screw axis are nearly prependicular with a dihedral angle of 101.6°, and thus the hydrophobic region exhibits typical herringbone packing. There are four hydrogen bonds in the hydrophilic region, providing full employment for the hydrogen atoms attached to N-1 and N-3, and comprising three hydrogen-bonding schemes. Two of these schemes are single head-to-tail links. One, N-1–H-1 → N-2, connects screw-related molecules. The other, N-3–H-9 → O-2, is between a pair related by lattice translation one unit cell diagonally along *a* and *b*. The third scheme is an infinite zig-zag chain ... → O-1 ← H-2–N-1–H-3) → O-1 ← ... along the *a* axis, between molecules screw related tail-to-tail. (Distances and angles are given in Table II.) The structure is thus held together in the *x* direction by the ribbon-like hydrogen bonding scheme, in the *z* direction by the N–N hydrogen bond and the hydrophobic interaction, and in the *y* direction by the N-3–O-2 hydrogen bond.

The standard deviations of the bond lengths are too great for this structure analysis to furnish evidence relevant to the postulate of Mighell and Riemann[19] that the longer C–N bond within the pyrazole ring should be the one to the protonated nitrogen.

Biological Implications

The hydrogen bond between N-3 and O-2 suggests that a similar bond could be formed between N-3 and a phosphate oxygen in the ribonuclease enzyme–substrate system. However, contrary to the stituation with histidine, donation of this hydrogen atom catalytically is not feasible at physiological pH, since N-2 is unlikely ($pK \approx 2$)[5] to be protonated. Hence, although binding of substrate could occur, catalysis and cleavage would be prohibited.

A comparison[20] of the structure of Pyr(3)Ala with a series of histidine crystal structures (Table V) demonstrates that, for most chemical purposes, Pyr(3)Ala is isosteric with histidine. However, Pyr(4)Ala (III) could possibly be a better substitute for histidine as a biochemical probe. Use of this isomer would avoid the insertion of a hydrophobic region in a place where it is not present in nature. This is due to the fact that if the ring of Pyr(4)Ala were divided along the C-3, C-4 vector (bond a, III), each side of the ring would have both a nitrogen and a carbon, and thus there would be no extensive hydrophobic region. Therefore, any potential hindrance of binding caused by the introduction of the hydrophobic region of Pyr(3)Ala would probably be avoided by substituting Pyr(4)Ala.

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Synthetic Peptides Related to Horse Heart Cytochrome *c*. VII. Synthesis and Inhibitory Properties of the 70–80 Undecapeptide[1]

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Contribution from the Department of Organic Chemistry, The Hebrew University of Jerusalem, and the Department of Biochemistry, Tel-Aviv University, Israel. *Received July 3, 1971.*

Abstract: The undecapeptide corresponding to the amino acid sequence 70–80 of cytochrome *c* was synthesized. The synthetic peptide inhibited the cytochrome oxidase mediated oxidation of ferrocytochrome *c*. The inhibition was not due to the overall positive electrostatic charge of the peptide, since the $N^{\alpha,\epsilon}$-acylated peptide was an even better inhibitor. No inhibition was observed when smaller peptides of the 70–80 sequence were tested.

Examination of the similarities and differences among the amino acid sequences of eukaryotic cytochrome *c* has led to tentative conclusions concerning structure–function relationships; the most remarkable constant segment of the polypeptide chain is that extending from residue 70 to residue 80, Asn-Pro-Lys-Lys-Tyr-Ile-Pro-Gly-Thr-Lys-Met, as contrasted to the next longest invariant segment which is no longer than two residues.[2] Experiments involving chemical modification and X-ray crystallographic analysis of horse ferricytochrome *c*[3] indicate that the area of residue 70–80 may represent a surface at which cytochrome oxidase binds to cytochrome *c*.

In order to explore this possibility, we synthesized the undecapeptide corresponding to the sequence 70–80 for cytochrome *c* as summarized in Figure 1. The two fragments VIII and XVI were synthesized in the stepwise elongation procedure from the respective C-terminal amino acid, and the *tert*-butyloxycarbonyl group was removed after each step of the elongation by using anhydrous trifluoroacetic acid.[4] The dipeptide X was

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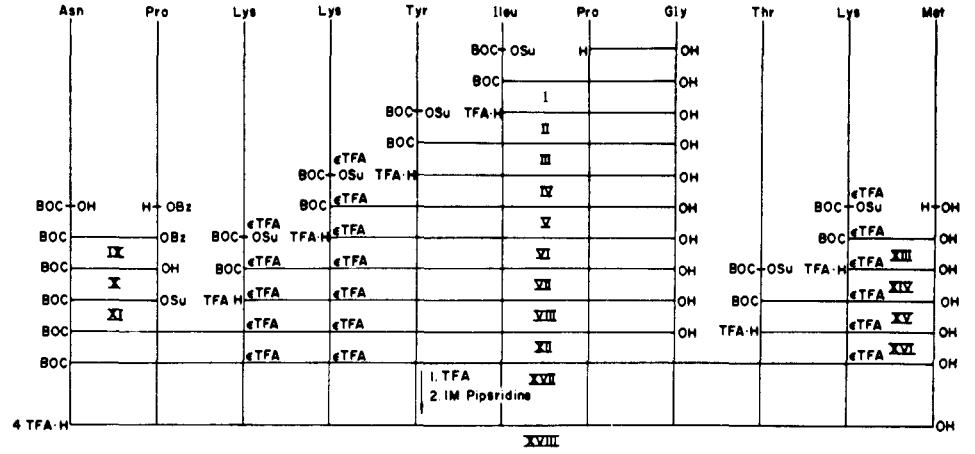

Figure 1. Schematic diagram of synthesis of undecapeptide (XVIII): BOC, *tert*-butyloxycarbonyl; TFA, trifluoroacetyl or trifluoroacetic acid; OSu, *N*-hydroxysuccinimide ester.

prepared by treating the mixed anhydride of *tert*-butyloxycarbonylasparagine and pivaloyl chloride[5] with benzyl prolinate and removing the benzyl ester by hydrogenation. The coupling of X with VIII was carried out using the hydroxysuccinimide ester method while coupling of XII and XVI was carried out by means of hydroxysuccinimide and dicyclohexylcarbodiimide.[6] The ε-amino groups of the three lysines were protected by the trifluoroacetyl group which was removed at the last stage of the synthesis by treatment with 1 *M* piperidine solution at 0°.[7] Peptide XVIII was treated with acetic anhydride at pH 7.5, resulting in acetylation of amino and phenolic groups. Treatment of the fully acetylated peptide with 0.2 *M* NH_2OH resulted in the removal of *O*-acetyl groups while the α and ε amino groups remained acetylated.

The effect of the 70–80 peptide on the cytochrome oxidase mediated oxidation of ferrocytochrome *c* was investigated. Figure 2 and Table I show the effect of various concentrations of the peptide on the enzymic reaction. At a molar concentration of 9.6×10^{-4} of the 70–80 peptide, the rate of the oxidation was reduced by 50%. No inhibition was observed when smaller peptides of the 70–80 sequence, such as XVI and VII and the peptide Thr-Lys-Met-Ile-Phe-Ala-Gly[8] corresponding to positions 78–84, were tested.

Baba and coworkers[9] showed that certain peptides obtained by proteolysis of yeast cytochrome *c* had an inhibitory effect on cytochrome oxidase. Notably, however, the peptide containing residues 65–79 caused no inhibition at all. There are two major differences between the procedures employed by Baba and by us. The first is that the time of incubation of the oxidase with the peptide was of 15 min in our experiments while it was of only 2 min in the other studies.[9] Furthermore, while the other work[9] was performed with a peptide concentration of 2×10^{-4} *M* we were using higher concentrations. Indeed, at a concentration of 3.8

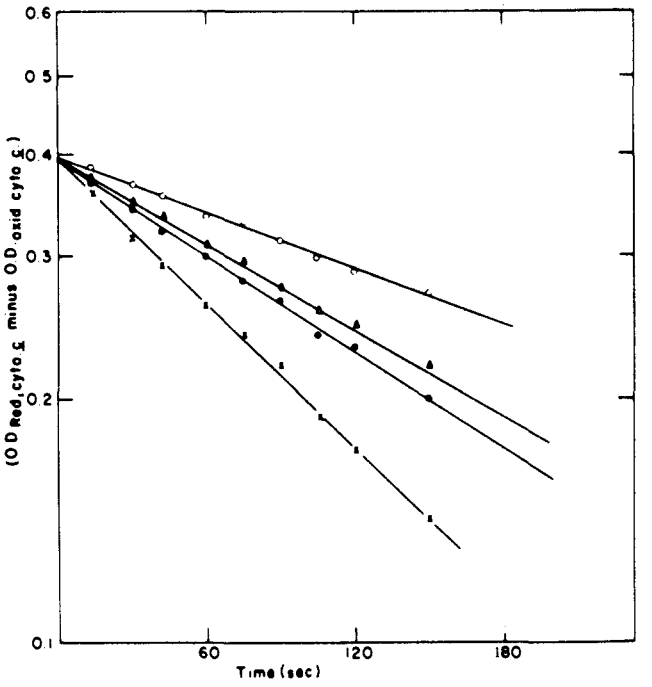

Figure 2. Effect of 5.7×10^{-4} *M* peptides, XVIII, and the acetylated derivatives on the oxidation of cytochrome *c* by cytochrome oxidase: ×–×, no inhibitor; Δ–Δ, undecapeptide XVIII; ●–● N,O-acetylated XVIII; ○–○, $N^{\alpha,\epsilon}$-acetylated XVIII.

$\times 10^{-4}$ *M* of the peptide, we also were unable to observe any inhibition.

Although the inhibitory effect of the 70–80 peptide could be taken as an indication of competition between this peptide and cytochrome *c* for a similar site in cytochrome oxidase, it was also possible that the inhibition was not specific but due to the overall positive electrostatic charge of the peptide. It is known that ferricytochrome *c*, a protein with highly positive charge, inhibits competitively the oxidation of ferrocytochrome *c* by cytochrome oxidase;[10] similar inhibitions are caused by polycations such as poly-L-lysine.[11] However, the acetylated 70–80 undecapeptide does inhibit the cyto-

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Table I. Inhibition of Cytochrome Oxidase Activity by the 70–80 Peptides

Peptide	Concn, m*M*	Inhibition, %
70–80	0.38	0
	0.57	38
	0.96	50
	1.3	64
$N^{\alpha,\epsilon}$,*O*-Acetyl 70–80	0.57	35
$N^{\alpha,\epsilon}$-Acetyl 70–80	0.57	60

chrome oxidase reaction with cytochrome *c*, as shown in Figure 2 and Table I. Hence, the effect of the 70–80 peptide does not appear to be due to its charge, but to a more specific feature of the peptide structure. It may be argued that the 70–80 portion of the cytochrome *c* protein may have a conformation different than the 70–80 free peptide. However, inspection of the 2.8-Å model of ferricytochrome *c*[2] shows that the 70–80 portion of the chain has few contacts with the rest of the protein and appears free enough to undergo conformational changes when interacting with another macromolecule.

Experimental Section

All the melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Ascending thin-layer chromatography was performed on silica gel G plates (E. Merck and Co., Darmstadt, Germany) with the following solvent systems: R_{fA}, Partridge system;[12] R_{fB}, *sec*-butanol–ammonia system.[13] Peptides bearing a free amino group were detected with 0.2% ninhydrin solution in 80% ethanol, followed by heating. Amino acid analyses of peptide derivatives were carried out with a Unichrome-Beckman amino acid analyzer on samples which had been hydrolyzed with constant boiling hydrochloric acid for 22 hr in an evacuated sealed tube at 110°. All the amino acids are of the L configuration.

Cytochrome *c* and Cytochrome Oxidase. Horse heart cytochrome *c* was obtained from Sigma Chemical Co. (grade II) and was purified by the method of Margoliash and Walasek.[14] Ferrocytochrome *c* was prepared using $Na_2S_2O_4$.[15] Cytochrome *c* oxidase was isolated and purified from beef heart muscle.[15]

Cytochrome *c* oxidase activity was measured spectrophotometrically by determining the decrease in absorbance of ferrocytochrome *c* at 550 mμ at 20° in 0.08 *M* phosphate buffer (K^+), pH 7.0, as described by Smith,[16] and expressed as the observed first-order rate constant (sec^{-1}). The following modifications were made. The concentration of ferrocytochrome *c* was 2×10^{-5} *M* and the final volume was 1 ml, containing 1 m*M* of EDTA. A few microliters of saturated potassium ferricyanide were always added after the completion of each assay to ensure that the cytochrome *c* was completely oxidized. Cytochrome oxidase was preincubated with the inspected peptides for 15 min at 20° before the addition of ferrocytochrome *c*.

***tert*-Butyloxycarbonylisoleucylprolylglycine (I).** A solution of *N*-hydroxysuccinimido *tert*-butyloxycarbonylisoleucinate (3.28 g, 10 mmol)[17] in dimethoxyethane (20 ml) was added to a solution of prolylglycine[18] (2.7 g, 15 mmol) in water (30 ml) containing sodium bicarbonate (2.1 g, 25 mmol). The reaction mixture was allowed to stir overnight and then filtered. The dimethoxyethane was removed *in vacuo* and the aqueous solution was cooled to 2° and acidified to pH 2 by the addition of solid citric acid. The product was extracted with ethyl acetate (three 70-ml portions); the organic solvent was dried and removed *in vacuo* to give an oil which contained about 20% *tert*-butyloxycarbonylisoleucine. The product was purified and characterized by making its dicyclohexylamine salt (2.6 g, 46%): mp 144–145°; $[\alpha]^{27}D$ −53.4° (*c* 1, MeOH). *Anal.* Calcd for $C_{18}H_{31}N_3O_6 \cdot C_{12}H_{23}N$: C, 63.57; H, 9.60; N, 9.88. Found: C, 63.53; H, 9.48; N, 10.11.

The desired pure compound was obtained as an oil by liberation of the salt in the usual manner. Amino acid analysis after acid hydrolysis indicated the composition: Pro, 1.0; Gly, 1.0; Ile, 0.95.

Isoleucylprolylglycine Trifluoroacetate (II). Compound I (1.15 g, 3 mmol) was dissolved in 5 ml of trifluoroacetic acid and the solution was allowed to stand for 15 min at room temperature. The trifluoroacetic acid was removed *in vacuo* and the ether was added to give the product (1.1 g, 90%): mp 190–191°; $[\alpha]^{27}D$ +32.4° (*c* 1, 0.2 *N* $NaHCO_3$); R_{fA} 0.63, R_{fB} 0.88. *Anal.* Calcd for $C_{18}H_{23}N_3O_4 \cdot CF_3COOH$: C, 45.10; H, 6.06; F, 14.27; N, 10.52. Found: C, 44.98; H, 6.17; F, 14.50; N, 10.39.

***N*-Hydroxysuccinimido *tert*-Butyloxycarbonyltyrosinate.** A solution of dicyclohexylcarbodiimide (1.03 g, 5 mmol) in ethyl acetate (20 ml) was added to a chilled solution of *tert*-butyloxycarbonyltyrosine[19] (1.4 g, 5 mmol) and *N*-hydroxysuccinimide (0.55 g, 5 mmol) in ethyl acetate (20 ml). After stirring 1 hr in the cold and 4 hr at room temperature a few drops of acetic acid were added. The dicyclohexylurea was removed by filtration, and the organic solvent was washed with 5% bicarbonate solution, water, and saturated sodium chloride solution, and dried over Na_2SO_4. After removal of most of the organic solvent, the product crystallized (1.55 g; 81%; mp 192–193°; $[\alpha]^{27}D$ −43.3° (*c* 1, dimethylformamide). *Anal.* Calcd for $C_{18}H_{22}N_2O_7$: C, 56.61; H, 5.86; N, 7.40. Found: C, 57.01; H, 5.92; N, 7.63.

***tert*-Butyloxycarbonyltyrosylisoleucylprolylglycine (III).** A solution of *N*-hydroxysuccinimido *tert*-butyloxycarbonyltyrosinate (1.9 g, 5 mmol) in dimethoxyethane (35 ml) was added to a solution of II (2 g, 5 mmol) in water (25 ml) containing sodium bicarbonate (1.26 g, 15 mmol), and the mixture was stirred overnight. The product (1.9 g, 70%) was isolated as described for I: mp 99–102°; $[\alpha]^{27}D$ −45.8° (*c* 1, dimethylformamide); amino acid analysis, after acid hydrolysis, indicated the composition, Tyr, 0.85; Ile, 1.05; Pro, 0.98; Gly, 1.0. *Anal.* Calcd for $C_{27}H_{40}N_4O_8$: C, 59.11; H, 7.33; N, 10.21. Found: C, 59.05; H, 7.69; N, 10.30.

Tyrosylisoleucylprolylglycine Trifluoroacetate (IV). The deprotection of III (2.75 g, 5 mmol) with trifluoroacetic acid was carried out as described for the preparation of II to give the product (2.1 g, 91%): mp 135°; $[\alpha]^{27}D$ −67.2° (*c* 1, 0.2 *N* $NaHCO_3$); R_{fA} 0.47, R_{fB} 0.78. *Anal.* Calcd for $C_{22}H_{32}N_4O_6 \cdot CF_3COOH$: C, 51.33; H, 5.74; F, 10.13; N, 9.96; Found: C, 51.19; H, 5.73; F, 10.52; N, 9.81.

N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysyltyrosylisoleucylprolylglycine (V). A solution of *N*-hydroxysuccinimido-N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysinate[20] (4.4 g, 10 mmol) in dimethoxyethane (40 ml) was added to a solution of IV (5.6 g, 30 mmol) in water (25 ml) containing sodium bicarbonate (2.52 g, 30 mmol). The product (5.6 g, 73%) was isolated as described above for I: mp 128°, $[\alpha]^{27}D$ −38.2° (*c* 1, dimethylformamide). Amino acid analysis after acid hydrolysis indicated the composition: Lys, 1.0; Tyr, 0.9; Ile, 0.9; Pro, 1.0; Gly, 1.0. *Anal.* Calcd for $C_{43}H_{62}F_6N_8O_{12}$: C, 51.80; H, 6.27; F, 11.43; N, 11.24. Found: C, 51.95; H, 6.32; F, 11.18; N, 11.30.

N^{ϵ}-Trifluoroacetyllysyltyrosylisoleucylprolylglycine Trifluoroacetate (VI). The deprotection of V (3.1 g, 4 mmol) with trifluoroacetic acid was carried out as described for the preparation of II to give the product (2.8 g, 90%): mp 138–139°; $[\alpha]^{27}D$ −46.2° (*c* 1, 0.2 *N* $NaHCO_3$); R_{fA} 0.59, R_{fB} 0.52. *Anal.* Calcd for $C_{30}H_{43}F_3N_6O_8 \cdot CF_3COOH$: C, 48.85; H, 5.64; F, 14.49; N, 10.68. Found: C, 48.69; H, 5.59; F, 14.72; N, 10.75.

N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysyl-N^{ϵ}-trifluoroacetyllysyltyrosylisoleucylprolylglycine (VII). A solution of *N*-hydrosuccinimido-N^{α}-*tert*-butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysinate (2.2 g, 5 mmol) in dimethoxyethane (50 ml) was added to a solution of VI (3.9 g, 5 mol) in water (30 ml) containing sodium bicarbonate (1.26 g, 15 mmol). The reaction mixture was treated as described for I to give the product (4.1 g, 82%): mp 131–133°; $[\alpha]^{27}D$ −30.8° (*c* 1, dimethylformamide). Amino acid analysis after acid hydrolysis indicated the composition: Lys, 1.9; Tyr, 0.9; Ile, 1.0; Pro, 1.0; Gly, 1.0.

N^{ϵ}-Trifluoroacetyllysyl-N^{ϵ}-trifluoroacetyllysyltyrosylisoleucylprolylglycine Trifluoroacetate (VIII). The deprotection of VII (3 g, 3 mmol) with trifluoroacetic acid was carried out as described

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for the preparation of II to give the product (2.9 g, 96%): mp 140–141°; $[\alpha]^{27}D$ −53.0° (*c* 1, 0.2 *N* $NaHCO_3$); R_{fA} 0.42, R_{fB} 0.60.

Anal. Calcd for $C_{38}H_{54}F_6N_8O_{10}\cdot CF_3COOH$: C, 47.52; H, 5.48; F, 16.92; N, 11.08. Found: C, 47.39; H, 5.62; F, 17.54; N, 10.82.

***tert*-Butylcarbonylasparaginylproline Benzyl Ester (IX).** To a solution of a mixed anhydride,[6] which was prepared in the usual manner for *tert*-butyloxycarbonylasparagine[21] (2.32 g, 10 mmol), triethylamine (1.44 ml, 10 mmol), pyridine (0.8 ml, 10 mmol), and pivaloyl chloride (1.22 g, 10 mmol) in dry chloroform (50 ml), a solution of proline benzyl ester hydrochloride (2.4 g, 10 mmol) in dry chloroform (50 ml) containing triethylamine (1.44 ml, 10 mmol) was added. The reaction mixture was stirred overnight, the solvent was evaporated to dryness *in vacuo*, and the residue was titrated with water, filtered off, washed with 5% sodium biacrbonate, 5% citric acid, and water, and crystallized from ethyl acetate–petroleum ether to give the product (3.6 g, 85%): mp 115–116°; $[\alpha]^{27}D$ −53.8° (*c* 1, methanol). *Anal.* Calcd for $C_{21}H_{29}N_3O_6$: C, 60.13; H, 6.97; N, 10.02. Found: C, 60.25; H, 6.58; N, 9.83.

***tert*-Butyloxycarbonylasparaginylproline (X).** A solution of *tert*-butyloxycarbonylasparaginylproline benzyl ester (4.2 g, 10 mmol) in methanol (50 ml) containing acetic acid (0.6 ml, 10 mmol) was hydrogenated over 10% palladium/charcoal. The catalyst was removed by filtration, the solvent was removed *in vacuo*, and the product crystallized from ethyl acetate–petroleum ether (3 g, 90%): mp 108–116°; $[\alpha]^{27}D$ −50° (*c* 1, methanol). *Anal.* Calcd for $C_{14}H_{23}N_3O_6$: C, 51.06; H, 7.04; N, 12.76. Found: C, 50.98; H, 7.19; N, 12.60.

***N*-Hydroxysuccinimido *tert*-Butyloxycarbonylasparaginylprolinate (XI).** A solution of dicyclohexylcarbodiimide (1.03 g, 5 mmol) in dioxane (20 ml) was added to a chilled solution of X (1.6 g, 5 mmol) and *N*-hydroxysuccinimide (0.55 g, 5 mmol) in dioxane (20 ml). After stirring 1 hr in the cold and 4 hr at room temperature a few drops of acetic acid was added and the organic solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and filtered and the organic solvent was washed with 5% bicarbonate solution, water, and saturated sodium chloride solution, and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a crystalline mass which was crystallized from ethyl acetate–petroleum ether to yield the product (1.9 g, 84%): mp 121°; $[\alpha]^{27}D$ −54.2° (*c* 1, ethyl acetate). *Anal.* Calcd for $C_{18}H_{26}N_4O_8$: C, 50.70; H, 6.15; N, 13.14. Found: C, 50.63; H, 6.29; N, 12.98.

***tert*-Butyloxycarbonylasparaginylprolyl-N^{ϵ}-trifluoroacetyllysyl-N^{ϵ}-trifluoroacetyllysyltyrosylisoleucylprolylglycine (XII).** A solution of XI (425 mg, 1 mmol) in dimethoxyethane (10 ml) was added to a solution of VIII (1.2 g, 1.2 mmol) in water (20 ml) containing sodium bicarbonate (277 mg, 3.4 mmol). After being stirred overnight, the reaction mixture was processed as described for I to give the product (1.05 g, 87%): mp 138–140°; $[\alpha]^{27}D$ −55.8° (*c* 1, dimethylformamide). Amino acid analysis after acid hydrolysis indicated the composition: Asp, 1.0; Pro, 2.0; Gly, 1.0; Ile, 1.1; Tyr, 0.9; Lys, 2.1. *Anal.* Calcd for $C_{52}H_{75}F_6N_{11}O_{15}$: C, 51.69; H, 6.26; F, 9.44; N, 12.75. Found: C, 51.75; H, 6.11; F, 9.08; N, 12.92.

N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysylmethionine (XIII). A solution of *N*-hydroxysuccinimido N^{α}-butyloxycarbonyl-N^{ϵ}-trifluoroacetyllysinate (8.8 g, 20 mmol) in dimethoxyethane (50 ml) was added to a solution of methionine (3 g, 20 mmol) in water (50 ml) containing sodium bicarbonate (3.36 g, 40 mmol). The reaction mixture was stirred overnight and was processed as described for I. An oil was obtained (7.6 g, 80%): $[\alpha]^{27}D$ +1.5° (*c* 1, ethyl acetate); attempts to crystallize the product failed. The compound was characterized as its dicyclohexylamine salt: mp 94–95°; $[\alpha]^{27}D$ −0.5° (*c* 1, methanol). *Anal.* Calcd for $C_{18}H_{30}F_3N_3O_6S\cdot C_{12}H_{23}N$: C, 55.04; H, 8.11; F, 8.71; N, 8.57; S, 4.90. Found: C, 55.16; H, 8.34; F, 8.54; N, 8.46; S, 4.40.

N^{ϵ}-Trifluoroacetyllysylmethionine Trifluoroacetate (XIV). The deprotection of XIII (4.7 g, 10 mmol) with trifluoroacetic acid (15 ml) was carried out as described for the preparation of II to give the product (9.2 g, 94%): mp 98–105° $[\alpha]^{27}D$ −3.2° (*c* 1, H_2O); R_{fA} 0.52; R_{fB} 0.75.

Anal. Calcd for $C_{13}H_{22}F_3N_3O_4S\cdot CF_3COOH$: C, 36.96; H, 4.75; F, 23.38; N, 8.62; S, 6.57. Found: C, 36.78; H, 4.92; F, 23.86; N, 8.51; S, 6.42.

***tert*-Butyloxycarbonylthreonyl-N^{ϵ}-trifluoroacetyllysylmethionine (XV).** A solution of *N*-hydroxysuccinimido *tert*-butyloxycarbonylthreoninate[22] (3.15 g, 10 mmol) in dimethoxyethane (30 ml) was added to a solution of XIV (4.87 g, 10 mmol) in water (30 ml) containing sodium bicarbonate (2.52 g, 30 mmol). After being stirred overnight, the reaction mixture was processed as in I to give an oil (8.7 g, 74%): $[\alpha]^{27}D$ +4.2° (*c* 1, ethyl acetate). Amino acid analysis after acid hydrolysis indicated the composition: Thr, 0.9; Lys, 1.0; Met, 1.0. All attempts to crystallize the tripeptide failed. The compound was characterized as its dicyclohexylamine salt: mp 147°; $[\alpha]^{27}D$ −75° (*c* 1, methanol). *Anal.* Calcd for $C_{22}H_{37}F_3N_4O_8S\cdot C_{12}H_{23}N$: C, 54.02 H, 8.00; F, 7.54; N, 9.26; S, 4.24. Found: C, 53.90; H, 8.18; F, 7.48; N, 9.02; S, 4.22.

Threonyl-N^{ϵ}-trifluoroacetyllysylmethionine Trifluoroacetate (XVI). The deprotection of XI (4.3 g, 7.5 mmol) with trifluoroacetic acid (10 ml) was carried out in the manner described for the preparation of II to give the product (4.2 g, 90%): mp 123–128°; $[\alpha]^{27}D$ −10.5° (*c* 1, H_2O); R_{fA} 0.61, R_{fB} 0.70. *Anal.* Calcd for $C_{17}H_{29}F_3N_4O_6S\cdot CF_3COOH$: C, 38.77; H, 5.14; F, 19.37; N, 9.52; S, 5.44. Found: C, 38.51; H, 5.27; F, 19.61; N, 9.48; S, 5.30.

***tert*-Butyloxycarbonylasparaginylprolyl-N^{ϵ}-trifluoroacetyllysyl-N^{ϵ}-trifluoroacetyllysyltyrosylisoleucylprolylglycylthreonyl-N^{ϵ}-trifluoroacetyllysylmethionine (XVII).** Dicyclohexylcarbodiimide (103 mg, 0.5 mmol) and *N*-hydroxysuccinimide (57 mg, 0.5 mmol) were added to a solution of XII (600 mg, 0.5 mmol), of XVI (588 mg, 1 mmol), and sodium bicarbonate (168 mg, 2 mmol) in dimethoxyethane–water (1:1), 30 ml. The reaction mixture was processed as described for I to give the product as an amorphous compound (615 mg, 73%): $[\alpha]^{27}D$ −46.6° (*c* 1, methanol). Amino acid analysis after acid hydrolysis indicated the composition: Asp, 1.0; Thr, 0.9; Pro, 2.0; Gly, 1.0; Met, 1.0; Ile, 1.0; Tyr, 0.9; Lys, 3.0. *Anal.* Calcd for $C_{69}H_{102}F_9N_{13}O_{20}S$: C, 49.78; H, 6.18; F, 10.27; N, 2.62; S, 1.93. Found: C, 49.51; H, 6.35; F, 9.88; N, 12.47; S, 1.52.

Asparaginylprolyllysyllysyltyrosylisoleucylprolylglycylthreonyllysylmethionine Tetraacetate Dihydrate (XVIII). Trifluoroacetic acid (2 ml) was added to XVII (166 mg, 0.1 mmol). After 20 min, ether was added and the resulting undecapeptide trifluoroacetate was filtered, washed with ether, dried over KOH and P_2O_5, and dissolved in 1 *M* piperidine solution (15 ml). The reaction mixture was left at 0° for 4 hr, acetic acid (1 ml) was added in the cold, and the solvent was removed by lyophilization. The product was incubated with 1% thioglycolic acid solution (50 ml) at 40° for 24 hr. The thioglycolic acid was removed by applying the reaction mixture to an Amberlite IRA-400 column (50 × 1.2 cm) in the acetate form and the product was eluted with 2% acetic acid. The desired compound was obtained by lyophilization, followed by dissolving the compound in 1% acetic acid (2 ml), applying it to a Sephadex G-15 column (120 × 1.2 cm) preequilibrated with 1% acetic acid, and eluting the product with 1% acetic acid to give 80 mg (50%): $[\alpha]^{27}D$ −24.8° (*c* 0.5, H_2O). Amino acid analysis after acid hydrolysis indicated the composition: Asp, 1.0; Thr, 0.9; Pro, 2.0; Gly, 1.0; Met, 1.0; Ile, 1.0; Tyr, 0.9; Lys, 3.0.

***N*-Acetylasparaginylprolyl-N^{ϵ}-acetyllysyl-N^{ϵ}-acetyllysyltyrosylisoleucylprolylglylcylthreonyl-N^{ϵ}-acetyllysylmethionine.** Acetylation of XVIII (10 mg) was carried out at pH 7.5 at 0° by adding a 150-fold molar excess of redistilled acetic anhydride, in three equal portions over the course of 1.5 hr. The pH was maintained at 7.5 by using a pH stat.

Deprotection of the *O*-acetyl group was carried out at room temperature by incubating the peptide solution (3 mg/ml) in 0.2 *M* hydroxylamine previously adjusted to pH 7.5 for 2 hr, and the reaction mixture was lyophilized. The residual material was dissolved in ammonium bicarbonate (0.2 *M*, pH 8), and desalting was achieved by chromatography on a Bio-Gel P-2 column (0.6 × 108 cm). The peptide was eluted with 0.02 *M* ammonium bicarbonate at a flow rate of 8 ml/hr, collecting fractions of 1 ml. The pooled fractions were collected and lyophilized several times. The concentration of the peptide (80% yield) was determined by amino acid analysis after acid hydrolysis. About 90% of the amino groups were acetylated as determined by the ninhydrin reaction.

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