Derivatives of a Novel Cyclopeptolide. 2. Synthesis, Activity against Multidrug Resistance in CHO and KB Cells *in Vitro*, and Structure–Activity Relationships

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A series of derivatives of the novel cyclopeptolide 1 was prepared, and their ability to chemosensitize multi drug resistant CHO and KB cells *in vitro* was evaluated. In contrast to the parent compound, several of the derivatives were found to be highly active. In particular, conversion of the *R*-lactic acid residue of 1 into its *S*-isomer via lactone ring cleavage and recyclization with inversion resulted in a marked enhancement of activity. Some of these derivatives (e.g., **15a**, SDZ 280.446) belong to the most potent resistance modulating compounds known so far.

Introduction

Multidrug resistance (MDR) in cancer cells[1] is a phenomenon known for over 20 years, and it still represents the most common obstacle encountered in cancer chemotherapy.[2] It is characterized by a decrease in cellular sensitivity to a broad range of anticancer drugs. Although some malignancies already exhibit *de novo* resistance, probably as a consequence of previous exposure to carcinogens, e.g., lung cancer, MDR is in most cases acquired during the course of chemotherapeutic anticancer treatment. This acquired resistance is associated with a strong increase in the amount of intrinsically anticancer drug resistant cells and/or the appearance of resistant clones after selective killing of the sensitive malignant cells by the antineoplastic agent. It was already published very early[3] that these emerging tumor cells show resistance against a wide variety of agents, unrelated in chemical structure, mode of action, and physicochemical properties, even though they have not been used in the treatment. Drugs typically involved are adriamycin, colchicine, vinblastine, actinomycin D, and other natural products.

The mechanism responsibe for MDR[4] is far from being completely understood. A considerable degree of variability is noted in the cross-resistant profiles when a variety of MDR cell lines of different origin, selected with different protocols of drug exposure, was examined. It is believed[4] that this pleiotropy does not in itself constitute an argument for involvement of multiple mechanisms and that a single genetic event is sufficient for the MDR phenotype.

Two principal mechanisms have been proposed in general to explain the reduced intracellular anticancer drug accumulation: reduced membrane permeability[3,5] and increased removal of drug from the cell via an energy-dependent efflux mechanism.[6] To some extent changes in intracellular compartmentalization were also observed.

Examination of changes in membrane architecture of MDR cells revealed the occurrence of a membrane associated high molecular weight (170 kDa) glycoprotein, the P-glycoprotein (gp-170).[7] It was shown that the level of expression of gp-170 correlated with the degree of drug resistance and that gp-170 was often overexpressed in MDR cell lines of hamster, mouse, and human origin, which allowed the comparison between rodent MDR cells and human MDR cells. In these cells, gp-170 rapidly eliminates the drug by active transport through the cell membrane, using ATP as energy source.[8]

While the association of gp-170 with MDR is well-documented,[4] a wide array of other changes has also been identified which distinguishes MDR cells from their drug-sensitive parent cells. In certain MDR cell lines which were selected by continuous growth in the presence of cytotoxic drugs for prolonged periods, alterations in DNA topoisomerase activity[4] and in glutathione metabolism[4,9] as well as the overexpression of sorcin,[4] a 22-kDa cytosolic protein, were found. The significance of these changes in certain MDR cells is not clear, but it seems to be of less importance for the MDR phenotype.

Glycoprotein gp-170 is a prominent member of the ATP binding cassette (ABC) superfamily of transport systems,[10,11] which now includes over 30 proteins that share extensive sequence similarity and domain organization. Besides the involvement of gp-170 in MDR, other representatives of this family of transport proteins are associated with cystic fibrosis, bacterial transport, and chloroquine resistance of malarian parasites.[10-12]

In the search for circumvention of gp-170-mediated tumor cell MDR, a variety of chemical substances was identified by screening in MDR cell lines. These compounds were able to restore partly or sometimes completely the normal sensitivity against antineoplastic agents *in vitro*. It is assumed but not proven in all cases that these chemosensitizers, also called "resistance-modulating" agents (RMA), act by interfering with gp-170, reversing the increase in drug efflux.[13] Typical RMA's are calcium channel blockers, calmodulin inhibitors, and noncytotoxic analogues of anthracyclines and vinca alkaloids.

Cyclosporin A (CyA Sandimmune) was found to be effective as RMA and was about 1 order of magnitude more active in vitro than other RMA's, known at that time.[14] Due to this result, other cyclosporins[15] and cyclosporin derivatives[16] were screened in order to find more potent RMA's lacking any immunosuppressive activity. Besides the nephrotoxicity, the immunosuppressive activity seems to be a major disadvantage for a clinical application of CyA in anticancer chemotherapy.

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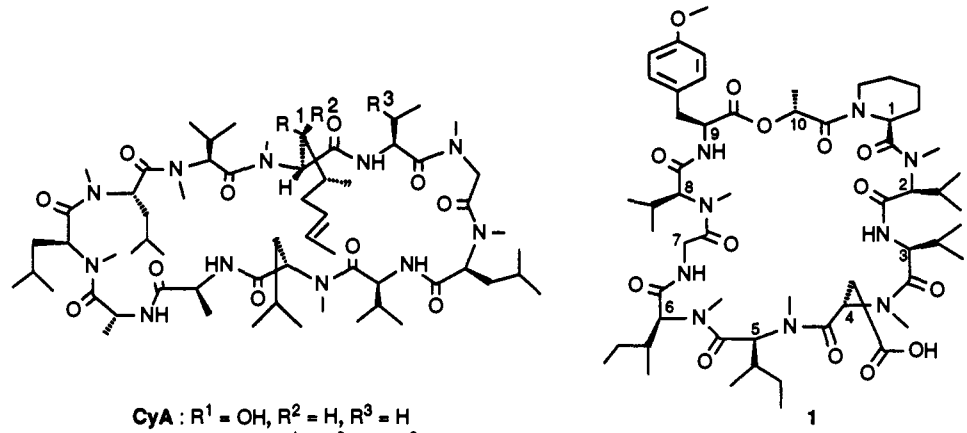
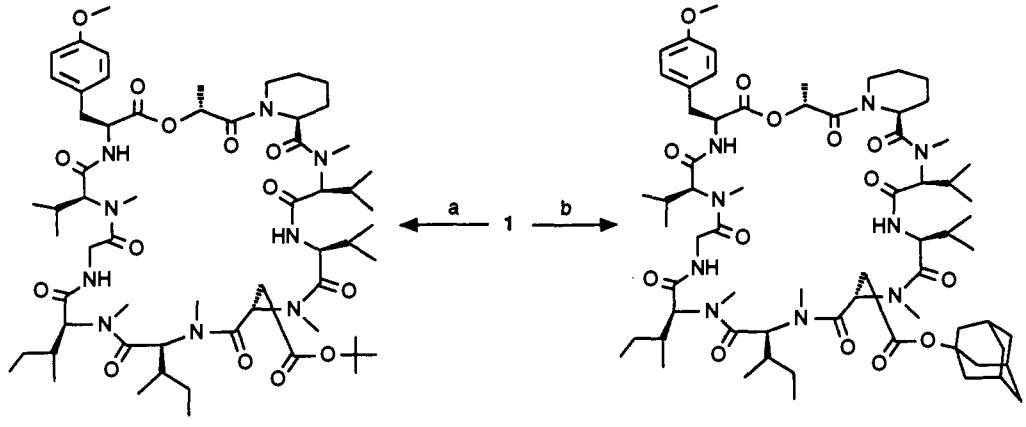
Scheme 1. Structures of Cyclosporin A, SDZ PSC 833, and Cyclopeptolide 1



CyA : R^1 = OH, R^2 = H, R^3 = H
SDZ PSC 833 : $R^1 + R^2$ = O, R^3 = CH_3

1

Scheme 2. Preparation of MeAsp[4] Esters[a]



2b **2f**

[a] (a) $(CH_3)_2NCH(OtBu)_2$, toluene, 100 °C; (b) 1-adamantanol, 2-Cl-1-Me-$C_5H_4N^+I^-$, nPr_3N, toluene, reflux.

Among the naturally occurring and the synthetic cyclosporins, the nonimmunosuppressive semisynthetic (3'-keto-Bmt[1])-(Val[2])-cyclosporin (formula in Scheme 1) could be identified as a very potent RMA.[17]

Consequently, novel cyclic and linear peptides were routinely tested in MDR-CHO cell lines. The cyclopeptolide 1 (Scheme 1), which was isolated from the fermentation broth of an imperfect Fungus (*Septoria* sp., NRRL 15761) due to its antifungal activity[18] did not show any interesting chemosensitizing effects. Some of its derivatives, however, which had been prepared with the aim to improve the antifungal activity,[19] turned out to be highly active in this assay. Therefore, derivatization of 1 was continued with a view to elucidate relationships between structure and chemosensitizing activity, resulting in the identification of SDZ 280.446 (**15a**), a compound which ranks under the most potent RMA's known so far.[20] Here we report on the chemical synthesis of SDZ 280.446 and other new derivatives of **1**, together with their *in vitro* effects on MDR-CHO and KB cells and the structure–activity relationships with regard to chemosensitizing activity.

Chemistry

The synthesis of some of the derivatives (**2a,b, 3e, 5, 7, 10, 11, 15a, 18, 19,** and **20**) starting from **1** is described in ref 19.

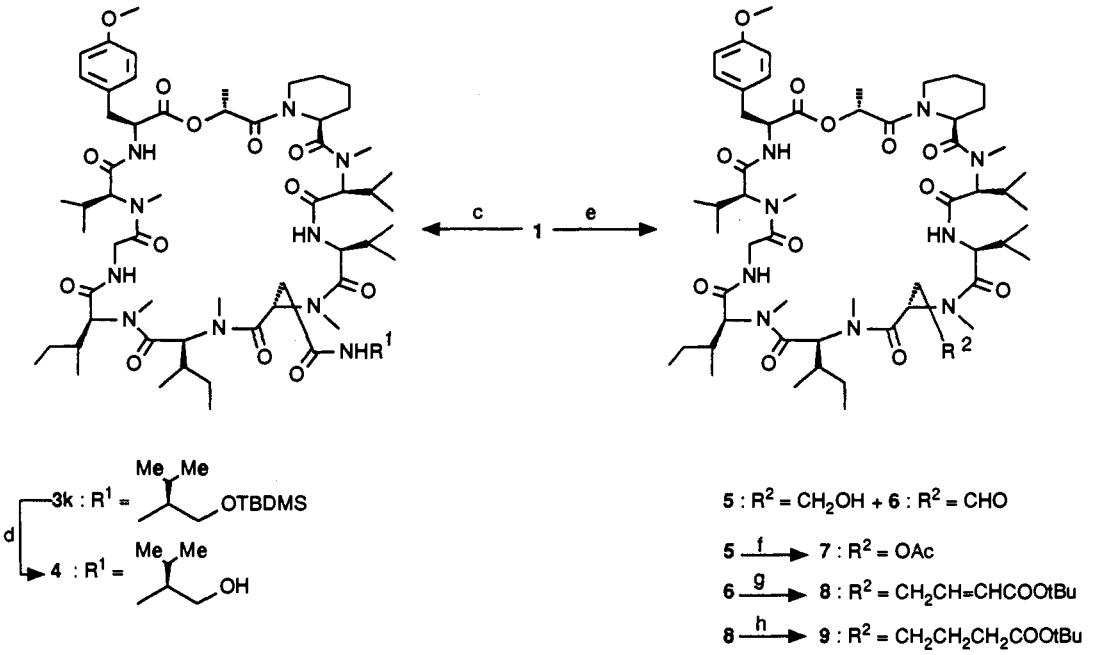
Modification of MeAsp[4]. Esters **2b-d** were prepared in analogy to a standard procedure[21] by heating **1** in toluene at 80 °C with the appropriate dimethylformamide dialkyl acetal as illustrated for the preparation of compound **2b** in Scheme 2. Dialkyl acetals with very bulky residues such as borneyl or adamantyl, however, failed to react with **1**, even when a large excess of reagent was used and the mixture was heated to reflux for several days. The problem was solved by application of the method described by Mukaiyama[22] for the preparation of esters and lactones using forced reaction conditions: treatment of **1** with the desired alcohol and 2-chloro-1-methylpyridinium idodide in the presence of tri-*n*-propylamine in toluene at reflux temperature (e.g., synthesis of **2f**, Scheme 2).

The amides **3a-n** were prepared from **1** in two steps as shown for the synthesis of compound **3k** in Scheme 3: (1) activation of the carboxyl group of MeAsp[4] in **1** with the Vilsmeier–Haack reagent (dimethylformamide imide chloride from DMF and oxalyl chloride); (2) treament of the active intermediate with the appropriate amine or amino acid derivative in pyridine.

Using the above protocol, we could not synthesize amide **4** by reacting cyclopeptolide **1** with unprotected valinol. Valinol *O-tert*-butyldimethylsilyl ether (valinol-*t*-BDMS), however, reacted smoothly to give the silyl derivative **3k**, deprotection of which using HF in acetonitrile resulted in **4** (Scheme 3).

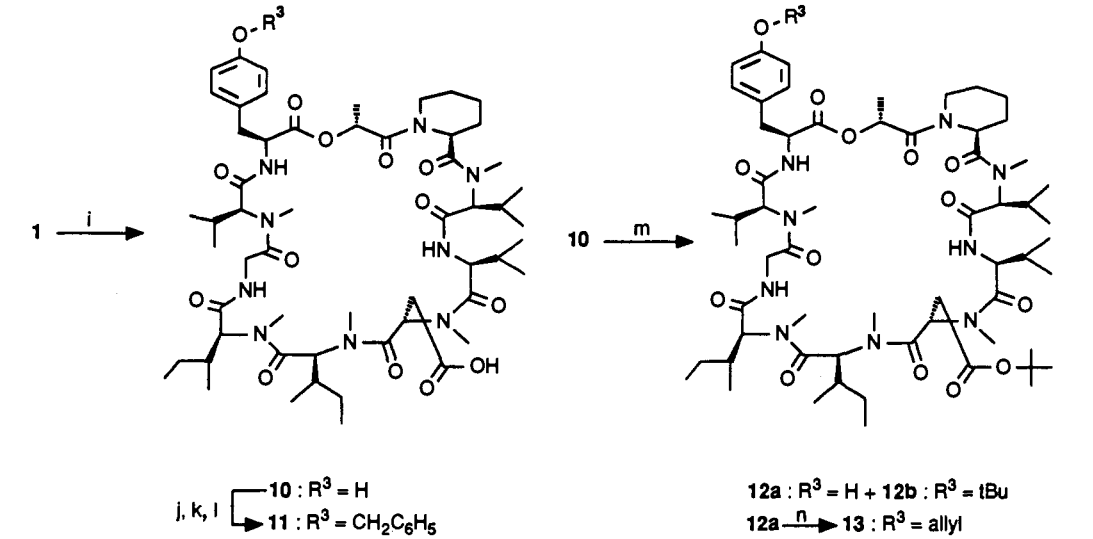
Reduction of the carboxyl group in cyclopeptolide **1** with sodium borohydride after activation with the Vilsmeier–Haack reagent[23] afforded alcohol **5** (formal replacement of MeAsp by MehSer). When the reduction was inter-

Scheme 3. Further Derivatization at MeAsp^4 [a]



[a] (c) DMF, $(COCl)_2$, $NH_2CH[CH(CH_3)_2]CH_2OtBDMS$, pyridine; (d) HF, CH_3CN; (e) DMF, $(COCl)_2$, $NaBH_4$; (f) Ac_2O, pyridine, DMAP; (g) $Ph_3PCHCOOtBu$; (h) H_2, Pd/C.

Scheme 4. Modification at Tyr(Me)^9 [a]



[a] (i) AlI_3, CS_2; (j) $(CH_3)_2NCH[OCH_2CH_2Si(CH_3)_3]_2$; (k) $C_6H_5CH_2Br$, K_2CO_3, acetone; (l) HF, CH_3CN; (m) $(CH_3)_2NCH(OtBu)_2$; (n) $CH_2{=}CHCH_2Br$, K_2CO_3, acetone.

rupted before completion, the corresponding aldehyde **6** was isolated as a byproduct. The alcohol **5** could be acetylated under standard conditions to give the acetate **7**. Treatment of the aldehyde **6** with [(*tert*-butoxycarbonyl)methylene]triphenylphosphorane led to the Wittig product **8** with an elongated unsaturated side chain. Catalytic hydrogenation (H_2, Pd/C) of the double bond in compound **8** resulted in the saturated derivative **9** (Scheme 3).

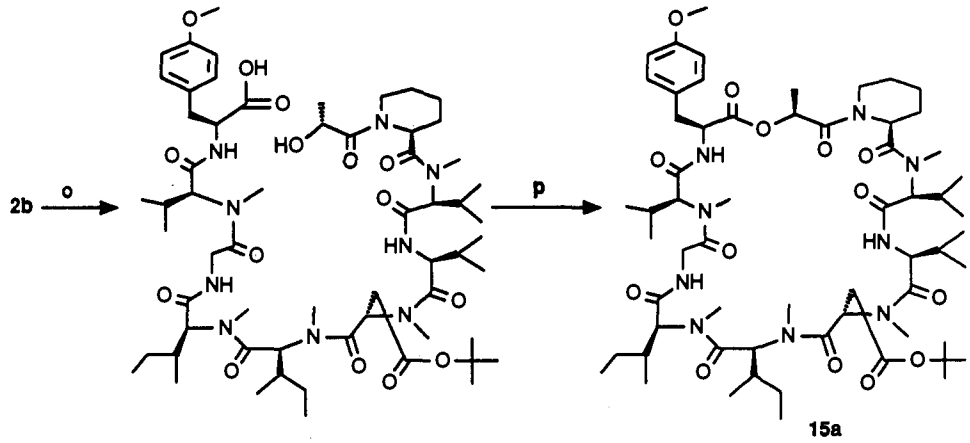
Modification of MeAsp^4 and Tyr(Me)^9. The methyl ether of Tyr(Me)^9 in **1** was cleaved with freshly prepared aluminum(III) iodide in carbon disulfide[19,24] to give the Tyr^9 analogue **10**. Treatment of **10** with dimethylformamide dibenzyl acetal afforded the corresponding MeAsp^4 benzyl ester **12c**. The *tert*-butyl ester **12a** was obtained similarly from **10** with dimethylformamide di-*tert*-butyl acetal. Prolonged heating with excess of reagent was, however, required, and the double-alkylated compound **12b** was formed as a byproduct.

O-Alkylation of the free phenol group in compound **12a** with allyl bromide under standard alkylation conditions resulted in the allyl derivative **13** (Scheme 4).

The aromatic ring of the *O*-methyltyrosine in compound **1** could be selectively oxidized with ruthenium(IV) oxide and sodium periodate[25] to give after treatment of the crude reaction product with an etherial solution of diazomethane the Asp(OMe)^9 analogue **14** (Scheme 6).

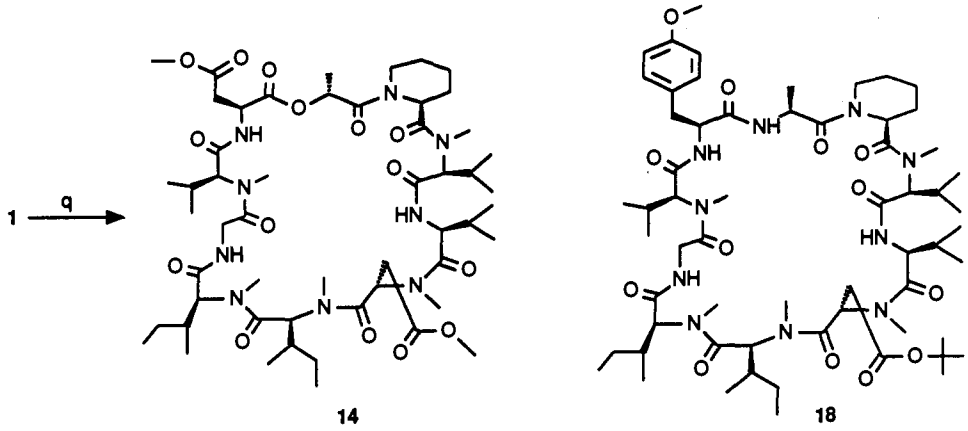
Modification of MeAsp^4 and *R*-Hypr^{10}. The esters **2b,c,h** and the amides **3b-f,j,k,m** were converted into the *S*-Hypr^{10} analogues **15a** (SDZ 280.446), **15b**, **15c**, and **16a-h** by lactone ring cleavage and recyclization under inversion of configuration in the lactic acid residue as illustrated in Scheme 5 for the synthesis of **15a**. Selective cleavage of the lactone proceeded smoothly with LiOH in THF. The

Scheme 5. Preparation of *S*-Hypr[10] Derivatives[a]



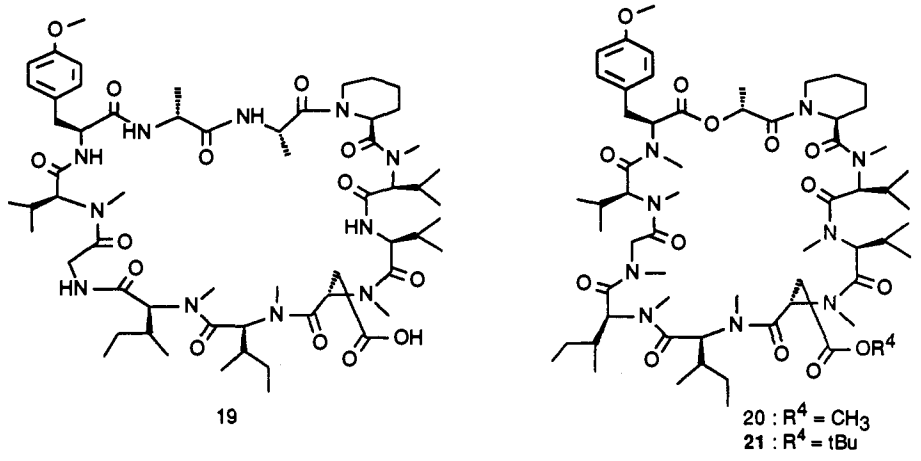
[a] (o) LiOH, THF/H_2O; (p) PPh_3, EtOCON=NCOOEt.

Scheme 6. Replacement of Tyr(Me)[9] by AspOMe and of *R*-Hypr[10] by *S*-Ala[a]



[a] (q) RuO_2, $NaIO_4$; CH_2N_2.

Scheme 7. Further Structural Variations: Ring Enlargement and (Amide)-N-permethylation



re-macrolactonization of the resulting seco acid was accomplished with triphenylphosphine and diethyl azodicarboxylate[19,26] in toluene under high dilution (1 mmol/L).

Under the above conditions, lactonization is induced by activation of the alcohol component, and it is known that such reactions proceed usually with complete inversion of configuration of the carbon to which the hydroxy group is attached[27] as a consequence of a clean S_N2 displacement with the carboxylate nucleophile. Indeed, no starting material could be detected in any of the ring opening/recyclization sequences described above. It is interesting to note that in spite of the facts that some of the compounds carried bulky ester or side chains on MeAsp[4] and that the reaction was generating a 30-membered ring, the yields of the reaction were relatively high.

Further Variations. The permethylated compounds **20** and **21** were prepared by treatment of the parent compound **1** or its *tert*-butyl ester **2b** with KH/18crown6 and iodomethane in THF at −20 °C (Scheme 7).

Biological Tests

Tumor Cell Lines. Two pairs of parental (PAR) and multidrug resistant (MDR) tumor cell lines were used: Chinese hamster ovary (CHO) fibroblastic carcinoma cell

lines[16] obtained from Dr. V. Ling (Ontario Cancer Institute, Toronto, Canada) and human KB naso-pharyngeal carcinoma cell lines[17] obtained from Dr. I. Pastan (National Cancer Institute, NIH, Bethesda, MD).

The PAR-KB (KB 3-1) and MDR-KB (KB-V1) cell lines were maintained in Dulbecco's modified Eagles medium containing 10% fetal calf serum and MIX (0.02 mg/L Asn, 1X αMEM vitamin, 100 IU/mL penicillin–streptomycin, 2 mmol Gln). The fully drug-sensitive PAR-KB cells did not detectably express P-glycoprotein, and their colchicine IC_{50} was 0.003 ± 0.001 μg/mL. The highly P-glycoprotein-expressing MDR-KB cells showed a colchicine IC_{50} of 1.5 ± 0.3 μg/mL, their colchicine resistance being thus 500-fold, relative to the PAR-KB cells.

The PAR-CHO (AUXB1, subclone ABISII) and MDR-CHO (CH^RC5, subclone C5S3.2) cell lines were grown in αMEM supplemented with 10% fetal calf serum and MIX. The highly P-glycoprotein expressing MDR-CHO cells showed a colchicine IC_{50} of 2.5 ± 0.7 μg/mL, while the colchicine IC_{50} of the PAR-CHO cells was 0.044 ± 0.010 μg/mL. The relative colchicine resistance of the MDR-CHO cells relative to the PAR-CHO cells was only 59-fold. However, the PAR-CHO line was not fully drug-sensitive: in comparison with various drug-sensitive cells whose colchicine IC_{50} was 0.003–0.004 μg/mL, the PAR-CHO cells were 11–15-fold resistant. The PAR-CHO resistance was due to its expression of small amounts of P-glycoprotein, and a variety of P-glycoprotein-directed chemosensitizers could restore its full anticancer drug sensitivity.[16,17,20]

The MDR cell lines were continuosly grown in the presence of the drug used for their selection: the KB-V1 in 1 μg/mL vinblastine and the CH^RC5 cells in 0.1 μg/mL colchicine. Eight to 24 hours before each experiment the culture medium of the MDR cell lines was removed and the cells were grown in drug-free medium.

Drugs. Stock solutions (1.0 and 0.1 mg/mL) of all test compounds were prepared in absolute ethanol. Colchicine was used as stock solution (1 mg/mL) in culture medium. For colorimetric assays, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, from *Sigma*) was dissolved at 5 mg/mL in medium.

Cell Growth Assays. Cell growth was measured in triplicate culture using the colorimetric MTT assay described earlier.[16] The cell suspension was dispensed into microculture plates. The number of cells per well were 5000 PAR-KB, 10 000 MDR-KB, 400 PAR-CHO, or 800 MDR-CHO. The microculture plates were incubated in a humidified incubator at 37 °C with 7% CO_2, for 3 days (KB cells) or 6 days (CHO cells). After this period, 100 μL of supernatant was discarded and 10 μL of a 5 mg/mL MTT solution in medium was added to each well. The microplates were further incubated for 2 h (KB cells) or 3 h (CHO cells) in the humidified incubator at 37 °C with 7% CO_2. Then, 100 μL of solvent (butan-2-ol, 2-propanol, 1 N HCl in a volume ratio 16/8/1) were added per well and the plates shaken until complete dissolution of the formazane crystals. The optical density was read at 540 nm with a multichannel spectrometer (Titertek Multiskan).

The different culture and assay conditions used for the four cell sublines had been selected in order to allow a sufficient number of cellular divisions, while remaining, for each cell subline, within the linear growth phase, below the saturation phase, and to perform the colorimetric readout in an adequate absorbance range (0.8–1.4 optical density).

Measurement of Colchicine-Mediated Cell Growth Inhibition. Concentration ranges of colchicine were tested for the parental lines (0.0001–0.3 μg/mL) and for the MDR lines (0.1–30 μg/mL). A down extension of the ranges of tested colchicine concentrations was done when necessary, i.e., when a chemosensitizer was strongly decreasing the colchicine IC_{50}. The cell growth was measured by the MTT colorimetric assay. The absorbances measured without colchicine were taken as representing 100% growth. The colchicine IC_{50}s were calculated from the dose–response curves by plotting the measured growth versus the colchicine concentration.

Measurement of Chemosensitization. The final concentrations of the chemosensitizing compounds in the cell cultures were, besides the ethanol solvent control, 1.0 and 3.0 μg/mL (KB cells only). The cell growth was measured by the MTT assay, the absorbances measured without colchicine being taken as 100% growth, and a full [cochicine dose/cell growth] curve being constructed at both tested concentrations of each chemosensitizer and in their absence.

Three colchicine IC_{50}s values were obtained for each tested compound at 0, 1.0, and 0.1 (or 3.0) μg/mL. Two values of increase in colchicine sensitivity (or grains of sensitivity) of the chemosensitizer-treated cells could then be calculated as the ratios of the IC_{50} measured in the absence of chemosensitizers (solvent control) to the IC_{50} measured in the presence of each of the two tested chemosensitizer concentrations: Colchicine gains at 1.0 μg/mL and at either 0.1 μg/mL (CHO cells) or 3.0 μg/mL (KB cells) of each chemosensitizing compound.

With the fully sensitive PAR-KB, no chemosensitization through P-glycoprotein inhibition was expected, and indeed the IC_{50} ratios were always close to 1 (not shown). With the MDR-KB cells, a complete restoration of colchicine sensitivity would correspond to a gain of sensitivity of about 500-fold. A complete sensitization would correspond to gains of 10–15-fold in the case of the PAR-CHO, but to 59-fold higher gains (600–900) in the case of the MDR-CHO cells.

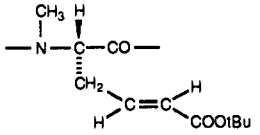
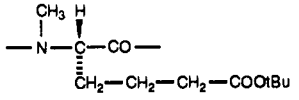
Results and Discussion

Biology. Restoration of Sensitivity of MDR-CHO Cells to Colchicine *in Vitro*. The sensitizing capacities of cyclopeptolide 1 and its derivatives for colchicine treatment of MDR-CHO and PAR-CHO cells were measured at two different concentrations (0.1 and 1 μg/mL). The gains, defined as the ratio of IC_{50} values for colchicine [IC_{50-}/IC_{50+}] determined in absence (IC_{50-}) or presence (IC_{50+}) of the test compound, are listed in Tables 1–3. Table 1 refers to compounds wherein $MeAsp^4$ is modified; Table 2 to compounds with $Tyr(Me)^9$ modifications. The results obtained with *S*-$Hypr^{10}$ derivatives, cyclopeptide analogues, and other miscellaneous derivatives are listed in Table 3. For comparison, data for Cyclosporin A is included (Table 3).

Table 4 presents the gains in colchicine sensitivity of MDR-KB cells observed with a set of compounds at concentrations of 1 and 3 μg/mL.

Structure–Activity Relationships. $MeAsp^4$ modification (Table 1). Whereas the parent compound is not active, almost all variants with modified $MeAsp^4$ proved

Table 1. Chemosensitizing Activity of Compounds in Colchicine Treatment of CHO Cells

	MeAsp[4] alteration				GAINS obtained			
					MDR-CHO		PAR-CHO	
compd	aa[4]	aa[9]	aa[10]	aa[11]	0.1[a]	1.0[a]	0.1[a]	1.0[a]
1[c]	MeAsp	Tyr(Me)	*R*-Hypr	–	1.1	1.8	2.2	7.6
2a[b]	MeAsp-OMe	Tyr(Me)	*R*-Hypr	–	1.9	84	6.4	8.4
2b[b]	MeAsp-OtBu	Tyr(Me)	*R*-Hypr	–	3.8	135	4.6	8.6
2c[b]	MeAsp-O$(CH_2)_2CH(CH_3)_2$	Tyr(Me)	*R*-Hypr	–	1.3	57	5.7	7.8
2d[b]	MeAsp-O$(CH_2)_2C(CH_3)_3$	Tyr(Me)	*R*-Hypr	–	1.9	95	7.5	8.7
2e[c]	MeAsp-O(borneyl)	Tyr(Me)	*R*-Hypr	–	1.2	42	4.2	6.1
2f[c]	MeAsp-O(1-adamantyl)	Tyr(Me)	*R*-Hypr	–	1.9	77	4.5	6.0
2g[c]	MeAsp-O(2-adamantyl)	Tyr(Me)	*R*-Hypr	–	1.1	29	3.4	6.1
2h[b]	MeAsp-OCH_2(1-adamantyl)	Tyr(Me)	*R*-Hypr	–	1.0	6.7	5.9	8.5
3a[c]	MeAsp-NH(allyl)	Tyr(Me)	*R*-Hypr	–	1.1	67	5.0	9.3
3b[b]	MeAsp-NHtBu	Tyr(Me)	*R*-Hypr	–	4.1	151	4.4	8.4
3c[b]	MeAsp-NH$(CH_2)_2CH(CH_3)_2$	Tyr(Me)	*R*-Hypr	–	3.7	126	5.8	8.1
3d[c]	MeAsp-NH$CH_2C(CH_3)_3$	Tyr(Me)	*R*-Hypr	–	11	155	7.4	12
3e[d]	MeAsp-NH$CH_2Si(CH_3)_3$	Tyr(Me)	*R*-Hypr	–	3.3	163	6.7	11
3f[c]	MeAsp-NH(1-adamantyl)	Tyr(Me)	*R*-Hypr	–	25	177	7.3	8.9
3g[d]	MeAsp-NHCH_2 (2-furyl)	Tyr(Me)	*R*-Hypr	–	1.6	91	6.8	10
3h[c]	MeAsp-NH$CH(C_5H_5)_2$	Tyr(Me)	*R*-Hypr	–	6.4	147	7.5	13
3i[b]	MeAsp-NMe_2	Tyr(Me)	*R*-Hypr	–	1.2	36	5.9	8.5
3j[b]	MeAsp-N(C_5H_{10})	Tyr(Me)	*R*-Hypr	–	3.2	137	7.1	10
3k[b]	MeAsp-valinol(TBDMS)	Tyr(Me)	*R*-Hypr	–	1.0	5.1	2.6	8.5
3l[c]	MeAspVal-O(benzyl)	Tyr(Me)	*R*-Hypr	–	1.6	61	4.3	9.7
3m[b]	MeAspVal-OtBu	Tyr(Me)	*R*-Hypr	–	15	187	6.0	8.5
3n[b]	MeAspPro-OtBu	Tyr(Me)	*R*-Hypr	–	10	222	6.8	8.2
4[b]	MeAsp-valinol	Tyr(Me)	*R*-Hypr	–	3.2	144	7.6	9.8
6[b]	MehSe-al	Tyr(Me)	*R*-Hypr	–	1.1	28	4.6	8.2
7[c]	MehSe(Ac)	Tyr(Me)	*R*-Hypr	–	3.8	170	7.2	11
8[b]		Tyr(Me)	*R*-Hypr	–	19	97	7.6	8.7
9[b]		Tyr(Me)	*R*-Hypr	–	4.2	114	6.8	8.2

[a] Concentration of compound in milligams per liter. [b] $3 < n < 5$. [c] $5 < n < 10$. [d] $10 < n < 15$. n = number of chemosensitizer-treated microcultures individually measured for calculating the gains.

Table 2. Chemosensitizing Activity of Compounds in Colchicine Treatment of CHO Cells

	Tyr(Me)[9] alteration/MeAsp[4] and Tyr(Me)[9] alteration				GAINS obtained			
					MDR-CHO		PAR-CHO	
	aa[4]	aa[9]	aa[10]	aa[11]	0.1[a]	1.0[a]	0.1[a]	1.0[a]
11[b]	MeAsp	Tyr(benzyl)	*R*-Hypr	–	1.3	2.1	1.1	3.9
12a[c]	MeAsp-OtBu	Tyr	*R*-Hypr	–	2.5	172	6.5	14
12b[b]	MeAsp-OtBu	Tyr(tBu)	*R*-Hypr	–	28	300	6.7	9
12c[b]	MeAsp-O(benzyl)	Tyr	*R*-Hypr	–	1.2	26	4.4	14
13[b]	MeAsp-OtBu	Tyr(allyl)	*R*-Hypr	–	1.5	9.6	5.8	11
14[b]	MeAsp-OMe	Asp-OMe	*R*-Hypr	–	1.1	3.5	1.9	5.6

[a] Concentration of compound in milligams per liter. [b] $3 < n < 5$. [c] $5 < n < 10$. n = number of chemosensitizer-treated microcultures individually measured for calculating the gains.

to be active. This is true even for the aldehyde analogue **6**, the derivative with the smallest structural change. The MeAsp methyl ether **2a** exhibiting a gain of 84 at a concentration of 1 μg/mL is superior to CyA by a factor of >4. Even higher activity (gain 135 at 1 μg/mL) is observed with the *tert*-butyl ester **2b**. A further increase in bulkiness of the ester residue results in lower activities (compounds **2c–h**), the 1-adamantylmethyl ester **2h** attaining a gain of only 6.7 for MDR-CHO cells which is in the same range as the gain in the parent CHO cells (8.5). In the series of MeAsp amides, high lipophilicity of the side chain appears again to be favorable. Among the simple amide derivatives carrying no further functional groups in the amide side chain, the optimum is found with the 1-adamantyl derivative **3f** (gain 177 at 1 μg/mL). Even higher activities (187 and 222) are observed with the MeAspVal- and MeAspPro-*tert*-butyl ester analogues **3m** and **3n**.

Surprisingly, in contrast to the unprotected MeAsp-valinol analogue **4**, its *O-tert*-butyldimethylsilyl derivative **3k** is almost inactive. In addition, a remarkable difference in activity is observed between the MeAsp[4]-1-adamantyl ester derivative **2f** and the corresponding adamantylmethyl ester derivative **2h** (77 versus 6.7 at 1 μg/mL). This data suggests that lipophilicity of the side chain is not the only factor which is decisive for biological activity of the cyclopeptolide analogues modified at MeAsp[4].

Tyr(Me)[9] Modification (Table 2). High activity is observed with compound **12b** (gain 300 at 1 μg/mL) which carries two *O-tert*-butyl groups (one in the Tyr residue

Table 3. Chemosensitizing Activity of Compounds in Colchicine Treatment of CHO Cells

	MeAsp[4] and R-Hypr[10] alteration/further variations				GAINS obtained			
					MDR-CHO		PAR-CHO	
	aa[4]	aa[9]	aa[10]	aa[11]	0.1[a]	1.0[a]	0.1[a]	1.0[a]
15a[d]	MeAsp-OtBu	Tyr(Me)	*S*-Hypr	-	66	333	5.4	7.0
15b[b]	MeAsp-O$(CH_2)_2$-C$(CH_3)_3$	Tyr(Me)	*S*-Hypr	-	17	292	6.7	7.9
15c[b]	MeAsp-OCH_2 (1-adamantyl)	Tyr(Me)	*S*-Hypr	-	1.4	78	6.9	7.4
16a[c]	MeAsp-NHtBu	Tyr(Me)	*S*-Hypr	-	70	343	8.4	12
16b[b]	MeAsp-N$H(CH_2)_2CH(CH_3)_2$	Tyr(Me)	*S*-Hypr	-	83	273	7.7	8.6
16c[c]	MeAsp-N$HCH_2C(CH_3)_3$	Tyr(Me)	*S*-Hypr	-	95	358	8.0	13
16d[c]	MeAsp-N$HCH_2Si(CH_3)_3$	Tyr(Me)	*S*-Hypr	-	81	336	7.4	11
16e[b]	MeAsp-NH (1-adamantyl)	Tyr(Me)	*S*-Hypr	-	92	290	7.7	8.0
16f[c]	MeAsp-N$H(C_5H_{10})$	Tyr(Me)	*S*-Hypr	-	72	286		
16g[b]	MeAsp-valinol (TBDMS)	Tyr(Me)	*S*-Hypr	-	2.1	106	7.9	8.5
16h[c]	MeAsp-Val-OtBu	Tyr(Me)	*S*-Hypr	-	115	344	8.6	11
17[b]	MeAsp-valinol	Tyr(Me)	*S*-Hypr	-	36	290	7.5	9
18[b]	MeAsp-OtBu	Tyr(Me)	*S*-Ala	-	5.2	144	4.6	6.2
19[b]	MeAsp	Tyr(Me)	*R*-Ala	*S*-Ala	1.1	1.3	1.4	2.7
20[b]	MeVal[3]-(MeAsp-OCH_3)[4]-Sar[7]-MeTyr(Me)[9]				1.1	5.5	1.3	4.6
21[c]	MeVal[3]-(MeAsp-OtBu)[4]-Sar[7]-MeTyr(Me)[9]				1.2	24	3.6	8.7
standard CyA[e]					1.1	19	4.2	8.8

[a] Concentration of compound in milligams per liter. [b] $3 < n < 5$. [c] $5 < n < 10$. [d] $10 < n < 15$. [e] $n > 15$. n = number of chemosensitizer-treated microcultures individually measured for calculating the gains.

Table 4. Chemosensitizing Activity of Compounds in Colchicine Treatment of MDR-KB Cells

compd	aa[10]	1.0[a]	3.0[a]
2b[c]	*R*-Hypr	1.1	1.8
3c[c]	*R*-Hypr	1.0	1.1
3d[c]	*R*-Hypr	1.1	2.3
3f[c]	*R*-Hypr	1.1	3.7
3m[c]	*R*-Hypr	1.2	124
15a[d]	*S*-Hypr	3.6	196
15b[c]	*S*-Hypr	1.7	25
15c[c]	*S*-Hypr	1.3	3.0
16a[c]	*S*-Hypr	1.5	113
16b[d]	*S*-Hypr	1.5	54
16c[d]	*S*-Hypr	2.2	82
16d[c]	*S*-Hypr	1.3	123
16e[c]	*S*-Hypr	3.7	772 (toxic)
CyA[e]		1.2	1.9

[a] Concentration of compound in milligrams per liter. [c] $5 < n < 10$. [d] $10 < n < 15$. [e] $n > 15$. n = number of chemosensitizer-treated microcultures individually measured for calculating the gains.

and the other at the carboxyl group of MeAsp). Replacement of Tyr(Me) in 1 by Asp(OMe) (14) results in loss of activity.

MeAsp[4] and *R*-Hypr[10] Modification. Formal substitution of *R*-Hypr[10] by its *S* enantiomer leads to a dramatic increase of activity, which is especially impressive at the low concentration (0.1 μg/mL). This is true for all pairs of Hypr epimers tested. An increase in activity is found with MeAsp[4] esters as well as with amides. *In vitro* at a concentration of 0.1 μg/mL, the highest gain in sensitivity to colchicine (115) is found with **16h**, the *S*-Hypr analogue of the MeAsp-Val-OtBu derivative. One of the derivatives, the MeAsp(O[t]Bu)[4]-*S*-Hypr[10] analogue **15a** (SDZ 280.446) was further evaluated in animal models and found to be highly active also *in vivo*, thus confirming the *in vitro* data.[20]

The *S*-Ala[10] derivative **18** has the same activity as the *R*-Hypr[10] analogue **2b**. Compound **19**, featuring an enlarged peptide ring and a free carboxyl group on MeAsp[4], is completely inactive.

As already observed in the evaluation of antifungal activity,[19] permethylation (compounds **20** and **21**) seems to have a negative effect.

MDR-KB cells are much less sensitive to some RMA's (e.g., Cyclosporin A) as compared with MDR-CHO cells.[17] This is also true for derivatives of the cyclopeptolide **1** (Table 4). For example, no effect is seen in MDR-KB cells with compound **2b** at 1 μg/mL, and at 3 μg/mL only a modest gain in sensitivity to colchicine is observed. However, some of the derivatives which show considerable activity in the MDR-CHO assay, e.g., **15a** (SDZ 280.446), are also found to be active in MDR-KB cells.

Summary

New derivatives of a novel antifungal cyclopeptolide (**1**) were synthesized from **1** by modification of the amino acids 4 (MeAsp) and 9 (Tyr(Me)) and of the hydroxy acid 10 (*R*-Hypr). The parent compound **1**, the new derivatives and a series of derivatives which had been prepared previously in order to study relationships between the structure and antifungal activity, were tested for their ability to restore sensitivity to multidrug resistant CHO cells *in vitro*. Almost all of the derivatives, but not the parent compound itself, were found to be active in this assay. High gains of chemosensitivity were observed with compounds carrying lipophilic ester and amide side chains at MeAsp[4], in particular when *R*-Hypr[10] was replaced by *S*-Hypr.

Experimental Section[28]

General Procedures for Esterification of MeAsp[4]: Method a. Dimethylformamide dialkyl acetal (4–6 mmol) was added dropwise to a solution of the cyclopeptolide **1** (1 mmol) in toluene (50 mL) at 80 °C, and the reaction mixture was kept at this temperature until completion of reaction (about 4 h) as checked by TLC. After cooling to room temperature, the reaction solution was washed five times with water and dried over Na_2SO_4. The solvent was evaporated *in vacuo*, and the residue was purified by chromatography on silica gel with hexane/ethyl acetate (1/5).

When not commercially available, the dimethylformamide acetals were prepared by gradual heating of dimethylformamide dimethyl acetal (13.7 mL, 0.1 mmol) and the desired alcohol (0.2 mmol) from room temperature to 120 °C (5 °C/min). Methanol which formed during the reaction was distilled off through a short Vigreux column, and the bath temperature was kept at 120 °C until 8 mL of methanol was distilled off. The residue was purified by distillation in vacuo.

Dimethylformamide diisopentyl acetal: bp 114–115 °C (14 Torr); 85% yield.

Dimethylformamide bis(3,3-dimethyl-1-butyl) acetal: bp 114–115 °C (11 Torr); 87% yield.

Dimethylformamide bis(trimethylsilylethyl) acetal: bp 125–127 °C (11 Torr): 75% yield.

Method b. A mixture of cyclopeptolide 1 (2.4 mmol), the desired alcohol (3 mmol), 2-chloro-1-methylpyridinium iodide (1.53 g, 6 mmol), tri-*n*-propylamine (2.25 mL, 12 mmol), and toluene (70 mL) was heated under reflux for 24 h. After cooling to room temperature, the reaction solution was diluted with ethyl acetate, washed with 0.1 N HCl and three times with water, dried with Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate (1/2 to 1/5) as eluant.

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-isopentyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2c): method a; 72% yield (colorless solid); HPLC 97.8%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 2.72, 2.78, 2.90, 2.92, 3.05 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.62, 2.78, 2.99, 3.03, 3.32 (NCH_3), 3.80 (OCH_3); FAB MS 1196 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(3,3-dimethyl-1-butyl)]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (2d): method a; 33% yield (colorless solid); HPLC 96.1%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/1.3, δ 0.88, 0.89 (tBu), 2.62, 2.72, 2.79, 2.79, 2.90, 2.92, 2.98, 3.03, 3.05, 3.34 (NCH_3), 3.75, 3.80 (OCH_3); FAB MS 1210 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-borneyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (2e): method b; 62% yield (colorless solid); HPLC 97.2%; ^{1}H NMR ($CDCl_3$) mixture of conformers 3/1, major conformer: δ 2.67, 2.80, 2.93, 2.96, 3.07 (NCH_3), 3.76 (OCH_3); minor conformer: δ 2.65, 2.79, 3.03, 3.05, 3.24 (NCH_3), 3.80 (OCH_3); FAB MS 1262 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(1-adamantyl)]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (2f): method b; 25% yield (colorless solid); HPLC 96.1%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 2.66, 2.78, 2.89, 2.95, 3.02 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.63, 2.78, 2.99, 3.01, 3.28 (NCH_3), 3.79 (OCH_3); FAB MS 1260 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(2-adamantyl)]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (2g): method b; 34% yield (colorless solid); HPLC 94.9%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 5/3, major conformer: δ 2.67, 2.79, 2.92, 2.93, 3.04 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.60, 2.78, 2.99, 3.01, 3.30 (NCH_3), 3.79 (OCH_3); FAB MS 1260 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(1-adamantyl)methyl]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (2h): method b; 60% yield (colorless foam); HPLC 96.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 2.73, 2.79, 2.90, 2.95, 3.05 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.63, 2.78, 3.01, 3.02, 3.50 (NCH_3), 3.79 (OCH_3); FAB MS 1274 (MH^+).

General Procedure for Amidation of MeAsp[4]. Cyclopeptolide 1 (2.25 g, 2 mmol) in CH_3CN (25 mL) and thereafter a solution of the amine (2.4–5 mmol) in dry pyridine (4 mL) or 2,4,6-trimethylpyridine (symm. collidine) were added dropwise 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). After stirring for 20 h at −20 °C the mixture was poured onto 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) to give the amide.

Cyclo-[Pec-MeVal-Val-MeAsp(β-allylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3a): 86% yield (colorless solid); HPLC 99.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.2/1, major conformer: δ 1.42 (d, J = 7 Hz, CH_3(*R*-Hypr)), 2.67, 2.78, 2.89, 3.01, 3.36 (NCH_3), 3.80 (OCH_3), 5.00, 5.09, 5.71 ($CH{=}CH_2$); minor conformer: δ 1.41 (d, J = 7 Hz, CH_3(*R*-Hypr)), 2.67, 2.78, 3.01, 3.36 (NCH_3), 3.80 (OCH_3), 5.00, 5.09, 5.71 ($CH{=}CH_2$); minor conformer: δ 1.41 (d, J = 7 Hz, CH_3(*R*-Hypr)), 2.67, 2.83, 2.89, 3.04, 3.09 (NCH_3), 3.75 (OCH_3), 5.00, 5.09, 5.71 ($CH{=}CH_2$); FAB MS 1165 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*tert*-butylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3b): 94% yield (colorless solid). An analytic sample was crystallized (C_2H_5OH): mp 161.5–164.5 °C; HPLC 99.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/1, δ 1.19, 1.23 (tBu), 2.67, 2.67, 2.78, 2.79, 2.90, 2.97, 3.03, 3.06, 3.06, 3.40 (NCH_3), 3.76, 3.79 (OCH_3); FAB MS 1181 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-isopentylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3c): 20% yield (colorless foam); HPLC 88.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 7/6, main conformer: δ 2.68, 2.78, 2.89, 3.08, 3.35 (NCH_3), 3.74 (OCH_3); minor conformer: δ 2.65, 2.77, 2.83, 3.00, 3.03 (NCH_3), 3.79 (OCH_3); FAB MS 1195 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-neopentylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3d): 80% yield (colorless solid): mp 165–168 °C; HPLC 99.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1/1, δ 0.80, 0.85 (tBu), 1.42, 1.43 (CH_3(*R*-Hypr)), 2.64, 2.67, 2.77, 2.78, 2.88, 2.92, 2.94, 3.04, 3.06, 3.38 (NCH_3), 3.75, 3.78 (OCH_3); FAB MS 1195 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-(1-adamantyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hyp} (3f): 87% yield (colorless solid). An analytical sample was crystallized (C_2H_5OH): mp 228–229 °C; HPLC 93.0%; ^{1}H NMR ($CDCl_3$) mixtures of conformers, 1.5/1, major conformer: δ 2.66, 2.78, 2.88, 2.96, 3.05 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.67, 2.77, 3.02, 3.05, 3.40 (NCH_3), 3.78 (OCH_3): FAB MS 1259 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-(2-furylmethyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (3g): 89% yield (colorless solid); HPLC 99.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 1.1/1, δ 2.63, 2.65, 2.78, 2.79, 2.84, 2.91, 2.98, 3.01, 3.10, 3.26 (NCH_3), 3.75, 3.80 (OCH_3), 6.10, 6.16, 6.25, 6.27, 7.24, 7.27 (furyl); FAB MS 1205 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp(β-(diphenylmethyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (3h): 93% yield (colorless solid); HPLC 94.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2/1, major conformer: δ 2.62, 2.74, 2.77, 2.99, 3.08 (NCH_3), 3.77 (OCH_3), 7.1–7.4 (C_6H_5); minor conformer: δ 2.62, 2.77, 2.87, 2.89, 2.92 (NCH_3), 3.73 (OCH_3), 7.1–7.4 (C_6H_5); FAB MS 1291 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-dimethylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3i): 75% yield (colorless solid); mp 150–155 °C; HPLC 96.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2.8/1, major conformer: δ 1.45 (d, J = 7 Hz, CH_3(*R*-Hypr)), 2.65, 2.75, 2.79, 2.95, 2.98, 3.01, 3.10 (NCH_3), 3.77 (OCH_3); minor conformer: δ 1.43 (d, J = 7 Hz, CH_3(*R*-Hypr)), 2.63, 2.77, 2.83, 3.01, 3.03, 3.09, 3.26 (NCH_3), 3.81 (OCH_3); FAB MS 1153 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-piperidineamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3j): 77% yield (colorless solid); mp 159–161 °C; HPLC 100.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 8/1, major conformer: δ 2.64, 2.80, 2.96, 3.01, 3.10 (NCH_3), 3.77 (OCH_3); minor conformer: δ 2.55, 2.77, 3.05, 3.08, 3.23 (NCH_3), 3.81 (OCH_3); FAB M 1193 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-(valinol *tert*-butyldimethylsilyl ether)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (3k): 63% yield (white crystals); mp 144 °C; HPLC 98.2%; ^{1}H NMR ($CDCl_3$) mixture of conformers 1.5/1, major conformer: δ 0.89 (tBu), 2.60, 2.77, 2.78, 3.00, 3.08 (NCH_3), 3.75 (OCH_3); minor conformer: δ 0.86 (tBu), 2.63, 2.87, 3.01, 3.04, 3.37 (NCH_3), 3.77 (OCH_3).

Cyclo-[Pec-MeVal-Val-MeAsp(β-Val-*O*-benzyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3l): 45% yield (colorless solid); HPLC 99.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers: 2/1, major conformer: δ 2.43, 2.77, 2.96, 2.96, 3.48 (NCH_3), 3.81 (OCH_3), 4.91, 5.03 ($COOCH_2Ph$), 7.2–7.4 (C_6H_5); minor conformer: δ 2.56, 2.79, 2.89, 2.99, 3.07 (NCH_3), 3.75 (OCH_3), 4.91, 5.03 ($COOCH_2Ph$), 7.2–7.4 (C_6H_5); FAB MS 1315 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-Val-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3m): 47% yield (colorless crystals, ether); mp 155–157 °C; HPLC 99.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 14/11, major conformer: δ 1.36 (tBu), 2.45, 2.78, 2.96, 2.97, 3.51 (NCH_3), 3.76 (OCH_3); minor conformer: δ 1.46 (tBu), 2.57, 2.79, 2.88, 3.02, 3.07 (NCH_3), 3.81 (OCH_3); FAB MS 1281 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-Pro-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (3n): 43% yield (colorless solid); mp 138–142 °C; HPLC 93.9%; ^{1}H NMR ($CDCl_3$) mixture of rotation isomers at room temperature, single component at 403 K in DMSO-d_6, δ 1.40 (tBu), 2.65, 2.73, 2.88, 2.99 (NCH_3), 3.73 (OCH_3); FAB MS 1279 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-valinolamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (4): 40% HF (0.5 mL) was added to a solution of compound **3k** (220 mg, 0.166 mmol)

in acetonitrile (10 mL) at 4 °C. The reaction solution was warmed to room temperature and kept for 1 h, poured on ice, and extracted three times with ethyl acetate. The organic phase was washed with $NaHCO_3$ solution and dried over Na_2SO_4, and the solvent was evaporated in vacuo. The crude product was purified by chromatography on silica gel with hexane/ethyl acetate (1/5) leading to 182 mg (90% yield) of **4** (colorless solid); mp 161–163 °C; HPLC 90.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer; δ 2.68, 2.78, 2.84, 2.93, 3.17 (NCH_3), 3.77 (OCH_3); minor conformer δ 2.58, 2.79, 2.99, 3.00, 3.43 (NCH_3), 3.81 (OCH_3); FAB MS 1211 (MH^+).

Cyclo-[Pec-MeVal-Val-MeHse-al-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (6). A solution of cyclopeptolide **1** (2.25 g, 2 mmol) in anhydrous acetonitrile (20 mL) and anhydrous THF (5 mL) was dropped to a freshly prepared suspension of (chloromethylene)dimethylammonium chloride (made from 2.56 mL of DMF and 0.96 mL of oxalyl chloride in 30 mL of dichloromethane) at −30 °C. The reaction mixture was stirred for 1 h between −30 °C and −10 °C, cooled to −70 °C, and treated with a suspension of $NaBH_4$ (225 mg, 6 mmol) in 10 mL of DMF. Stirring was continued for 1 h at −70 °C and then brought to −20 °C in 15 min. After this period 5 mL of saturated NH_4Cl solution was added, and the reaction mixture was diluted with water and extracted three times with ethyl acetate. Evaporation of the solvent *in vacuo* gave 2.6 g of a yellow resin which was purified by column chromatography (silica gel, hexane/ethyl acetate, 1/5) to yield 600 mg (27% yield) of alcohol **5** and 900 mg (41% yield) of the aldehyde **6** (colorless solid): HPLC 100.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 1.5/1, major conformer: δ 2.63, 2.78, 3.02, 3.04, 3.39 (NCH_3), 3.81 (OCH_3), 9.70 (dd, J_1 = 3.5 Hz, J_2 = 1.5 Hz, CHO); minor conformer: δ 2.69, 2.78, 2.88, 2.90, 3.08 (NCH_3), 3.75 (OCH_3), 9.75 (d, br, J = 3 Hz, CHO); FAB MS 1110 (MH^+).

Cyclo-{Pec-MeVal-Val-Sar-[(*S*,*E*)-(3-*tert*-butoxycarbonyl)prop-2-enyl]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (8). A mixture of aldehyde **6** (107 mg, 0.096 mmol) and [(*tert*-butoxycarbonyl)methylene]triphenylphosphorane (50 mg, 0.13 mmol) was stirred for 20 h in toluene (4 mL) at room temperature. The solvent was evaporated *in vacuo* and the residue purified by column chromatography (silica gel, hexane/ethyl acetate, 1/4) and crystallized (ether) to give 93 mg (80% yield) of **8** (colorless crystals): mp 176–178 °C; HPLC 100.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 1.39 (tBu), 2.65, 2.78, 3.01, 3.04, 3.38 (NCH_3), 3.79 (OCH_3), 5.83 (d, J = 15 Hz, CH=CHCOO); minor conformer: δ 1.40 (tBu), 2.71, 2.79, 2.83, 2.85, 3.08 (NCH_3), 3.73 (OCH_3), 5.80 (d, J = 15 Hz, CH=CHCOO).

Cyclo-{Pec-MeVal-Val-Sar-[(*S*)-(3-*tert*-butoxycarbonyl)propyl]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr} (9). The unsaturated compound **8** (35 mg, 0.029 mmol) in ethanol (3 mL) was treated with hydrogen in the presence of 10% Pd/C for 18 h. The catalyst was filtered off and the solution evaporated to dryness. The residue was purified by treatment with diethyl ether/petroleum ether to yield 32 mg (91% yield) of **9** as a colorless solid: mp 159–162 °C; HPLC 100.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 4/1, major conformer; δ 1.39 (tBu), 2.59, 2.79, 2.96, 2.99, 3.40 (NCH_3), 3.79 (OCH_3); minor conformer: δ 1.39 (tBu), (tBu), 2.69, 2.84, 2.86, 3.02, 3.11 (NCH_3), 3.74 (OCH_3); FAB MS 1210 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr-*R*-Hypr] (12a). Via the general procedure for esterification of MeAsp[4] (method a), compound **10** (111 mg, 0.1 mmol) was treated with DMF di-*tert*-butyl acetal (0.11 mL, 0.4 mmol) in refluxing benzene (6 mL) for 4 h to give 63 mg (55% yield) of **12a** (colorless foam): HPLC 99.5%; ^1H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 1.31 (tBu), 2.72, 2.81, 2.85, 2.99, 3.04 (NCH_3); minor conformer: δ 1.36 (tBu), 2.57, 2.79, 2.96, 3.05, 3.34 (NCH_3); FAB MS 1168 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*R*-Hypr] (12b). Via the general procedure for esterification of MeAsp[4] (method a), compound **10** (555 mg, 0.1 mmol) was treated with DMF di-*tert*-butyl acetal (0.55 mL, 2.0 mmol) in refluxing benzene (6 mL) for 18 h. Then 290 mg (50% yield) of compound **12a** and 73 mg of compound **12b** (12% yield) were obtained after separation and purification by column chromatography (silica gel, hexane/ethyl acetate, 1/2). Compound **12b**: HPLC 96.0%; ^1H NMR ($CDCl_3$) mixture of conformers, 4/1, major conformer: δ 2.70, 2.79, 2.96, 2.96, 3.05 (NCH_3); FAB MS 1224 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsP(β-*O*-benzyl)-MeIle-MeIle-Gly-MeVal-Tyr-*R*-Hypr] (12c). Compound **10** (67 mg, 0.06 mmol) was reacted with dimethylformamide dibenzyl acetal (0.058 mL, 0.2 mmol) in refluxing benzene for 3 h. Workup as described in the general esterification procedure afforded after chromatography 60 mg (83% yield) of **12c** (colorless foam): HPLC 88.5%; ^1H NNR ($CDCl_3$) mixture of conformers 1.2/1, major conformer: δ 2.69, 2.79, 2.82, 2.98, 3.06 (NCH_3), 5.06, 5.10 ($COOCH_2$), 7.20–7.40 (C_6H_5); FAB MS 1202 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(allyl)-*R*-Hypr] (13). *tert*-Butyl ester **12a** (174 mg, 0.15 mmol) was heated with K_2CO_3 (42 mg, 0.3 mmol) and allyl bromide (0.126 mL, 1.5 mmol) in acetone (9 mL) under reflux for 4 h. After cooling to room temperature the solvent was evaporated in *vacuo* and the residue taken up in ethyl acetate. After washing with 0.1 N HCl, water, and brine, the solution was dried over Na_2SO_4 and the solvent evaporated *in vacuo*. Column chromatography (silica gel, hexane/ethyl acetate, 1/5) gave 170 mg (93% yield) of **13** (colorless foam): HPLC 96.4%; ^1H NMR ($CDCl_3$) mixture of conformers, 2.6/1, major conformer: δ 1.31 (tBu), 2.70, 2.79, 2.91, 2.96, 3.04 (NCH_3), 5.27, 5.41, 6.02 ($CH=CH_2$); minor conformer: δ 1.36 (tBu), 2.70, 2.78, 3.02, 3.05, 3.22 (NCH_3), 5.27, 5.41, 6.05 ($CH=CH_2$); FAB MS 1208 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Asp(β-OMe)-*R*-Hypr] (14). 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), $NaIO_4$ (88 mg, 0.41 mmol) and a catalytical amount of RuO_2 were added, and the reaction mixture was heated to 60 °C for 2 h. After cooling to room temperature CH_2Cl_2 was added, and the organic phase was washed with water and dried over Na_2SO_4. After evaporation of the solvent *in vacuo*, the residue was purified by column chromatography (CH_2Cl_2/CH_3OH, 9/1), leading to a colorless solid: ^1H NMR ($CDCl_3$) major conformer: δ 2.80, 3.00 3.04, 3.18, 3.21 (NCH_3). The solid which was dissolved in 3 mL of methanol, cooled to 0 °C, and treated with an excess of an etheric solution of diazomethane. After the mixture was stirred for 1 h, the solvent was evaporated and the residue purified by column chromatography (silica gel, hexane/ether acetate, 1/5). A 67-mg (62%) yield of dimethyl ester **14** in the form of a colorless solid was obtained: FAB MS 1092 (MH^+).

General Procedure for Lactone Cleavage and Relactonization. One millimole of one of the ester derivatives **2b,d,f** or of the amides **3b-f,j,k,m** was dissolved in 40 mL of a 1:1 mixture of THF and water. $LiOH \cdot H_2O$ (84 mg, 2 mmol) was added under stirring. After 18 h, the solution was acidified (pH = 3) with 1 N HCl, saturated with NaCl, and extracted three times with ethyl acetate. The residue, obtained after evaporation of the solvent and coevaporation with toluene, was used for the following cyclization step without further purification. It was dissolved in anhydrous toluene (150 mL) together with triphenylphosphine (525 mg, 2 mmol), and a solution of diethyl azodicarboxylate (DEAD) (0.314 mL, 2 mmol) in toluene (150 mL) was added over a period of 5 h under vigorous stirring. After 20 h, the solvent was evaporated *in vacuo* and the crude product purified by chromatography on silica gel (hexane/ethyl acetate, 1/5). In cases where the triphenylphosphine oxide formed during the reaction process was difficult to separate from the product, a second chromatography on Sephadex LH 20 with dichloromethane as eluant was performed.

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(3,3-dimethylbutyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (15b): 22% yield from **2d** (colorless solid); HPLC 87.9%; ^1H NMR ($CDCl_3$) mixture of conformers, 1/2/2/4, δ 3.73, 3.75, 3.79, 3.80 (s, OCH_3); FAB MS 1210 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-*O*-(1-adamantyl)methyl]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (15c): 36% yield from **2d** (colorless solid); HPLC 93.8%; ^1H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 2.75, 2.78, 2.87, 3.06, 3.09 (NCH_3), 3.76 (OCH_3); FAB MS 1274 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-*tert*-butylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (16a): 38% yield from **3b** (colorless solid); mp 160–162.5 °C; HPLC 98.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2.5/1, major conformer: δ 1.22 (tBu), 1.39 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.71, 2.75, 2.94, 3.03, 3.04 (NCH_3), 3.75 (OCH_3); minor conformer: δ 1.26 (tBu), 1.43 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.58, 2.74, 3.03, 3.07, 3.26 (NCH_3), 3.79 (OCH_3); FAB MS 1181 (MH^+).

Cyclo-[Pec-MeVal-Val-MeAsp(β-isopentylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (16b): 28% yield from **3c** (colorless solid); HPLC 92.6%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2/1, major conformer: δ 2.64, 2.76, 2.90, 2.96, 3.07 (NCH_3), 3.74 (OCH_3); minor conformer: δ 2.55, 2.75, 3.00, 3.05, 3.31 (NCH_3), 3.79 (OCH_3); FAB MS 1195 (MH^+).

Cyclo[Pec-MeVal-Val-MeAsp(β-neopentylamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (16c): 52% yield from **3d** (colorless solid): mp 164–166 °C; HPLC 98.5%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2/1, major conformer: δ 0.82 (tBu), 1.39 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.65, 2.76, 2.92, 3.00, 3.05 (NCH_3), 3.74 (OCH_3); minor conformer: δ 0.86 (tBu), 1.44 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.55, 2.75, 3.03, 3.05, 3.28 (NCH_3), 3.79 (OCH_3); FAB MS 1195 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-[(trimethylsilyl)methyl]-amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (16d): 26% yield from **3e** (colorless foam): HPLC 90.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 2/1, major conformer: δ 0.01 ($SiCH_3)_3$, 1.40 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.67, 2.76, 2.90, 2.99, 3.06 (NCH_3), 3.74 (OCH_3); minor conformer: δ 0.03 ($Si(CH_3)_3$, 1.44 (d, J = 7 Hz, CH_3(*S*-Hypr)), 2.56, 2.75, 3.02, 3.05, 3.31 (NCH_3), 3.80 (OCH_3); FAB MS 1211 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-(1-adamantyl)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (16e): 47% yield from **3f** (colorless solid); mp 184–185 °C; HPLC 97.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 4/1, major conformer: δ 2.67, 2.75, 2.95, 3.01, 3.05 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.58, 2.75, 3.04, 3.07, 3.28 (NCH_3), 3.79 (OCH_3); FAB MS 1259 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-piperidylamide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (16f): 52% yield from **3j** (colorless solid); HPLC 94.8%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 2.69, 2.75, 2.95, 3.08, 3.09 (NCH_3), 3.76 (OCH_3); minor conformer: δ 2.54, 2.73, 3.05, 3.13, 3.18 (NCH_3), 3.81 (OCH_3); FAB MS 1193 (MH^+).

Cyclo-{Pec-MeVal-Val-MeAsp[β-(valinol *tert*-butyldimethylsilyl ether)amide]-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr} (16g): 54% yield from **3k** (colorless solid): mp 143–145 °C; HPLC 100.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers, 3/1, major conformer: δ 2.67, 2.75, 2.95, 3.03, 3.08 (NCH_3), 3.75 (OCH_3); minor conformer: δ 2.56, 2.75, 3.04, 3.07, 3.29 (NCH_3), 3.78 (OCH_3).

Cyclo-[Pec-MeVal-Val-MeAsp(β-Val-*O*-*tert*-butyl)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (16h): 43% yield from **3m** (colorless solid); mp 185–186 °C; HPLC 89.0%; ^{1}H NMR ($CDCl_3$) mixture of conformers 1.2/1, δ 1.37, 1.45 (tBu), 2.40, 2.62, 2.76, 2.76, 2.95, 2.98, 3.02, 3.02, 3.06, 3.45 (NCH_3), 3.75, 3.80 (OCH_3).

Cyclo-[Pec-MeVal-Val-MeAsp(β-valinolamide)-MeIle-MeIle-Gly-MeVal-Tyr(Me)-*S*-Hypr] (17). Compound **16g** (250 mg, 0.189 mmol) was treated with HF in acetonitrile as described for the preparation of compound **4** to give 167 mg (55% yield) of product **17** (colorless solid): HPLC 97.0%; ^{1}H NMR ($CDCl_3$ mixture of conformers, 3/1, major conformer: δ 2.58, 2.61, 2.63, 3.09, 3.18 (NCH_3), 3.80 (OCH_3); minor conformer: δ 2.76, 2.82, 2.83, 2.87, 3.06 (NCH_3), 3.76 (OCH_3); FAB MS 1211 (MH^+).

Cyclo-[Pec-MeVal-MeVal-MeAsp(β-*O*-tert-butyl)-MeIle-MeIle-Sar-MeVal-MeTyr(Me)-*R*-Hypr] (21). Ester **2b** (350 mg, 0.3 mmol) and 18crown6 (320 mg, 1.2 mmol) in dry THF (10 mL) were added to KH (240 mg of a commercially available 20% suspension in oil were made essentially oil-free by washing with hexane under argon) under argon at −20 °C followed by CH_3I (0.25 mL, 4 mmol). After 20 h at −20 °C, the reaction mixture was carefully poured onto ice/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 chromatography (silica gel, ethyl acetate) to yield 290 mg (80% yield) of **21** (colorless foam): HPLC 84.0%; ^{1}H NMR ($CDCl_3$) δ 1.43 (tBu), 2.46, 2.73, 2.76, 2.84, 2.91, 2.98, 3.02, 3.08 (NCH_3), 3.81 (OCH_3); FAB MS 1224 (MH^+).

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