbody>
<tr>
<td>DpOC-Tyr-OH</td>
<td>DpOC-Gln-Leu-Glu-Asn-Tyr-Cys-Asn-OTMB (III)</td>
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L-Asparagine 2,4,6-trimethylbenzyl ester hydrochloride (IV) was coupled via the N-hydroxysuccinimide method to tert-butylxycarbonyl-S-trityl-l-cysteine dicyclohexylamine salt (V). The O-BOC group of the dipeptide VI was subsequently removed by the action of boron trifluoride in acetic acid, and S-trityl-l-cysteinyl-l-ascparagine 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 characterisation 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 Tyr19, 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-diphenylisopropylxycarbonyl)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 &10 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-buty1-l-tyrosine was cleanly acylated by the action of [2-(p-diphenylisopropyl)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-diphenylisopropylxycarbonyl)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 NCI 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.6 The free base XII was obtained as a ninhydrin-positive solid, homogeneous on NCI; 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-nitrophenylsulfonyl-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.,11 the coupling reaction between XII and XIVa

proceeded readily in DME to provide N-o-nitrophenylsulfonyl-L-asparginyl-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 thioglycolic acid in DME gave no reaction; similar results were also obtained using o-nitrothiophenol. Complete cleavage was finally observed using exactly 1 equiv of o-nitrophenylsulfonyl chloride in the presence of β-mercaptoethanol. 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 color impurities due to S-trityl cleavage were not observed. The salt XVIIa was converted to the free base XVIIb and coupled with N-2-(p-diphenyl)isopropylxoycarbonyl-γ-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)isopropylxoycarbonyl-γ-tert-butyl-L-glutamyl-L-asparginyl-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. 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 the N-trityl derivative, was considered. In order to avoid any cleavage of the DpOC group, 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)isopropylxoycarbonyl-O-tert-butyl-L-tyrosyl-L-glutaminyl-L-leucyl-γ-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 established that the DpOC group was stable to excess hydrogen chloride in THF-DMF mixtures at low temperatures (−20 to −40°C). The coupling between the azide, prepared by the Rudinger method, 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

N-o-Nitrophenylsulfonyl-L-asparagine.—L-Asparagine (79.2 g, 0.6 mol) was dissolved in 750 ml of dioxane, cooled to 5°C, and treated with 300 ml of 2 N sodium hydroxide solution. The clear solution was treated simultaneously with 126 g (10% excess) of nitrophenylsulfonyl 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°C, homogeneous (system B) (lit. mp 165−166°C).

N-o-Nitrophenylsulfonyl-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·······


(13) Melting points are uncorrected. Combustion analyses were performed at the Micro-Tech Laboratories, Skokie, III. \(\text{Amino acid analyses were determined on a Beckman Model 116 amino acid analyzer and have not been corrected for destruction during hydrolysis. Thin layer chromatography (tlc) was conducted on silicon gel G 60 with the following solvent systems: (A) chloroform-methanol (9:1); (B) chloroform-methanol-acetic acid (9:1:1); (C) 1-butanol-acetic acid-water (10:1:3); (D) n-heptane tert-butyl alcohol-acetic acid-water-pyridine (25:70:6:24:30); (E) ace-butyl alcohol-3% ammonium hydroxide (7:3); (F) 1-butanone-acetic acid-water-pyridine (60:6:24:90). Unless otherwise stated, products were dried in vacuo over phosphorus pentoxide and sodium hydroxide pellets.\)
ethylamine and 27.6 g (0.16 mol) of molten 2,4,6-trimethylbenzyl chloroform. The crude product was filtered and washed with water; recrystallization from a cyclohexane–chloroform mixture provided 4.94 g (56%) of a light yellow solid, homogeneous (system D), mp 173–174° (lit. 18 mp 173–174°).

L-Aspartic acid 2,4,6-trimethylbenzyl ester hydrochloride (IV) was prepared by the procedure of Stewart in 69% yield, homogenous (system B), mp 198–199° (lit. 19 194.5–195.5°).

Dicyclohexylamine salt (IX).—A solution containing 4.7 g (0.02 mol) of anhydrous oxalic acid. The salt was precipitated with 2 ml of DMAc; the filtrate was poured into 45.2 g (67.6%) of yellow solid, homogeneous (system D), mp 173–174° (lit. 20 mp 173–174°).

The tripeptide XI could also be obtained from the oxalate salt (VIII).—To a solution of 6.99 g (0.01 mol) of VII in 20 ml of DMAc 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 mixture. The yellow solid was filtered, washed with cold isopropanol alcohol and dried in vacuo, mp 182–183°, homogeneous (system A).

Anal. Caled for C_{60}H_{66}N_7O_4: 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.

O-t-Butyl-L-tyrosyl-L-tryptophanyl-L-glutaminyl-L-phenylsulfenyl-L-aspartyl-2,4,6-trimethylbenzyl ester (XIVa).—A solution containing 11.41 g (0.04 mol) of N-o-nitrophenylsulfenyl-l-aspartyl-2,4,6-trimethylbenzyl ester (XIIIa) in 40 ml of DMAc was cooled to 0° and treated with 4.61 g (0.04 mol) of N-hydroxyaspartimide 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 evaporated. The obtained oil was carefully dried in vacuo, dissolved in 15 ml of DMF, and treated with 6.6 g (0.02 mol) of [diphenylisopropyl] 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 and treated with 3.2 g of sodium bicarbonate solution, and washed with water and brine. The solution was vigorously stirred at room temperature for 17 hr. The solution was poured into 200 ml of cold brine and the product was partitioned between 100 ml of ethyl acetate and 10% sodium bicarbonate solution, and washed with water and brine, and dried. Removal of the solvent and recrystallization from isomyl alcohol provided 2.69 g (97.9%) of white solid, mp 182–183°, homogeneous (system A). [α]_D +26.8° (c 0.90, DMF).

Anal. Caled for C_{60}H_{66}N_7O_4: C, 64.57; H, 6.06; N, 8.93; S, 5.84.

Found: C, 64.41; H, 5.91; N, 6.00; S, 4.26. Found: C, 74.74; H, 8.71; N, 4.22.

N-2-(N-Diphenylisopropyl)oxycarbonyl-L-tyrosyl-L-phenylsulfenyl-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 N-hydroxyaspartic acid 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 tetratrapeptide, mp 204–206°, homogeneous (system A), [α]_D +22.8° (c 0.90, DMF).

Anal. Caled for C_{60}H_{66}N_7O_4S: C, 64.57; H, 6.06; N, 8.93; S, 5.84. Found: C, 64.51; H, 5.85; N, 5.88; S, 5.92.

N-2-(N-Diphenylisopropyl)oxycarbonyl-L-tyrosyl-2,4,6-trimethylbenzyl ester (XIIIb).—A solution containing 6.97 g (0.0157 mol) of N-2-(N-nitrophenylsulfenyl-est-2,4,6-trimethylbenzyl ester (XIIIa) in 40 ml of DMAc was cooled to 0° and treated with 4.61 g (0.04 mol) of N-hydroxyaspartimide 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 evaporated. The obtained oil was carefully dried in vacuo, dissolved in 15 ml of DMF, and treated with 6.6 g (0.02 mol) of [diphenylisopropyl] 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 and treated with 3.2 g of sodium bicarbonate solution, and washed with water and brine. The solution was vigorously stirred at room temperature for 17 hr. The solution was poured into 200 ml of cold brine and the product was partitioned between 100 ml of ethyl acetate and 10% sodium bicarbonate solution, and washed with water and brine, and dried. Removal of the solvent and recrystallization from isomyl alcohol provided 2.69 g (97.9%) of white solid, mp 182–183°, homogeneous (system A). [α]_D +26.8° (c 0.90, DMF).

Anal. Caled for C_{60}H_{66}N_7O_4S: 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.


ether mixture to yield 12.0 g (65%) of the salt, mp 136–138°, [α]D +2.9° (c 1.7, methanol).

Anal. Calcd for C19H20O11N: C, 55.90; H, 7.34; N, 11.01. Found: C, 55.9; H, 7.25; N, 10.98. 

N-2-(p-Diphenylisopropoxycarbonyl)-L-tyr butyl-L-glutamyl-L-asparaginyl-2,4,6-Trimethylbenzyl Ester (XVIII).—A solution containing 2.2 g (2.0 mmol) of the protected dipeptide derivative in 20 ml of 1,2-dimethoxyethane was cooled to 0° and treated with 0.25 ml of DCC. The reaction mixture was stirred 3 hr at 0° and stirred at room temperature, filtered, and evaporated.

The solid was triturated with ether and the resulting hydrochloride salt (1.8 g), homogenous in system A, was used directly in the following coupling reaction.

N-2-(p-Diphenylisopropoxycarbonyl)-L-tyr butyl-L-glutamyl-L-asparaginyl-O-tet butyl-L-tyrosyl-S-trityl-L-cysteyinyl-L-asparaginyl-2,4,6-Trimethylbenzyl Ester (XIX).—The blocked pentapeptide XVIII (2.3 g, 1.58 mmol) was dissolved in 20 ml of glacial acetic acid and after 24 hr diluted with 20 ml of water. The reaction mixture was stirred 3 hr at room temperature, and diluted with 200 ml of brine; the resulting semisolid was isolated by decantation. The product was dissolved in 20 ml of 1,2-dimethoxyethane and the solution was filtered. The solid was washed with water and then triturated with ether to yield a gelatinous solid, 2.5 g (93%), mp 160° (crystallized from chloroform-n-hexane), homogenous (system A), [α]D +7.3° (c 1, methanol).

Anal. Calcd for C29H84O8N2: C, 64.67; H, 6.36; N, 5.20. Found: C, 64.75; H, 6.28; N, 5.12.

S-trityl-L-glutaminyl-L-leucine Benzyl Ester (XX).—A solution containing 7.92 g (0.02 mol) of the active ester and 0.9 ml of o-nitrophenylsulfenyl chloride in 40 ml of chloroform was washed 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-Nitrophenylsulfonyl-L-glutaminyl-N-methylmorpholine (XXI).—A solution of 5.5 g (0.011 mol) of the dipeptide derivative in 10 ml of DMF was treated with 0.568 g (3 mmol) of o-nitrophenylsulfenyl chloride in 40 ml of DMAC 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 148–149°, [α]D +55.8° (c 2.0, DMF) (lit. mp 142–143°).

N-o-Nitrophenylsulfonyl-L-glutaminyl-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 20 ml of 1,2-dimethoxyethane was stirred 24 hr at room temperature, filtered, 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 0.82 g (75%) of the crude product, homogenous (system B). The crude solid was used directly in the next step.

N-2-(p-Diphenylisopropoxycarbonyl)-L-tyr butyl-L-glutamyl-L-asparaginyl-O-tet butyl-L-tyrosyl-S-trityl-L-cysteyinyl-L-asparaginyl-2,4,6-Trimethylbenzyl Ester Hydrochloride Salt (XIXa).—A suspension of 2.2 g (2.0 mmol) of the protected tetrapeptide in 80 ml of chloroform, containing 1 ml of β-mercaptoethanol, was stirred vigorously and treated with 0.68 g (3 mmol) of o-nitrophenylsulfonyl chloride in 40 ml of chloroform, containing 0.5 ml of β-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), homogenous in system A, was used directly in the following coupling reaction.

N-2-(p-Diphenylisopropoxycarbonyl)-L-tyr butyl-L-glutamyl-L-asparaginyl-O-tet butyl-L-tyrosyl-S-trityl-L-cysteyinyl-L-asparaginyl-2,4,6-Trimethylbenzyl Ester Hydrochloride Salt (XIXa).—A suspension of 1.7 g (1.8 mmol) of the crude tetrapeptide hydrochloride salt XIXa 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, filtered. The solid was washed with water and then triturated with ether to yield a gelatinous solid, 2.5 g (93%), mp 160° (crystallized from chloroform-n-hexane), homogenous (system A), [α]D +7.3° (c 1, methanol).

Anal. Calcd for C19H20O11N: C, 55.90; H, 7.34; N, 11.01. Found: C, 55.9; H, 7.25; N, 11.01.

N-2-(p-Diphenylisopropoxycarbonyl)-L-tyr butyl-L-glutamyl-L-glutaminyl-L-leucine Benzyl Ester Hydrochloride Salt (XX).—A solution of 5.9 g (0.01 mol) of the dipeptide derivative in 20 ml of 1,2-dimethoxyethane was stirred 24 hr at room temperature, filtered, and the resulting gum was dissolved 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°, [α]D +10.6° (c 1, methanol).

Anal. Calcd for C29H84O8N2: C, 64.67; H, 6.36; N, 5.20. Found: C, 64.75; H, 6.28; N, 5.12.

L-Glutaminyl-L-leucine Benzyl Ester Hydrochloride Salt (XXI).—A solution of 10.6° (c 1, methanol).
solution cooled to yield the diketopiperazine derivative, 0.73 g absolute methanol. The reaction mixture was stirred for 3 min, extracted with chloroform and washed with 10% NaHCO₃ solution, and extracted with hygroscopic solid. The semisolid was dissolved in 5 ml of H₂O, the remaining oil on trituration with ether provided a white yield 2.5 g (0.008 mol) of L-glutamine methyl ester XXII (0.8 g, 1.0 mmol) was dissolved in 10 ml of dry 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 solution of the azide at —40° was treated with a precooled hydrogen chloride in tetrahydrofuran solution. —A mixture containing 3.2 g (0.008 mol) of XIX and 1.5 ml of N-methylmorpholine. The solution of the hydrazide —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 solution to yield the diketopiperazine derivative, 0.73 g (44%), mp 239–240° (recrystallized from methanol-chloroform). —A solution of 2.13 g (0.005 mol) of XVIII, 33532-10-4; X, 33527-03-6; XV, 25461-15-8; XVI, 33527-10-5; XVIII, 33527-06-9; XIVa, 21753-83-3; XlVb, 33527-12-7; XXI, 33527-13-8; XXII, 33527-11-6; XX, 33527-12-7; XXI, 33527-13-8; XXII, 33527-14-9; 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; XIIIb, 33527-15-0; XIV, 33527-16-1; XXXV diketopiperazine, 33527-17-2; XXVI, 33527-18-3.

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; XIIIb, 33527-15-0; XIV, 33527-16-1; XXXV diketopiperazine, 33527-17-2; XXVI, 33527-18-3.