with acetic acid.

Anal. Calcd for $C_{62}H_{78}N_{17}O_{14}\cdot CH_3COO^-\cdot CH_3COOH\cdot 6H_2O$: C, 52.41; H, 6.47; N, 15.74. Found: C, 52.57; H, 6.63; N, 15.72.

Acknowledgments. The authors gratefully acknowledge financial support for this work by a grant from National Institutes of Health (USPHS AM 15410). They also wish to thank Dr. W. Vale of the Salk Institute for testing the LRF analogues, R. Schroeder of the Salk Institute for the Amino acid analyses, and Mr. W. A. Moller of the Department of Biology (UCSD) for the help he provided in the use of the ultracentrifuge. We are also grateful to Professor Th. Wieland for his helpful suggestions in checking the homogeneity of the cyclic compound and to Dr. M. S. Verlander for his helpful comments on the manuscript.

Registry No.—N^{α}-Trifluoroacetyl-L-ornithine, 5123-49-9; N^{α}-*t*-Boc-N^{α}-trifluoroacetyl-L-ornithine, 63865-89-4; *tert*-butyloxycarbonyl azide, 1070-19-5; *p*-Glu-His-Trp-Glu-Tyr-D-Ala-Orn-Orn(TFA)-Pro-Gly-$NH_2\cdot CH_3CO_2H$, 63865-91-8; *p*-Glu-His-Trp-*cyclo*(Glu-Tyr-D-Ala-Orn)-Orn(TFA)-Pro-Gly-$NH_2\cdot CH_3CO_2H$, 63904-16-5; *p*-Glu-His-Trp-*cyclo*(Glu-Tyr-D-Ala-Orn)-Orn(TFA)--Pro-Gly-$NH_2\cdot 2HCl$, 63865-92-9; *p*-Glu-His-Trp-*cyclo*(Glu-Tyr-D-Ala-Orn)-Orn-Pro-Gly-NH_2, 63865-93-0; *p*-Glu-His-*cyclo*(Trp-Glu-Tyr-D-Ala-Orn)-Nva($Nic^+CH_3COO^-$)-Pro-Gly-$NH_2\cdot CH_3CO$-OH, 63904-76-7; *p*-Glu-His-*cyclo*(Trp-Glu-Tyr-D-Ala-Orn)-Nva(Nic^+Cl^-)-Pro-Gly-NH_2, 63865-97-4; Z-*p*-Glu-His(Tos)-Trp-Glu(OBzl)-Tyr(OBzl)-D-Ala-Orn(Z)-Orn(TFA)-Pro-Gly-NH_2, 63915-17-3; 2,4-dinitrophenylnicotinamidium chloride, 53406-00-1.

References and Notes

(1) Abbreviations used in the text are: LRF, luteinizing hormone-releasing factor (also called luliberin); Z, benzyloxycarbonyl; TFA, trifluoroacetyl; Bzl, benzyl; Tos, toluenesulfonyl; HF, hydrogen fluoride; CMC, carboxymethylcellulose; BPyA, 1-butanol/pyridine/0.1% acetic acid in water (5:3:11); BAW, 1-butanol/acetic acid/water (4:1:5); G-25, Sephadex G-25 fine; DEAE-cellulose, diethylaminoethylcellulose; DCC, dicyclohexylcarbodiimide; *N*-HSu, *N*-hydroxysuccinimide; Nic^+, nicotinamidium; Ac, acetyl; Me_2SO, dimethyl sulfoxide; Boc, *tert*-butyloxycarbonyl.
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Synthesis of Tentoxin and Related Dehydro Cyclic Tetrapeptides[1,2]

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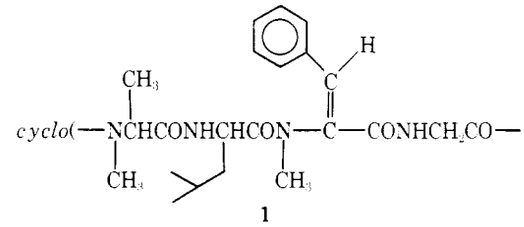
Three methods are reported for synthesizing the dehydro cyclic tetrapeptide tentoxin, *cyclo*(-L-MeAla-L-Leu-MePhe[(*Z*)Δ]-Gly-), a plant toxin that inhibits chloroplast coupling factor 1. Boc-MeAla-Leu-Phe(3-SBzl)-Gly-OMe was prepared by solid-phase synthesis, oxidized to the sulfoxide, dehydrosulfenylated, and *N*-methylated to give Boc-MeAla-Leu-MePhe[(*Z*)Δ]-Gly-OMe. Boc-MeAla-Leu-MePhe(3-SBzl)-Gly-OMe was prepared stepwise in solution from *erythro*-Boc-MePhe(3-SBzl) and converted by dehydrosulfenylation to the dehydro tetrapeptide which was also prepared in good yield by coupling Boc-MeAla with H-Leu-MePhe[(*Z*)Δ]-Gly-OMe. The synthesis of the cyclic tetrapeptides (-X-Leu-MePhe[(*Z*)Δ]-Gly-), where X = L-MeAla, D-MeAla, L-Pro, D-Pro, L-Me[2,3-^{3}H]Ala, L-*N*-[^{13}C-Me]MeAla, and D-*N*-[^{13}C-Me]MeAla, was achieved using the trichlorophenyl ester method. Saponification of Boc-*N*-methyldehydrophenylalanyl peptides led to hydantoin formation with loss of *tert*-butyl alcohol.

The cyclic tetrapeptide, tentoxin, *cyclo*(*N*-methyl-L-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycyl) (**1**) is a phytotoxin produced by the plant pathogenic fungus *Alternaria tenuis*.[3] When applied to germinating seedlings tentoxin causes chlorosis in some species but has little apparent effect on others.[3,4] This selectivity has been linked to the presence of a single tentoxin binding site on chloroplast coupling factor 1 (CF_1), a key protein involved in ATP synthesis.[5] CF_1 from sensitive species bind tentoxin strongly ($K_{assn} = 10^8$), while CF_1 from insensitive species binds tentoxin weakly ($K_{assn} \leq 10^4$). Tentoxin is the only inhibitor of CF_1 reported to exhibit such species specificity.

Tentoxin contains two structural features not commonly found in peptides, the 12-membered cyclic tetrapeptide ring system and the α,β-unsaturated amino acid, *N*-methyldehydrophenylalanine, MePhe[(*Z*)Δ].[2,6] Although several naturally occurring biologically active cyclic tetrapeptides have been identified in nature recently,[7] and a number of peptides containing dehydro residues have been reported,[8] tentoxin



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remains the only peptide isolated that contains both structural features. We report here methods to synthesize tentoxin and several tentoxin analogues that are required for biochemical and conformational studies in progress.

Results and Discussion

(I) Synthesis of Linear Tetrapeptides. The synthesis of peptides containing dehydro amino acid residues may be complicated by the chemical reactivity of the double bond. The simplest unit, dehydroalanine, rapidly adds anhydrous

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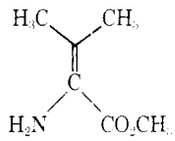
Scheme I

Boc-Gly-O polymer
→ (solid-phase synthesis with: 1. Boc-Phe(3-SBzl) (7); 2. Boc-Leu; 3. Boc-MeAla; 4. MeOH/TEA) Boc-MeAla-Leu-Phe(3-SBzl)-Gly-OMe (12)
→ ($NaIO_4$, $MeOH/H_2O$) Boc-MeAla-Leu-Phe[3-S(O)Bzl]-Gly-OMe (13)
→ (xylene, reflux) Boc-MeAla-Leu-Phe (Δ)-Gly-OMe (14a [(Z)Δ]-, 14b [(E)Δ]-)
→ (K_2CO_3, CH_3I, DMF) Boc-MeAla-Leu-MePhe[(Z)Δ]-Gly-OMe (15a)
→ (1. 1 N KOH/EtOH; 2. Tcp/DCC; 3. pyridine) Boc-MeAla-Leu-MePhe[(Z)Δ]-Gly-OR (16a, R = H; 16b, R = Tcp)
→ (1. 1 N HCl/HOAc; 2. Pyridine (18%)) *cyclo*(-L-MeAla-L-Leu-MePhe[(Z)Δ]-Gly-) (1)

hydrobromic acid, hydrochloric acid,[9] or water. This reactivity has been exploited to effect specific cleavages of protein chains[10] and removal of peptides from polystyrene resins,[11] but the acid lability severely limits the methods that can be used to synthesize dehydro peptides. Substitution at the β position of a dehydro amino acid increases its stability toward electrophilic reagents, and two analogues, dehydrovaline methyl ester **2**[12] and dehydrophenylalanine anilide **3**,[13] are sufficiently stable to have been isolated.



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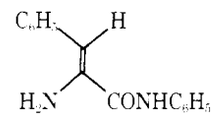

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Dehydro amino acids also may be difficult to couple using standard peptide-coupling procedures. While testing several routes to the synthesis of tetoxin precursors, we found that the C-terminal carboxyl group of Boc-leucyldehydrophenylalanine (**4**) was hard to activate. Dipeptide **4** did not couple with glycine ethyl ester when dicyclohexylcarbodiimide (DCC) was used. When **4** was converted to the acid chloride **5** by reaction with triphenylphosphine–carbon tetrachloride,[14] only low yields of tripeptide, Boc-Leu-MePhe(Δ)-GlyOMe (**6**), could be obtained. These results, and those reported for the reaction of electrophilic and nucleophilic reagents with dehydro peptides,[9-11] suggested that the synthesis of tentoxin precursors, e.g., **15a**, should be designed so that the α,β-double bond would be introduced into the molecule as late as possible in the synthesis.

A synthesis[15] of the protected linear tetrapeptide **15a** was carried out first using the solid-phase method[16] (Scheme I). The nonmethylated, 3-benzylthiophenylalanine derivative **7** was chosen for this synthesis, rather than the *N*-methyl derivative **8**, because it avoided the possible formation of *cyclo*[Leu-MePhe(3-*S*-benzyl)]. Diketopiperazine formation is a side reaction which occasionally occurs with *N*-methyl- or prolylamides,[17,18] and which appeared to be particularly troublesome in preliminary studies of related model systems. For example, attempts to synthesize Boc-MeAla-Leu-MePhe-GlyOMe from **9**, either in solution or on the solid phase,[16] gave, predominantly, the dipeptide **10** and the diketopiperazine **11**.[19] The use of amino acid **7** also made possible the assignment by ultraviolet spectroscopy of the

Boc-MeAla + H-Leu-MePhe-Gly-OR (**9**)
↓ (DCC or the *p*-nitrophenyl ester)
Boc-MeAla-Gly-OR (**10**) + *cyclo*-(-Leu-MePhe-) (**11**)

stereochemistry of the *E* and *Z*[20] isomers of tetrapeptides **14a** and **14b**, and this information could be used to confirm the *Z* configuration proposed[6] for the double bond in tentoxin.

Tetrapeptide **12** was prepared by solid-phase synthesis[16] and removed from the resin by methanolysis. Oxidation with sodium metaperiodate gave the sulfoxide **13** which undergoes thermolytic dehydrosulfenylation[21] at 140 °C to give the dehydrophenylalanine isomers **14a,b**. These could be separated by chromatography on silica gel.

Peptide **14a** (λ_{max} 284, ϵ 18 400) was assigned the *Z* configuration and peptide **14b** (λ_{max} 282, ϵ 9080) assigned the *E* configuration on the basis of the greater intensity of absorbance observed for the trans isomers of cinnamic acids.[23]

Peptide **14a** was converted to the MePhe[(*Z*)Δ] tetrapeptide **15a** by treatment with methyl iodide and anhydrous potassium carbonate in DMF or acetonitrile,[24] which methylates only the dehydrophenylalanine nitrogen without isomerizing the double bond. Complete methylation of the dehydrophenylalanine nitrogen is difficult to achieve consistently and requires that these reagents be dried carefully. However, when 18-crown-6-ethers[25] are added to the reaction mixture (0.05 equiv), the methylation proceeds in spite of small amounts of contaminating moisture. When cesium carbonate was used instead of potassium carbonate, acyl–glycine bonds were methylated at about the same rate as the dehydroamide bond, and the remaining amide nitrogens were methylated more slowly. Thus, cesium carbonate in DMF is not suitable for methylating dehydro residues selectively. The potassium carbonate–methyl iodide reagent did not methylate the *E* isomer of dehydropeptide **14b**.

Peptide **15a** was converted to the acid **16a** and then to the trichlorophenyl ester **16b**, deprotected with 1 N hydrochloric acid in acetic acid, and cyclized in pyridine to tentoxin (**1**) in 18% yield overall. The NMR, IR, UV, and mass spectral properties of synthetic **1** were identical with those of natural tentoxin and the biological potencies on germinating lettuce seedlings were indistinguishable. Thus, the proposed structure of tentoxin[6] is correct insofar as sequence and olefin configuration are concerned.

Several labeled analogues of tentoxin were required for our biological and conformational studies. The synthesis described in Scheme I is not efficient for preparing labeled derivatives because an excess of each protected amino acid is required for each solid-phase coupling reaction, and because dehydrosulfenylation of sulfoxide **13** produces 20–30% of the *E* isomer which can't be used to prepare **15a** and which must be discarded. For these reasons, a more economical route for the synthesis of labeled derivatives of **15a** was developed.

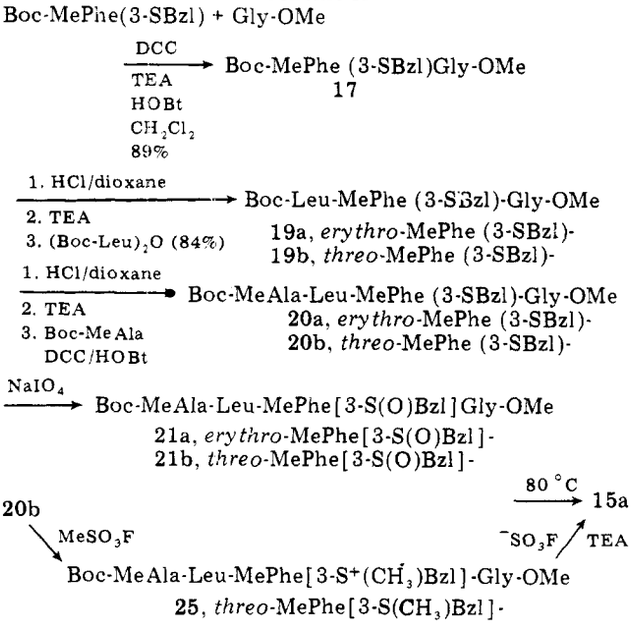
Tetrapeptide **15a** was prepared stepwise in solution starting with the N-methylated amino acid, Boc-MePhe(3-SBzl) (**8**) (Scheme II). Using DCC/1-hydroxybenzotriazole (HOBt),[26] **8a** was coupled with glycine methyl ester to give dipeptide **17** in 89% yield. When HOBt was omitted from the reaction solution a 60% yield of *N*-acylurea was obtained. Following deprotection and neutralization, the free dipeptide, MePhe(3-SBzl)-Gly-OMe (**18**), was coupled with Boc-Leu using the symmetrical anhydride method.[27] The tripeptide **19a** was obtained in 84% yield. We have found that the symmetrical anhydride method gives higher yields of product than use of either DCC or DCC with HOBt for acylation of secondary amino acids (e.g., *N*-methyl-, prolyl-, or thiazolidinecarboxylic

Table I

Compd	Boc-X-L-Leu-MePhe[(*Z*)Δ]-Gly-OMe (X =)	Compd	*cyclo*(-X-L-Leu-MePhe[(*Z*)Δ]-Gly-) (X =)	Cyclization % yield
15a	L-MeAla	**1**	L-MeAla	26–32
27	D-MeAla	**41**	D-MeAla	44–46
28	L-Pro	**42**	L-Pro	30
29	D-Pro	**43**	D-Pro	48
30	L-Me[2,3-^{3}H]Ala	**44**	L-Me[2,3-^{3}H]Ala[a]	26
31	L-*N*-[Me-^{13}C]MeAla	**45**	L-*N*-[Me-^{13}C]MeAla	32
32	D-*N*-[Me-^{13}C]MeAla	**46**	D-*N*-[Me-^{13}C]MeAla	46

[a] Data for this compound has been reported in reference 5.

Scheme II



acid derivatives). In the present case, inclusion of HOBt in the reaction led to products formed by elimination of the thiobenzyl group.

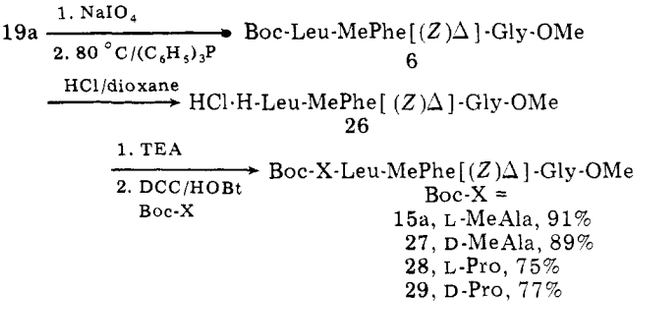
Tripeptide **19a** was deprotected, neutralized, and coupled with *tert*-butyloxycarbonyl-*N*-methylalanine using DCC/HOBt to give tetrapeptide **20a** in 85% yield. The tetrapeptide sulfide **20a** was oxidized with sodium metaperiodate to the sulfoxide **21a** which eliminated sulfenic acid slowly at 25 °C and rapidly at 80 °C. Unsaturated tetrapeptide **15a** was isolated in 70% yield.

The stereochemistry of the product obtained from dehydrosulfenylation of **21a** was predominantly *Z*. Since dehydrosulfenylation of β-alkylsulfinyl amino acids is stereospecific,[22] the MePhe(3-SBzl) precursor employed was predominantly the erythro isomer **8a**. The diastereomers **8a,b** were prepared by addition of benzyl mercaptan to *N*-acetyl-*N*-methyldehydrophenylalanine methyl ester. The adduct was hydrolyzed and the free amino acids were converted to the mixture of diastereomeric Boc derivatives by reaction with *tert*-butyl azidoformate. The diastereomers could be separated by precipitation of **8a** from hexane.

By changing the method used to generate the double bond, diastereomer **8b** also could be used to prepare **15a** and the corresponding threo linear peptides **20b** and **21b**. Dehydrosulfenylation of sulfoxide **21b** gave, as expected, the *E* isomer **15b**. However, when the *threo*-sulfide **20b** was converted to the sulfonium salt **25** by reaction with methyl fluorosulfonate, and then subjected to β elimination using triethylamine,[28] the *Z* isomer **15a** was obtained in 40% yield (Scheme II). No attempt was made to optimize the yield of this reaction.

The successful conversion of dehydro tetrapeptide **15a** into

Scheme III



tentoxin (Scheme I) established that the *N*-methyldehydrophenylalanine residue was not destroyed by the acidic conditions needed to remove the Boc group nor by the basic conditions used for saponification or cyclization. As a result, tetrapeptide **15a** could be synthesized from tripeptide **26** which contained a preformed *N*-methyldehydrophenylalanyl residue (Scheme III). Oxidation of tripeptide **19a** followed by dehydrosulfenylation gave the *Z* dehydro peptide **6** in 61% yield. The *E* isomer, when present, could be removed by chromatography but neither the *E* nor the *Z* isomer could be crystallized. However, after removal of the Boc group, the tripeptide hydrochloride salt **26** could be crystallized from chloroform–hexane mixtures.

Condensation of Boc-MeAla or Boc-Pro with tripeptide **26** using DCC/HOBt gave good yields of the protected tetrapeptides **15a** and **27–32** (75–91%) (Scheme III). No formation of the diketopiperazine, *cyclo*(-Leu-MePhe[(*Z*)Δ]-), was detected. Thus, peptide **26** is an excellent intermediate for the synthesis of analogues of **15a** substituted in the 1 position. Using this intermediate, analogues **27–32** (Table I) were synthesized by replacing Boc-L-MeAla with the appropriate Boc amino acid.

The conformational and biological properties of cyclic tetrapeptides **41–46** will be reported separately. However, attention should be drawn to the analogue, [1-D-methylalanine[1] tentoxin (**41**)] which is a mixture of two conformers at room temperature. Our recent results have established that these conformers are in equilibrium with each other at 25 °c but can be separated and isolated at 4 °C. This work plus assignment of their conformations and biological activities will be reported in another communication.[37]

Not all *N*-methyldehydrophenylalanyl peptides are stable to the conditions used to saponify peptide esters. During the

Boc-MePhe[(*Z*)Δ]-Gly-MeAla-Leu-OMe
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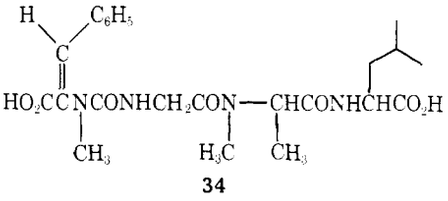

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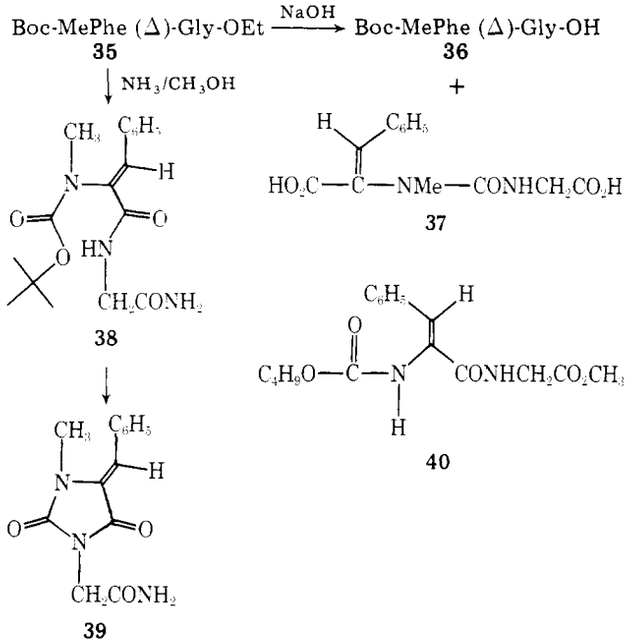
Scheme IV



synthesis of other linear sequences of tentoxin, an unusual side reaction was encountered when Boc-MePhe(Δ) was at the N-terminus of the chain. Saponification of the N-terminal Boc-*N*-methyldehydrophenylalanyl peptide **33** with alcoholic sodium hydroxide gave, exclusively, a ninhydrin-negative product, which did not contain a *tert*-butyl group. The product, identified as diacid **34**, probably was formed by hydrolysis of an intermediate hydantoin. Although N-terminal carbamates, e.g., the benzyloxycarbonyl group, are known to undergo hydantoin formation under basic conditions,[29] the more hindered Boc group does not. Thus, we decided to study this reaction more closely using Boc-MePhe(Δ)-Gly-OEt (**35**) (Scheme IV) to determine the parameters affecting hydantoin formation.

Saponification of dipeptide ester **35** gave the Boc acid **36** in 80% yield along with a small amount of diacid **37**. Because abstraction of the glycyl amide proton would be less likely to occur when the glycyl nitrogen is adjacent to a carboxylate anion (as it is in **36**) than to an amide group (as it is in **33**), an attempt was made to convert dipeptide ester **35** to the amide **38**. However, ammonolysis of **35** led rapidly to the formation of hydantoin **39**, which was isolated in 75% yield. In contrast, no hydantoin was formed when the non-N-methylated dehydrophenylalanyl peptide **40** was treated with either sodium hydroxide or methanolic ammonia. These results suggest that the dehydrophenylalanyl nitrogen must be methylated for hydantion formation or occur. No attempt has been made to determine if peptides containing other *N*-methyl dehydro amino acids will undergo hydantoin formation as readily as the *N*-methyldehydrophenylalanyl peptides. The unusual lability of this system, in comparison with normal N-terminal Boc peptides, probably is caused by the five adjacent trigonal centers which place the glycyl nitrogen adjacent to the Boc carbonyl group when the phenylalanine nitrogen is methylated and can assume the *cis*-amide conformation shown in structure **38**. The *cis*-amide conformation is less likely to occur in **40** because secondary amide bonds are predominantly trans.

The results reported here for linear, *N*-methyldehydrophenylalanine-containing peptides indicate that tripeptides, such as **6**, are the smallest *N*-methyldehydrophenylalanyl unit which can be used conveniently to prepare larger peptides using standard synthetic procedures. The linear peptides we studied that contained N-terminal Boc-MePhe[(*Z*)Δ] residues, e.g., **33**, **35**, **38**, are suceptible to base-catalyzed hydantoin formation, and peptides containing C-terminal MePhe[(*Z*)Δ]-OR residues, e.g., Boc-Leu-MePhe(Δ)-OH (**4**) or the acid chloride **5**, did not react well with glycine ethyl ester and may be difficult to couple. Once in the center of a tripeptide, e.g., **6**, the *N*-methyldehydrophenylalanyl residue is stable toward the acidic and basic conditions used for the synthesis of the linear and cyclic tetrapeptides reported here. However, the successful use of tripeptides containing dehydro residues may be limited to more stable dehydro residues, e.g., dehydrophenylalanine, which are much more stable to acid than aliphatic dehydro amino acids.[9,13]

Cyclization of Linear Tetrapeptides (Scheme I). Tetrapeptide **15a** was saponified and converted to the 2,4,5-trichlorophenyl ester **16** by reaction with Tcp and DCC in pyridine. After precipitation from hexane to remove DCU and Tcp, the Boc group was removed using HCl/dioxane. We obtained better yields using the hydrochloride salt of the linear tetrapeptide during the cyclization reaction than the trifluoroacetate salt. The hydrochloride was carefully dried, dissolved in DME, and added slowly to preheated pyridine to effect cyclization under dilute conditions (10^{-4} M). Systematic variation of the reaction temperature established that the highest yields were obtained at temperatures near 90 °C. A similar temperature was reported to be optimal for the synthesis of *cyclo*(ProGly)$_2$.[30] No cyclic tetrapeptides were detected from cyclizations run at temperatures below 50 °C. No attempt was made to isolate or characterize other products of the reaction. Slow addition of the peptide in DMF to the pyridine solution also was important, and this was accomplished using a motor-driven syringe. The use of other active esters, e.g., *p*-nitrophenyl ester or 2-thiopyridyl ester, did not lead to increased yields. Using the general cyclization procedure, cyclic tetrapeptides **1** and **41–46** were synthesized from the linear peptides in 26–48% yield (Table I). Linear peptides containing a D-amino acid in position 1 (e.g., **27**, **29**, **32**) gave better yields of cyclic tetrapeptide (44–48%) than the corresponding peptides containing an L-amino acid in this position (26–32%).

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected.

The ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer or a Bruker HX-90E-pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit. The mass spectrometer employed was Finnigan 1015. Ultraviolet data were taken with a Cary-14 ultraviolet spectrometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Thin-layer chromatography (TLC) was performed on silica gel G plates using the following solvent sysetms: (1) 6% methyl in benzene; (2) 20% ethyl acetate in benzene; (3) 35% ethyl acetate in benzene; (4) 50% ethyl acetate in benzene; (5) 5% ethanol in ethyl acetate; (6) 10% ethanol in ethyl acetate.

General Workup Procedure for Boc Amino Acids and Peptides. After removal of reaction solvent by evaporation, the organic residue was dissolved in ethyl acetate and washed three times with 1 N citric acid. The organic layer was separated, washed three times with saturated sodium chloride solution, dried ($MgSO_4$), and evaporated in vacuo.

Methyl *N*-Acetyl-2-*N*-methylaminocinnamate. Following the procedure developed for methylating carbamyl amino acids,[31] reaction of *N*-acetyldehydrophenylalanine (12.4 g) in 200 mL of DMF with sodium hydride (7.64 g, 57% dispersion) and methyl iodide (56.5 mL) for 30 min gave, after distillation (bp 159–163 °C, 10 mm), 13.57 g (94%) of the *N*-methyl ester: R_f (1) 0.375; NMR δ 1.98 (3 H, s), 3.068 (3 H, s), 3.86 (3 H, s), 7.46 (5 H, s), 7.63 (1 H, s).

***N*-Acetyl-*N*-methyl-3-benzylthiophenylalanine Methyl Ester (47).** To a solution of ester **46** (12.77 g) in anhydrous methanol (50 mL) were added benzyl mercaptan (9.63 mL) and sodium methoxide (100 mg). The solution was stirred at 25 °C for 7 days. Disappearance of **46** was followed by TLC. The solvent was evaporated in vacuo, and the ester **47**, after purification by chromatography over silica gel

eluting with ethyl acetate–benzene (1:9), was isolated in 80% yield (12.6 g): R_f (1) 0.47. Anal. ($C_{20}H_{23}NO_3S$): C, H, N.

***N*-Methyl-3-*S*-benzylthiophenylalanine (48).** A solution of **47** (7.15 g) in 240 mL of hydrochloric acid, 80% formic acid, and water (1:1:1) was refluxed for 6 h. The solution was cooled, diluted with water (200 mL), neutralized with ammonium hydroxide, and cooled. The product which crystallized was collected, washed with water and acetone, and dried. Recrystallization from refluxing acetic acid gave 3.74 g (67%) of **48**: mp 216–218 °C. Anal. ($C_{17}H_{19}NO_2S$): C, H, N.

***N*-*tert*-Butyloxycarbonyl-*N*-methyl-3-*S*-benzylthiophenylalanine (8a,b).** Finely powdered acid **48** (3.0 g) was suspended in DMF (30 mL), and tetramethylguanidine[32] (4.6 g, 4 equiv) was added followed by *tert*-butyloxycarbonyl azide (5.72 g, 4 equiv). The solution was stirred for 3 days. Precipitated starting material was collected and resubjected to the carbamylating conditions until free amino acid no longer precipitated. The solutions were kept at 4 °C for an additional 72 h, and then evaporated in vacuo. The residue was suspended in ethyl acetate (50 mL) and acidified with 1 N citric acid. The organic layer was separated, washed three times with 1 N citric acid (50 mL) and three times with saturated sodium chloride solution (30 mL), dried ($MgSO_4$), and evaporated. The residue was suspended in petroleum ether (50 mL) at room temperature for 24 h and then filtered to give erythro diastereomer **8a** (2.24 g, 56%): mp 133–136 °C; NMR δ 1.45 (s, 9 H), 2.6–2.7 (3 H, m), 3.65 (s, 2 H), 4.3 (1 H, m), 5.1 (1 H, m), 7.2 (10 H), 9.7 (NH); mass spectrum *m/e*, M^+ 401. Anal. ($C_{22}H_{27}NO_4S$): C, H, N, S.

Evaporation of the filtrate from **8a** gave the threo diastereomer **8b**: NMR δ 1.47 (9 H, d), 2.6–2.7 (3 H, m), 3.2 (2 H, d), 4.1 (1 H, m), 4.7 (1 H, m), 7.0 (10 H, d), 9.7 (1 H, s); mass spectrum *m/e*, M^+ 401. Anal. ($C_{22}H_{27}NO_4S$): C, H, N, S.

***N*-*tert*-Butyloxycarbonyl-*N*-methyl[2,3-^{3}H]alanine (49).** L-[2,3-^{3}H]Alanine (2.5 mCi, sp act. 31 Ci/mmol) was added to L-alanine (4.6 mg) in water (85 μL). Triethylamine (32 μL) and *tert*-butyl azidoformate (10 μL) in dioxane (85 μL) were added. The mixture was stirred for 16 h. The solvent was removed by evaporation and the residue dissolved in ether. Standard workup gave Boc-L-[2,3-^{3}H]Ala (80% yield), which was dissolved in THF (1 mL) and treated at −78 °C with sodium hydride (20 mg, 50% dispersion) and methyl iodide (300 μL) under nitrogen. The mixture was warmed to room temperature with stirring and worked up in the usual manner to give **49** (88% yield).

***N*-*tert*-Butyloxycarbonyl-L-*N*-[Me-^{13}C]methylalanine (50).** Boc-L-Ala (570 mg) and [^{13}C]methyl iodide (99% ^{13}C, 0.86 g) in 5 mL of tetrahydrofuran at 0 °C were treated with sodium hydride (84 mg, 50% dispersion). The mixture was stirred for 18 h (it gels). Solvent was added (5 mL) along with 0.5 mL of methyl iodide. The mixture was stirred for 5 days and then worked up in the normal manner to give 558 mg (91%) of **50**: $[\alpha]_D$ (MeOH) −30.0° (lit.[33], −29°). Analysis by NMR and mass spectrometry showed that L-MeAla was 80 atom-% ^{13}C: NMR δ 1.35 (3 H, d, J = 5 Hz), 1.54 (9 H, s), 2.85 (2.4 H, d, J = 135 Hz, and 0.6 H, s, unlabeled N-CH_3), 4.7 (1 H, m), and 11.3 (1 H); mass spectrum M^+ 204/203 = 4.

***N*-*tert*-Butyloxycarbonyl-D-*N*-[Me-^{13}C]methylalanine (51).** This compound was synthesized in 90% yield and 75 atom-% ^{13}C following the procedure described for the L isomer.

Methyl *N*-*tert*-Butyloxycarbonyl-*N*-methyl-3-*S*-benzylthiophenylalanylglycinate (17). To a solution of Boc-MePhe(3-SBzl) **8a** (2 g), glycine methyl ester hydrochloride (0.625 g), 1-hydroxybenzotriazole (76 mg) in 5 mL methylene chloride at 4 °C were added triethylamine (0.632 mL) and dichclohexylcarbodiimide (1.03 g) and 5 mL of CH_2Cl_2. The mixture was stirred at 4 °C for 8 h. The solvent was evaporated in vacuo. The residue was worked up normally to give a white solid, shown by TLC to be essentially one product. Crystallization from EtOAc/hexane gave pure **17** (2.2 g, 89%): R_f (2) 0.48; NMR δ 1.5 (9 H, s), 2.8 (3 H, s), 3.36 (2 H, d, J = 10.5 Hz), 3.7 (3 H, s), 3.7–3.9 (2 H, m), 4.3 (1 H, d, J = 11.5 Hz), 5.05 (1 H, d, 11.5 Hz), 6.45 (1 H, br s), 7.15–7.5 (10 H, m); mass spectrum M^+ 472. Anal. ($C_{25}H_{32}N_2O_5S$): C, H, N.

Methyl *N*-*tert*-Butyloxycarbonyl-L-leucyl-*N*-methyl-3-*S*-benzylthiophenylalanylglycinate (19). The dipeptide **17** (1.828 g) was dissolved in 5 mL of 4 N HCl–dioxane, and the solution was stirred for 30 min. The solvent was evaporated in vacuo and the residue was dried in vacuo over KOH. The dried hydrochloride was dissolved in CH_2Cl_2 (5 mL) and cooled to −78 °C. The symmetrical anhydride of Boc-L-Leu (1.9 g) (prepared by reaction of 1.99 g of Boc-Leu with 2.47 g of DCC for 7 h at 5 °C in CH_2Cl_2) was added followed by 0.556 mL of triethylamine. The solution was stirred at 4 °C for 12 h. Workup in the usual manner gave **19** as an uncrystallizable semisolid (1.96 g, 84%) which was homogeneous by TLC: R_f (3) 0.45; NMR δ 0.65–1.1 (6 H, m), 1.2–2 (15 H, m), 2.8 (3 H, s), 3.65 (2 H, s), 3.7 (3 H, s), 3.8–5.7 (5 H, m), 7.1–7.4 (12 H, m). This material was used in the next step without further purification.

Methyl *N*-*tert*-Butyloxycarbonyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (6). To the tripeptide **19** (1.9 g) in methanol (5 mL) was added sodium metaperiodate (852 mg) in 3 mL of water at 4 °C. The oxidation was followed by TLC and was complete within 12 h. The solvent was evaporated and the residue extracted with ether. The ether layer was washed, dried, and evaporated. The residue was heated in refluxing toluene for 8 h. Solvent was evaporated and the residue chromatographed on silica gel eluting with a gradient of 20 to 50% ethyl acetate in benzene. Tripeptide **6** (1.22 g, 64%) was isolated as a noncrystalline solid: R_f (4) 0.4; NMR δ 0.5–0.7 (6 H, m), 0.9–1.55 (12 H, contains nine proton singlet for Boc and β and γ protons of Leu), 3.2 (3 H, s), 3.75 (3 H, s), 4.2 (2 H, d, J = 6 Hz), 6.7 (1 H, br s), 7.25 (5 H, s), 7.75 (1 H, S, vinyl proton).

Hydrochloride of L-Leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycine Methyl Ester (26). Dehydrotripeptide **6** (1.6 g) was treated with 4 N HCl/dioxane for 30 min and the solvent was evaporated. The residue was dried in vacuo and crystallized from chloroform–hexane to give pure **6** (885 mg, 86%). Anal. ($C_{19}H_{27}N_3O_4$·HCl): C, H, N.

Methyl *N*-*tert*-Butyloxycarbonyl-*N*-methyl-L-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (15a). Boc-MeAla (102 mg) was added to a solution of the tripeptide **6** (200 mg) in CH_2Cl_2 (3 mL). The solution was cooled (4 °C), DCC (105 mg), triethylamine (50.5 mg), and 1-hydroxybenzotriazole (10 mg) were added, and the solution was stirred for 8 h. After the normal workup, the tetrapeptide **15a** was isolated (246 mg, 91%): R_f (4) 0.36; NMR δ 0.5–0.7 (6 H, d, J = 9 Hz), 1.49 (9 H, s), 1.35 (3 H, d, J = 6.6 Hz), 0.9–1.2 (3 H, m), 2.82 (3 H), 3.2 (3 H, s), 3.7 (3 H, s), 4.15 (2 H, d, J = 6 Hz), 6.4 (1 H, br s), 7.38 (5 H, s), 7.7 (1 H, s) and 8.5 (1 H, br s); UV ($CHCl_3$) λ_{max} 277 (ϵ 18 400); IR 3400, 3300, 2960, 1680–1650, 1525 cm^{-1}. Anal. ($C_{28}H_{42}N_4O_7$): C, H, N.

Methyl *N*-*tert*-Butyloxycarbonyl-*N*-methyl-D-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (27). Tetrapeptide **27** was prepared in 89% yield from **6** using Boc-D-MeAla: R_f (3) 0.50; NMR ($CDCl_3$) δ 0.60 (6 H, d, J = 6 Hz), 1.28 (3 H, d, J = 7 Hz), 1.2–1.7 (3 H, m), 1.48 (9 H, s), 2.83 (3 H, s), 3.2 (3 H, s), 3.73 (3 H, s), 4.15 (2 H, d, J = 6.5 Hz), 4.5 (1 H, m), 4.7 (1 H, m), 6.5 (NH, br s), 7.4 (5 H, s), 7.7 (1 H, s), 8.5 (NH, m); mass spectrum, *m/e*, M^+ 547, 485, 458, 384, 346, 248 (58), 132 (32), 116 (12), 102 (48), 86 (75), 57 (100).

Methyl *N*-*tert*-Butyloxycarbonyl-L-prolyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (28). Following the procedure developed for the analogue **15a**, tetrapeptide **28** was prepared in 75% yield using Boc-L-Pro: R_f (3) 0.44; NMR ($CDCl_3$) δ 0.55 (6 H, d, J = 6 Hz), 1.30 (3 H, d, J = 7 Hz), 1.41 (9 H, s), 1.2–1.7 (3 H, m), 1.7–2.2 (4 H, m), 3.21 (3 H, s), 3.05–3.55 (2 H, m), 3.72 (3 H, s), 4.0–4.35 (4 H, m), 7.2–7.4 (2 NH, m), 7.44 (5 H, s), and 7.74 (1 H, s); mass spectrum, *m/e*, M^+ 559, 459, 458, 414, 297, 298, 248, 132 (33), 131 (22), 116 (12), 114 (35), 86 (49), **70** (100), 56 (40).

Methyl *N*-*tert*-Butyloxycarbonyl-D-prolyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (29). Following the procedure developed for the synthesis of analogue **15a**, the tetrapeptide **29** was prepared using Boc-D-Pro in 77% yield: R_f (3) 0.43; NMR ($CDCl_3$) δ 0.55, 0.63 (6 H, dd, J = 3.5 Hz), 1.40 9 H, s), 0.8–1.4 (3 H, m), 1.7–2.2 (4 H, m), 3.2 (3 H, s), 3.35–3.55 (2 H, m), 3.62 (3 H, s), 4.0–4.45 (4 H, m), 7.38 (5 H, s), 7.71 (1 H, s); mass spectrum, *m/e*, M^+ 559, 459, 458, 414, 299, 298, 248, 132 (30), 131 (26), 116 (15), 114 (39), 86 (52), **70** (100), 56 (43).

Ethyl *N*-*tert*-Butyloxycarbonyl-*N*-methyl-L-[2,3-$^3H_2$]-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (30). For this tetrapeptide, the ethyl ester was used. Following the general procedure described, Boc-L-Me[2,3-$^3H_2$]Ala **49** in 600 μL of CH_2Cl_2, tripeptide **6** (21.2 mg), triethylamine (7.2 mg), and dicyclohexylcarbodiimide (10.5 mg) at 0 °C for 15 h gave a 50% yield of tetrapeptide **30** which was purified by preparative TLC on silica gel and identified by direct comparison with unlabeled analogue **15a**.

Methyl *N*-*tert*-Butyloxycarbonyl-L-*N*-[Me-^{13}C]methylalanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (31). Starting with 100 mg of ^{13}C-labeled Boc-MeAla **50**, the tetrapeptide was synthesized in 91% yield. Analytical data for **31** are the same as for **15a**, except for the NMR of the *N*-methyl group: δ 2.82 (2.4 H, d, J = 138 Hz, and 0.6 H, s), 80 atom-% ^{13}C.

Methyl *N*-*tert*-Butyloxycarbonyl-D-*N*-[Me-^{13}C]methylalanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate (32). Labeled tetrapeptide **32** was prepared in 89% yield from **6** and Boc-D-*N*-[Me-^{13}C]MeAla **51**: R_f (3) 0.50. Analytical data for **32** are the same as for the unlabeled compound **27** except for the NMR of the alanyl *N*-methyl group: δ 2.81 (2.4 H, d, J = 138 Hz, and 0.6 H, s),

80 atom-% ^{13}C.

General Procedure for Saponification. *N*-*tert*-Butyloxycarbonyl-*N*-methyl-L-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycine (16a). The tetrapeptide **15a** was dissolved in 95% ethanol and 1.5 equiv of ethanolic potassium hydroxide solution was added. The reaction mixture was stirred at 25 °C for 30–45 min and the saponification monitored by TLC. The solvent was evaporated, suspended in ether, and acidified with 1 N citric acid solution. The ether layer was removed, and the aqueous layer washed with ethyl acetate. The organic layers were combined, dried, and evaporated. The NMR of **16a** was essentially the same as that of **15a**, except the methyl signal at 3.7 ppm was absent.

General Procedure for Cyclization of Tetrapeptides. A solution of Boc tetrapeptide acid **16a** (0.3 mmol) and 2,4,5-trichlorophenol (0.36 mmol) in pyridine (3–4 mL) was stirred at 4 °C under nitrogen. DCC (0.36 mmol) was added and the reaction was allowed to proceed for 12 h at 4 °C. The pyridine was removed in vacuo and the residue dissolved in ethyl acetate. The solution was cooled (−78 °C) and filtered to remove DCU. Solvent was removed and the residue treated with 4 N HCl in dioxane for 30 min at 25 °C. The solvent was evaporated in vacuo. The hydrochloride was dissolved in DMF (1–2 mL) and this solution was added dropwise, using a motor-driven syringe, to pyridine (1 L) preheated to 90 °C. After 8 h, the solvent was evaporated at 40 °C and the residue was dissolved in ethyl acetate. The solution was washed three times with cold water, dried ($MgSO_4$), and evaporated. The residue was purified by chromatography on silica gel eluting with a gradient of 0 to 30% ethanol in ethyl acetate. Fractions containing product **1** were pooled, concentrated, and crystallized from chloroform–ether or, occasionally, further purified by preparative TLC on silica gel eluting with 5% ethanol in ethyl acetate. Using this method, the tentoxin analogues **40–45** were prepared in 25–40% yield.

Tentoxin. *cyclo*(*N*-Methyl-L-alanyl-L-leucyl-*N*-methyldehydrophenylalanylglycyl) (1). Applying the general saponification and cyclization procedures, tentoxin **1** was prepared in 26% yield from tetrapeptide **15a**: mp 173–175 °C (lit.[6], 172–175 °C); R_f (5) 0.356, (6) 0.52; UV λ_{max} (CH_3OH), 282 (ϵ 20 700); NMR ($CDCl_3$) δ 0.52, 0.71 (6 H, m), 1.15–1.25 (3 H, m), 1.52 (3 H, d, J = 8 Hz), 2.77 (3 H, s), 3.24 (3 H, s), 3.52 (1 H, dd, J = 2, 15 Hz), 4.16 (1 H, m), 4.30 (H, q), 5.07 (1 H, dd, J = 10.1, 15 Hz), 7.2 (1 H, d, 8.9 Hz), 7.27 (5 H, s), 7.74 (H, s), and 7.97 (1 H, d, 10.1 Hz); mass spectrum, *m/e*, 414. Anal. ($C_{22}H_{30}N_4O_4$): C, H, N.

***cyclo*(*N*-Methyl-D-alanyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycyl). D-MeAla[1]-tentoxin (41).** Following the standard saponification and cyclization procedures, D-MeAla[1]-tentoxin was prepared in 44% yield: mp 158–162 °C; UV λ_{max} (CH_3OH) 278 (ϵ 19 700); NMR ($CDCl_3$) shows signals for two conformers: conformer A δ 0.77–0.53 (6 H, m), 1.55–1.15 (3 H, m), 1.52 (3 H, d, J = 8 Hz), 3.04 (3 H, s), 3.18 (3 H, s), 3.7 (1 H, d, J = 16 Hz), 4.17 (1 H, m), 4.48 (1 H, q, J = 8 Hz), 5.19 (1 H, dd, J = 10, 16 Hz), 6.94 (1 H, d, J = 10 Hz), 6.0 (1 H, d, J = 6 Hz), 7.4 (5 H, s); conformer B: δ 0.67–0.53 (6 H, m), 1.4–1.1 (3 H, m), 1.54 (3 H, d, J = 7 Hz), 3.07 (3 H, s), 3.26 (3 H, s), 3.5 (1 H, m), 4.24 (1 H, q, J = 7 Hz), 4.52 (1 H, m), 4.9 (1 H, br s), 7.25 (1 H), 6.34 (1 H, s), and 7.4 (5 H, s). Mass spectrum *m/e* (% base peak) 414 (5), 301 (3), 216 (3), 215 (3), 214 (3), 132 (12), 131 (15), 116 (19), 114 (12), 86 (11), 81 (10), 58 (100). Anal. ($C_{22}H_{30}N_4O_4$): C, H, N.

***cyclo*(L-Prolyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycyl). L-Pro[1]-tentoxin (42).** Starting with tetrapeptide **28** and following standard procedure for saponification and cyclization, L-Pro-tentoxin **42** was obtained in 30% yield: mp 173–175 °C; R_f (5) 0.153, R_f (6) 0.29; UV λ_{max} (CH_3OH) 282 (19 100); NMR ($CDCl_3$) δ 0.63–0.51 (6 H, m), 1.25–1.11 (3 H, m), 1.98–1.59 (3 H, m), 2.41 (1 H, 3.19 (3 H, s), 3.54 (1 H, d, 14.8 Hz), 3.54 (2 H, m), 4.40 (1 H, dd, 7 and 6 Hz), 4.49 (1 H, m), 5.12 (1 H, dd, 8 and 14.8 Hz), 7.41 (5 H, s), 7.93 (1 H, d, J = 8 Hz), 7.3 (1 H, d, J = 7 Hz), 7.76 (1 H, s); mass spectrum *m/e* (% of base) 427 (1.2), 426 (1.5), 216 (1), 189 (1), 188 (4), 187 (3.5), 132 (7), 131 (8), 130 (3), 117 (5), 116 (10), 91 (5), 89 (5), 86 (5), 82 (5), 70 (100). Anal. ($C_{23}H_{30}N_4O_4$): C, H, N.

***cyclo*(D-Prolyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycyl). D-Pro[1]-Tentoxin (43).** Following the procedure for the L isomer, D-Pro[1]-tentoxin **43** was prepared in 48% yield: mp >300 °C (sublimes); UV λ_{max} (CH_3OH) 282 (19 100); NMR ($CDCl_3$) δ 0.77–0.54 (6 H, m), 1.53–1.08 (3 H, m), 1.93 (3 H, m), 2.2 (1 H, m), 3.2 (3 H, s), 3.6 (2 H, m), 3.72 (1 H, d, J = 16 Hz), 4.34 (1 H, d), 4.48 (1 H, m), 5.06 (1 H, dd, 10 and 16 Hz), 6.42 (1 H, d, J = 6.8 Hz), 7.17 (1 H, s), 7.2 (1 H, d, J = 10 Hz), 7.35 (5 H, s), 7.74 (1 H, s); mass spectrum *m/e* (% of base), 427 (2.5), 426 (2.5), 216 (2.5), 189 (1), 188 (2), 187 (2), 132 (10), 131 (12), 130 (3), 117 (5), 116 (12), 91 (6), 89 (4), 86 (4), 83 (6), 70 (100). Anal. ($C_{23}H_{30}N_4O_4$): C, H, N.

***cyclo*(*N*-[Me-^{13}C]Methyl-L-alanyl-L-leucyl-*N*-methyldehydrophenylalanylglycyl-) (45).** Compound **45** was prepared in 32% yield from linear tetrapeptide **31** following the procedures developed for the unlabeled compound. Compound **45** was 80 atom-% in ^{13}C in the methyl group of *N*-methylalanyl residue in position one: mass spectrum *m/e* (% of base), M^+ 415 (7), 414 (6.3), 302 (2), 301 (1), 217 (1.6), 216 (2.4), 215 (2.0), 214 (2.2), **58** (100).

***cyclo*(*N*-[Me-^{13}C]Methyl-D-alanyl-L-leucyl-*N*-methyldehydrophenylalanylglycyl) (46).** Compound **46** was prepared in 46% yield from the tetrapeptide **32** following the procedures developed for the unlabeled compound. Compound **46** was 75 atom-% ^{13}C in the *N*-methyl-D-alanine residue: mass spectrum *m/e* (% of base), M^+ 415 (7), 414 (6.5), 302 (3), 301 (1), 217 (1.5), 216 (2.2), 215 (2.1), 214 (2.2), 58 (100).

Saponification of Methyl *tert*-Butyloxycarbonyl-*N*-methyldehydrophenylalanylglycyl-*N*-methylalanylleucinate. Compound **33** (12.5 mg, 0.023 mmol) was dissolved in methanol (1 mL), and 1 N sodium hydroxide (0.025 mL, 0.025 mmol) was added and the solution was stirred at 25 °C for 30 min. The methanol was removed by evaporation and the solution was acidified to pH 3 and washed three times with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated to give the diacid **34** (9 mg, 83%): R_f (EtOAc) 0.55; UV λ_{max} 277.5 (ϵ 14 400); NMR δ 0.93 (6 H, dd), 1.35 (3 H, d, J = 7 Hz), 1.63 (3 H, m), 3.0 (3 H, s), 3.04 (3 H, s), 4.14 (1 H, m), 4.60 (2 H, m), 5.1 (1 H, m), 7.45, 7.68 (6 H, m). Anal. ($C_{23}H_{32}N_4O_7$): C, H, N.

Ethyl *tert*-Butyloxycarbonyl-*N*-methyldehydrophenylalanylglycinate (35). Ethyl *tert*-butyloxycarbonyl-*N*-methyl-3-benzylthiophenylalanylglycinate (mp 86–87 °C, 470 mg, 0.96 mmol) was oxidized in methanol (25 mL) and water (10 mL) with sodium periodate (226 mg, 1.05 mmol) to the sulfoxide. After the usual workup, the residue was dissolved in benzene and heated at reflux for 1 h. Workup, followed by chromatography on silica gel eluting with 40% ethyl acetate in benzene, gave pure **35** (300 mg, 87%): R_f (3) 0.22; NMR δ 1.3 (9 H, s), 1.3 (3 H, t, J = 7 Hz), 3.0 (3 H, s), 4.4 (4 H, m, q), 7.25 (1 H, s), 7.32 (5 H, s), 6.7 (NH). Anal. ($C_{19}H_{26}N_2O_5$): C, H, N.

Ammonolysis of 35. Formation of Hydantoin 39. A solution of **35** (100 mg) and 10 mL of methanol was saturated with anhydrous ammonia at 0 °C for 40 min. The solution was stirred for 3.5 h and then the solvent was evaporated. Crystallization from acetone gave pure hydantoin **39** (77%): mp 197–203 °C; IR 5.65, 5.82, 5.95, 6.05, 6.12 μm; NMR (Me_2SO-d_6) 2.88 (3 H, s), 4.07 (2 H, s), 6.84 (1 H, s), and 7.45 (5 H, s). Anal. ($C_{13}H_{13}N_3O_3$): C, H, N.

Solid-Phase Synthesis of *N*-*tert*-Butyloxycarbonyl-L-*N*-methylalanyl-L-leucyl-3-*S*-benzylthiophenylalaninylglycine Methyl Ester (12). The solid-phase synthesis was carried out on a Beckman Model 990 peptide synthesizer. Removal of the *N*-Boc protecting group, neutralization of the peptide resin salt, and addition of the next amino acid followed a program previously reported[34,35] for the synthesis of oxytocin. The resin (1% divinyl benzene) contained 0.6 mmol of Gly/g. The protected peptide was cleaved from the peptide resin (5.9 g) by stirring in freshly prepared anhydrous methanol (60 mL) and dimethoxyethane (60 mL) containing triethylamine (15 mL).[36] The flask was wired shut and stirred at room temperature for 24 h. The suspension was filtered and the resin resuspended in identical amounts of the above solvent for an additional 24 h. After filtration, the filtrates were combined and rotary evaporated to give a crude product which was dissolved in ethyl acetate and washed with water, citric acid, water, and sodium bicarbonate, and dried ($MgSO_4$). The residue was chromatographed on silica gel eluting with a gradient of benzene to 50% ethyl acetate in benzene. This material was further purified by gel filtration through LH-20 in methanol to give 1.48 g (64%) of pure **12**: R_f (4) 0.38; NMR ($CDCl_3$) δ 0.85 (6 H, m), 1.0–1.40 (3 H, m), 1.18 (3 H, d, J = 7 Hz), 1.36 (9 H, s), 2.76 and 2.69 (3 H, two s), 3.58 (2 H, d), 3.70 (3 H, s), 3.94 (3 H, m), 4.2 (1 H, m), 4.41 (1 H, m), 4.85 (1 H, m), 6.52 (3 H, m), 7.27 (5 H, s), 7.29 (5 H, s). Anal. ($C_{34}H_{48}N_4O_7S$): C, H, N, S.

***N*-*tert*-Butyloxycarbonyl-L-*N*-methylalanyl-L-leucyldehydrophenylalanylglycine Methyl Ester (14a,b).** The sulfide **12** (176 mg) was dissolved in methanol (5 mL), and sodium periodate (60 mg) and water (3 mL) were added. The reaction mixture was stirred at 0–5 °C for 24 h. The solvent was removed under reduced pressure. Ethyl acetate and water were added to the residue, and the aqueous layer was separated and washed with ethyl acetate. The organic extracts were combined, washed with water, and dried ($MgSO_4$), and solvent was removed to give sulfoxide **13** (180 mg, 94%) which was used without further purification.

Sulfoxide **13** (180 mg) was refluxed in xylene (18 mL) under nitrogen for 30 h. The solvent was removed in vacuo and the residue chromatographed on LH-20 in methanol to give a mixture of *E* and *Z* isomers in 60% yield.

The isomers were separated by chromatography on a column of silica gel packed in 30% ethyl acetate–benzene, eluting with a gradient to 50% ethyl acetate.

Boc-L-MeAla-L-Leu-Phe[(*Z*)Δ]-Gly-OMe (**14a**): R_f (EtOAc) 0.62; UV λ_{max} 276 (ε 18 400); NMR ($CDCl_3$) δ 0.91 (6 H, m), 1.27 (3 H, d, *J* = 7 Hz), 1.42 (9 H, s), 1.1–1.3 (3 H, m), 2.77 (3 H, s), 3.71 (3 H, s), 4.05 (2 H, d, *J* = 6 Hz), 4.4 (1 H, m), 4.5 (1 H, m), 6.73 (1 H, br s), 7.37 (5 H, m), 8.3 (1 H, br s). Anal. ($C_{28}H_{42}N_4O_7$): C, H, N.

Boc-L-MeAla-L-Leu-Phe[(*E*)Δ]-Gly-OMe (**14b**): R_f (EtOAc) 0.81; UV λ_{max} 282 (ε 9080); NMR ($CDCl_3$) δ 0.92 (6 H, m), 1.36 (3 H, d, *J* = 7 Hz), 1.2–1.4 (3 H, m), 1.48 (9 H, s), 2.79 (3 H, s), 3.64 (3 H, s), 3.91 (2 H, d, *J* = 6 Hz), 4.50 (1 H, m), 4.56 (1 H, m), 6.29 (1 H, m), 6.67 (1 H, m), 7.34 (5 H, m), 7.90 (1 H, s). Anal. ($C_{28}H_{42}N_4O_7$): C, H, N.

N-Methylation of Ester 14a to Give 15a. The procedure reported earlier[24] gave **15a** in 89% yield. Traces of moisture retard the methylation. However, addition of 18-crown-6-ether (0.05 equiv) to potassium carbonate in either dimethylformamide or acetonitrile gave reproducible and comparable yields of product.

Registry No.—**1**, 28540-82-1; **6**, 64044-93-5; **8a**, 64044-94-6; **8b**, 64044-95-7; **12**, 64070-04-8; **13**, 64091-06-1; **14a**, 55478-19-8; **14b**, 55528-34-2; **15a**, 55478-20-1; **16a**, 55478-21-2; **17**, 64044-96-8; **19**, 64044-98-0; **26**, 64044-97-9; **27**, 64070-05-9; **28**, 64044-99-1; **29**, 64070-06-0; **30**, 64045-00-7; **31**, 64045-01-8; **22**, 64070-07-1; **33**, 55477-73-1; **34**, 64044-92-4; **35**, 64044-81-1; **39**, 64044-82-2; **41**, 64070-01-5; **42**, 64044-83-3; **43**, 64070-02-6; **45**, 64044-84-4; **46**, 64070-03-7; **47**, 64044-85-5; **48**, 64044-86-6; **49**, 64070-50-4; **50**, 64044-87-7; **51**, 64044-88-8; methyl-*N*-acetyl-2-*N*-methylamino cinnamate, 64044-89-9; *N*-acetyldehydrophenylalanine, 5469-45-4; methyl iodide, 74-88-4; benzyl mercaptan, 100-53-8; *tert*-butyloxycarbonyl azide, 1070-19-5; L-[2,3-^{3}H]alanine, 56877-49-7; Boc-L-[2,3-^{3}H]Ala, 64044-90-2; Boc-L-Ala, 15761-38-3; [^{13}C]methyl iodide, 4227-95-6; Boc-D-Ala, 7764-95-6; glycine methyl ester hydrochloride, 5680-79-5; Boc-L-Leu anhydride, 51499-91-3; Boc-Leu, 13139-15-6; Boc-MeAla, 16948-16-6; Boc-D-MeAla, 19914-38-6; Boc-L-Pro, 15761-39-4; Boc-D-Pro, 37784-17-1; ethyl *tert*-butyloxycarbonyl-*N*-methyl-3-benzylthiophenylalanylglycinate, 64044-91-3; ethyl *N*-*tert*-butyloxycarbonyl-L-leucyl-*N*-methyl-(*Z*)-dehydrophenylalanylglycinate, 64044-80-0.

References and Notes

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(2) All amino acids except glycine are of the L configuration unless noted. Standard abbreviations for amino acids, protecting groups, and peptides as recommended by the IUPAC–IUB Commission on Biochemical Nomenclature [*J. Biol. Chem.*, **247**, p 977 (1972)] are used. Additional abbreviations are: MePhe(*Z*Δ), *N*-methyl-(*Z*)-dehydrophenylalanine; MeAla, *N*-methylalanine; Boc, *tert*-butoxycarbonyl; MePhe(3-SBzl), *N*-methyl-3-*S*-benzylthiophenylalanine; MePhe[3-S(O)Bzl], *N*-methyl-3-benzylsulfinylphenylalanine; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TEA, triethylamine; TMG, tetramethylguanidine; Tcp, trichlorophenyl; DCU, dicyclohexylurea.

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