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Tyrosine Protecting Groups: Minimization of Rearrangement to 3-Alkyltyrosine during Acidolysis[1,2]

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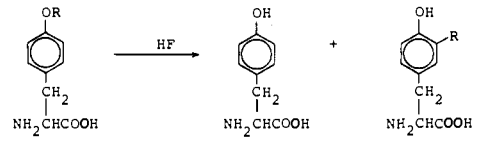
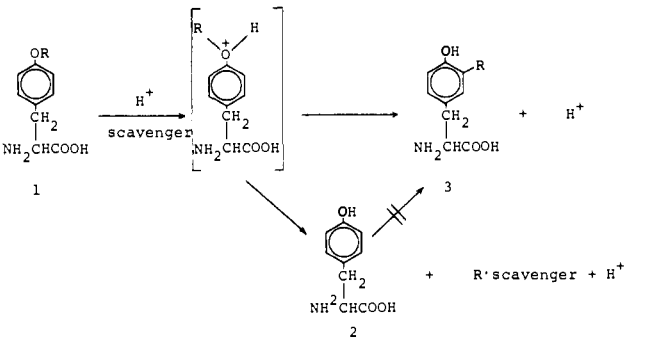
Abstract: In an effort to avoid the rearrangement of tyrosine ethers to 3-alkyl derivatives that occurs during acidolysis with hydrogen fluoride four new tyrosine protecting groups were designed and tested under conditions used in peptide synthesis. *O*-Isobornyltyrosine and *O*-[1-(5-pentamethylcyclopentadienyl)ethyl]tyrosine gave no rearrangement product. However, they were far too labile toward acidolysis in 50% TFA/CH_2Cl_2 to be used in the standard procedures of peptide synthesis, although they might be valuable in different schemes. The other two derivatives, *O*-isopropyltyrosine and *O*-cyclohexyltyrosine, both were sufficiently stable in acid and underwent minimal rearrangement. The cyclohexyl group was readily cleaved from Tyr(c-Hex) under standard cleavage conditions with HF (0 °C, 30 min) and is therefore an especially suitable protecting group for peptide synthesis. Its value was demonstrated by a synthesis of angiotensin II in which fully active hormone was obtained in high yield without the formation of significant amounts (<0.3%) of the cyclohexyltyrosyl rearrangement product.

Many side chain protecting groups used in peptide synthesis are cleaved in a final deprotection step by acidolysis. For the protection of the phenolic side chain of tyrosine several groups with such properties are available.[3] However, none is entirely satisfactory for prolonged stepwise peptide synthesis which is, for example, the usual strategy in solid-phase peptide synthesis.[4] Some of these protecting groups are too sensitive to acids[5] or to nucleophiles[6] and others undergo rearrangements during deprotection in strong acids to form 3-alkyltyrosine (**3**) by-products.[7]



Tyrosine benzyl ether (Tyr(Bzl)) has frequently been used in combination with N^{α}-Boc protection for stepwise syntheses employing selective acidic deprotection of the N^{α} group, after coupling of each Boc amino acid. The degree of selectivity of Boc removal by trifluoroacetic acid was greatly improved by the introduction of derivatives containing electron-withdrawing halogen substituents such as *m*-bromobenzyltyrosine[8] or 2,6-dichlorobenzyltyrosine.[7] The latter substitution also reduced the 3-alkylation side reaction in the final deprotection stage from levels of 10–70% to about 5%. While this level of rearrangement is tolerable for small peptides containing few tyrosines, it is unsatisfactory for large peptides with many tyrosine residues.

It is now well established that the acid-catalyzed rearrangement of aryl ethers to ring-substituted phenols proceeds via both intermolecular and intramolecular pathways.[9] To minimize or avoid the alkylation reaction several points must be considered. On the one hand it is possible to suppress the intermolecular pathway by scavengers such as anisole[7] or benzene[9] which compete with starting material and reaction products for the alkylating species. However, it is more difficult to interfere with the intramolecular pathway. Thus, the solvent



and the acid used for the cleavage reaction have only a small effect on the magnitude of the alkylation side reaction.[7,9] Data derived from the acidolysis of benzyltyrosine in trifluoroacetic acid or hydrogen fluoride suggest that the intramolecular pathway is less favored in the presence of HF.[7] However, the nature of the leaving group should have more influence on the intramolecular reaction. For example, benzyl phenyl ether is rapidly converted by aluminum bromide in chlorobenzene to a mixture of phenol and *o*-benzylphenol,[10] whereas *sec*-butyl phenyl ether is cleaved to both ortho and para products.[9,11] This indicates a dominant intramolecular pathway in the case of the cleavage of the benzyl ether whereas in the latter example both intra- and intermolecular reactions occur.[9,11] It is also expected that a group yielding upon acidolysis a cation that could rearrange to a neutral, nonalkylating product would have the desired properties:

$$\text{Tyr(R)} \xrightarrow{\text{HF}} \text{Tyr} + \text{R}^+ \rightarrow \text{R} + \text{H}^+$$

Finally, it should be noted that steric effects should also play a role on the ratio of the intra- and intermolecular reactions. These data suggest the suitability of alkyl ethers for the protection of the phenolic hydroxyl of tyrosine. This is supported by the fact that H-Tyr(Bu-*t*)-OH shows less than 0.05% rearrangement during HF acidolysis.[12] However, this protecting group is much too labile[5] to be used in prolonged stepwise

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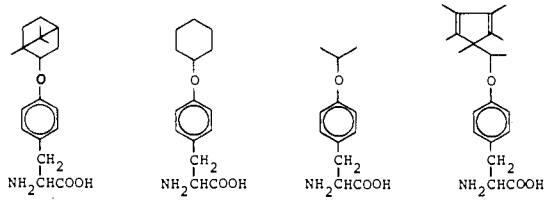
Table I. Product Distribution (%) after HF cleavage (30 min, 0 °C) of Tyrosine Ethers in the Presence and Absence of Cation Scavengers

Compd	HF[b]			HF/anisole[b]			HF/phenol		
	Tyr	Tyr[R]	3-R-Tyr	Tyr	Tyr[R]	3-R-Tyr	Tyr	Tyr[R]	3-R-Tyr
H-Tyr(*i*-Bor)	100	0	0	100	0	0			
H-Tyr(c-Ppe)	100[a]	0	0						
H-Tyr(c-Hex)	93.5	0	6.5	99.5	0	<0.5	97.6	0	2.4
H-Tyr(*i*-Pr)	96.0	0	4.0	96.5	0	3.5			

[a] Cleavage in 50% TFA/CH_3COOH. [b] The tyrosine-containing products were determined quantitatively in six separate cleavages. The averages were summed and the percent distribution was calculated. The range of each value was within ±0.3%.

peptide synthesis. *sec*-Alkyl ethers, on the other hand, were expected to have suitable stability.

Based on these considerations the following four compounds were synthesized and characterized. They are *O*-isobornyl-L-tyrosine (**4**), *O*-cyclohexyl-L-tyrosine (**5**), *O*-isopropyl-L-tyrosine (**6**), and *O*-[1-(5-pentamethylcyclopentadienyl)-



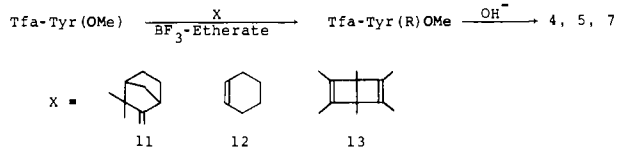
4 5 6 7

ethyl]-L-tyrosine (**7**). These four derivatives were chosen as representatives of secondary alkyl ethers, with an expected stability toward acidolysis increasing in the order **7** < **4** < **5** < **6**. The substituted cyclopentadiene group in **7**, after acidolysis to the cation **8**, should rapidly rearrange, via **9**, to the neutral hexamethylbenzene **10**[13] without attacking the aromatic ring of tyrosine.

8 9 10

Results and Discussion

A. Synthesis. The synthesis of **4, 5,** and **7** followed the same scheme. The reaction of the *N*-trifluoroacetyltyrosine methyl ester with boron trifluoride and either camphene (**11**), cyclo-



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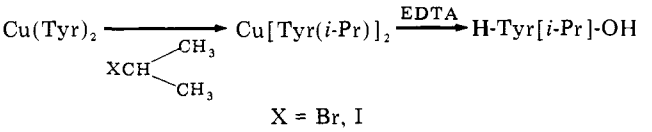
hexene (**12**), or hexamethyl(Dewar benzene) (**13**) in methylene chloride yielded a mixture of Tfa-Tyr(R)-OMe, Tfa-3-R-Tyr(R)-OMe, Tfa-3-R-Tyr-OMe, and unreacted starting material. After extraction of the reaction mixture with 1 N NaOH and evaporation of the solvents the resulting oils were purified by column chromatography or PLC[2] to yield pure Tfa-Tyr(R)-OMe. Saponification gave H-Tyr(R)-OH, and in the case of the cyclohexyl derivative reaction with $(Boc)_2O$ produced the corresponding Boc-Try(R)-OH in an overall yield of 40% from Tfa-Tyr-OMe.[14]

Formation of the cyclopentadiene derivative **7** from hexamethyl(Dewar benzene) (**13**) and Tfa-Tyr-OMe can be rationalized as proceeding by way of a BF_3-generated bicyclo[3.1.0]hexene cation.[15] The known competing isomerization to hexamethylbenzene[13] can account for the low yield of **7**.

Tyr(*i*-Pr) (**6**)[16] could be obtained in low yield via reaction of the copper complex of tyrosine with isopropyl bromide or iodide:



X = Br, I

The structures of all new compounds were confirmed by NMR spectra and elemental analyses as well as by the identification of tyrosine after acid cleavage of the protecting groups.

B. Characterization. To test the suitability of these four compounds (**4, 5, 6,** and **7**) to synthetic strategies involving final HF deprotection of the assembled peptide, they were treated with hydrogen fluoride under various conditions. To assess their stability in prolonged stepwise synthesis involving repeated acidolytic cleavage of the N^{α} protecting group the apparent first-order constants for loss of the phenolic protecting group in 50% TFA/CH_2Cl_2 were determined. The stability of the derivatives toward nucleophiles such as diisopropylethylamine, benzylamine, and thiophenol was also established. Finally, it was shown that there was no racemization during synthesis of the tyrosine derivatives.

1. Treatment with HF and 5.3 N HBr/CH_3COOH. As shown in Table I all the ethers were readily cleaved by treatment with HF at 0 °C for 30 min. As predicted, rearrangement to 3-alkyl derivatives was reduced. In the case of Tyr(*i*-Bor) and Tyr(c-Ppe) no rearrangement was observed even in the absence of the scavenger anisole. On the other hand, Tyr(*i*-Pr) and Tyr(c-Hex) gave rise to some 3-alkyltyrosine in neat HF, but its formation could be considerably reduced by adding the scavenger to the reaction mixture.

According to the literature no rearrangement of Tyr(Bzl) was observed following deprotection in concentrated HBr/CH_3COOH in the presence of anisol.[17,18] Therefore, we compared the removal of the cyclohexyl and the benzyl group under similar conditions with the aid of a sensitive chromatographic system (Table II). In our experiments the rearrangement products from both Tyr(Bzl) and Tyr(c-Hex) were readily detectable, although they were reduced (1 and 0.3%, respectively). The acidolysis of the benzyl ether in 5.3 N HBr/CH_3COOH was complete after 1 h, whereas in the case of Tyr(c-Hex) 1% of starting material was still present after 2 h.

2. Treatment with 50% TFA/CH_2Cl_2. Cleavage of the ether bond in the protected tyrosine derivatives by a large molar excess of 50% TFA/CH_2Cl_2 follows the rate law $kt = \ln [1 + (Y_t/X_t)]$, where k is the apparent first-order rate constant, X_t is the concentration of the protected amino acid remaining at time t, and Y_t is the concentration of tyrosine and 3-alkyltyrosine at the same time. The measured rate constants are given in Table III together with those of Tyr(Bzl) and Tyr(2,6-Cl_2Bzl), two derivatives of tyrosine often used in solid phase peptide synthesis. Also shown are the percent loss of protecting group per cycle of deprotection, based on a 20-min deprotection time. Tyr(*i*-Bor) and Tyr(c-Ppe) were much less stable than Tyr(Bzl) against acidolysis in 50% TFA. They were completely deprotected after only 5 min. Tyr(c-Hex) and Tyr(*i*-Pr) were

Table II. Product Distribution after Cleavage of Boc-Tyr(Bzl) and Boc-Tyr(c-Hex)·DCHA in 5.3 N HBr/CH_3COOH and Anisole at Room Temperature

Compd	Deprotection time, h	Product, % Tyr	Tyr(R)	3-R-Tyr
Boc-Tyr(Bzl)	1	99.3		0.7
	2	98.6		1.4
Boc-Tyr(c-Hex)·DCHA	1	89.5	10.3	0.25
	2	98.8	1.0	0.2
	4	99.7	≤0.01	0.3
	24	99.7	≤0.01	0.3

Table III. Apparent First-Order Rate Constants for the Reaction Tyr(R) + 50% $TFA/CH_2Cl_2 \rightarrow$ Tyr + R^+ and Loss of the Protecting Groups per Cycle

Compd	k_1, s^{-1}	k_{rel}	Loss per cycle,[b] %
Tyr(c-Ppe)	$\sim 1 \times 10^{-1}$	$\sim 1.5 \times 10^4$	100
Tyr(*i*-Bor)	$\sim 2 \times 10^{-2}$	$\sim 1.5 \times 10^3$	100
Tyr(Bz;)[a]	6.4×10^{-6}	1	0.77
Tyr(c-Hex)	5.1×10^{-8}	8×10^{-3}	0.006
Tyr(*i*-Pr)	1.5×10^{-9}	2.3×10^{-4}	0.000 18
Tyr(2,6-Cl_2Bzl)[a]	1.2×10^{-9}	1.9×10^{-4}	0.000 14

[a] From ref 7. [b] Based on a 20-min deprotection cycle.

not as stable as the recently developed *O*-(2,6-dichlorobenzyl)tyrosine,[7] but would still be suitable for the synthesis of a large peptide. For example, the synthesis of ribonuclease A, with 124 amino acid residues including six tyrosines, would require exposure of protected tyrosine to a total of 472 deprotection periods. This means that using the c-Hex group $[1 - 0.000\ 06]^{472} = 0.972 = 97.2\%$ of the molecules would still have all six tyrosine protecting groups intact, and only 2.8% of the chains would have one tyrosine residue deprotected.

3. Treatment with Nucleophiles. To determine the stability of the newly synthesized tyrosine ethers **4, 5,** and **6** toward nucleophiles sometimes used in peptide chemistry, they were treated with thiophenol, diisopropylethylamine, and benzylamine. Even after 24-h treatment with 12% solutions in CH_2Cl_2 of these agents no loss of protection could be detected.

4. Summary of Stability Data. From these data it is obvious that two derivatives, **4** and **7,** are an order of magnitude too labile to be used in an solid-phase peptide synthesis that is based on differential acid stability of α-amino, side chain, and C-terminal protecting groups. Although *O*-isopropyltyrosine has good acid stability, the rearrangement could not be suppressed sufficiently. Tyr(c-Hex) has the best combination of properties for use in the usual protocol of stepwise peptide synthesis. It has satisfactory stability in 50% TFA/CH_2Cl_2 and it is unaffected by nucleophiles; the cyclohexyl group is readily removed after 0.5 h in hydrogen fluoride, and, most importantly, the rearrangement to 3-alkyl derivatives is sufficiently suppressed.

C. Synthesis of Angiotensin II. To demonstrate the efficacy of *O*-cyclohexyltyrosine in an actual peptide synthesis, it was compared with the often used Tyr(2,6-Cl_2Bzl) in the solid-phase synthesis of the octapeptide hormone angiotensin II, H-Asp-Arg-Val-Tyr-Val-His-Pro-Phe-OH. Several solid-phase syntheses have been reported using Tyr(Bzl).[19] In these studies a by-product (13–20%) was found which lacked tyrosine but contained one residue of 3-benzyltyrosine.[19b] In the present study angiotensin II was assembled stepwise, but after Val^5 had been coupled to the peptide resin the sample was divided in half and the synthesis was continued separately using

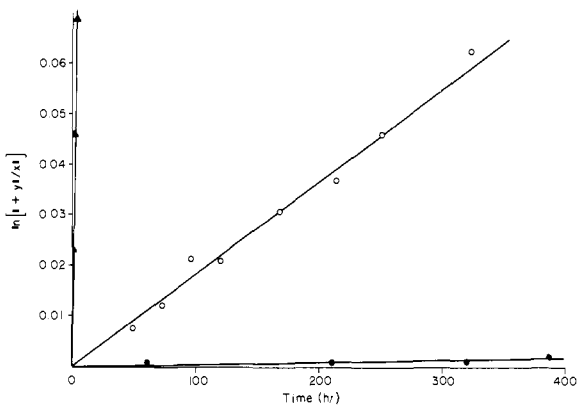


Figure 1. Plot for the apparent first-order loss of protecting groups in TFA/CH_2Cl_2 (v/v 1:1) at 20 °C: (○) Tyr(c-Hex); (●) Tyr(i-Pr); (▲) Tyr(Bzl). Data for Tyr(Bzl) from ref 7.

Tyr(c-Hex) for run A and Tyr(2,6-Cl_2Bzl) for run B. After completion of the synthesis the protected peptides were cleaved from the resin (HF/10% anisole, 0 °C, 0.5 h). An amino acid analysis of the crude peptide mixture showed in the case of run A less than 0.3% of 3-cyclohexyltyrosine, whereas in the case of run B 2.5% of 3-(2,6-dichlorobenzyl)tyrosine was found. The products were then purified on a 0.9 × 13 cm Aminex column using 1 N pyridine acetate buffer, pH 5.2. In both runs pure angiotensin II with full biological activity and devoid of the rearrangement product was isolated in high yield from the main peak. The octapeptides containing 3-substituted tyrosine derivatives were isolated from a slow-moving peak.

Experimental Section

Instrumentation. Melting points were taken on a Thomas-Hoover capillary melting point apparatus. They are uncorrected. Nuclear magnetic resonance (NMR) spectra were observed with a Varian A-60 spectrometer. Amino acid and peptide analyses were conducted with Beckman Model 120B or 121 amino acid analyzers. The optical rotation was measured on a Cary CD spectrometer. Elemental analysis was performed by Mr. S. T. Bella of the Microanalytical Laboratory, The Rockefeller University.

Materials. All solvents and bulk chemicals were reagent grade. Dichloromethane was distilled from sodium carbonate and stored in amber bottles. The HBr/CH_3COOH solution was prepared by bubbling hydrogen bromide (Matheson) through a solution of resorcinol in trifluoroacetic acid and then through cooled acetic acid. The normality was determined by Volhard titration. Diisopropylethylamine (Aldrich Chemical Co., bp 126–129 °C) was distilled from sodium hydride. The borate buffer (4 L) contained 12 g of boric acid, 8 g of NaOH, and 35 g of NaCl. The pH was adjusted by adding 10 N NaOH (3–5 drops). Peptide chromatography was performed on a 0.9 × 13 cm column of Aminex 50 W-X4 (Bio-Rad Laboratories) resin with pyridine/acetate buffer at a flow rate of 46 mL/h at room temperature. The buffer was 1 M in pyridine. The pH was adjusted by adding acetic acid to the pyridine-water solution. Thin layer chromatograms (TLC) were run on precoated silica gel GF plates (Analtech, 250 μ). Preparative layer chromatography (PLC) was performed using precoated silica gel GF plates (Analtech, 2000 μ) or 30 × 30 × 0.5 cm plates[20] prepared with silica gel PF-254 containing $CaSO_4$ (Brinkmann Instruments). The following solvents were used for thin layer and preparative layer chromatography (in parts by volume): I, chloroform; II, chloroform (50)/hexane (50); III, chloroform (85)/methanol (10)/acetic acid (5); IV, 1-butanol (8)/acetic acid (1)/water (1). Amino acids were visualized with a ninhydrin or tolidine spray. Trifluoroacetyltyrosine methyl ester (Tfa-Tyr-OMe) was prepared by conventional procedures.[21] For the retention times of the amino acids on ion exchange columns see Table IV.

Kinetic Experiments. The protected amino acid (12 μmol) was dissolved in 50 mL of 50% TFA/CH_2Cl_2 and kept at 20 °C. At certain intervals 2-mL aliquots were taken, freed of the solvent by a gentle stream of nitrogen, and dissolved in 2 mL of citrate buffer pH 2.2 within 2 min. The buffer solution (1 mL) was applied to the column

Table IV. Conditions for Amino Acid Analysis

Tyrosine derivative	Column temp, °C	Retention time, min		
		Tyr(R)	3-(R)Tyr	Tyr
H-Tyr(*i*-Bor)-OH[a]	75	42		15
H-Tyr(c-Hex)-OH[b]	57	103	76	21
H-Tyr(*i*-Pr)-OH[c]	57	94	81	52
H-Tyr(Bzl)-OH[b]	86	82	44	18

[a] 0.9 × 35 cm column of sulfonated polystyrene (Durrum DC-6A), borate buffer; pH 10.0; 70 mL/h. [b] 0.9 × 10.5 cm column of sulfonated polystyrene (Beckman PA-35), citrate buffer, pH 6.4; 60 mL/h. [c] 0.9 × 58 cm column of sulfonated polystyrene (Beckman AA-15), citrate buffer, pH 7.0; 60 mL/h.

to analyze for free, rearranged, and protected amino acid. In the case of Tyr(c-Ppe) 3 μmol was dissolved in 1 mL of 50% TFA/CH_2Cl_2. From this solution 50-μL samples were analyzed on a TLC plate with fluorescence indicator and developed in solvent system IV.

Deprotection in HF. The cleavage of the side chain protected amino acid to the free amino acid, and resin bound angiotensin II to the unprotected peptide, was carried out in a fluorocarbon vessel. The deprotection was done either in the presence or absence of 10% anisole at 0 °C for 0.5 h. After this time the HF was evaporated under water aspiration. Finally, traces of HF were removed under high vacuum. The anisole was dissolved in dry ether and decanted. The deprotected amino acids were taken into TFA (0.2 mL) and diluted to 2 mL with citrate buffer pH 2.2. Angiotensin was dissolved in 10% acetic acid/water and lyophilized.

Deprotection in 5.3 N HBr/CH_3COOH. Boc-Tyr(c-Hex)-DCHA (100 mg) was weighed into a 100-mL flask and 10 mL of 5.3 N HBr/CH_3COOH and 1 mL of anisole were added. The mixture was stirred at room temperature. At intervals of 1–24 h, 2-mL samples were removed and evaporated to dryness. The residue was evaporated four times from CH_2Cl_2. It was dissolved in water, adjusted to pH 12–13 with 1 N NaOH and allowed to stand overnight to hydrolyze any *O*-acetyltyrosine. The solution was then brought to pH 2 with 1 N HCl, diluted to 20 mL with citrate buffer, pH 2.2, and run on the 120B analyzer. Boc-Tyr(Bzl) was treated similarly.

Stability against Nucleophiles. A solution of the side chain protected amino acid (2.5 mM) and the nucleophile (12%) (diisopropylethylamine, benzylamine, or thiophenol) in CH_2Cl_2 was kept at room temperature for up to 60 h. An aliquot was taken, freed of solvent, and analyzed for free amino acid.

***O*-(Cyclohexyl)-L-tyrosine.** A suspension of 20 g (0.069 mol) of Tfa-L-Tyr-OMe in 100 mL of CH_2Cl_2 and 50 mL of cyclohexene (Aldrich Chemical Co., Inc.) and 2 mL of boron trifluoride etherate (0.49 mol) (Aldrich Chemical Co., Inc.) was heated at reflux for 24 h to give a clear brown solution. After 12 h an additional 25 mL of cyclohexene and 1 mL of boron trifluoride etherate were added. The progress of the reaction was followed by TLC (solvent system I) where four spots could be observed: (1) unreacted starting material (R_f 0.03); (2) Tfa-3-(c-Hex)-Tyr-OMe (R_f 0.1); (3) Tfa-Tyr(c-Hex)-OMe (R_f 0.23); (4) Tfa-3-(c-Hex)Tyr(C-Hex)-OMe (R_f 0.32).

The solution was extracted with 1 N NaOH, 1 N HCl, and with water (three times each). The organic layer was dried over magnesium sulfate and the solvent was evaporated to yield a viscous, yellow oil. The mixture was diluted with 30 mL of $CHCl_3$ and applied to a dry chromatography column[22] (silica gel Woelm for dry column chromatography, ICN Pharmaceuticals) (5 × 56 cm, solvent system II). The purification gave three fractions. The first fraction contained Tfa-3-(c-Hex)Tyr(c-Hex)-OMe (1.3 g), whereas fraction 3 consisted of pure Tfa-Tyr(c-Hex)-OMe (yellow oil, 5.3 g). Fraction 2 was a mixture of both derivatives, and was purified further (twice) by dry column chromatography to give another two crops of 6.4 and 3.5 g of pure Tfa-Tyr(c-Hex)-OMe. The combined yield was 59%. [NMR ($CDCl_3$) δ 6.88 (4 H, CH aromatic, m), 4.97 (1 H, $C^{\alpha}H$, q), 4.37 (1 H, CH cyclohexyl), 3.95 (3 H, CH_3, s), 3.34 (2 H, $C^{\beta}H_2$, d), 2.3–1.3 (10 H, CH_2 cyclohexyl, m)]. From the previous two runs another 1.5 g of a yellow oil consisting of Tfa-3-(c-Hex)Tyr(c-Hex)-OMe was obtained [NMR ($CDCl_3$) δ 6.86 (3 H, aromatic, m), 4.86 (1 H, $C^{\alpha}H$, q), 4.21 (1 H, CH cyclohexyl, m), 3.85 (3 H, CH_3, s), 3.20 (2 H, $C^{\beta}H_2$, d), 3.00 (1, CH cyclohexyl, m), 2.2–1.1 (20 H, CH_2 cyclohexyl, m)].

Hydrolysis of Tfa-Tyr(c-Hex)-OMe. Tfa-Tyr(c-Hex)-OMe (9.34 g, 0.025 mol) was dissolved in 130 mL of 0.4 N NaOH and 130 mL of tetrahydrofuran (THF) and stirred for 12 h at room temperature. After 6 h another 60 mL of THF and 60 mL of 0.4 N NaOH were added. The mixture was brought to pH 5–6 with 1 N HCl and evaporated. The precipitate was recrystallized from 30% THF/H_2O, washed thoroughly with water, and dried to yield 5.5 g (84% yield) of H-Tyr(c-Hex)-OH, $[\alpha]^{20}_D$ +5° (*c* 1, CH_3COOH), mp 195 °C dec, R_f 0.69 (solvent system IV).

Anal. ($C_{15}H_{21}NO_3$) C, H, N.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*O*-(cyclohexyl)-L-tyrosine.** Boc-Tyr(c-Hex)-OH was prepared by the reaction of H-Tyr(c-Hex)-OH (7.1 g, 0.027 mol) with *O,O'*-di-*tert*-butylcarbonic acid anhydride (di-*tert*-butyl dicarbonate, Fluka AG). The procedure of Moroder[14] was followed except that 1.5 equiv of the anhydride was used. The reaction time was 12 h. The compound was isolated as the DCHA salt and recrystallized from hexane (yield 11.8 g, 80%, mp 142 °C, $[\alpha]^{20}_D$ 36.5° (*c* 1, CH_3OH)). A similar yield was obtained without isolating H-Tyr(c-Hex)-OH.

Anal. ($C_{32}H_{52}N_2O_5$) C, H, N.

To test the optical purity of Boc-Tyr(c-Hex)-OH it was deprotected in HF and coupled with Boc-Glu(OBu-*t*)-OSu.[23,24] The solvent-freed reaction mixture was treated for 1 h in trifluoroacetic acid. The acid was evaporated and the crude mixture was submitted to an ion exchange column to separate L-Glu-L-Tyr from its enantiomer. No L-Glu-D-Tyr (<0.1%) could be detected.

3-(c-Hex)Tyr-OH. Tfa-Tyr(c-Hex)-OMe (1 g, 2.67 mmol) was dissolved in 3 mL of dichloromethane and 1 mL (8 mmol) of boron trifluoride etherate and heated at reflux for 3 h. The reaction mixture was applied directly to two thick layer plates (Analtech, 2000 μ, 20 × 20 cm) and eluted with solvent system III to give 0.324 g of a yellow oil (32.4%) [NMR ($CDCl_3$) δ 6.9 (3 H, CH aromatic, m), 5.08 (1 H, $C^{\alpha}H$, q), 4.06 (3 H, CH_3, s), 3.34 (2 H, $C^{\beta}H_2$, d), 3.1 (1 H, CH cyclohexyl, m), 2.4–1.5 (10 H, CH_2 cyclohexyl, m). The oil was saponified in 0.2 N NaOH in water/THF (1:1) and precipitated by adding a few drops of 1 N HCl (yield 0.19 g, 85%, mp 197 °C). Identical material could be obtained from the sodium hydroxide extraction of the previous experiment.

Anal. ($C_{15}H_{21}NO_3$) C, H, N.

H-Tyr(*i*-Bor)-OH. Treatment of Tfa-Tyr-OMe (1 g, 3.4 mmol) and 0.45 g (3.3 mmol) of camphene (Aldrich Chemical Co., Inc.) in 10 mL of CH_2Cl_2 at 4 °C for 1 h with 0.25 mL (2.0 mmol) of boron trifluoride etherate resulted in an 85% yield of Tfa-Tyr(*i*-Bor)-OMe. Less than 1% of the ring-substituted product was observed by TLC. The reaction mixture was extracted with 1 N NaOH, 1 N HCl, and water (three times each). The organic layer was dried over $MgSO_4$, the solvent was evaporated, and the product was purified by thick layer chromatography (Analtech, 2000 μ, 20 × 20 cm) (50% $CHCl_3$/hexane (v/v)). The remaining oil (1.2 g, 83%) [^{1}H NMR ($CDCl_3$) δ 6.88 (4 H, CH aromatic, m), 4.87 (1 H, $C^{\alpha}H$, q), 4.05 (1 H, CH, *i*-Bor, m), 3.80 (3 H, CH_3, s), 3.17 (2 H, $C^{\beta}H_2$, d), 1.95–0.8 (16 H, H-*i*-Bor s and m)] was hydrolyzed in 0.4 N NaOH/THF (1:1 v/v) for 2 h at room temperature. The solution was brought to pH 5–6 with 1 N HCl and the gel-like precipitate was collected and recrystallized from THF/water (1:1 v/v) and washed with water, yield 0.76 g, 85%, $[\alpha]^{24}_D$ −6° (*c* 1, CH_3COOH), mp 185 °C dec, R_f 0.75 (solvent system IV).

Anal. ($C_{19}H_{27}NO_3$) C, H, N.

H-Tyr(c-Ppe)-OH. Tfa-Tyr-OMe (0.300 g, 1.0 mmol), 1 mL (5.0 mmol) of hexamethyl(Dewar benzene) (Aldrich Chemical Co., Inc.), and 2 mL of CH_2Cl_2 were treated with 0.2 mL (1.6 mmol) of boron trifluoride etherate. With a delay time of ca. 10 min a vigorous reaction occurred, to produce mainly hexamethylbenzene. The reaction mixture was worked up as usual. Final purification by thick layer chromatography (Analtech, 2000 μ, 20 × 20 cm, solvent system II)

Table V

	Asp (1)	Arg (1)	Val (2)	Tyr (1)	His (1)	Pro (1)	Phe (1)
Run A found:	1.15	0.99	1.95	0.97	0.96	1.02	0.96
Run B	1.13	0.97	1.96	0.97	0.95	1.02	0.97

gave 10 mg of Tfa-Tyr(c-Ppe)-OMe as a yellow oil [^{1}H NMR ($CDCl_3$) δ 6.8 (4 H, CH aromatic, m), 3.75 (3 H, CH_3, s), 3.10 (2 H, $C^\beta H_2$, d), 2.21 (3 H, CH_3 ethane, d), 2.05, 1.98, and 1.92 (3 × 3 H, 3-CH_3 cyclopentadiene, s), 1.15 (2 × 3 H, 2-CH_3 cyclopentadiene, s)]. Saponification by 1:1 0.4 N NaOH/THF (v/v), and recrystallization from 1:2 water/THF (v/v) yielded 5 mg of H-Tyr(c-Ppe)-OH. After treatment for 1 h with 50% TFA/CH_2Cl_2, tyrosine was recovered and quantitated by amino acid analysis.

H-Tyr(i-Pr)-OH. Tyrosine (10 g, 0.055 mol) was dissolved in 56 mL of 2 N NaOH. To this solution 6.4 g (0.082 mol) of $CuSO_4 \cdot H_2O$ in 28 mL of water was added. The mixture was warmed to 50 °C for 1 h and then cooled to room temperature. After 20 g (0.16 mol) of isopropyl bromide was added the solution was kept for 11 days at room temperature. A blue precipitate was formed which was isolated and washed with methanol/water (3:1), methanol, and acetone. To destroy the copper complex the precipitate and 0.4 g of EDTA were suspended in 100 mL of EtOH/H_2O (1:1). The suspension was briefly brought to the boiling point whereupon most of the solid material went into solution. It was filtered and the solution was kept for 2 days at 4 °C. A light blue precipitate was formed which was recrystallized from EtOH/H_2O (1:1), yield 0.47 g (4%), mp 195 °C dec [NMR (TFA) δ 6.69 (4 H, CH aromatic, q), 2.98 (2 H, $C^\beta H_2$, d), 0.94 (6 H, 2 CH_3, d)].

Anal. ($C_{12}H_{17}NO_3$) C, H, N.

[Val^5]-Angiotensin II. The peptide was prepared by the solid-phase method.[4] Boc-Phe-OCH_2-polystyrene (LAB system, 1% DVB, 200–400 mesh, 2.8 g, 0.25 mmol Phe/g resin) was converted into the tetrapeptide resin Val-His-Pro-Phe-Resin by successive addition of the three amino acids. Here the peptide resin was divided into two parts and the synthesis was carried on either with Boc-Tyr(c-Hex)-OH (peptide A) or Boc-Tyr(2,6-Cl_2Bzl)-OH (peptide B). The side chain protecting groups were 4-toluenesulfonyl for arginine and histidine, benzyl for aspartic acid, and 2,6-dichlorobenzyl or cyclohexyl for tyrosine. The protocol for one synthetic cycle was (1) deprotection with 50% TFA/CH_2Cl_2 (v/v) for 5 and 20 min; (2) neutralization two times 5 min with 5% diisopropylethylamine/CH_2Cl_2 (v/v); (3) addition of the Boc amino acid (4 equiv) and shaking for 10 min; (4) addition of DCC and shaking for 2 h; (5) coupling steps (2, 3, and 4) were repeated once. After all eight amino acids were assembled on the resin, the octapeptide, Asp-Arg-Val-Tyr-Val-His-Pro-Phe, was cleaved from the resin (0.50 g, 125 μmol Phe) in HF–anisole (0.5 h and 0 °C), extracted with 10% acetic acid, and lyophilized. Finally, the peptide was purified on an Aminex 50W-X2 column (0.9 × 13 cm) with pyridine/acetate buffer (1.0 M, pH 5.2, flow rate 46 mL/h); retention times 169 min for angiotensin II and 217 min for the c-Hex derivative. Yield 81 mg, 63% from run A; 76 mg, 59% from run B. Amino acid analyses are given in Table V.

Both peptides had full pressor activity.[26] The assay method used was that of Pickens et al.[25]

References and Notes

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(2) Abbreviations used: EDTA, ethylenediaminetetraacetic acid; TFA, trifluoroacetic acid; THF, tetrahydrofuran; c-Hex, cyclohexyl; *i*-Bor, isobornyl; *i*-Pr, isopropyl; c-Ppe, pentamethylcyclopentadienylethyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DCHA, dicyclohexylamine; NMR, nuclear magnetic resonance; TLC; thin layer chromatography; PLC, preparative layer chromatography. Other nomenclature and symbols follow the Tentative Rules of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **241**, 2491 (1966); **242**, 555 (1967); **247**, 977 (1972).

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