13. Cyclic Octa- and Decapeptides as Ionophores for Magnesium

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Cyclic octa- and decapeptides have been prepared, and their ion selectivity in solvent polymeric membranes was studied. Cyclo(-LPro-DLeu-)$_5$ behaves as an ionophore which selects Mg^{2+} over Ca^{2+} by a factor of 100. Cyclo(LPro-LLeu)$_5$ induces selectivity in membranes for Mg^{2+} over Li^+, Na^+ and K^+ by a factor of 400, 200 and 10, respectively, while selectivity of Mg^{2+} over Ca^{2+} is poor. Some evidence is presented which indicates that certain ionophores exhibit high selectivity for monovalent magnesium complexes.

Introduction. – Some cyclooligopeptides and cyclooligodepsipeptides behave as ionophores and induce very high selectivities for alkali metal cations when incorporated in bulk artificial membranes [1–3]. So far, no such compounds with high membrane selectivity for alkaline earth metal ions have been described. There are, however, reports on the interaction of cyclooligopeptides and cyclooligodepsipeptides with alkaline earth metal cations [1–3]. Structures of complexes such as a 1:2 sandwich of Ca^{2+} with the cyclic decapeptide antamanide [4], a 1:1 adduct of Mg^{2+} with cyclo(-DPhe-Pro-Gly-DAla-Pro-) [5] and a 1:2 sandwich of Mg^{2+} with cyclo(-Gly-Pro-Pro-Gly-Pro-Pro-) [6] have been elucidated. On the other hand, the monocarboxylic polyether antibiotic-*6016* has been reported to be a Mg^{2+}-selective ionophore which displays preferential extraction from an aqueous into an organic phase of Mg^{2+} over Ca^{2+} and Ba^{2+} [7]. We were, however, unable to detect potentiometrically such selectivities when the antibiotic was incorporated in solvent polymeric membranes [8]. In these membranes, Ca^{2+} was clearly favoured over Mg^{2+}. Despite major efforts, it was impossible to prepare membranes showing a high selectivity for Mg^{2+} over Ca^{2+} until recently. Here, we report the preparation of new cyclic peptides and their behaviour in membranes with respect to A-cations. Some are attractive ionophores with high selectivity for Mg^{2+}.

Results and Discussion. – The potentiometrically determined selectivity factors induced in solvent polymeric membranes by the peptides **1–8** are depicted in *Figs. 1* and *2*. The selectivity factors given were obtained by separate EMF measurements in pure aqueous solutions of the metal chlorides [9]. The values are reported relative to Mg^{2+} (K^{Pot}_{MgM}) and, therefore, represent the membranes preference for the interfering ion M^{z+} relative to Mg^{2+}. In the absence of lipophilic anionic sites (potassium tetrakis (*p*-chlorophenyl)borate (KT*p*ClPB)), most of the peptides only slightly affected the selectivity

cyclo(-LPro-LLeu-)$_4$	**1**
cyclo(-LPro-LPro-LLeu-LLeu-)$_2$	**2**
cyclo(-LPro-LLeu-)$_5$	**3**
cyclo(-LPro-LPro-LLeu-LLeu-LLeu-)$_2$	**4**
cyclo(-LPro-LPro-LLeu-DLeu-LLeu-)$_2$	**5**
cyclo(-LPro-DLeu-)$_5$	**6**
cyclo(-LPro-DLeu-LPro-LLeu-LPro-LLeu-DPro-LLeu-LPro-LLeu-)	**7**
cyclo(-LPro-DLeu-LPro-DLeu-LPro-LLeu-DPro-LLeu-D-Pro-LLeu-)	**8**

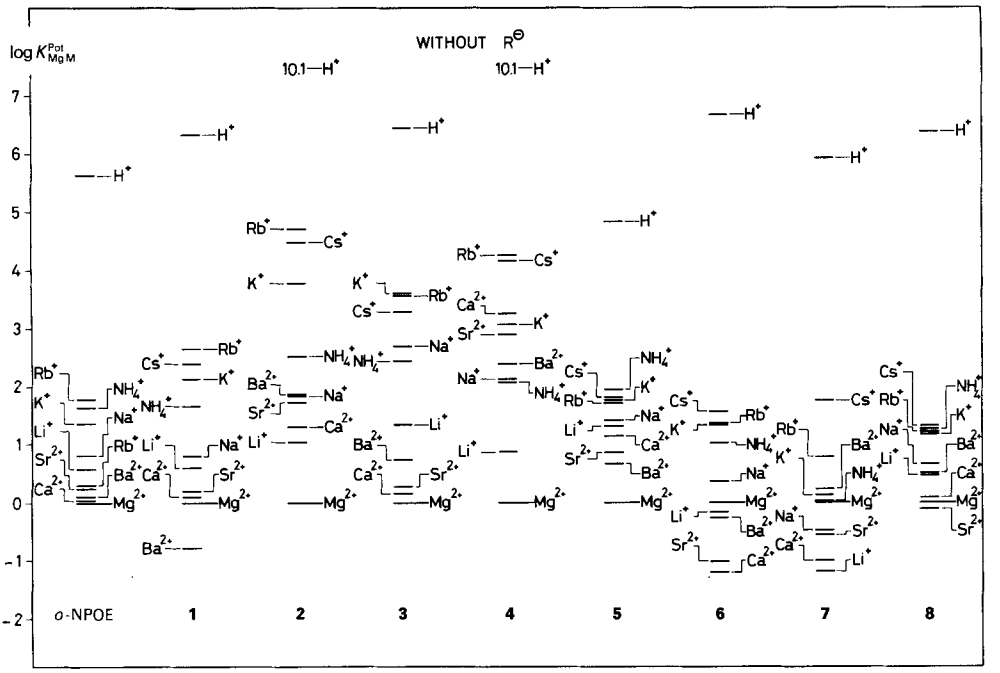

Fig. 1. *Selectivity factors, log* $K^{Pot}_{Mg,M}$, *of solvent polymeric membranes based on the cyclopeptides* **1–8**. *Column 1:* membrane without peptide.

of the membrane (*cf. columns 2–6, 9* with *column 1* (blank membrane) in *Fig. 1*). The cyclodecapeptides **6** and **7** (*columns 7* and *8*) lead to some preference for Mg^{2+} with respect to the other alkaline earth cations. The configuration of the amino acids obviously has a remarkable effect on the selectivity (see **3** and **6–8**). Through a change in the sequence of the amino acids, a drastic preference for larger alkaline earth cations than Mg^{2+} was induced (see **4** in *Fig. 1*).

Certain ligands behave as carriers only if lipophilic anionic sites are simultaneously present in the membrane phase [10–12]. This effect is especially pronounced for carriers **3** and **8** (*Fig. 2, columns 4* and *9*). The membrane with **3** and KT*p*ClPB exhibits selectivity for divalent over monovalent cations. Unfortunately, the Mg^{2+}/Ca^{2+} selectivity is rather poor ($K^{Pot}_{MgCa} \approx 1$). The drastic effect of R^- in membranes with **8** becomes obvious by the high Ba^{2+}/Mg^{2+} selectivity. When incorporated in membranes with KT*p*ClPB, the carriers **6** and **7** induce an enhanced Mg^{2+}/Ca^{2+} selectivity ($K^{Pot}_{MgCa} \approx 0.01$) as com-

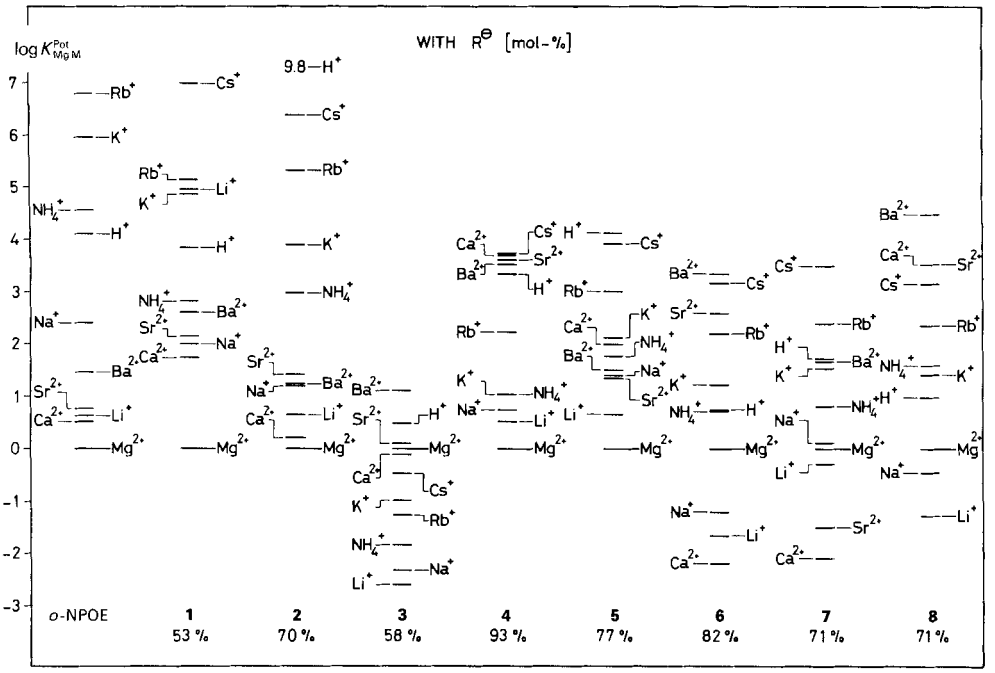

Fig. 2. *Selectivity factors, log* $K^{Pot}_{Mg,M}$, *of solvent polymeric membranes based on the cyclopeptides* **1–8** *and on lipophilic anionic sites* R^-. *Column 1:* membrane without peptide.

pared to membranes without R^- *(cf. columns 7* and *8* in *Fig. 2* to the same columns in *Fig. 1)*. By comparing membranes containing the cyclopeptides **6–8** with one another, it again becomes obvious that changes in the configuration of the ionophores can lead to drastic changes in the preference for cations of different size (see selectivity of Mg^{2+} relative to Ca^{2+} and Sr^{2+}).

The carrier properties of the ligands **3** and **6–8** in membranes with anionic sites are corroborated in *Fig. 3*. All the Mg^{2+}-electrode response functions show a nearly theoretical slope for divalent cations and are linear to at least 10^{-5} M $MgCl_2$. However, when the Mg^{2+}-electrode response is measured in buffered solutions at increased pH values (pH = 7.5, *Fig. 4*), drastic deviations from both the theoretical *Nernst* slope and linearity are observed *(Fig. 4)*. At high Mg^{2+} activities, the response curves approach slopes expected for monovalent cations (see especially electrode functions for **3** and **7**).

Magnesium forms complexes with various anions such as OH^- and Cl^- *(Fig. 5* and *Table 1)*. At constant activities of $MgCl_2$ and at high pH values, the $MgCl^+$ activity remains constant. It increases at low pH values due to the addition of HCl to the sample solution to lower its pH. The $MgOH^+$ activity increases with increasing pH values and reaches an upper limit because of the formation of $Mg(OH)_2$. *Figs. 4* and *5* show results which indicate that monovalent cations ($MgOH^+$ and $MgCl^+$) are indeed involved as permeating species in the membranes discussed. A similar behaviour has been observed for carrier membranes with selectivity for lead [13], cadmium [14], and uranyl [15]. To evaluate the contributions by the different Mg species as well as other interfering ions J^{z+}, the electrode response was described by the following equation:

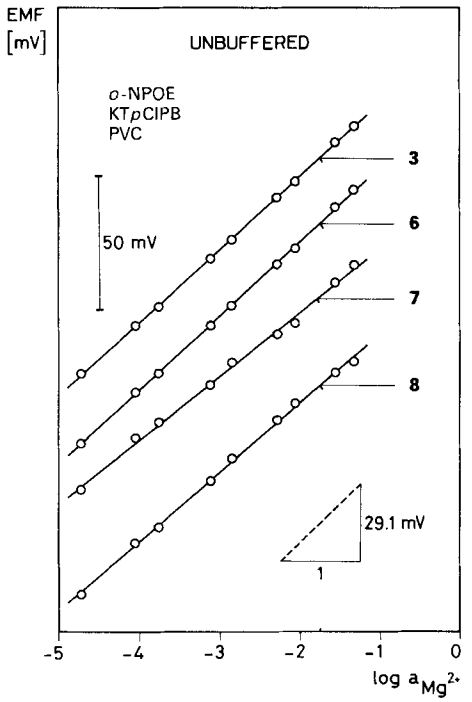

Fig. 3. *EMF response of liquid membrane electrodes based on the cyclopeptides* **3**, **6–8** *to unbuffered aqueous solutions of* $MgCl_2$

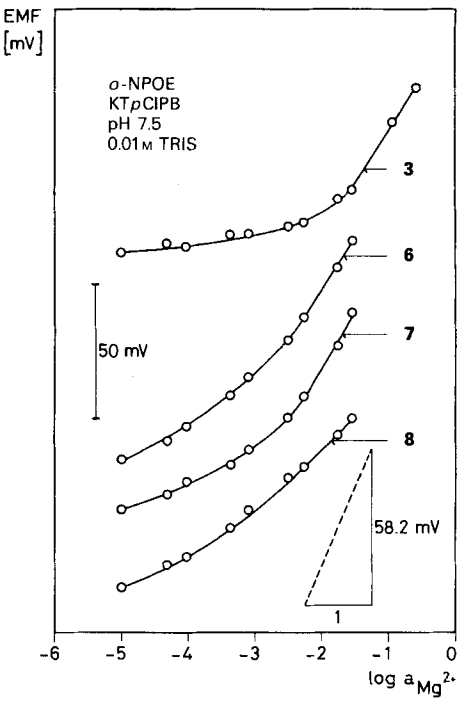

Fig. 4. *EMF response of liquid membrane electrodes based on the cyclopeptides* **3**, **6–8** *to buffered aqueous solutions of* $MgCl_2$

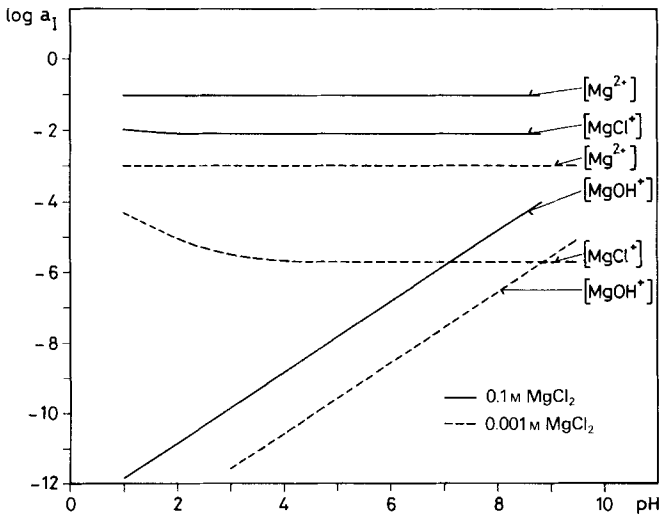

Fig. 5. *Activity of* Mg^{2+}, $MgCl^+$ *and* $MgOH^+$ *for* $MgCl_2$ *solutions as a function of the sample pH*

Table 1. *Thermodynamic Formation Constants,* K, *for the 1:1 Association of* Mg^{2+} *with Different Anions* [16] [17]

Anion	log K_1	Anion	log K_1
Cl^-	0.08	$HOOC-COO^-$	2.55
OH^-	2.5	$H_2N-CH_2-COO^-$	3.44
HPO_4^{2-}	2.5	$HOOC-CH(OH)-CH_2-COO^-$	0.77
CH_3COO^-	0.51		

$$E = E_o + \frac{2.303\ RT}{F} \log\left(a_{MgOH} + K^{Pot}_{MgOH,MgCl} \cdot a_{MgCl} + K^{Pot}_{MgOH,Mg} \cdot a_{Mg}^{1/2} + \sum_J K^{Pot}_{MgOH,J} \cdot a_J^{1/zJ}\right) \quad (1)$$

Since both $MgOH^+$ and Mg^{2+} are present in all of the aqueous solutions studied and since the slopes of the electrode-response functions *(Fig. 4)* tend towards values for monovalent ions, $MgOH^+$ was chosen as the reference ion. This does not imply that $MgOH^+$ represents the preferred ion for each situation studied (type of carrier, composition of solution). For the determination of selectivity factors by the separate solution method the measured EMF values are then discussed by:

$$E_J = E_o + \frac{2.303\ RT}{F} \log(K^{Pot}_{MgOH,J} \cdot a_J^{1/zJ}) \quad (2)$$

$$E_{Mg} = E_o + \frac{2.303\ RT}{F} \log(a_{MgOH} + K^{Pot}_{MgOH,MgCl} \cdot a_{MgCl} + K^{Pot}_{MgOH,Mg} \cdot a_{Mg}^{1/2}) \quad (3)$$

The combination of *Eqns. 2* and *3* yields an expression for the selectivity factor $K^{Pot}_{MgOH,J}$:

$$K^{Pot}_{MgOH,J} = \frac{a_{Mg}'}{a_J^{1/zJ}} \cdot 10^{\left(\frac{F \cdot (E_J - E_{Mg})}{2.303\ RT}\right)} \quad (4)$$

where

$$a_{Mg}' = a_{MgOH} + K^{Pot}_{MgOH,MgCl} \cdot a_{MgCl} + K^{Pot}_{MgOH,Mg} \cdot a_{Mg}^{1/2} \quad (5)$$

Thus, a_{Mg}' represents the sum of the activities of all contributing charged Mg species. The selectivity factors $K^{Pot}_{MgOH,MgCl}$ and $K^{Pot}_{MgOH,Mg}$, however, are experimentally not directly accessible. They may be estimated by fitting a response curve to the experimentally determined values. *Fig. 6* indicates that a satisfactory fit (dashed lines) is obtained even for sample solutions with different ion-backgrounds and pH values. For the calculation of the activities of the ions involved, the *Debye-Hückel Eqn. 6,*

$$\log \gamma_i = \frac{-0.51 \cdot z_i^2 \cdot I^{1/2}}{1 + 0.33 \cdot a \cdot I^{1/2}} \quad (6)$$

$$I = 0.5 \sum_i c_i z_i^2 \quad (7)$$

using the parameters a given in *Table 2* and the thermodynamic formation constants of *Table 1,* was used. The potentiometric selectivity factors for the best fit are given in *Table 3.* For metal ions which tend to form rather stable hydroxy or other anion complexes, there will be a competition of the various species for the permeating ion complexes in the membrane phase. The present study demonstrates that even with Mg^{2+}, which forms relatively weak complexes with anions, monovalent complexes

Table 2. *Values of the Parameter a, as Used in Eqn.* **6**

Ion	a
OH^-, Cl^-	3.0
Mg^{2+}	8.0
$MgCl^+$, $MgOH^+$	5.0[a])
Na^+	4.0
K^+	3.0
Ca^{2+}	6.0

[a]) Estimated.

Table 3. *Potentiometric Selectivity Factors, log* $K^{Pot}_{MgOH,J}$, *for a Liquid-Membrane Electrode Based on Ligand* **3**

J	log $K^{Pot}_{MgOH,J}$
$MgCl^+$	−2.3
Mg^{2+}	−3.2
H^+	3.4
Na^+	−4.0
K^+	−3.3
Ca^{2+}	−2.9

heavily influence the electromotive behavior of solvent polymeric membranes. This has significant consequences for the analytical relevance of such sensors. Although the membranes based on the carrier **3** exhibit a useful Mg^{2+} response at a typical extracellular ion-background *(Fig. 6)*, the presence of other complexing anions may lead to serious interferences (see *Table 1*). This requires a calibration which takes care of all of the relevant sample anions that undergo complex formation with Mg^{2+}. Obviously, this drawback can be partly circumvented by the design of carriers with high selectivity for Mg^{2+} relative to monovalent Mg complexes.

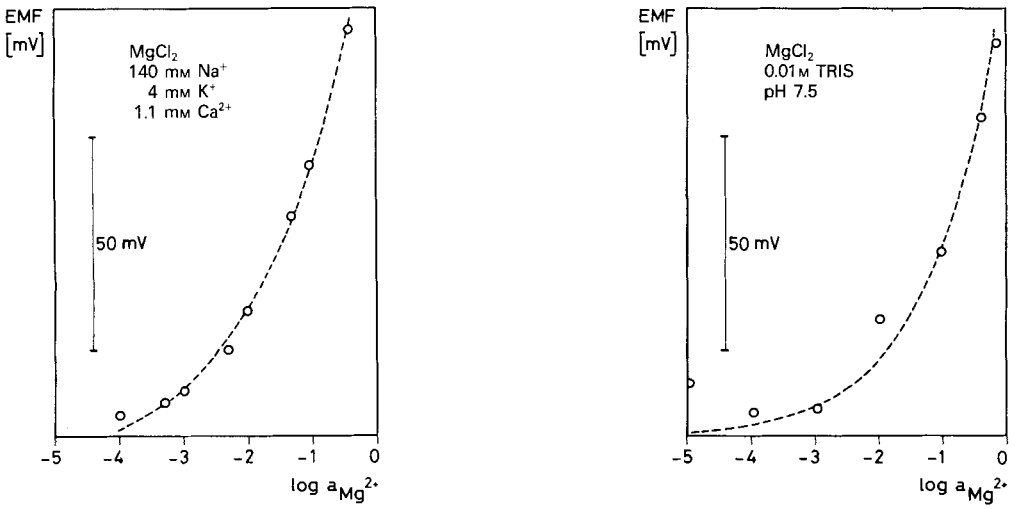

Fig. 6. *EMF response of liquid membrane electrodes based on the cyclopeptide* **3** *and on KTpClPB to aqueous solutions of* $MgCl_2$. *Left:* Extracellular ion background, unbuffered. *Right:* Buffered solutions of $MgCl_2$. *Dashed lines:* Calculated response curves using the selectivity factors given in *Table 3*. The parameters given in *Table 1* and *2* were taken for the calculation of the Mg^{2+}, $MgCl^+$ and $MgOH^+$ activities.

Experimental Part

General. – Chemicals and Solvents. DCC: *Fluka, puriss.;* Boc-proline: *Bachem;* Boc-leucine: *Bachem;* Nps-leucine dicyclohexylamine salt: *Fluka;* diphenylphosphoryl azide: *EGA-Chemie;* ODS (Me_2)-silica: was prepared according to [18]. PVC: *Lonza AG,* Visp, S *704* hochmolekular; *o*-NPOE: *Fluka, purum;* KT*p*ClPB: *Fluka, purum, p.a.;* Tris: *Fluka, puriss. p.a.;* metal chlorides in highest purity available. HPLC: *Spectra Physics 8000 B* equipped with 0.46 × 25 cm columns. Prep. HPLC was performed on columns with axial compression. MS: *Perkin-Elmer 270, Kratos AEI MS-50, Varian MAT 311A.* FAB-MS: *Kratos AEI MS-50* fitted with *M*-scan FAB system; Ar bombardment at 8–10 kV. *EMF* measurements: deionized H_2O, doubly distilled from quartz vessels; 16-channel electrode monitor, each channel was equipped with a FET operational amplifier *AD 515 KH* (*Analog Devices,* Norwood, MA). Data acquisition was performed with an *Intel Data System* (*Intel Corp.,* Santa Clara, CA) and our own software. The pH determination was carried out with a *Philips GA100* glass electrode. The external reference electrode was a double-junction calomel electrode, *Philips R11. Abbreviations.* PVC, poly(vinyl chloride); Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; KT*p*ClPB, potassium tetrakis(*p*-chlorophenyl)borate; *o*-NPOE, *o*-nitrophenyloctylether; Leu, leucine; Pro, proline; Nps, 2-nitrophenylthio; Boc, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; ODS($Me)_2$, octadecyldimethylsilyl.

EMF Measurements. Solvent polymeric membranes were prepared [19] which contained 1% (w/w) ligand (**1–8**), 66% (w/w) *o*-NPOE and 33% (w/w) PVC. Membranes with lipophilic anionic sites contained approximately 70% (mol) KT*p*ClPB. Throughout, cell assemblies of the type Hg; Hg_2Cl_2, KCl (satd.) | 3M KCl | sample solution || solvent polymeric membrane || 0.1M $MgCl_2$, 0.01MTris (pH 7.5) AgCl; Ag were used. Activity coefficients and complexation equilibria for ionic species were calculated as described above. Experimental data were corrected for changes in the liquid-junction potential using the *Henderson* formalism [20]. The selectivity factors were determined by the separate solution method [9] in 0.1M aq. metal chloride solutions using 0.01MTris buffer or 0.01M $Na_2B_4O_7$ buffer of pH 7.5 and pH 8.8, respectively. All measurements were performed at 20° ± 0.5°.

Syntheses. The cyclopeptides **1–8** were obtained from the corresponding open-chain peptide sequences by intramolecular amide bond formation in dilute soln. with the aid of either dicyclohexylcarbodiimide [21] [22] or diphenylphosphoryl azide [23]. The cyclization yields ranged from 4% to 18%, after purification by prep. HPLC. The structures were verified by MS, M^+ being observed in all cases, and by quant. amino-acid analysis. The open-chain peptides were synthesized automatically by the *Merrifield* solid-phase method [24] in nearly quant. yield. Quant. amino-acid analysis gave the expected amino-acid ratios. The purity of the crude products was at least 95%, as judged by HPLC, and they were used directly for the cyclizations.

Open-Chain Peptides. Nps-Leuresin and Boc-Proresin, obtained by esterification of Nps-L-leucine and Boc-L-proline with chloromethylated *Bio-Beads S-X2* by the method of *Gisin* [25], and containing 0.4 and 0.9 mmol amino acid/g, were subjected to repeated cycles of deprotection and coupling [24] in a peptide synthesizer (*SYN 1,* The Danish Institute of Protein Chemistry). The syntheses were carried out on a 1–4 mmol scale, using 2 h coupling with 3 equiv. of DCC and Boc-amino acid, and 0.5 h deprotection with 1MHCl in AcOH. Each peptide was cleaved from the resin by treatment for 0.8 h with HBr in CF_3COOH [24] at r.t. The peptide hydrobromide, remaining after evaporation of the solvent, was dissolved in DMF, and precipitated by addition of dry Et_2O. The crude products were obtained in yields of 90–95% of theory. Anal. HPLC was performed on an *SP 8000B,* using ODS($Me)_2$-silica (10 μm) as stationary phase, and a mobile phase, consisting of a gradient of MeCN (30–100%) in H_2O acidified with 0.4% CF_3COOH (detection performed by UV at 215 nm). Integration indicated purities of 95–98%. Quant. amino-acid analysis gave the expected ratios accordingly, the peptides were used for cyclization without purification.

Cyclization. Cyclization was carried out on a 1-mmol scale by several different procedures. Thus, **1** and **3** were cyclized with DCC/*N*-hydroxysuccinimide in DMF/CH_2Cl_2 according to [22], while **2** and **4** were cyclized with DCC in pyridine according to [21] (*N*-hydroxysuccinimide was not used in this procedure). The yields obtained, after purification by prep. HPLC as described below, were: **1** 10%, **2** 10%, **3** 15%, **4** 5%. Cyclization of **4** by diphenylphosphoryl azide according to [23] instead of DCC, increased the yield to 10%. This method was adopted for the subsequent cyclizations of **5–8**, for which the yields were: **5** 18%, **6** 11%, **7** 4%, **8** 12%.

Chromatographic Purification. The crude cyclization product was dissolved in MeOH, and subjected to reversed-phase chromatography on ODS($Me)_2$-silica (10 μm) (column: 4 × 18 cm), using a gradient of MeOH (50–100%) in H_2O, which was acidified with either 0.4% CF_3COOH or 10 mM triethylammonium formate to pH 3. The optical rotation of the effluent was recorded at 365 nm with a *Perkin-Elmer 141* photoelectric polarimeter equipped with a flow-through cell. All the cyclization products gave several major peaks from

which the one belonging to the target peptide could only be conclusively assigned by MS (because of the similarity in amino-acid composition). Final purification was achieved by chromatography on unsubstituted silica (*Si-60*, 12 μm) (column: 4 × 25 cm), using a gradient of MeOH (0–10%) in $CHCl_3$. The products were obtained as glassy or crystalline residues upon evaporation of the solvents.

Analysis. The purity of the synthesized cyclic octa- and decapeptides was assayed by analytical HPLC in the same system as used for the open-chain precursors **1–8** and was judged to be at least 98%. Quant. amino-acid analysis after 24- or 48-h hydrolysis in 6N HCl at 110° yielded the correct Leu/Pro ratios (1:1 or 3:2). The amino-acid content was close to the theoretical value with slight deviations being due to the varying amount of the solvent of crystallization present in the crystals. MS yielded the expected sequence information as well as the molecular ion in all cases ($M^+ = 840$, 1049, and 1066). MS(FAB): **1**: 863 (6, $M\text{Na}^+$); 842 (45); 841 (100, $M\text{H}^+$); 784 (5, $M\text{H}^+ - \text{CH}_2\text{CH}(\text{CH}_3)_2$); 744 (2, $M\text{H}^+ -$ Pro); 728 (3, $M\text{H}^+ -$ Leu); 631 (3, $M\text{H}^+ -$ Leu-Pro); 603 (2, $M\text{H}^+ -$ Leu-Pro-CO); 534 (1, $M\text{H}^+ -$ Pro-Leu-Pro); 518 (5, $M\text{H}^+ -$ Leu-Pro-Leu); 421 (29, $M\text{H}^+ -$ (Pro-Leu)$_2$); 211 (91, $M\text{H}^+ -$ (Pro-Leu)$_3$); *etc.* **3**: 1052 (62); 1051 (100, $M\text{H}^+$); 994 (8, $M\text{H}^+ - \text{CH}_2\text{CH}(\text{CH}_3)_2$); 954 (1, $M\text{H}^+ -$ Pro); 938 (1, $M\text{H}^+ -$ Leu); 841 (4, $M\text{H}^+ -$ Pro-Leu); 813 (2, $M\text{H}^+ -$ Pro-Leu-CO); 744 (1, $M\text{H}^+ -$ Pro-Leu-Pro); 728 (1, $M\text{H}^+ -$ Leu-Pro-Leu); 631 (16, $M\text{H}^+ -$ (Pro-Leu)$_2$); 603 (8, $M\text{H}^+ -$ (Pro-Leu)$_2$-CO); *etc.* **4**: 1089 (56, $M\text{Na}^+$); 1068 (50); 1067 (78, $M\text{H}^+$); 1010 (8, $M\text{H}^+ - \text{CH}_2\text{CH}(\text{CH}_3)_2$); 954 (6, $M\text{H}^+ -$ Leu); 841 (2, $M\text{H}^+ -$ Leu$_2$); 760 (6, $M\text{H}^+ -$ Pro-Leu-Pro); 744 (5, $M\text{H}^+ -$ Leu-Pro-Leu); 647 (11, $M\text{H}^+ -$ Pro-Leu-Leu-Pro); 534 (70, $M\text{H}^+ -$ Leu-Pro-Leu-Leu-Pro); 421 (100, $M\text{H}^+ -$ (Leu-Pro-Leu)$_2$); *etc.* **6**: 1074 (8); 1073 (6, $M\text{Na}^+$); 1052 (74); 1051 (100, $M\text{H}^+$); 994 (23, $M\text{H}^+ - \text{CH}_2\text{CH}(\text{CH}_3)_2$); 954 (3, $M\text{H}^+ -$ Pro); 938 (2, $M\text{H}^+ -$ Leu); 841 (7, $M\text{H}^+ -$ Pro-Leu); 813 (5, $M\text{H}^+ -$ Pro-Leu-CO); 799 (2); 744 (3, $M\text{H}^+ -$ Leu-Pro-Leu); 728 (4, $M\text{H}^+ -$Leu-Pro-Leu); 631 (40, $M\text{H}^+ -$ (Pro-Leu)$_2$); 603 (19, $M\text{H}^+ -$ (Pro-Leu)$_2$-CO); *etc.*

Some of the data presented here have been reported at the 17th European Peptide Symposium in Prague, Czechoslovakia, 1982 and at the 18th European Peptide Symposium in Stockholm, Sweden, 1984 (see also [26] and [27]).

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