Reaction of 2,4(5)-Dinitroimidazole with Ethylene Oxide. A solution of **2** (1.0 g, 6.3 mmol) in 50 mL of EtOH was heated at 70 °C and 7 mL (0.14 mol) of ethylene oxide was added to the solution. The reaction mixture was refluxed for 2 h, an additional 7 mL of ethylene oxide was added, and then the mixture was stirred overnight at room temperature. The solvent was removed in vacuo to leave a residual oil consisting of a mixture of **4** and **5**. A solution of the residual oil in 5 mL of EtOAc was applied on a silica gel (50 g) column (2.5 × 30 cm) and initially eluted with $CHCl_3$. The first six fractions of 50 mL each were collected, and the solvent was removed in vacuo at 20 °C to leave an oil which was crystallized from Et_2O in a cold room to yield 0.175 g (18%) of **5** in colorless crystals: mp 107–108 °C; IR (KBr) 1500 and 1330 cm^{-1} (NO_2); ^1H NMR ($CDCl_3$) 7.65 (s, C_6-H), 5.22 (t, CH_2O), 4.57 ppm (t, NCH_2); mass spectrum *m/e* 155 (M^+), 139 (M − O), 109 (M − NO_2). Anal. ($C_5H_5N_3O_3$) C, H, N.

Compound **4** was obtained from the silica gel column by changing the solvent to EtOAc. The EtOAc fractions (3 × 50 mL) were collected, and solvent was removed in vacuo to leave a residue, which was crystallized (EtOAc/$CHCl_3$) to yield 0.64 g (50%) of **4**: mp 102–103 °C; IR (KBr) 3340 (OH), 1530 and 1310 cm^{-1} (NO_2); ^1H NMR (CD_3OD) 8.44 (s, C_5-H), 4.71 (t, CH_2O), 3.96 ppm (t, NCH_2); mass spectrum *m/e* 156 (M − NO_2). Anal. ($C_5H_6N_4O_5$) C, H, N.

1-(2-Hydroxyethyl)-4-nitro-5-chloroimidazole (6). A solution of **3** (0.15 g, 1 mmol) in 25 mL of absolute EtOH was heated to 70 °C, and 30 mg of NaOH was added followed by 7 mL of ethylene oxide. The mixture was refluxed for 7 h and then allowed to stir at room temperature overnight. The solvent was removed in vacuo, and the residue was recrystallized (EtOAc) to yield 75 mg (39%) of **6**: mp 138–140 °C; IR (KBr) 3350 (OH), 1500 and 1330 cm^{-1} (NO_2); ^1H NMR (Me_2SO-d_6) 8.46 (s, C_2-H), 4.12 (t, CH_2O), 3.75 ppm (t, NCH_2). Anal. ($C_5H_6ClN_3O_3$) C, H, N.

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Synthesis of a Proposed Thymic Factor

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<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn, a proposed serum thymic factor, has been synthesized. The protected precursor, <Glu-Ala-Lys(*i*-Noc)-Ser(Bzl)-Gln-Gly-Gly-Ser(Bzl)-Asn, was prepared by a combination of solid phase and solution methods. The benzyl blocking groups were removed by HF and the *i*-Noc blocking group was removed by catalytic hydrogenation.

Bach and associates[1,2] have proposed the amino acid sequence V for a serum thymic factor (STF) isolated in their laboratory. We report herein the synthesis of a compound having this peptide sequence. The nonapeptide V was prepared by a fragment-coupling procedure as indicated in Scheme I. The protected heptapeptide <Glu-Ala-Lys(*i*-Noc)-Ser(Bzl)-Gln-Gly-Gly-OMe[3] (Ia) was prepared using the solid-phase method of peptide

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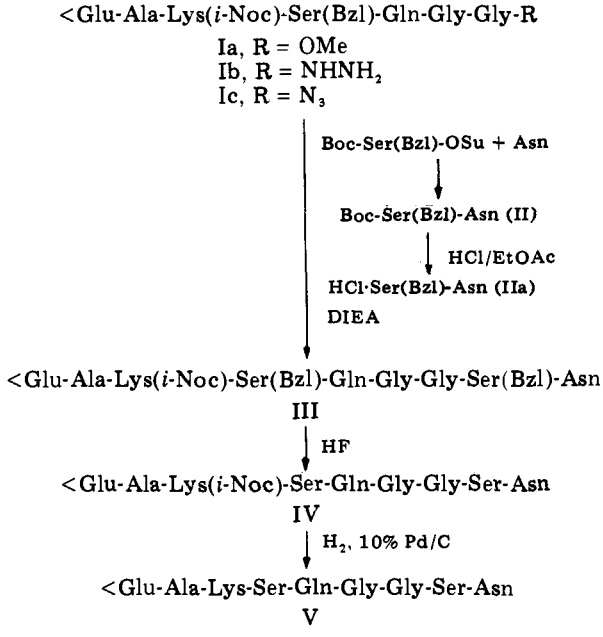
Scheme I



synthesis, followed by removal of the bound peptide from the polymeric support by transesterification with methanol catalyzed by triethylamine. I was coupled to IIa via the azide Ic to give III. We have found frequently that the removal of *O*-benzyl ether blocking groups by catalytic hydrogenation is slow. So, prior to purification, the benzyl protecting groups were removed from the two serine residues of III by the action of anhydrous HF at 0 °C. After purification of the partially protected nonapeptide IV by silica gel chromatography, the isonicotinyloxycarbonyl protecting group was removed readily by catalytic hydrogenation to give V. The ^1H NMR spectrum at 300 MHz showed normal resonances for all of the amino acids. Sequence analysis after tryptic hydrolysis confirmed the order of the amino acids. Amino acid analysis of the nonapeptide after acid hydrolysis was also consistent with the structure V. Taken together, these data establish the structure of the synthetic product.

The fragment-coupling route was chosen in preference to a total synthesis by the solid-phase method in order to avoid any potential problem of imide formation and consequent loss of peptide which might result from asparagine in the carboxy-terminal position esterified to the polymeric support.[4]

This synthesis gives the first detailed example of the application of the isonicotinyloxycarbonyl protecting group in the synthesis of a peptide.[5,6]

The product, V, has been reported to be active in the sheep cell rosette θ conversion assay at a concentration of 10^{-14} M, comparable to that of the isolated factor.[1,2] The biological properties and comparison of our peptide with the natural product are described by Bach and associates.[1,2,7,8] Further detailed in vivo studies have been presented by Martinez et al.[9]

Experimental Section

<Glu-Ala-Lys(*i*-Noc)-Ser(Bzl)-Gln-Gly-Gly-OMe (Ia). The peptide was prepared by standard solid-phase methodology[10] using a Beckman Model 990 peptide synthesizer to carry out all of the operations. The starting polymer was Boc-Gly esterified to 2% cross-linked polystyrene–divinylbenzene (2 mmol, 2.14 g). The N^{α}-Boc derivatives of Gly, Gln, Ser(Bzl), Lys(*i*-Noc), and Ala followed by unprotected <Glu were coupled using dicyclohexylcarbodiimide. Two couplings of 30 min each (2.5 equiv of Boc amino acid) were used for each amino acid. Trifluoroacetic acid (25% in CH_2Cl_2) was used for removal of the Boc protecting group. An equimolar quantity of HBT was used in the couplings of Boc-Gln and <Glu. The detailed schedule for the solid-phase procedure is given in Table I, with program 1 used for the first coupling and program 2 for the second coupling. Solvents and volumes for the protected amino acids are given in Table II. The finished peptide resin was dried in vacuo (4.67 g). The peptidoresin (4.67 g) was suspended in 187 mL of MeOH, and 33 mL of triethylamine was added. The mixture was stirred for 51 h, and DMF (50 mL) and 50% AcOH (50 mL) were added. The spent resin was removed by filtration and washed with a small volume of 50% AcOH, and the combined filtrates were taken to dryness in vacuo. The residue was suspended in 25 mL of EtOAc, filtered, washed two times with 10 mL of EtOAc, and dried in vacuo to give Ia (1.57 g, 87% yield): TLC R_f (silica gel K1, Whatman; EtOAc–Pyr–AcOH–H_2O, 10:5:1:3), 0.42, R_f (silica gel K1, Whatman; *n*-BuOH–AcOH–H_2O, 65:10:25), 0.27, R_f (silica gel K1, Whatman; $CHCl_3$–CH_3OH–H_2O–NH_4OH, 80:20:0.4:0.6) 0.39, R_f (silica gel Q1, Quantum, Inc.; $CHCl_3$–CH_3OH–H_2O, 80:20:2) 0.50; $[\alpha]^{22}_{589}$ −5.6° (*c* 1.1, DMF). Amino acid analysis after hydrolysis (6 N HCl, 20 h): Gly, 2.10; Glu, 1.95; Ser, 0.91; Lys, 1.01; Ala, 1.06.

Table I. Schedule of Steps for 2 mmol Run

step	solvent/reagent	vol, mL	mix time, min
program 1			
1	$CHCl_3$	2 × 40	5
2	25% TFA in CH_2Cl_2	1 × 40	2
3	25% TFA in CH_2Cl_2	1 × 40	25
4	$CHCl_3$	3 × 40	2
5	10% TEA in CH_2Cl_2	2 × 40	5
6	$CHCl_3$	3 × 40	2
7	CH_2Cl_2	3 × 40	2
8	Boc-AA		5
9	0.5 M DCC in CH_2Cl_2	10	5
10	coupling		30
11	DMF	2 × 40	2
12	MeOH	1 × 40	2
13	DMF	1 × 40	2
14	MeOH	1 × 40	2
15	$CHCl_3$	2 × 40	2
program 2			
steps 4 (1×) to 15 of program 1			

Table II

protected amino acid	solvent
Boc-Gly	25 mL of CH_2Cl_2
Boc-Gln + HBT	25 mL of DMF
Boc-Ser(Bzl)	25 mL of CH_2Cl_2
Boc-Lys(*i*-Noc)	11 mL of DMF
	14 mL of CH_2Cl_2
Boc-Ala	25 mL of CH_2Cl_2
<Glu + HBT	25 mL of DMF

Boc-Ser(Bzl)-Asn (II). To a solution of L-asparagine (0.6 g, 4 mmol) and sodium bicarbonate (0.672 g, 8 mmol) in 12 mL of water and 3 mL of DMF was added Boc-Ser(Bzl)-OSu[11] (1.88 g, 4.8 mmol) dissolved in 5 mL of DMF. The reaction was stirred at room temperature for 5 h and then concentrated to an oil in vacuo at 25 °C. The oil was dissolved in water, the pH of the solution was adjusted to 3 with 2.5 N HCl, and the aqueous layer was extracted three times with 50-mL portions of EtOAc. The combined EtOAc extracts were washed twice with 50 mL of water and dried over Na_2SO_4. The Na_2SO_4 was removed by filtration and the EtOAc concentrated to dryness in vacuo to yield 1.68 g of crude II. The crude product was dissolved in the minimum volume of $CHCl_3$–CH_3OH–H_2O (70:30:3) and the solution applied to a column of silica gel 60 (260 g) packed in the same solvent. The column was eluted with the same solvent (10-mL fractions). The appropriate fractions, as determined by TLC, were combined and concentrated to dryness in vacuo to yield 1.26 g (76.8%) of II: TLC R_f (silica gel Q1, Quantum, Inc.; $CHCl_3$–CH_3OH–H_2O, 70:30:3) 0.40; $[\alpha]^{22}_{589}$ −11.8° (*c* 1.5, EtOH). Amino acid analysis after acid hydrolysis (6 N HCl, 20 h): Asp, 1.01; Ser, 0.98.

Ser(Bzl)-Asn·HCl (IIa). II (1.0 g, 2.4 mmol) was suspended in 10 mL of anhydrous EtOAc and cooled to 0 °C, and anhydrous HCl was bubbled into the solution vigorously for 10 min, at which time a clear solution resulted. The reaction mixture was purged with N_2 at 0 °C for 45 min and a precipitate formed. The solid was filtered, washed with cold EtOAc, and dried in vacuo to yield 0.88 g of Ser(Bzl)-Asn·HCl (IIa; 96% yield): TLC R_f (silica gel Q1, Quantum, Inc.; EtOAc–Pyr–AcOH–H_2O, 10:5:1:3) 0.08, R_f (silica gel Q1, Quantum, Inc.; $CHCl_3$–CH_3OH–NH_4OH, 70:30:3) 0.27, R_f (silica gel Q1, Quantum, Inc.; *n*-BuOH–AcOH–H_2O, 65:10:25) 0.29; $[\alpha]^{22}_{589}$ −1.92° (*c* 0.75, 5% AcOH); ^1H NMR spectrum at 90 MHz was consistent with the structure. Amino acid analysis after acid hydrolysis (6 N HCl, 20 h): Asp, 1.00; Ser, 0.99.

<Glu-Ala-Lys(*i*-Noc)-Ser-Gln-Gly-Gly-Ser-Asn (IV). The protected heptapeptide methyl ester Ia (1.57 g, 1.75 mmol) was dissolved in 4 mL of MeOH containing 2 mL of anhydrous hydrazine and stirred at room temperature for 15 min. The solution was evaporated to dryness in vacuo, suspended two times in MeOH, and taken to dryness each time. The residue was triturated with MeOH, and the resultant solid was isolated by filtration, washed with a small volume of cold MeOH, and dried to yield 0.555 g of the hydrazide Ib. The combined filtrate and washing were concentrated to dryness in vacuo, and the residue was triturated with ether to yield, after drying, an additional 0.437 g of Ib (total yield 0.99 g): TLC R_f (silica gel Q1, Quantum, Inc.; $CHCl_3$–CH_3OH–H_2O, 50:40:10) 0.72.

To 10 mL of freshly degassed DMF was added the blocked heptapeptide hydrazide Ib (0.99 g, 1.10 mmol). The mixture, under a N_2 atmosphere, was cooled to −25 °C and 1.05 mL of HCl in THF (5.24 N) was added. To the clear solution was added isoamyl nitrite (0.148 g, 1.26 mmol) over a 2.5-h period. The resulting yellow-orange solution of Ic was cooled to −35 °C and Ser(Bzl)-Asn·HCl (0.383 g, 1.0 mmol) dissolved in 1 mL of DMF was added. The "pH" of the reaction was adjusted to 7.6–7.8 with diisopropylethylamine (as measured by moistened indicator paper). The solution was kept at −20 °C for 24 h, the pH was adjusted to 7.6 by the further addition of tertiary amine, and the solution was maintained at −20 °C for an additional 48 h. The solution was concentrated in vacuo (35 °C bath) to a viscous oil and triturated with MeOH. The resulting solid was isolated by filtration, washed with cold MeOH, and dried in vacuo to yield 1.3 g of crude protected nonapeptide III.

III (0.9 g, 0.766 mmol) was mixed with 2 mL of anisole and the mixture was cooled in a dry ice–acetone bath. HF (20 mL) was added by distillation and the solution was stirred for 1 h at 0 °C. The HF was removed by evaporation in vacuo at 0 °C and the residue triturated with EtOAc (35 mL). The resultant solid was isolated by filtration, washed with EtOAc, and dried in vacuo to yield 0.722 g of IV (94%). IV was purified by chromatography on a silica gel 60 column (171 g) prepared in $CHCl_3$–CH_3OH–H_2O (50:40:10). The crude IV (0.68 g) was applied to the column by dissolution in a mixture of 6.5 mL of H_2O, 30 mL of MeOH, and 40 mL of $CHCl_3$. Insolubles were removed by centrifugation (0.056 g). The column was eluted with $CHCl_3$–CH_3OH–H_2O (50:40:10); fractions (5 mL each) containing homogeneous IV, as determined by TLC, were combined and concentrated in vacuo, and the residue was dissolved in H_2O and freeze-dried to yield 0.224 g of IV: TLC R_f (silica gel, Analtech; $CHCl_3$–CH_3OH–H_2O–NH_4OH, 60:30:4:6) 0.25, R_f (silica gel, Analtech; EtOAc–Pyr–AcOH–H_2O, 5:5:1:3) 0.59; ^1H NMR spectrum at 300 MHz consistent with the structure. Amino acid analysis after acid hydrolysis (6 N HCl, 20 h): Lys, 0.98; Asp, 0.98; Ser, 2.01; Glu, 2.03; Gly, 2.02; Ala, 0.98.

<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn (V). IV (0.22 g, 0.22 mmol) was dissolved in 10 mL of 5% AcOH, and 10% Pd/C (0.2 g) was added. The system was purged with N_2, and then H_2 was bubbled in for 30 min. The reaction vessel walls were washed periodically with 5% AcOH to return the catalyst to the reaction. The mixture was filtered through Supercel, and the cake was washed with 50 mL of 5% AcOH. The combined filtrate and washings were concentrated to a small volume in vacuo and then lyophilized to yield 0.188 g of crude V.

Crude V (0.1 g) was dissolved in 2 mL of $CHCl_3$–CH_3OH–H_2O–AcOH (40:47:13:1) and applied to a silica gel 60 column (5 g) prepared and eluted in the same solvent system. The appropriate fractions (1 mL each), as determined by TLC, were combined, concentrated in vacuo, diluted with water, and lyophilized to give 0.043 g of V: TLC R_f (silica gel, Analtech; $CHCl_3$–CH_3OH–H_2O–AcOH, 40:47:13:1) 0.173, R_f (silica gel, Analtech; $CHCl_3$–CH_3OH–NH_4OH, 50:50:22.5) 0.18, R_f (silica gel Q1, Quantum, Inc.; *n*-BuOH–EtOAc–AcOH–H_2O, 1:1:1:1) 0.04, R_f (silica gel Q1, Quantum, Inc.; 2-propanol–CH_3OH–H_2O–NH_4OH, 40:40:18:2) 0.12. Total impurities were judged to be less than 2%. Detection limits were established to be <1% by application at a level such that the same volume of the solution after 100-fold dilution gave a detectable spot:[12] $[\alpha]^{24}_{589}$ −59.3° (*c* 0.25, 5% AcOH); ^1H NMR spectrum at 300 MHz consistent with the structure. Amino acid analysis after hydrolysis (6 N HCl, 20 h): Asp, 1.03; Ser, 1.90; Gly, 1.99; Glu, 2.06; Lys, 1.01; Ala, 1.02.

Sequence Analysis of V. A 4 h tryptic digest (10 mM Tris-HCl buffer adjusted to pH 9.1) of 500 nmol of V was separated into two components on a 0.9 × 90 cm Bio-Gel P-2 column using 0.025 M NH_4HCO_3 as the eluent. The component which was eluted last from the column was treated with dansyl chloride and, after acid hydrolysis (6 N HCl, 4 h), showed only ε-dansyllysine. Amino acid analysis: Glu, 0.99; Ala, 1.00; Lys, 1.01. Amino acid analysis of the first eluted fragment: Asp, 1.02; Ser, 2.20; Glu, 0.92; Gly, 1.93. Application of the Edman dansyl method of Gray and Smith[13] to this fragment showed the sequence to be Ser-Glx-Gly-Gly-Ser-Asn (free Asn was isolated after step 5).

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