Expedited Articles

Derivatives of a Novel Cyclopeptolide. 1. Synthesis, Antifungal Activity, and Structure–Activity Relationships

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Received August 10, 1993®

The synthesis of a series of derivatives of the novel antifungal cyclopeptolide 1, which consists of nine *S*-amino acids and *R*-lactic acid, is described. Besides functional group variation of MeAsp[4] (esters **2a–d**, amides **3a–d**, alcohol **4**, and its derivatives) and Tyr(Me)[9] (demethyl derivative **8**, ethers **12a–f**, **13**, and oxidative degradation of the phenyl group to **14**), opening of the lactone by LiOH in THF/H_2O allowed manipulation of the hydroxy group of *R*-Hypr[10] in the resulting acyclic peptide **15**. Recyclization of **15** under Mitsunobu conditions followed by deprotection led to the *S*-Hypr[10] analogue **17** of **1**. Cyclic decapeptides **33** and **34** as well as cyclic undecapeptides **35** and **36** were obtained via the corresponding modified linear peptides **23**, **24**, **27**, and **28** by cyclization. Methylation of all secondary amide groups by CH_3I and KH/18crown6 gave the permethylated compound **37**. Two of the derivatives (**17** and **34**) showed superior activities against yeasts in vitro at pH 6.5 as compared to **1**, but not at a lower pH (4.5).

Introduction

The cyclopeptolide **1** (Figure 1) was isolated from the fermentation broth of an imperfect fungus (*Septoria* sp., NRRL 15761) due to its antifungal activity.[1] It is composed of nine *S*-amino acids and *R*-lactic acid. The structure was determined by X-ray analysis of its bromoanilide derivative[2] **2**, and the NMR spectra in $CDCl_3$ were fully analyzed by 1D and 2D spectroscopy.[3] Compound **1** was found to be active against yeasts and yeast-like fungi, but not against filamentous fungi. The activity against yeasts was confirmed in several experimental infections of rodents with *Candida albicans*. Interestingly, the compound was effective not only after topical or intraperitoneal application but also when given orally. A series of derivatives of **1** was prepared with the aim to improve the antifungal activity.

The structure of **1** was modified mainly at three sites: amino acid 4 (MeAsp), amino acid 9 (TyrMe), and hydroxy acid 10 (*R*-Hypr). In addition, a few analogues were prepared in which *R*-Hypr was replaced by a dipeptide. The chemistry leading to the derivatives starting from **1**, as well as antifungal activities of the new compounds, is described.

Cyclopeptolide **1** has a remote similarity to the immunosuppressive undecapeptide Cyclosporin A (CyA, Sandimmun). Since Cyclosporin A was reported[4] to decrease the anticancer drug resistance of multidrug-resistant (MDR) cells, all derivatives of **1** were also tested routinely for resistance modulation. Several derivatives were indeed found to be potent chemosensitizers of tumor cells whose multidrug resistance is due to the P-glycoprotein mediated drug efflux. These activities could also be confirmed with one compound (SDZ 280-446, compound **16**) *in vivo*.[5] The results on multidrug resistance modulation are presented and discussed in the accompanying publication.[6]

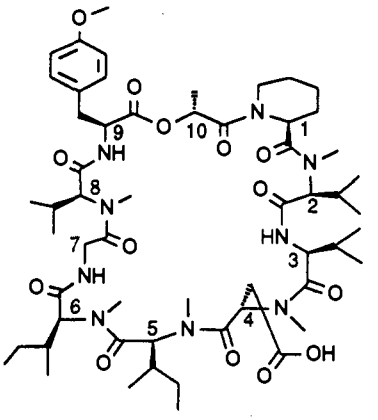

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Figure 1. Structure of cyclopeptolide **1** and numbering of amino acids.

Chemistry

Modification of MeAsp[4]. The β-carboxyl group of MeAsp[4] of cyclopeptolide **1** was easily esterified or converted into an amide (Scheme 1). Depending on the alcohol component, three different methods (A, B, C) were used for esterification of **1**. Treatment of **1** with diazomethane in diethyl ether led to the methyl ester **2a** (method A). The allyl ester **2b** was obtained by reacting **1** with allyl bromide in acetone in the presence of potassium carbonate (method B). All other esters were prepared by treating **1** with the appropriate dimethylformamide dialkyl acetal in toluene at 80 °C (method C). For amide formation the carboxyl group was activated with the Vilsmeier–Haack reagent (dimethylformamide imide chloride obtained from DMF and oxalyl chloride) and the resulting intermediate reacted with different amines to give compounds **3a–d**. Treatment of **1** with the Vilsmeier–Haack reagent followed by sodium borohydride[7] resulted in derivative **4**, wherein MeAsp[4] is reduced to MehSer. The hydroxy group of the methylhomoserine residue in **4** could be esterified (com-

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® Abstract published in *Advance ACS Abstracts*, June 1, 1994.

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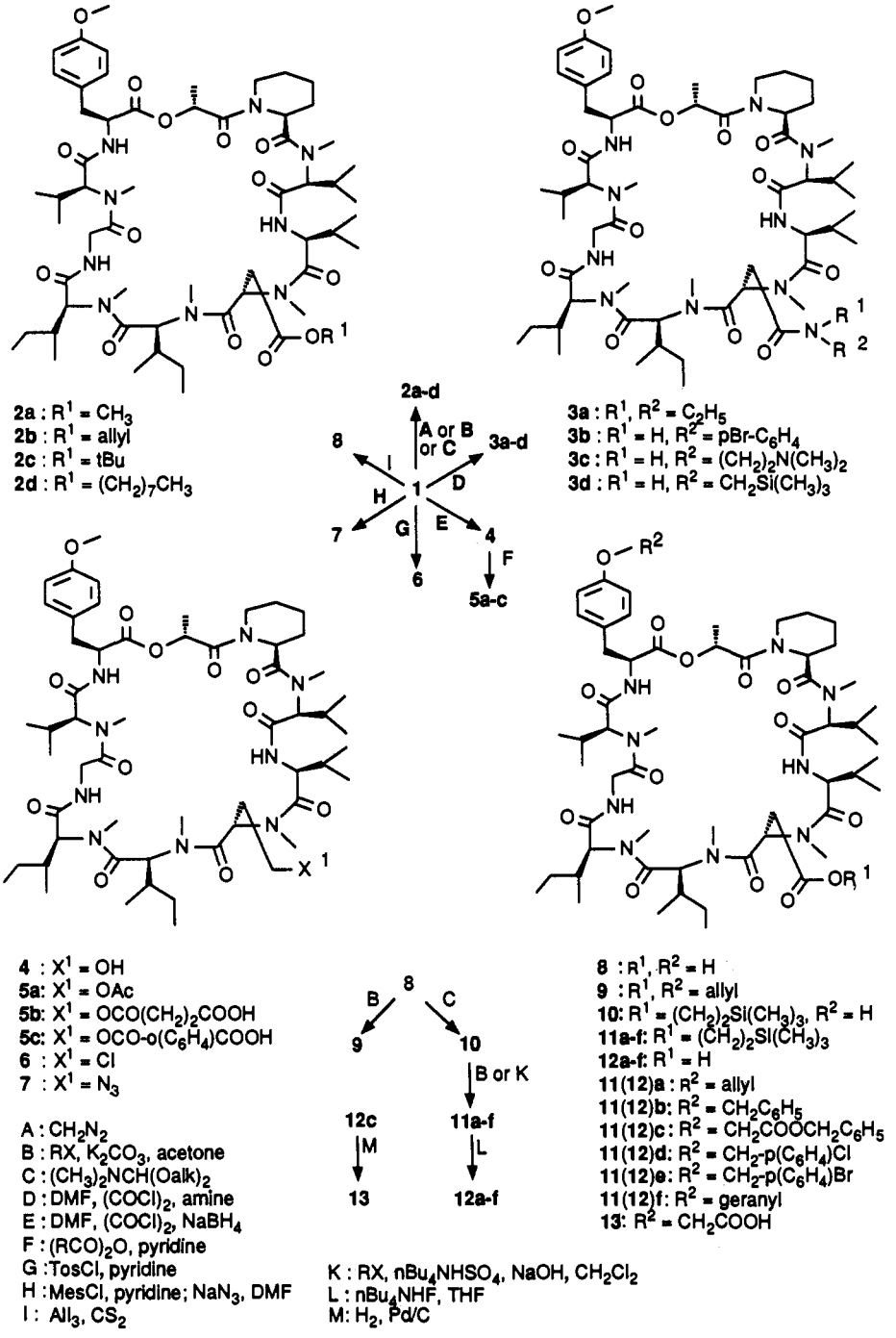
Scheme 1. Modifications at MetAsp[4] and Tyr(Me)[10]



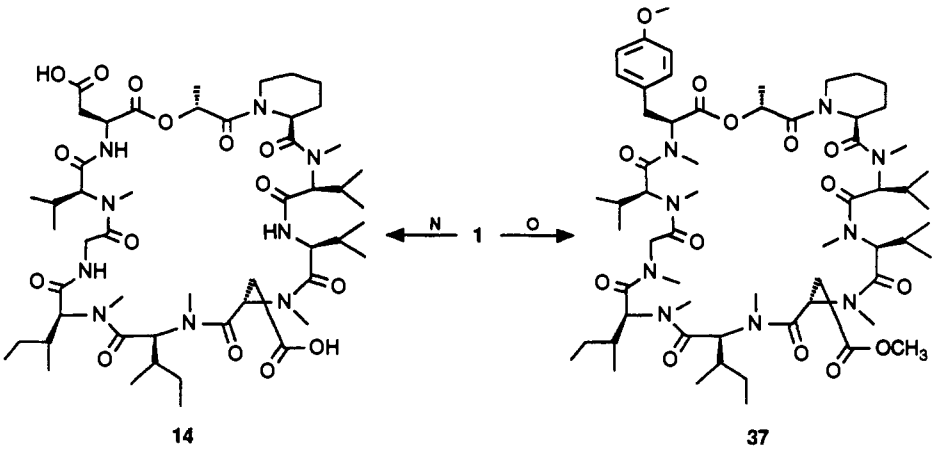
pounds **5a–c**) or converted to the chloride **6** and the azide derivative **7** by standard procedures (Scheme 1).

Modification of Tyr(Me)[9]. The methyl ether of the *O*-methyltyrosine residue in **1** could be cleaved smoothly with freshly prepared aluminum iodide in carbon disulfide[8] to yield the tyrosine derivative **8**. Treatment of **8** with allyl bromide in acetone in the presence of potassium carbonate led to alkylation of both the phenol and the carboxyl groups producing compound **9**. For selective alkylation of the phenol group in compound **8**, the free carboxyl group of the *N*-methylaspartic acid residue had to be protected as an easily removable ester prior to alkylation. The (trimethylsilyl)ethyl ester **10**, which was prepared from compound **8** with DMF bis(trimethylsilylethyl) acetal applying method C, proved to be appropriate. **10** could be alkylated with reactive halides using potassium carbonate in acetone or under phase-transfer conditions ($NaOH/CH_2Cl_2$, nBu_4NHSO_4) to give compounds **11a–f**, the deprotection of which with tetrabutylammonium fluoride in THF resulted in Tyr[9]-*O*-alkyl derivatives **12a–f** with free MeAsp[4]. The free diacid **13** was obtained from the *O*-benzyl derivative **12c** by hydrogenolysis (Scheme 1).

Transformation of Tyr(Me)[9] into Asp[9]. The aromatic ring system of the *N*-methyltyrosine residue in compound **1** was selectively oxidized with ruthenium dioxide/sodium periodate,[9] yielding compound **14** (Scheme 2), wherein Tyr(Me)[9] is replaced by Asp.

Transformation of *R*-Hypr[10] into *S*-Hypr[10]. The β-carboxyl group of Me-Asp[4] had to be suitably protected in order to allow selective manipulation of the linear peptide, which in turn might be obtained through hydrolytic cleavage of the lactone under basic conditions. The *tert*-butyl ester **2c**, obtained from **1** under neutral

Scheme 2. Oxidative Degradation of the Aromatic Ring of Tyr(Me)[9] and (Amide)-N-permethylation of 1



N : RuO_2, $NaIO_4$; O : KH, 18crown 6, MeI.

conditions with dimethylformamide di-*tert*-butyl acetal[10] according to method C, turned out to be stable enough to permit selective lactone cleavage with lithium hydroxide. Other procedures such as the Mukaiyama[11] protocol or the method described by Stadler[12] gave less satisfactory results. Thus, cleavage of the lactone **2c** proceeded smoothly with 1.5 equiv of lithium hydroxide in aqueous THF resulting in the linear peptide **15** which was used without further purification in the following step. In order to re-macrolactonize with inversion of configuration[13] at the α-position of the lactic acid residue, compound **15** was reacted with triphenylphosphine and diethyl azodicarboxylate (DEAD) in high dilution (~1 mM) to give cyclic compound **16** in 67% yield. Deprotection of the β-carboxyl group with trifluoroacetic acid ultimately led to the *S*-Hypr[10] analogue **17** (Scheme 3).

Replacement of *R*-Hypr[10] by *R*-Ala[10], *S*-Ala[10], *R*-Ala[10]*R*-Ala[11], or *R*-Ala[10]*S*-Ala[11]. For further manipulation of the hydroxy group of the lactic acid residue in compound **15**, the terminal carboxyl group was protected as benzyl ester to allow for selective deprotection in the presence of the Asp-*tert*-butyl ester in a later step. The linear peptide **18** (Scheme 3) was converted into the azide derivative **20** via the mesylate **19** using sodium azide in dimethylformamide (DMF). On the other hand, the azide could also be introduced starting from **18** with double inversion (resulting in net retention of configuration) in two steps: (1) tosylation under Mitsunobu conditions (triphenylphosphine/DEAD/zinc tosylate);[14] (2) displacement by azide (sodium azide/DMF). Catalytic hydrogenation of the epimeric azides **20** and **22** proceeded with concomitant removal of the benzyl (ester) groups to give amines **23** and **24**. Chain extension by attachment of an *R*-alanine residue on the N-terminus of peptides **23** and **24** using the *p*-nitrophenyl ester of N-protected *R*-alanine led to derivatives **25** and **26**, which upon hydrogenolytic deprotection afforded the linear peptides **27** and **28**. Macrocyclization of compounds **23**, **24**, **27**, and **28** was brought about with dicyclohexylcarbodiimide/pentafluorophenol in dichloromethane under high dilution leading to the cyclic analogues **29–32** in satisfactory yields (37–59%). It may be noted that the length of the peptide chain did not have much influence on the efficacy of the macrocyclization. Deprotection with trifluoroacetic acid resulted in the final products **33–36** (Scheme 4).

Transformation of Val[3]Gly[7]Tyr(Me)[9] into MeVal[3]-Sar[7]MeTyr(Me)[9]. In addition to the β-carboxylic acid function of the *N*-methylaspartic acid residue, the nitrogen of all secondary amide groups could be methylated using methyl iodide/potassium hydride/18crown6/THF to give the permethylated compound **37** (Scheme 2).

Biological Tests

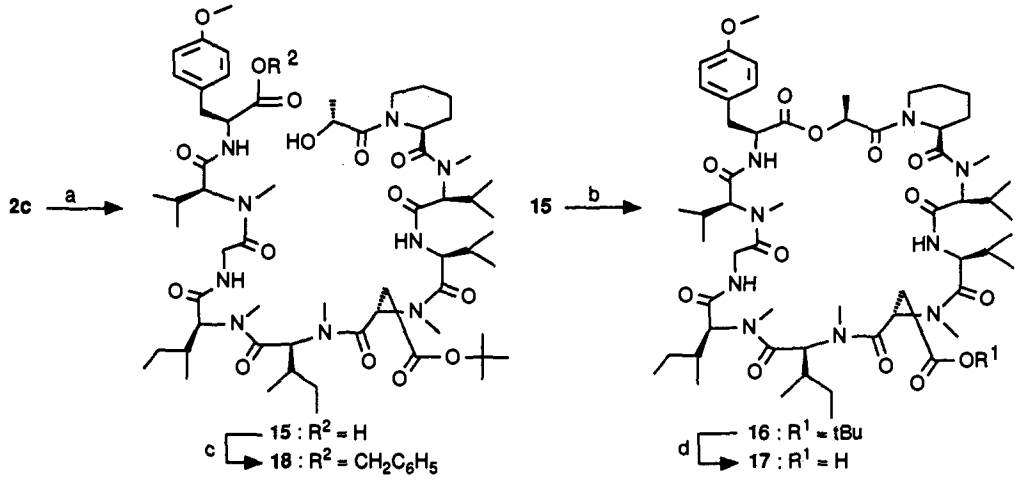
Test Organisms. Test cells of *Candida albicans* (strains no. 3, 4, 9, 26, 88, 90, 124), *Candida guillermondii* (no. 80), *Candida krusei* (no. 132), and *Candida tropicalis* (no. 43) were used. The cultures grown in Sabouraud's glucose broth had been stored in liquid nitrogen[15] and were used immediately after thawing at appropriate dilutions for *in vitro* and *in vivo* studies.

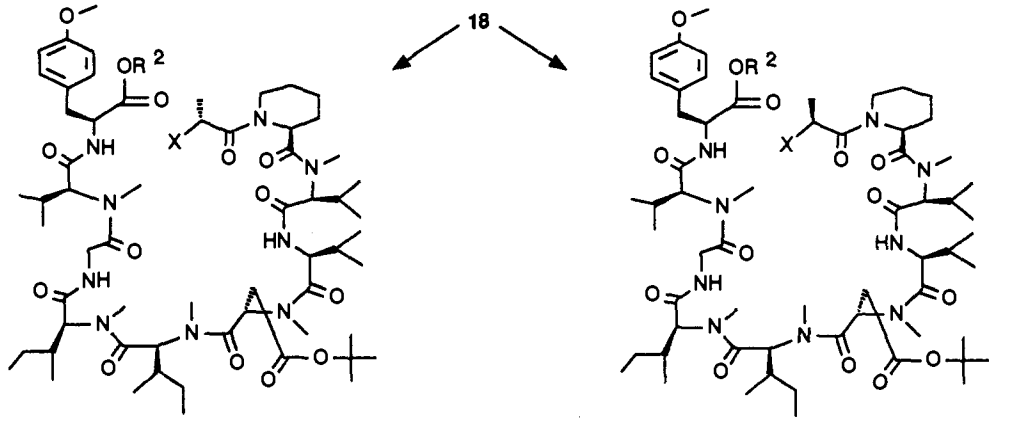
Laboratory Animals. Female NMRI mice weighing 18–20 g and female Wistar rats with a body weight of approximately 150 g were obtained from Charles River Wiga (FRG). The animals were housed under standardized environmental conditions (22 ± 1 °C room temperature, 55 ± 5% humidity, 12 h day/night cycle, and 8 air changes per hour) and had free access to pelleted food and tap water.

Serial Dilution Test. Antifungal activity of the test compounds was determined in cultures grown in malt extract broth (E. Merck, initial pH 4.5 or 6.5) in microtiter trays (Greiner no. 655101) which had been inoculated with 1×10^3 colony forming units (CFU's) per 0.2 mL/well. Stock solutions of the test compounds (0.08%) were prepared in 5% DMSO/0.2% Tween 80 (v/v) and serially diluted with growth medium, yielding final concentrations of 0.05–100 μg/mL. Fungal growth was macroscopically examined after incubation for 48 h at 30 °C. Efficacy of the test compounds was determined by the minimal inhibitory concentrations (MIC) as well as the minimum subinhibitory concentrations (MSIC). MIC was defined as the lowest compound concentration at which no fungal growth could be detected whereas MSIC was defined as the lowest compound concentration inducing a visible growth inhibition in at least two of three triplicates. Cultures treated with the reference compound ketoconazole were used for comparison, and cultures containing the vehicle alone served as negative controls.

Vaginal Candidiasis in Mice and Rats. Vaginal infections with *C. albicans*, no. 124, were established in hormonally conditioned animals (10 per group) as described previously[16] by inoculation of 50 μL of Sabouraud's glucose broth containing 3.5×10^4 CFU's. The compounds

Scheme 3. Synthesis of *S*-Hypr[10] Analogue **17** and of Linear Precursors **23**, **24**, **27**, and **28**





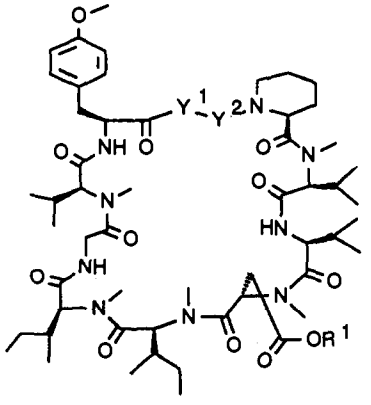
19 : $R^2 = CH_2C_6H_5$, X = OMes
22 : $R^2 = CH_2C_6H_5$, $X = N_3$
24 : R² = H, $X = NH_2$
26 : R² = H, X = $pNO_2C_6H_5OCONHCH(CH_3)CONH$
28 : R² = H, X = $H_2NCH(CH_3)CONH$

20 : $R^2 = CH_2C_6H_5$, $X = N_3$
21 : $R^2 = CH_2C_6H_5$, X = OTs
23 : R² = H, $X = NH_2$
25 : R² = H, X = $pNO_2C_6H_5OCONHCH(CH_3)CONH$
27 : R² = H, X = $H_2NCH(CH_3)CONH$

18 —e→ **19** —g→ **20** —h→ **23** —i→ **25** —h→ **27**
18 —f→ **21** —g→ **22** —h→ **24** —i→ **26** —h→ **28**

a: LiOH, THF, H_2O; b: PPh_3, DEAD;
c: $N(CH_3)_2CH(OCH_2C_6H_5)_2$; d: CF_3COOH;
e: MesCl, Pyr.; f: PPh_3, DEAD, Zn-tosylate;
g: NaN_3, DMF; h: H_2, Pd/C;
i: $pNO_2C_6H_5CH_2OCO$-(R)-ala-O-pNO_2-C_6H_5

Scheme 4. Syntheses of Cyclopeptides **33–36** (Cyclization Reaction)



23 —k→ **29** —d→ **33**
24 —k→ **30** —d→ **34**
27 —k→ **31** —d→ **35**
28 —k→ **32** —d→ **36**

k: DCI, pentafluorophenol
d: CF_3COOH

29 : R^1 = tBu, Y^1 = (S)ala, Y^2 = bond
30 : R^1 = tBu, Y^1 = (R)ala, Y^2 = bond
31 : R^1 = tBu, Y^1 = (R)ala, Y^2 = (S)ala
32 : R^1 = tBu, Y^1 = (R)ala, Y^2 = (R)ala
33 : R^1 = H, Y^1 = (S)ala, Y^2 = bond
34 : R^1 = H, Y^1 = (R)ala, Y^2 = bond
35 : R^1 = H, Y^1 = (R)ala, Y^2 = (S)ala
36 : R^1 = H, Z^1 = (R)ala, Y^2 = (R)ala

were dissolved in 10% DMSO/0.2% Tween (v/v) for systemic treatment. Topical treatment was performed with a gel formulation prepared of 40% DMSO, 50% poly(ethylene glycol) 400, and 10% aerosil (v/v). Rats were treated orally or intraperitoneally twice, 2 h before and 18 h after infection. Mice were dosed four times (per gavage or intraperitoneally), 2 h before and 2, 18, and 24 h after infection. Intravaginal treatment of rats with 0.2 mL of

Table 1. Minimum Inhibitory Concentrations (MIC, μg/mL)[a] and Minimum Subinhibitory Concentration (MSIC, μg/mL) of Cyclopeptolide 1 against *Candida* spp. in Comparison to ketoconazole

		cyclopeptolide 1				ketoconazole; pH 6.5	
		pH 4.5		pH 6.5			
strain	no.	MIC[a]	MSIC	MIC	MSIC	MIC	MSIC
C. albicans	3	6.25	0.78	>100	6.25	100	0.19
	4	6.25	0.78	100	12.50	>100	<0.05
	9	12.50	0.78	>100	12.50	100	<0.05
	26	6.25	0.78	>100	6.25	>100	<0.05
	88	12.50	0.78	100	3.12	>100	<0.05
	90	3.12	0.78	100	3.12	>100	0.39
	124	1.56	0.40	50	3.12	>100	0.19
C. guillermondii	80	6.25	0.8	>100	12.50	>100	0.19
C. krusei	132	>100	1.56	>100	3.12	>100	0.78
C. tropicalis	43	1.56	0.80	100	6.25	>100	6.25

[a] For details, see the section on biological tests/serial broth dilution test.

Table 2. Efficacy of Cyclopeptolide 1 in Experimental Vaginal or Systemic Candidosis after Oral, Intraperitoneal, or Topical Treatment[a]

			cure rate (%)	
model	dose (mg kg^{-1} bw)	application	cyclopeptolide 1	ketoconazole
vaginal candidosis (rat)	2 × 300	po	56	100
	2 × 150	po	0	100
	2 × 100	ip	76	
	2 × 50	ip	0	
	9 × 1%[b]	top.	100	
	9 × 0.1%[b]	top.	78	73
vaginal candidosis (mouse)	4 × 300	po	64	
	4 × 150	po	31	100
	4 × 100	ip	14 (toxic)	
	4 × 50	ip	7	
systemic candidosis (mouse)	8 × 200	po	90	100
	8 × 100	po	40	100
	8 × 100	ip	100	
	8 × 50	ip	20	
	8 × 25	ip	20	

[a] Abbreviations: body weight, bw; oral, po; intraperitoneal, ip; topical, top.; not tested, nt. [b] Percent drug concentration.

gel was started 2 h after inoculation and continued twice daily for 4 consecutive days (i.e., nine aplications). Efficacy was assessed one day after the last treatment by plating vaginal swabs onto Sabouraud's glucose agar with streptomycin (0.1 mg/mL) which were incubated for 48 h at 37 °C. Animals were considered to be cured when reisolation of *C. albicans* failed.

Systemic Candidiasis in Mice. Septicemic infections in mice (10 per group) were initiated by intravenous injection of 10^6 CFU of *C. albicans* (no. 124) via the lateral tail vein. This inoculum proved to cause death within 4 days. The compounds were prepared as above for systemic treatment and administered orally or intraperitoneally twice daily on 4 consecutive days, starting 2 h before inoculation. Efficacy was assessed by the survival rate until day 9 after infection. Controls were used as above.

Results and Discussion

Antifungal Activities. In serial dilution tests the parent cyclopeptolide 1 was found to be active against yeasts (Table 1) but showed no effects against filamentous fungi such as *Trichophyton* spp., *Aspergillus fumigatus*, or *Sporothrix schenkii* in concentrations up to 100 μg/mL (data not shown). Activity against yeasts was primarily dependent on the pH of the growth medium and secondly on the species and strain used. Much higher potency (assessed by MIC and MSIC) of compound 1 was observed when the initial pH of the growth medium was 4.5 than at pH 6.5. In contrast to *C. albicans*, *C. krusei*, and *C. tropicalis*, cells of *C. guillermondii* proved to be resistant to 1 (MIC >100 μg/mL). *In vivo*, compound 1 exhibited dose-dependent antimycotic activity after oral, parenteral, or topical treatment in rodent models of localized (intravaginal) or systemic *C. albicans* infections. In comparison with ketoconazole, compound 1 was less active after systemic application but equaled ketoconazole when both compounds were applied topically at 0.1% in rat vaginal candidiasis.

Table 3. *In Vitro* Activities of 1 (MIC, μg/mL) and Derivaties of 1 against *Candida albicans* 124 (*C. a.*), *Candida guillermondii* 80 (*C. g.*), *Candida krusei* 132 (*C. k.*), and *Candida tropicalis* 43 (*C. t.*) at pH 4.5 and 6.5, MIC (MSIC)

compd	pH 4.5				pH 6.5			
no.	*C. a.*	*C. g.*	*C. k.*	*C. t.*	*C. a.*	*C. g.*	*C. k.*	*C. t.*
1	1.56 (0.8)	6.25 (0.8)	>100 (1.56)	1.56 (0.8)	50 (6.25)	>100 (12.5)	>100 (3.12)	100 (6.25)
2c	>100	>100	>100 (12.5)	>100	>100	>100 (25)	>100 (25)	>100
2d	>100	>100	>100 (25)	>100	>100	>100 (50)	>100 (25)	>100 (25)
3a	>100	>100	>100	>100	>100	>100	>100	>100
3b	>100	>100	>100	>100	>100	>100	>100	>100
3c	>100	>100	>100	>100	>100	>100	>100	>100
3d	>100 (25)	>100	>100	>100	>100	>100	>100	>100
4	>100	>100	>100 (12.5)	>100	>100 (12.5)	>100 (25)	>100 (12.5)	>100 (50)
5a	12.5	>100	>100	100 (25)	50 (3.13)	>100	>100	>100 (6.25)
5b	>100	>100	>100	>100	>100	>100	>100	>100
5c	>100	>100	>100	>100	>100	>100	>100	>100
6	>100 (25)	>100	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100 (25)	>100 (50)	>100	>100	>100 (100)
8	>100	>100	>100	>100	50 (12.5)	100	100	100 (50)
9	>100	>100	>100	>100	>100	>100	>100	>100
12a	3.13 (0.8)	100 (6.25)	100	100 (12.5)	6.25 (3.13)	100 (25)	>100	100 (50)
12b	50	>100	>100	>100	>100	>100	>100	>100
12d	100	>100	>100	>100	>100	>100	>100	>100
12e	>100	>100	>100	>100	>100	>100	>100	>100
12f	>100	>100	>100	>100	>100	>100	>100	>100
13	12.5	25	>100	25	100	>100	>100	>100
14	100	>100	>100	>100	>100	>100	>100	>100
17	3.13	12.5	25	6.25	12.5	100	50	25
31	>100	>100	>100	>100	nt[a]	nt	nt	nt
33	25	>100	>100	>100	100	>100	>100	>100
34	3.13	6.25	25	6.25	6.25	25	50	25
35	12.5	50	>100	100	>100	>100	>100	>100
36	>100	>100	>100	>100	>100	>100	>100	>100

[a] nt = not tested.

Those derivatives of 1 which were active showed the same profiles of antifungal activities as the parent compound. Therefore, data for selected derivatives are given only for four *Candida* strains (Table 3). As with the parent compound, different activities were observed at initial pH 4.5 and 6.5. With the exception of compound 8, higher activities were again found at the lower pH.

Structure–Activity Relationships: Modifications of *N*-MeAsp[4]. Esterification of the free carboxyl group (compounds **2a–d**) resulted in decreased *in vitro* antifungal activity in all cases. Conversion to amides (compounds **3a–d**) led to a complete loss of *in vitro* activities. Surprisingly, however, the diethylamide **3a** turned out to retain some activity *in vivo* (systemic candidiasis in mice, 100 mg/kg ip).

Modification of Tyr(Me)[9]. When the *O*-methyl group was replaced by *O*-allyl (compound **12a**), *in vitro* activity was preserved. Introduction of larger groups resulted in inactive analogues (**12b-f**). A more extensive modification of the molecular structure, i.e., formal replacement of Tyr-(Me)[9] by Asp by oxidative degradation (compound **14**) also led to loss of activity.

Modifications of *R*-Hypr[10]. An increase of antifungal activity *in vitro* (pH = 6.5) was observed when *R*-Hypr[10] was replaced by *S*-lactic acid (compound **17**). Replacing *R*-Hypr with *R*-alanine (compound **34**) resulted in even greater enhancement of activity. Comparison of the ^{1}H-NMR spectra (CD_3OD) suggested that **34** might have a conformation very similar to that of the parent compound **1**. Metabolic stability and therefore bioavailability *in vivo* was expected to be higher for **34** as compared to **1** due to replacement of the ester function (lactone) by the more stable amide isostere. The cyclic peptide **34**, however, turned out to be completely inactive *in vivo* (100 mg/kg ip, systemic candidosis in mice). Replacement of *R*-Hypr by *S*-Ala (compound **33**) or *R*-Ala-*S*-Ala (compound **35**) led to higher MIC values, and the replacement by *R*-Ala-*R*-Ala (compound **36**) resulted in complete loss of antifungal activity.

Summary

The newly isolated cyclopeptolide **1** exhibits interesting antifungal activities, especially against *Candida* strains *in vitro* and *in vivo*. A series of new semisynthetic derivatives was synthesized starting from **1** and evaluated *in vitro* and in a few cases also *in vivo*. Two of the derivatives were more active than **1** *in vitro* (pH = 6.5), but none was superior *in vivo*.

Experimental Section

Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter (concentration in g/100 mL). IR spectra were obtained with a Perkin-Elmer 198 photometer. ^{1}H NMR spectra were recorded at 250 MHz (Bruker WM 250) and at 500 MHz (Bruker AMX 500). Due to hindered rotation around the amide bonds and to internal H-bonds, at room temperature most of the compounds existed in solution ($CDCl_3$) in two or more conformers. When crystalline compounds were dissolved, it took a certain time (about 10–20 min) until an equilibrium state of conformers was obtained. Measurement was performed after the equilibrium was reached, and only data for the prominent conformers are given. All mass spectra are fast atom bombardment (FAB) spectra (matrix: nitrobenzylic alcohol) and were recorded on a VG 70-SE instrument (VG Analytical) operating at an 8-kV accelerating voltage. Column chromatography was accomplished on silica gel 60 (0.063–0.2 mm, Merck) under hydrostatic pressure or on commercially available columns (Lobar Fertigsäule, filled with LiChroprep Si 60, 0.04–0.063 mm, Merck) using pressures up to 5 bars. Furthermore, separation from low molecular weight reaction byproducts was effected by column chromatography on Sephadex LH 20 with CH_2Cl_2 as mobile phase. Thin-layer chromatography was performed on silica gel 60 F_{254} (Merck). The spots were visualized either by quenching of UV fluorescence (λ_{max} = 254 nm) or by staining with ninhydrin, followed by heating, or with potassium permanganate. The purity of the products was checked by high-performance liquid chromatography (Beckman 114M) on a column (250 × 4.6 mm) of Nucleosil 5C_8 (Merck) or on a column (250 × 4.6 mm) of Hypersil CN (Merck) with a water/acetonitrile gradient (20–100% CH_3CN in 40 min, flow rate 1 mL/min) and a Beckman 165 UV detector (detection wavelength, 220 nm).

Tetrahydrofuran (THF) was dried over $LiAlH_4$ under an argon atmosphere. All other solvents were reagent-grade quality and dried by storing over molecular sieves (0.4 nm). (4-Nitrobenzyloxycarbonyl)-*R*-Ala 4-nitrophenyl ester was prepared according to literature.[17,18] Dimethylformamide dioctyl acetal and dimethylformamide bis(trimethylsilyl)ethyl acetal were prepared from dimethylformamide dimethyl acetal and the appropriate alcohols, following a general literature procedure for the synthesis of DMF acetals,[19] and were distilled in vacuo before use.

Ketoconazole, which was used as antifungal standard, was synthesized according to the literature procedure.[20]

Esterification: Method A: Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-methyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2a). An ethereal solution of diazomethane was added to a solution of **1** (200 mg, 0.177 mmol) in methanol (20 mL) under stirring until the yellow color persisted. After 10 min the solvents were evaporated *in vacuo*. The crude product was purified by column chromatography (ethyl acetate), giving 200 mg (99% yield) of methyl ester **2a** (colorless solid): HPLC 98.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/1, δ 2.59, 2.74, 2.78, 2.78, 2.86, 2.88, 2.96, 3.05, 3.08, 3.36 (NCH_3), 3.61, 3.63 ($COOCH_3$), 3.74, 3.80 (OCH_3); FAB MS 1140 (MH^+).

Method B: Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-allyl)-MeIle-(MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2b). Potassium carbonate (140 mg, 1 mmol) and allyl bromide (0.42 mL, 4.9 mmol) were added to a solution of **1** (560 mg, 0.5 mmol) in acetate (25 mL), and the mixture was refluxed for 3 h. After cooling to room temperature, the solvent was evaporated *in vacuo*, and the residue was taken up in ethyl acetate and 0.1 N HCl. The organic layer was washed three times with brine and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography (ethyl acetate) to give 560 mg (96%) of **2b** as a colorless solid: HPLC 99.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.3/1, major conformer: δ 2.73, 2.78, 2.89, 2.92, 3.06 (NCH_3), 3.75 (OCH_3), 4.47 ($COOCH_2$), 5.16, 5.21, 5.84 ($CH{=}CH_2$); minor conformer: δ 2.60, 2.78, 3.00, 3.04, 3.32 (NCH_3), 3.80 (OCH_3), 4.47 ($COOCH_2$), 5.16, 5.21, 5.84 ($CH{=}CH_2$); minor conformer: δ 2.60, 2.78, 3.00, 3.04, 3.32 (NCH_3), 3.80 (OCH_3), 4.47 ($COOCH_2$), 5.16, 5.21, 5.84 ($CH{=}CH_2$); FAB MS 1166 (MH^+), 1188 (MNa^+).

Method C. Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2c). Dimethylformamide di-*tert*-butyl acetal (30 mL, 125 mmol) was added dropwise to a solution of cyclopeptolide **1** (25 g, 22.2 mmol) in 900 mL of anhydrous toluene, which was preheated to 100 °C. The solution was kept at this temperature until the reaction was complete (about 4 h, checked by TLC). The solution was then allowed to cool to room temperature, washed five times with water, and dried over Na_2SO_4. The oil obtained after evaporation of the solvent *in vacuo* was purified by chromatography on silica gel with hexane/ethyl acetate (1/5). Then, 18.54 g (71% yield) of the ester (colorless foam) was isolated: HPLC 95.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 3.5/1, major conformer: δ 1.31 (tBu), 2.70, 2.80, 2.91, 2.96, 3.04 (NCH_3), 3.76 (OCH_3); minor conformer: δ 1.36 (tBu), 2.67, 2.79, 3.02, 3.04, 3.23 (NCH_3), 3.80 (OCH_3); FAB MS 1182 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*n*-octyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2d). Cyclopeptolide **1** (300 mg, 0.27 mmol) was treated with dimethylformamide di-*n*-octyl acetal as described for the preparation of **2c**, leading to 156 mg (47% yield) of **2d** (colorless solid): HPLC 96.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.6/1, major conformer: δ 2.73, 2.79, 2.90, 2.93, 3.05 (NCH_3), 3.75 (OCH_3), 3.92 ($COOCH_2$); minor conformer: δ 2.64, 2.79, 3.00, 3.03, 3.31 (NCH_3), 3.80 (OCH_3), 3.92 ($COOCH_2$); FAB MS 1238 (MH^+).

Amidation. Cyclo-[Pec-MeVal-Val-MeAsp(β-diethylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3a). To a suspension of (chloromethylene)dimethylammonium chloride (prepared from 2.56 mL of DMF and 0.96 mL of oxalyl chloride in 16 mL of CH_3CN at −30 °C) was added dropwise a solution of cyclopeptolide **1** (2.25 g, 2 mmol) in CH_3CN (25 mL) followed by a solution of diethylamine (0.52 mL) in dry pyridine (4 mL). After stirring for 20 h at −20 °C the mixture was poured onto 0.1 N HCl and extracted three times with ethyl acetate. The organic layer was washed three times with water and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was chromatographed (silica gel, hexane/ethyl acetate, 1/5), giving 1.7 g (72% yield) of pure **3a** (colorless

amorphous solid). An analytical sample was prepared by crystallization (C_2H_5OH): mp 234–236 °C; $[\alpha]^{20}_D = -211°$ (c = 1.0, CH_3OH); ^1H NMR ($CDCl_3$) mixture of conformers; 1/4, major conformer: δ 2.60, 2.78, 2.92, 3.02, 3.10 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.64, 2.76, 3.03, 3.08, 3.21 (NCH_3), 3.79 (OCH_3); FAB MS 1181 (MH^+).

The following three compounds were prepared similarly.

Cyclo-[Pec-MeVal-Val-MeAsp(β-4-bromoanilide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3b): 2.0 g (78% yield) from 2.25 g (2 mmol) of 1. Crystallization from C_2H_5OH gave white crystals: mp 198–201 °C; $[\alpha]^{20}_D = -217°$ (c = 1.0, $CHCl_3$).

Cyclo-[Pec-MeVal-Val-MeAsp[β-(2-(dimethylamino)-ethyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3c): 1.55 g (65% yield) of 3c as the hydrochloride from 2.25 g (2 mmol) of 1. Crystallization from C_2H_5OH gave white crystals: mp 190 °C dec.

Cyclo-[Pec-MeVal-Val-MeAsp[β-((trimethylsilyl)-methyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3d): 5.2 g (72% yield) from 6.75 g (6 mmol) 1. Colorless solid: mp 173–175 °C; HPLC 96.6%; ^1H NMR ($CDCl_3$) mixture of conformers, 1.2/1, δ 0.00 ($Si(CH_3)_3$), 2.65, 2.66, 2.78, 2.79, 2.87, 2.92, 3.00, 3.02, 3.06, 3.37 (NCH_3), 3.75, 3.79 (OCH_3); FAB MS 1211 (MH^+), 1233 (MNa^+).

Cyclo-[Pec-MeVal-Val-MehSe-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (4). (Chloromethylene)dimethylammonium chloride (prepared from 0.5 mL of DMF and 0.25 mL of oxalyl chloride in 15 mL of CH_2Cl_2) and cyclopeptolide 1 (375 mg, 0.33 mmol) were stirred in anhydrous THF (5.5 mL) and anhydrous CH_3CN (1.5 mL) for 1 h at 0–4 °C. A solution of $NaBH_4$ (76 mg, 2 mmol) in DMF (4 mL) was added dropwise at −70 °C, and stirring was continued for 1.5 h at −70 °C. The temperature of the reaction mixture was allowed to rise to −10 °C. Then, 4 mL of a saturated aqueous solution of ammonium chloride was added, and the reaction mixture was extracted with ethyl acetate. The organic phase was washed with water and dried over Na_2SO_4 and the solvent evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, ethyl acetate) to give 277 mg (0.25 mmol, 75% yield) of 4: colorless solid; HPLC 98.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 2.50, 2.78, 2.87, 2.95, 3.44 (NCH_3), 3.78 (OCH_3).

Cyclo-[Pec-MeVal-Val-MehSe(Ac)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (5a). A solution of 4 (123 mg, 0.11 mmol) in anhydrous pyridine (3 mL) was treated with acetic acid anhydride (0.1 mL) and (dimethylamino)pyridine (1 mg) for 15 h. The reaction solution was concentrated, diluted with ethyl acetate, and washed with 0.1 N HCl and water. After drying over Na_2SO_4 and evaporation of the solvent, 123 mg (0.106 mmol, 96% yield) of acetate 5a (white crystals) was obtained: mp 184–195 °C; HPLC 98.7%; ^1H NMR ($CDCl_3$) mixture of conformers, 4/1, major conformer: δ 1.38 (CH_3(*R*-Hypr)), 1.97 (CH_3COO), 2.58, 2.77, 2.95, 3.00, 3.37 (NCH_3), 3.79 (OCH_3); minor conformer: δ 1.41 (CH_3(*R*-Hypr)), 2.04 (CH_3COO), 2.73, 2.77, 2.80, 2.86, 3.09 (NCH_3), 3.74 (OCH_3). Anal. ($C_{59}H_{95}N_9O_{14}$) C, H, N.

Cyclo-[Pec-MeVal-Val-MehSe(hemisuccinate)-MeIle-MeIle-Gly-MeVal-Val-Tyr(Me)-*R*-Hypr] (5b). 4 (200 mg, 6.18 mmol) was reacted with succinic acid anhydride (225 mg, 2.25 mmol) as described for the preparation of 5a, leading to 220 mg (0.18 mmol, 100% yield) of hemisuccinate 5b (colorless solid): HPLC 95.0%; ^1H NMR $CDCl_3$) mixture of conformers, 4/1, major conformer: δ 2.57 ($COCH_2CH_2CO$), 2.78, 2.78, 2.98, 3.03, 3.30 (NCH_3), 3.80 (OCH_3). Anal. ($C_{61}H_{97}N_9O_{16}$) C, H, N.

Cyclo-[Pec-MeVal-Val-MehSe(*o*-hemiphthalate)-MeIle-MeIle-Gly-MeVal-Val-Tyr(Me)-*R*-Hypr] (5c). 4 (200 mg, 0.18 mmol) was reacted with *o*-phthalic acid anhydride (337 mg, 2.25 mmol) as described for the preparation of 5a, leading to 233 mg (0.18 mmol, 100% yield) of the *o*-phthalate 5c (colorless solid): HPLC 84%; ^1H NMR ($CDCl_3$) mixture of conformers, 1/1, δ 2.57, 2.59, 2.79, 2.79, 2.81, 2.88, 2.89, 3.00, 3.06, 3.08 (NCH_3), 3.74, 3.80 (OCH_3), 7.2–7.4 (Ar). Anal. ($C_{65}H_{97}N_9O_{16}$) C, H, N.

Cyclo-[Pec-MeVal-Val-MeAbu(γ-chloro)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (6). 4 (87 mg, 0.078 mmol) was reacted with 4-toluenesulfonyl chloride (154 mg, 0.8 mmol) in anhydrous pyridine (7 mL) at 60 °C for 64 h. After the mixture was cooled to room temperature, water (10 mL) was added, and the solution was concentrated under reduced pressure to about 2 mL and extracted with ethyl acetate. The organic phase was successively washed with water, 0.1 N HCl, saturated $NaHCO_3$ solution, and water and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by chromatography (SiO_2, ethyl acetate) to yield 25 mg (0.022 mmol, 25% yield) of the chloride 6 (colorless resin): HPLC 86%; ^1H NMR ($CDCl_3$) mixture of conformers, 2/1, major conformer: δ 2.60, 2.78, 2.93, 3.02, 3.37 (NCH_3), 3.80 (OCH_3); minor conformer: δ 2.72, 2.78, 2.81, 2.86, 3.08 (NCH_3), 3.74 (OCH_3).

Cyclo-[Pec-MeVal-Val-MeAbu(γ-azido)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (7). 4 (624 mg, 0.55 mmol) wasstirred with (dimethylamino)pyridine (5 mg) and methanesulfonyl chloride (0.44 mL, 0.56 mmol) in dry pyridine (9 mL) at 0 °C for 3 h. The solution was concentrated *in vacuo*, treated with water (5 mL), and extracted with ethyl acetate. After the mixture was dried over Na_2SO_4, the solvent was evaporated *in vacuo*. The residue was dissolved in 9 mL of DMF, 155 mg (2.38 mmol) NaN_3 was added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with ethyl acetate and washed five times with water. After drying over Na_2SO_4 and evaporation of the solvent, the residue was chromatographed (SiO_2, hexane/ethyl acetate, 1:2) to give 347 mg (0.31 mmol, 54% yield) of the azide 7 (colorless crystals): mp 216–223 °C. Anal. ($C_{57}H_{91}N_{12}O_{12}$) C, H, N.

Demethylation. Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr-*R*-Hypr] (8). A fresh solution of aluminum iodide was prepared by boiling pieces of aluminum foil (2.5 g) and iodine (19 g) in CS_2 (100 mL) for 3.5 h. After cooling to room temperature, cyclopeptolide 1 (5.6 g, 5 mmol) was added and refluxing was continued for 3 h. The solution was cooled in an ice bath and added carefully to an ice-cold aqueous NH_4Cl solution. After extraction with ethyl acetate, the organic phase was washed with aqueous sodium thiosulfate and water and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the crude product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH/(i\text{-}C_3H_7)_2O$, 10/1/4) to give 5.4 g (97% yield) of 8: mp 217–219 °C; HPLC 96.0%; $[\alpha]^{20}_D = -228°$ (c = 1.0, CH_3OH); ^1H NMR ($CDCl_3$) mixture of conformers, 6/1, major conformer: δ 2.42, 2.79, 2.91, 3.00, 3.52 (NCH_3), 3.80 (7-αH, 7-α′H), 6.65, 6.94 (Ar); FAB MS 1112 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-allyl)-MeIle-MeIle-Gly-MeVal-Tyr(allyl)-*R*-Hypr] (9). Cyclopeptolide 8 (333 mg, 0.3 mmol) was treated with allyl bromide (0.48 mL, 6 mmol) and K_2CO_3 (168 mg, 1.2 mmol) as described for the preparation of 2a to yield 250 mg (85% yield) of the diallyl derivative 9 (colorless solid): HPLC 93.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 1.2/1, major conformer: δ 2.73, 2.79, 2.90, 2.92, 3.06 (NCH_3), 4.47 ($COOCH_2$), 4.53 (OCH_2), 5.16, 5.21, 5.84 ($CH{=}CH_2$), 5.27, 5.40, 6.04 ($CH{=}CH_2$); minor conformer: δ 2.64, 2.78, 3.00, 3.04, 3.31 (NCH_3), 4.47 ($COOCH_2$), 4.53 (OCH_2), 5.16, 5.21, 5.84 ($CH{=}CH_2$), 5.27, 5.47, 6.04 ($CH{=}CH_2$); FAB MS 1192 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr-*R*-Hypr] (10). Via the procedure given for the preparation of 2c (method C), cyclopeptolide 8 (2.22 g, 2 mmol) was treated with dimethylformamide bis-(trimethylsilyl)ethyl acetal (2.2 mL, 4 mmol) to give 1.94 g (80% yield) of (trimethylsilyl)ethyl ester 10 (colorless foam): HPLC 88.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.72, 2.79, 2.90, 2.92, 3.07 (NCH_3), 3.76 (OCH_3), 3.90 ($COOCH_2$); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.64, 2.79, 2.99, 3.05, 3.33 (NCH_3), 3.80 (OCH_3), 3.90 ($COOCH_2$).

O-Alkylation. Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr(*O*-allyl)-*R*-Hypr] (11a). Ester 10 (240 mg, 0.2 mmol) was treated with allyl bromide (0.084 mL, 1 mmol) and K_2CO_3 (28 mg, 0.2 mmol) as described for the preparation of 2b to give 200 mg (80% yield) of 11a (colorless solid): HPLC 93.6%; ^1H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.73, 2.79, 2.91, 2.91, 3.02 (NCH_3), 4.10 ($COCH_2$), 4.48 (OCH_2), 5.27, 5.41, 6.05 ($CH{=}CH_2$); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.67, 2.79, 3.00, 3.05, 3.33 (NCH_3), 4.10 ($COOCH_2$), 4.53 (OCH_2), 5.27, 5.46, 6.05 ($CH{=}CH_2$).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr(*O*-benzyl)-*R*-Hypr] (11b). Ester 10 (2.42 mg, 2 mmol) was treated with benzyl bromide (2.38 mL, 20 mmol) and K_2CO_3 (553 mg, 4 mmol) as described above, leading to 2.2 g (85% yield) of 11b (colorless solid): mp 127–130

°C; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.64, 2.79, 2.91, 2.93, 3.07 (NCH_3), 4.10 ($COOCH_2$), 5.03 (OCH_2), 7.3–7.5 (Ar); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.63, 2.78, 3.00, 3.06, 3.34 (NCH_3), 4.10 ($COOCH_2$), 5.10 (OCH_2), 7.3–7.5 (Ar).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr(*O*-(benzyloxycarbonyl)methylene)-*R*-Hypr] (11c). Ester **10** (363 mg, 0.3 mmol) was treated with benzyl bromoacetate (0.47 mL, 3 mmol) and K_2CO_3 (84 mg, 0.6 mmol) as described above, leading to 326 mg (81% yield) of **11c** (colorless solid): HPLC 93.4%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.72, 2.79, 2.82, 2.91, 3.06 (NCH_3), 4.10 ($COOCH_2$), 4.65 (OCH_2CO), 5.14 ($COOCH_2$), 7.36 (Ar); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.64, 2.79, 2.91, 2.93, 3.07 (NCH_3), 4.10 ($COOCH_2$), 4.70 (OCH_2CO), 5.16 ($COOCH_2$), 7.36 (Ar).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-(trimethylsilyl)ethyl-MeIle-MeIle-Gly-MeVal-Tyr(*O*-4-chlorobenzyl)-*R*-Hypr] (11d). Ester **10** (605 mg, 0.5 mmol) was treated with 4-chlorobenzyl bromide (0.65 mmol) and K_2CO_3 (140 mg, 1 mmol) as described above, leading to 640 mg (96% yield) of **11d** (colorless solid): ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.2/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.65, 2.79, 2.90, 2.91, 3.06 (NCH_3), 4.10 ($COOCH_2$), 4.98 (OCH_2), 7.3–7.5 (Ar); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.61, 2.78, 2.96, 3.05, 3.34 (NCH_3), 4.10 ($COOCH_2$), 5.06 (OCH_2), 7.3–7.5 (Ar).

Cyclo-[Pec-MeVal-Val-MeAsp-(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr(*O*-4-bromobenzyl-*R*-Hypr] (11e). Ester **10** (360 mg, 0.3 mmol) was treated with 4-bromobenzyl bromide (0.76 mg, 3 mmol) and K_2CO_3 (84 mg, 0.6 mmol) as described above, to give 340 mg (82% yield) of **11e** (colorless foam): ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.1/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 2.64, 2.78, 2.89, 2.90, 3.05 (NCH_3), 4.10 ($COOCH_2$), 4.96 (OCH_2), 7.33, 7.52 (Ar); minor conformer: δ 0.01 ($Si(CH_3)_3$), 2.60, 2.78, 2.95, 3.04, 3.34 (NCH_3), 4.10 ($COOCH_2$), 5.04 (OCH_2), 7.42, 7.50 (Ar).

Cyclo-[Pec-MeVal-Val-MeAsp-(β-*O*-(trimethylsilyl)ethyl)-MeIle-MeIle-Gly-MeVal-Tyr(*O*-*E*-3,7-dimethyl-2,6-octadien-1-yl)-*R*-Hypr] (11f). To a solution of ester **10** (121 mg, 0.1 mmol) in anhydrous 1,2-dichloroethane (5 mL) were added geranyl bromide (900 mg, 0.4 mmol), $(n\text{-}C_4H_9)_4NHSO_4$ (20 mg), and 0.1 N aqueous NaOH (2 mL), and the mixture was heated to reflux under stirring for 18 h. The reaction mixture was cooled, and diluted with CH_2Cl_2, and the organic phase was separated and washed with 0.1 N HCl and brine. After drying over Na_2SO_4 and evaporation of the solvent *in vacuo*, the residue was chromatographed on silica gel (hexane/ethyl acetate, 1/2), leading to 70 mg (57% yield) of **11f** (colorless foam): ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 0.00 ($Si(CH_3)_3$), 1.62, 1.69 ($C{=}C(CH_3)_2$), 2.75, 2.80, 2.90, 2.93, 3.07 (NCH_3), 4.10 ($COOCH_2$), 4.47 ($OCH_2C{=}C$); minor conformer: δ 0.01 ($Si(CH_3)_3$), 1.62, 1.69 ($C{=}C(CH_3)_2$), 2.69, 2.79, 3.00, 3.05, 3.32 (NCH_3), 4.10 ($COOCH_2$), 4.13 ($OCH_2C{=}C$).

Deprotection: Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*)-allyl)-*R*-Hypr] (12a). A solution of **11a** (230 mg, 0.18 mmol) in THF (6 mL) was cooled to −20 °C and treated with a commerical 1 M solution of $(n\text{-}C_4H_9)_4NHF$ in THF (0.4 mL, 0.4 mmol) for 4 h. The solution was concentrated *in vacuo*, and the residue was taken up in ethyl acetate, washed with water and brine, and dried over Na_2SO_4. Adter evaporation of the solvent *in vacuo*, the residue was chromatographed (silica gel, $CH_2Cl_2/CH_3OH/(i\text{-}C_3H_7)_2O$, 10/1/4) to give 162 mg (78% yield) of **12a** (colorless foam): HPLC 89%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/4, major conformer: δ 2.53, 2.81, 2.92, 3.02, 3.46 (NCH_3), 4.52 (OCH_3), 5.30, 5.45, 6.06 ($CH{=}CH_2$); FAB MS 1151 (MH^+), 1173 (MNa^+).

The following compounds were obtained by the same procedure.

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-benzyl)-*R*-Hypr] (12b): 94% yield from **11b** (colorless foam); HPLC 86%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/3, major conformer: δ 2.45, 2.80, 2.91, 3.02, 3.45 (NCH_3), 5.03 + 5.09, J_{AB} = 12 Hz (OCH_2Ar), 7.3–7.5 (Ar); FAB MS 1202 (MH^+), 1224 (MNa^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-(benzyloxycarbonyl)methylene)-*R*-Hypr] (12c): 57% yield from **11c**; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/4, major conformer: δ 2.52, 2.80, 2.90, 2.99, 3.44 (NCH_3), 4.66 (OCH_2CO), 5.26 ($COOCH_2$), 7.37 (Ar).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-4-chlorobenzyl)-*R*-Hypr] (12d): 72% yield from **11d** (colorless foam); HPLC 94%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/4, major conformer: δ 2.45, 2.79, 2.90, 2.99, 3.46 (NCH_3), 4.98 + 5.06, J_{AB} = 12 Hz (OCH_2Ar), 7.3–7.5 (Ar); FAB MS 1237 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-4-bromobenzyl)-*R*-Hypr] (12e): 64% yield from **11e** (colorless foam); HPLC 95.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/4, major conformer: δ 2.45, 2.79, 2.90, 2.99, 3.46 (NCH_3), 4.97 + 5.05, J_{AB} = 12 Hz (OCH_2Ar), 7.3–7.5 (Ar); FAB MS 1281 (MH^+), 1303 (MNa^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-*E*-3,7-dimethyl-2,6-octadien-1-yl)-*R*-Hypr] (12f): 51% yield from **11f** (colorless foam); ^{1}H NMR ($CDCl_3$) major conformer: δ 1.61, 1.69, 1.74 ($C{=}CCH_3$), 2.52, 2.80, 2.91, 3.02, 3.43 (NCH_3); FAB MS 1248 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(*O*-(hydroxycarbonyl)methylene)-*R*-Hypr] (13). **12c** (140 mg, 0.11 mmol) was hydrogenated in C_2H_5OH on 10% Pd/C as described for the preparation of **23** to give 90 mg (70% yield) of **13** (colorless solid) after chromatography (SiO_2, CH_2Cl_2/CH_3OH, 9/1): HPLC 95.0%; FAB MS 1170 (MH^+).

Oxidative Degradation of the Aromatic System: Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Asp-*R*-Hypr] (14). $NaIO_4$ (88 mg, 0.41 mmol) and a catalytic amount of RuO_2 were added to a stirred solution of cyclopeptolide **1** (112.5 mg, 0.1 mmol) in CCl_4 (0.5 mL), CH_3CN (0.5 mL), and water (0.8 mL), and the reaction mixture was heated at 60 °C for 2 h. After cooling to room temperature, CH_2Cl_2 was added and the organic phase washed with water and dried over Na_2SO_4. After evaporation of the solvent *in vacuo*, the crude product was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH, 9/1), leading to 87 mg (82% yield) of **14** (colorless solid): HPLC 88.0%; ^{1}H NMR ($CDCl_3$) major conformer: δ 2.80, 3.00, 3.04, 3.18, 3.21 (NCH_3); FAB MS 1064 (MH^+), 1102 (MK^+).

Cleavage of Lactone: H-*R*-Hypr-Pec-MeVal-Val-MeAsp-(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH (15). **2c** (18.0 g, 15.2 mmol) was dissolved in a mixture of THF (400 mL) and water (400 mL), LiOH (730 mg, 30.4 mmol) was added, and stirring continued for 18 h at room temperature. The solution was acidified by addition of 1 N HCl to pH 3. Solid NaCl was added until saturation, and the mixture was extracted three times with ethyl acetate. After evaporation of the organic solvent, 18.04 g (99% yield) of **15** (colorless foam) was obtained. The crude product was used without further purification for the next step: FAB MS 1200 (MH^+), 1222 (MNa^+).

Relactonization: Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (16). A solution of diethyl azodicarboxylate (DEAD) (2.37 mL, 15 mmol) in toluene (500 mL) was added under vigorous stirring to a solution of peptide **15** (6.0 g, 5 mmol) and $P(C_6H_5)_3$ (4.08 g, 15 mmol) over a period of 24 h. The solvent was evaporated *in vacuo*, and the crude product was chromatographed on silica gel with hexane/ethyl acetate, 1/5. The product was freed from traces of triphenylphosphine oxide through column chromatography on Sephadex LH 20 with CH_2Cl_2 to give 4.02 g (67% yield) of **16** (colorless solid): mp 146–148 °C; $[\alpha]^{20}_D$ = −221° (*c* = 1.0, CH_2Cl_2); HPLC 98.1%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 5/1, major conformer: δ 1.33 (tBu), 2.75, 2.76, 2.90, 3.04, 3.08 (NCH_3), 3.76 (OCH_3).

Cleavage of *tert*-Butyl Ester. Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (17). Compound **16** (103 mg, 0.087 mmol) was dissolved in freshly distilled CF_3COOH (3 mL), precooled to −20 °C, and kept at this temperature for 3.5 h. After removal of the trifluoroacetic acid *in vacuo*, the residue was purified by column chromatography on silica gel with $CH_2Cl_2/CH_3OH/(iC_3H_7)_2O$, 10/1/4, to yield 60 mg (61% yield) of **17** (colorless solid): HPLC 98.0%; ^{1}H NMR ($CDCl_3$) mixture of three conformers (10 min after dissolution): conformer a: δ 2.40, 2.78, 2.92, 2.98, 3.43 (NCH_3), 3.78 (OCH_3): conformer b: δ 2.54, 2.79, 2.96, 3.06, 3.11 (NCH_3), 3.72 (OCH_3); FAB MS 1126 (MH^+), 1148 (MNa^+).

Synthesis of Linear Precursors 18–28 for Cyclopeptides 29–36. H-*R*-Hypr-Pec-MeVal-Val-MeAsp(*β*-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*O*-benzyl (18). Via the procedure given for the preparation of the ester **2c** (method C), linear peptide **15** (5.3 g, 4.4 mmol) was reacted with dimethylformamide dibenzyl acetal (4.8 mL, 18.6 mmol) to give 4.25 g (75% yield) of **18** (colorless foam) after chromatography on silica gel (hexane/ethyl acetate, 1/5): FAB MS 1291 (MH^+), 1313 (MNa^+).

***R*-Hypr-(*O*-methylsulfonyl)-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*O*-benzyl (19).** Methanesulfonic acid chloride (0.87 mL, 11.2 mmol) was added dropwise to a solution of **18** (7.0 g, 5.4 mmol) in dry pyridine (55 mL) at 0 °C. After 3.5 h at 0 °C, the excess pyridine was removed *in vacuo* and the residue partitioned between ethyl acetate and water. After separation, the aqueous phase was extracted with ethyl acetate, and the combined organic phases were successively washed with 1 N HCl, 2% aqueous $NaHCO_3$, and brine. After drying over Na_2SO_4, the solvent was evaporated *in vacuo* and the residue was purified by trituration with $(i\text{-}C_3H_7)_2O$ to give 6.3 g (85% yield) of **19** (colorless solid): FAB MS 1368 (MH^+), 1390 (MNa^+).

(*S*)-2-Azidopropionyl-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*O*-benzyl (20). NaN_3 (1.24 g, 19 mmol) was added to a solution of the mesylate **19** (5.3 g, 3.87 mmol) in DMF (55 mL), and the reaction mixture was stirred for 6 h at 60 °C. After cooling to room temperature, the mixture was diluted with ethyl acetate, washed five times with water and once with brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel (CH_2Cl_2/ethyl acetate, 1/2 to 1/5), leading to 4.5 g (88% yield) of **20** as a colorless resin: IR 2120 cm^{-1}; FAB MS 1316 (MH^+), 1338 (MNa^+).

***S*-Hypr(*O*-tolylsulfonyl)-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*O*-benzyl (21).** Diester **20** (2.38 g, 1.84 mmol) in anhydrous toluene (40 mL) was treated with $P(C_6H_5)_3$ (0.97 g, 3.7 mmol), zinc(I) tosylate (0.94 g, 2.2 mmol), and DEAD (0.63 mL, 3.7 mmol). After 2 and 5 h of reaction time, two further portions of $P(C_6H_5)_3$ (0.97 g) and DEAD (0.63 mL) were added. After 48 h of reaction, the solvent was evaporated and the residue chromatographed on silica gel (hexane/ethyl acetate, 1/5) to give 1.32 g (56% yield) of the tosylate **21** (colorless solid).

(*R*)-2-Azidopropionyl-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*O*-benzyl (22). **21** (4.6 g, 3.18 mmol) was treated with NaN_3 (1.03 g, 16 mmol) in DMF (100 mL) as described for the preparation of azide **20** to give after chromatography 3.56 g (85% yield) of azide **22** as a colorless solid: IR 2105 cm^{-1}; FAB MS 1316 (MH^+), 1338 (MNa^+).

H-Ala-Pec-MeVal-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH (23). Azide **20** (1.2 g, 0.91 mmol) was dissolved in ethanol (50 mL) and hydrogenated for 2.5 h at normal pressure using 10% Pd/C. Filtration of the catalyst and evaporation of the solvent gave 970 mg (91% yield) of the decapeptide **23**: HPLC 85.0%; FAB MS 1199 (MH^+).

H-*R*-Ala-Pec-MeVal-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH (24). Azide **22** (1.42 g, 1.08 mmol) was hydrogenated, as described for the preparation of **23**, leading to 1.23 g (95% yield) of **24** (colorless solid): FAB MS 1199 (MH^+).

(4-Nitrobenzyloxycarbonyl)-*R*-Ala-Ala-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr-(Me)-OH (25). To a solution of decapeptide **23** (2.6 g, 2.16 mmol) in DMF (20 mL), $N(C_2H_5)_3$ (0.28 mL, 2 mmol) and (4-nitrobenzyloxycarbonyl)-*R*-alanine 4-nitrophenyl ester (779 mg, 2 mmol) were added under stirring. After 4 days the reaction solution was diluted with ethyl acetate and washed five times with water, followed by brine. After drying over Na_2SO_4, the solvent was evaporated *in vacuo* and the residue chromatographed on silica gel (CH_2Cl_2/CH_3OH, 9/1 to 4/1) to give 2.4 g (77% yield) of **25** as a colorless solid.

(4-Nitrobenzyloxycarbonyl)-*R*-Ala-*R*-Ala-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr-(Me)-OH (26). **24** (700 mg, 0.58 mmol) was treated as described above for the synthesis of **25**, leading to 590 mg (70% yield) of **26** (colorless solid).

H-*R*-Ala-Ala-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH (27). Protected undecapeptide **25** (500 mg, 0.34 mmol) was hydrogenated as described in the preparation of **23**, leading to 320 mg (74% yield) of **27** (colorless solid): HPLC 94.0%; FAB MS 1252 ($M - OH^+$), 1270 (MH^+), 1308 (MK^+).

H-*R*-Ala-*R*-Ala-Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-OH (28). Protected undecapeptide **26** (540 mg, 0.37 mmol) was hydrogenated as described in the preparation of **23**, leading to 408 mg (87% yield) of **28** colorless solid).

Synthesis of Cyclopeptides 29–32 (cyclizations). Cyclo-[Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-Ala] (29). Pentafluorophenol (156 mg, 0.85 mmol) and dicyclohexylcarbodiimide (176 mg, 0.85 mmol) were added under stirring to a solution of the linear peptide **23** (500 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (1.5 L). After 2 days at room temperature, the solution was concentrated to 150 mL, washed successively with 0.2 N aqueous sodium hydroxide, water, and brine, and dried over Na_2SO_4. The remaining solvent was evaporated under reduced pressure, and the residue was triturated with ether, filtered, and washed twice with ether. The filtrate was evaporated to dryness and the residue chromatographed over silica gel with ethyl acetate to give 180 mg (37% yield) of the cyclic decapeptide **29** (colorless solid): ^1H NMR ($CDCl_3$), major conformer: δ 1.32 (tBu), 2.64, 2.77, 2.92, 2.97, 3.12 (NCH_3), 3.79 (OCH_3).

The following compounds were obtained by the same procedure.

Cyclo-[Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala] (30): 59% yield from **24** (colorless solid); ^1H NMR ($CDCl_3$) mixture of conformers, 2.5/1, major conformer: δ 1.32 (tBu), 2.79, 2.80, 2.96, 3.06 (NCH_3), 3.76 (OCH_3).

Cyclo-[Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala-Ala] (31): 56% yield from **27** (colorless solid); HPLC 97.0%; ^1H NMR ($CDCl_3$) major conformer: δ 1.23 (CH_3(Ala)), 1.33 (tBu), 2.72, 2.86, 2.88, 3.10, 3.20 (NCH_3), 3.76 (OCH_3), 4.60, 4.95 (α-H(Ala)).

Cyclo-[Pec-MeVal-Val-MeAsp(*β*-*O*-tert-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala-*R*-Ala] (32): 46% yield from **28** (colorless foam); HPLC 93.0%; ^1H NMR ($CDCl_3$) major conformer: δ 1.34 (tBu), 2.78, 2.85, 2.92, 3,01, 3.15 (NCH_3), 3.78 (OCH_3); FAB MS 1252 (MH^+), 1274 (MNa^+).

Deprotection of Cyclopeptides 29–32: Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-Ala] (33). **29** (300 mg, 0.24 mmol) was treated with CF_3COOH (10 mL) at −20 °C for 6 h, worked up, and purified as described for the preparation of **17**, leading to 149 mg (55% yield) of **33** (colorless solid): ^1H NMR ($CDCl_3$) δ 2.67, 2.78, 2.90, 3.03 3.05 (NCH_3), 3.75 (OCH_3): FAB MS 1125 (MH^+), 1147 (MNa^+).

The following compounds were obtained by the same procedure.

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala] (34): 28% yield from **30** (colorless solid); HPLC 95.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 1/4, major conformer: δ 1.32 (Ala), 2.35, 2.78, 2.96, 3.01, 3.39 (NCH_3), 3.78 (OCH_3); FAB MS 1125 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala-Ala] (35): 42% yield from **31** (colorless solid); HPLC 95.0%; ^1H NMR ($CDCl_3$) δ 2.35, 2.81, 2.99, 3.00, 3.05 (NCH_3), 3.69 (OCH_3); FAB MS 1196 (MH^+), 1218 (MNa^+).

Cyclo-[Pec-MeVal-Val-MeAsp-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Ala-*R*-Ala] (36): 53% yield from **32** (colorless solid); HPLC 95.0%; ^1H NMR ($CDCl_3$) δ 2.72, 2.80, 2.89, 2.98, 3.11 (NCH_3), 3.77 (OCH_3); FAB MS 1196 (MH^+), 1218 (MNa^+).

Permethylation: Cyclo-[Pec-MeVal-MeVal-MeAsp(*β*-*O*-methyl)-MeIle-MeIle-Sar-MeVal-MeTyr(Me)-*R*-Hypr] (37). KH (1.2 mmol, 240 mg of a commercial 20% suspension in oil was made essentially oil-free by trituration with hexane prior to use) was added to **1** (337 mg, 0.3 mmol) and 18crown6 (317 mg, 1.2 mmol) in anhydrous THF (10 mL) under argon at −20 °C. After 5 min of stirring at −20 °C, CH_3I (0.25 mL, 4 mmol) was added dropwise. After 48 h at −20 °C, the reaction mixture was carefully poured onto ice-cold water, acidified with 0.1 N HCl, and extracted with ethyl acetate. The organic phase was washed

with water and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (ethyl acetate) to give 220 mg (62% yield) of permethylated compound **37** (colorless foam): ^1H NMR ($CDCl_3$) δ 2.46, 2.73, 2.76, 2.83, 2.91, 2.97, 3.03, 3.09 (NCH_3), 3.62 ($COOCH_3$), 3.81 (OCH_3); FAB MS 1182 (MH^+).

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