Development of a Novel Class of Cyclic Hexapeptide Oxytocin Antagonists Based on a Natural Product†

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A new structural class of cyclic hexapeptide oxytocin antagonists derived from *Streptomyces silvensis* and typified by L-365,209 (cyclo-[L-prolyl[1]-D-phenylalanyl[2]-L-isoleucyl[3]-D-dehydropiperazyl[4]-L-dehydropiperazyl[5]-D-(*N*-methyl)phenylalanyl[6]]) was recently reported. In this paper we further delineate the structure–activity profile for this new class by systematic study of L-365,209 analogs obtained by total synthesis. The optimal combination of cyclic amino acid ring sizes at positions 1, 4, and 5 and the role of the *N*-alkyl substituent at position 6 was elucidated. The lipophilic amino acids at positions 2 and 3 and the unusual amino acid D-dehydropiperazic acid at position 4 were found to be the most critical residues for obtaining good oxytocin receptor affinity. Analogs containing a basic side chain at the less critical 5- and 6-positions maintained good receptor affinity and also had useful levels of water solubility for intravenous formulation. By combining potency- and solubility-enhancing substitutions, several analogs were identified that have the desired combination of properties in vitro (**22**, cyclo-[L-prolyl-D-tryptophanyl-L-isoleucyl-D-pipecolyl-L-pipecolyl-D-histidyl]; **25**, cyclo-[L-prolyl-D-2-naphthylalanyl-L-isoleucyl-D-pipecolyl-L-pipecolyl-D-histidyl]; **26**, cyclo-[L-prolyl-D-tryptophanyl-L-isoleucyl-D-dehydropiperazyl-L-pipecolyl-D-histidyl]; **33**, cyclo-[L-prolyl-D-tryptophanyl-L-isoleucyl-D-pipecolyl-L-piperazinylcarboxy-D-(*N*-methyl)phenylalanyl]; **34**, cyclo-[L-prolyl-D-phenylalanyl-L-isoleucyl-D-dehydropiperazyl-L-ornithyl-D-(*N*-methyl)phenylalanyl]). In general, this class exhibited good selectivity for binding to the oxytocin receptor versus the arginine vasopressin V1a and V2 receptor subtypes, although increased V2 receptor affinity was observed in one case (**32**, cyclo-[L-prolyl-D-2-naphthylalanyl-L-isoleucyl-D-pipecolyl-L-lysyl-D-(*N*-methyl)phenylalanyl]). Unexpectedly, compound **33** was found to stimulate contractions of the isolated rat uterus via activation of the uterine bradykinin receptor. Compounds **22**, **25**, **26**, **33**, and **34** were found to be potent antagonists of oxytocin-stimulated contraction of the rat uterus in vitro and in vivo. Compounds **22** and **25** were additionally characterized as potent antagonists of oxytocin-stimulated uterine contractions in the near-term pregnant rhesus monkey. These studies thus demonstrate the selectivity and efficacy of certain members of this novel class of antagonists and suggest their use as pharmacological tools in further defining the role of oxytocin in both term and preterm labor.

Introduction

The neurohypophyseal hormone oxytocin (OT; Figure 1) is well known for its contractile effects on the uterus and mammary tissue. In conjunction with several other endogenous factors, e.g., estrogen, progesterone, and prostaglandins, OT plays an important role in regulating uterine motility during parturition.[1,2] The concept of OT receptor blockade as a mechanistically novel method for treating preterm labor[3] has long been recognized and has gained support from several recent studies in which OT antagonists have been shown to inhibit both spontaneous and OT-induced uterine contractions in pregnant rodents and primates.[4] Encouraging clinical results have also been

† Presented in honor of Dr. Ralph F. Hirschmann on the occasion of his 70th birthday. As a mentor and friend he has offered invaluable guidance and support throughout his years of leadership in the Merck Sharp & Dohme Research Laboratories. He played a key role in developing a leading peptide research group in these laboratories. His insights in methods of peptide synthesis and in the use of cyclic peptides toward the solution of medicinal chemistry problems were pioneering, showing the path for studies such as the one reported here.

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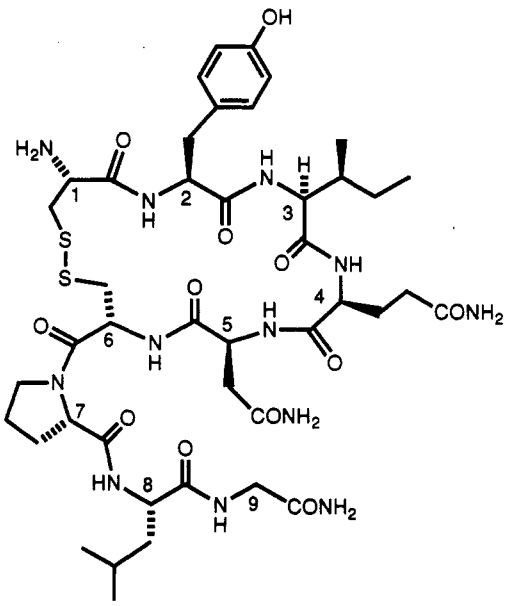
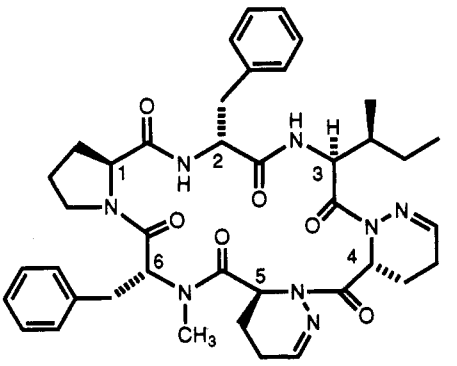


Oxytocin (OT)



L-365,209

[^{3}H]OT: K_i = 7.3 nM (rat uterus)

[^{3}H]AVP: K_i = 370 nM (rat liver V1a)
820 nM (rat kidney V2)

Figure 1.

found using the OT antagonist RWJ 22164 (Atosiban) for reducing uterine contractility in women with preterm labor.[5] Given the organ specificity of OT late in gestation, OT receptor blockade may offer a highly selective method of tocolysis, perhaps with fewer mechanism-related side effects compared to the commonly employed tocolytics such as magnesium sulfate and β-adrenergic agonists.[6]

The pioneering total syntheses of the structurally related neurohypophyseal hormones OT and arginine vasopressin (AVP) by du Vigneaud and colleagues nearly 40 years ago[7] ushered in the era of peptide hormone structure–function study. Over the last several decades the study of OT and AVP analogs has resulted in the discovery of many selective agonists and antagonists.[8] A number of these analogs have contributed enormously to our understanding of the physiological roles of OT and AVP,[8a,b] as well as providing a general understanding of the molecular basis of hormone–receptor interactions.[8c-e] Our interest in this area began with the discovery of a structurally novel class of cyclic hexapeptide OT antagonists derived from *Streptomyces silvensis*.[9] Representative of this class is L-365,209 (Figure 1), which in radioligand binding assays displays good affinity for the rat uterine OT receptor and selectivity with respect to the AVP-V1a (rat liver) and AVP-V2 (rat kidney) receptor subtypes. L-365,209 was also characterized as a potent and relatively long-acting OT antagonist in the rat, with no evidence of agonist properties. In this paper we describe structure–activity studies of L-365,209 analogs that have defined key structural features for obtaining good receptor affinity and selectivity, as well as substitutions that increase aqueous solubility to facilitate intravenous formulation.[10]

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Results and Discussion[11]

Chemical Methods. The linear hexapeptide precursors were prepared using either solution-phase or solid-phase methods. From initial solution-phase studies using *N*-Boc amino acids, it was found that many of the intermediates were not stable to the acidic conditions required to remove the Boc group[12] and thus *N*-Fmoc amino acids were employed. Because of the difficulty in acylating pipecolic acid and the various other secondary amino acids used in the sequences, special carboxyl group activation was required, including the BOP-Cl method of Tung and Rich,[13] the Fmoc amino acid chloride method of Carpino and co-workers,[14] and for especially difficult cases, newly developed methodology employing an Fmoc amino acid chloride in the presence of silver cyanide.[15,18] Efficient cyclization of the linear hexapeptides occurred upon formation of the amide bond between positions 1 and 2,[16] using DPPA[17] as illustrated in Scheme I with the synthesis of compound 1. The success of this approach in solution led to the development of a solid-phase method utilizing *N*-Fmoc amino acid chlorides (Scheme I).[18] The linear hexapeptides prepared on solid phase were cleaved from the resin with hydrazine and were cyclized via the acyl azide.[19] With the exception of the five compounds noted below, all of the analogs described herein were prepared in a linear fashion and cyclized between positions 1 and 2 as shown in Scheme I. Piperazic acid (Piz) was prepared and resolved using a variation on the procedure described by Hassal et al.[20] The differentially protected derivative, D-(N^{δ}-Cbz,N^{α}-Fmoc)Piz, was used in coupling reactions.[21] Piperazinecarboxylic acid (Ppz) was prepared and resolved using a modification of the procedure of Felder et al.[22] The absolute configuration was established by correlating the optical rotation of Ppz obtained by resolution with (+)-10-camphorsulfonic acid with a sample prepared from cyclo-(L-Ser-Gly). Formation of the copper(II) carboxylate salt allowed selective protection to obtain L-(N^{δ}-Cbz,N^{α}-Fmoc)Ppz used in coupling reactions.[23] Preparation of cyclic hexapeptides containing the unusual amino acid dehydropiperazic acid (Δ-Piz), was accomplished by either of two methods. Compound **20** was prepared using D-(N^{δ}-Cbz,N^{α}-Fmoc)Piz in step c of the linear approach shown in Scheme I. The D-Piz[4] cyclic hexapeptide that resulted was oxidized to **20** in modest yield using *tert*-butyl hypochlorite in pyridine. A more efficient strategy was developed that involves fragment coupling of the Fmoc-Ile[3]-D-Δ-Piz[4] dipeptide, the preparation of which is given in Scheme II. The latter approach allows for incorporation of other oxidation sensitive functionality in the cyclic hexapeptide as is found in compounds **26**, **34**, and **35**. The linear hexapeptide precursors to the position 1 substituted analogs **5** and **9** were prepared by fragment coupling of the 2–6 pentapeptide pivaloyl mixed anhydride with the benzyl esters of L-Pip and L-(*N*-Me)Ala, respectively.

Biological Methods. The high-affinity binding of [^{3}H]-OT to uterine tissue and [^{3}H]AVP to liver (AVP-V1a site) and kidney medullary (AVP-V2 site) formed the basis for competition experiments to determine receptor affinities of the test compounds listed in Tables I–VI. Uterine tissue was taken from pregnant rats (day 22–23 of gestation) or from DES-pretreated rats. Uterine tissue (myometrium) was also taken from near-term pregnant rhesus (day 140–157 of gestation) and from nonlabor pregnant women with informed consent undergoing caesarian section (six at week 38–39 of gestation and one at week 32 of gestation). Liver and kidney medulla were taken from male rats. Receptor affinities in Tables I–V are expressed as IC_{50} values, the molar concentration of test compound required to inhibit binding of the radioligand by 50% to the receptor of interest. Competition studies were conducted at equilibrium using 1 nM [^{3}H]OT or 0.5 nM [^{3}H]AVP. K_i values in Table VI were calculated by the method of Cheng and Prusoff,[24] where $K_i = IC_{50}/[1 + c/K_d]$, and [^{3}H]OT K_d = 0.69 nM (rat uterus), 0.93 nM (rhesus myometrium), 1.1 nM (human myometrium). Procedures for these radioligand binding assays have been described in detail.[9,25]

(11) List of abbreviations: AVP = arginine vasopressin; Boc = *tert*-butyloxycarbonyl; BOP = (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate; BOP-Cl = bis(2-oxo-3-oxazolidinyl)-phosphinic chloride; BuOH = *n*-butanol; Cbz = benzyloxycarbonyl; DCM = dichloromethane; DES = diethylstilbesterol dipropionate; DIEA = diisopropylethylamine; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; DPPA = diphenyl phosphorazidate; EtOAc = ethyl acetate; Fmoc = (9-fluorenylmethoxy)carbonyl; 1-Nal = 3-(1-naphthyl)alanine; 2-Nal = 3-(2-naphthyl)alanine; Nle = norleucine = 2-aminohexanoic acid; Orn = ornithine = 2,5-diaminopentanoic acid; OT = oxytocin; PAM = [4-(hydroxymethyl)phenylacetamido]methyl poly(styrene-*co*-divinylbenzene) resin; PI = phosphatidylinositol; Pip = pipecolic acid = piperidine-2-carboxylic acid; Piz = piperazic acid = hexahydropyridazine-3-carboxylic acid; Δ-Piz = dehydropiperazic acid = 2,3,4,5-tetrahydropyridazine-3-carboxylic acid; Ppz = piperazine-2-carboxylic acid; TFA = trifluoroacetic acid; THF = tetrahydrofuran. Standard three-letter abbreviations are used for the 20 common amino acids.

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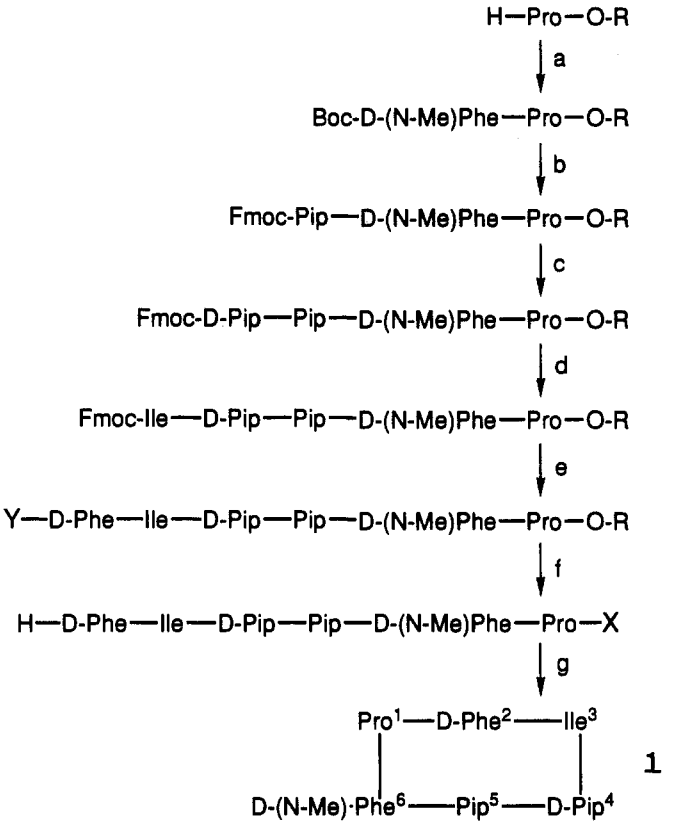
Scheme I



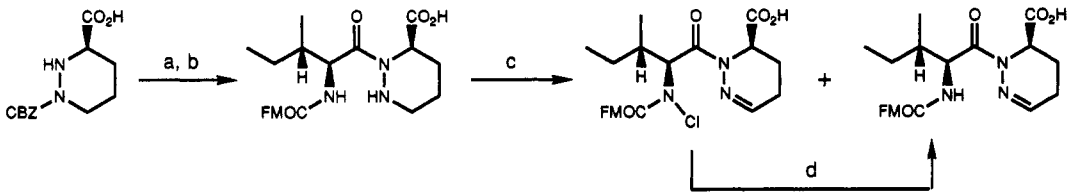
Solution Phase Synthesis: R = CH_2Ph

(a) Boc-D-(N-Me)Phe-OH, BOP-Cl, DIEA, DCM, 0°C (b) i. HCl-EtOAc; ii. Fmoc-Pip-Cl, DIEA, DCM, 0°C (c) i. Et_2NH-CH_3CN; ii. Fmoc-D-Pip-Cl, AgCN, toluene, 80°C (d) i. Et_2NH-CH_3CN; ii. Fmoc-Ile-Cl, AgCN, toluene, 80°C (e) i. Et_2NH-CH_3CN; ii. Cbz-D-Phe-OH, BOP, DIEA, CH_3CN, 25°C (Y=Cbz) (f) H_2 (55 psig), $Pd(OH)_2$, EtOH (X=OH) (g) DPPA, $NaHCO_3$, DMF, 0°C (0.003M)

Solid Phase Synthesis: R = PAM-resin

(a) Boc-D-(N-Me)Phe-OH, BOP, DIEA, DMF (b) i. HCl-dioxane; ii. Fmoc-Pip-Cl, DIEA, DCM, 5°C (c) i. piperidine-DMF; ii. Fmoc-D-Pip-Cl, DIEA, DCM, 25°C (d) i. piperidine-DMF; ii. Fmoc-Ile-Cl, DIEA, DCM, 25°C (e) i. piperidine-DMF; ii. Fmoc-D-Phe-Cl, DIEA, DCM, 25°C (Y=Fmoc) (f) i. piperidine-DMF; ii. NH_2NH_2, MeOH, 50°C (X=$NHNH_2$) (g) i. i-$C_5H_{11}ONO$, pH 3, -20°C, DMF; ii. DMF to 0.003M; iii. DIEA to pH 8

Scheme II [a]



[a] Reagents: (a) Me_3SiCl, CH_2Cl_2; Fmoc-Ile-Cl, iPr_2NEt; H_3O^+; (b) 1 atm H_2, 10% Pd/C, EtOH; (c) Me_3SiCl, pyridine; *t*-BuOCl; H_3O^+; (d) Bu_4NI, NaI, $Na_2S_2O_3$, H_2O–CH_2Cl_2.

Results for antagonism of OT-stimulated contraction of the isolated rat uterus are listed in Table VI. Antagonist potencies were determined by the method of Schild[26] and are expressed as pA_2 values, the negative logarithm of the molar concentration of antagonist that causes a 2-fold rightward shift in the dose–response curve of an antagonist (i.e., a dose ratio of 2). Uteri were isolated from DES-pretreated rats and were prepared for recording of contractile responses as previously described.[9,25]

OT-stimulated phosphatidylinositol turnover in the rat uterus was estimated by measuring the accumulation of total [^{3}H]inositol phosphates in uterine slices prelabeled with [^{3}H]myoinositol as described previously in detail.[27]

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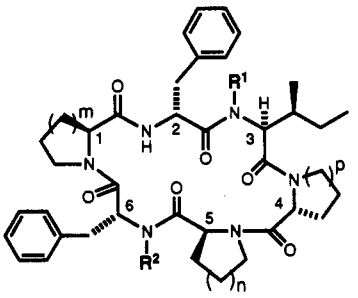
Table I. Cyclic Amino Acid Ring Size and Amide Bond Alkylation



no.	m	n	p	R^1	R^2	IC50 (nM) or % inhibn at 10 μM[a] OT	V1a	V2
L365,209[b]						18	1300	2300
1	1	2	2	H	CH_3	200	3200	3800
2	1	2	1	H	CH_3	540	23%	43%
3	1	1	2	H	CH_3	670	33%	39%
4	1	1	1	H	CH_3	3400	3300	81000
5	2	2	2	H	CH_3	660	51%	48%
6	1	2	2	H	H	340	5700	11000
7	1	2	2	H	$CH_2CH{=}CH_2$	190	45%	77%
8	1	2	2	CH_3	CH_3	25000	18%	19%

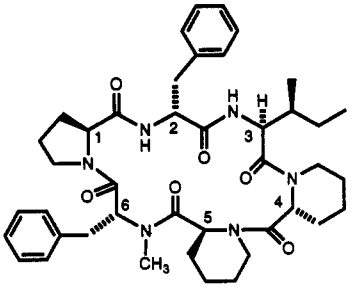
[a] IC_{50} values refer to the molar concentration of test compound that results in 50% displacement of [^{3}H]OT from the rat uterine receptor (OT column), or 50% displacement of [^{3}H]AVP from the rat liver and rat kidney receptors (V1a and V2 columns, respectively). For complete experimental details, see Pettibone, et al.[9,25] Percent inhibition values refer to the percent displacement of radioligand observed at a concentration of 10 μM of test compound. [b] See Figure 1 for structure.

IC_{50} values in Table VI refer to the concentration of test compound that reduces by 50% the accumulation of total [^{3}H]inositol phosphates stimulated by exposure of the tissue to 3 nM OT.

Results for antagonism of OT-stimulated contraction of the in situ rat uterus are given in Table VI. DES-pretreated rats were prepared for recording of isometric contraction of the uterus in situ as described previously in detail.[4e,9] OT was injected iv every 35 min (1 μg/kg; approximately an ED_{50} dose) for a total of eight times. The contractile response obtained after the third injection was set as 100%. Fifteen minutes before the fourth injection of OT, vehicle with or without antagonist was infused iv over a 10-min period. AD_{50} values, the dose of antagonist required to reduce the response to OT by 50%, were calculated from the contractile response obtained after the fourth injection of OT (i.e., 5 min postinfusion of test compound). Percentage antagonism relative to the concurrent vehicle-treated group was determined for each dose of antagonist, and the AD_{50} values were determined by regression analysis.

Structure–Activity Studies Using Radioligand Binding Assays. An early goal in the structure–activity studies was to simplify analog synthesis by replacing the dehydropiperazic acids at positions 4 and 5 in L-365,209 with more readily available amino acids. Incorporation of the isosteric pipecolic acids at these positions led to a 10-fold reduction in OT receptor affinity (1, Table I). Despite its reduced affinity, compound 1 has served as a useful benchmark for evaluating other structural changes in this chemically simplified series. The importance of cyclic amino acid ring size at positions 1, 4, and 5 was investigated with compounds 2–5 (Table I). All of these variations led to a reduction in OT receptor affinity relative to 1. The N^{α}-methyl substituent at position 6 in compound 1 had relatively little impact on OT receptor affinity. Removal of the methyl group produced only a modest drop in OT receptor affinity (6) and enlarging from methyl to propenyl had essentially no effect (7). To contrast this, methylation of the α-amino group at position 3 resulted in a profound drop in OT receptor affinity (8).

Table II. Alanine Point Substitutions



no.	substitution	IC50 (nM) or % inhibn at 10 μM[a] OT	V1a	V2
1		200	3200	3800
9	L-(N-Me)Ala[1]	930	34%	37%
10	D-Ala[2],D-Phe[6]	0%	0%	4%
11	L-Ala[3]	30000	3%	2%
12	D-(N-Me)Ala[4]	1500	20%	17%
13	L-(N-Me)Ala[5]	2700	25%	34%
14	D-(N-Me)Ala[6]	1200	20%	12%

[a] Receptor binding affinities as defined in Table I, footnote a.

Alanine point substitution analogs of 1 were prepared in order to determine the relative importance of each of the amino acids for receptor binding (Table II). To minimize the conformational perturbation of alanine substitution, the chirality of the amino acid being replaced was preserved by substitution with the corresponding alanine enantiomer, and N^{α}-methylalanine was incorporated at the three cyclic amino acid positions and also at position 6. The greatest drop in OT receptor affinity occurred with compounds 10 and 11, thus demonstrating the importance of the 2- and 3-position amino acids. The similarity of the circular dichroism spectra obtained for 1, 10, and 11 suggests that the reduced potency of the latter two compounds is not the result of a gross change in backbone conformation, and it is therefore inferred that the 2- and 3-position amino acids are important because of their side-chain interactions with the receptor.

Introduction of polar functionalities to enhance water solubility was then undertaken at the less critical positions (Table III). An acidic side chain at position 5 reduced affinity (15), whereas incorporation of amino acids having a basic side chain at either position 5 or position 6 were better tolerated (16–19). The histidine analog 19 was optimal in this regard, having improved OT receptor affinity and selectivity versus the AVP-V1a and AVP-V2 sites compared to 1, and having useful levels of aqueous solubility (5.1 mg/mL at pH 5.2).

Increasing the potency of 1 by 1 order of magnitude could be accomplished in either of two ways. From the studies on the direct chemical modification of L-365,209 it was found that D-Δ-Piz at position 4 was required for high levels of OT receptor affinity.[10b] This result held true in the synthetic series as determined with compound

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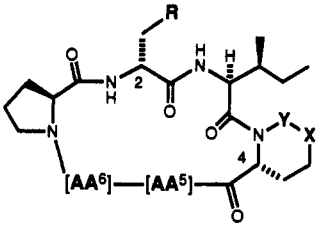
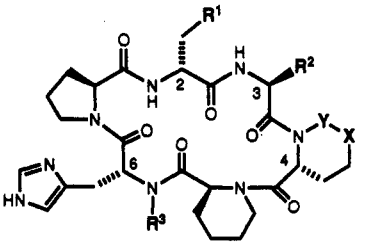
Table III. Introduction of Charged Groups at Positions 5 and 6, and Potency-Enhancing Substitutions at Positions 2 and 4



no.	R	X–Y	AA^6	AA^5	IC_{50} (nM) or % inhibn at 10 μM[a] OT	V1a	V2
15	phenyl	CH_2CH_2	D-(*N*-Me)Phe	L-Asp	1500	0%	12%
16	phenyl	CH_2CH_2	D-(*N*-Me)Phe	L-Lys	160	2000	1400
17	phenyl	CH_2CH_2	D-(*N*-Me)Phe	L-His	220	66%	65%
18	phenyl	CH_2CH_2	D-Lys	L-Pip	410	1100	7400
19	phenyl	CH_2CH_2	D-His	L-Pip	88	7300	19000
20	phenyl	CH=N	D-(*N*-Me)Phe	L-Pip	15	3500	1200
21	3-indolyl	CH_2CH_2	D-(*N*-Me)Phe	L-Pip	20	12000	750

[a] Receptor binding affinities as defined in Table I, footnote *a*.

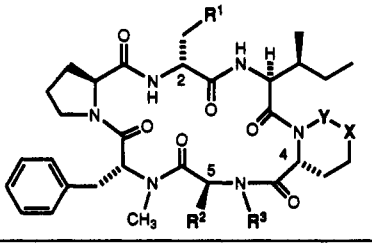
Table IV. Combination of Potency-Enhancing Modifications with a Solubilizing Group at Position 6, and Substitutions at Position 3



no.	R^1	R^2	R^3	X–Y	IC_{50} (nM)[a] OT	V1a	V2	aq solubility, mg/mL (pH)[b]
22	3-indolyl	2(*S*)-butyl	H	CH_2CH_2	15	7400	5200	2.0 (5.0)
23	1-CH_3-3-indolyl	2(*S*)-butyl	H	CH_2CH_2	10	3700	1300	nd
24	1-naphthyl	2(*S*)-butyl	H	CH_2CH_2	8.3	750	720	nd
25	2-naphthyl	2(*S*)-butyl	H	CH_2CH_2	2.2	2600	910	1.5 (5.0)
26	3-indolyl	2(*S*)-butyl	H	CH=N	6.0	4000	1100	1.9 (5.6)
27	3-indolyl	2(*S*)-butyl	CH_3	CH_2CH_2	18	14000	4300	nd
28	3-indolyl	isobutyl	H	CH_2CH_2	190	3000	11000	nd
29	3-indolyl	*n*-butyl	H	CH_2CH_2	72	1300	1700	nd

[a] Receptor binding affinities as defined in Table I, footnote *a*. [b] nd = not determined.

Table V. Combination of Potency-Enhancing Modifications with a Solubilizing Group at Position 5



	R^1	R^2	R^3	X–Y	IC_{50} (nM)[a] OT	V1a	V2	aq solubility, mg/mL (pH)[b]
30	3-indolyl	$(CH_2)_4NH_2$	H	CH_2CH_2	65	1600	410	nd
31	1-naphthyl	$(CH_2)_4NH_2$	H	CH_2CH_2	45	510	150	nd
32	2-naphthyl	$(CH_2)_4NH_2$	H	CH_2CH_2	13	1300	110	1.7 (5.2)
33	3-indolyl	$-CH_2NHCH_2CH_2-$		CH_2CH_2	8.4	32000	690	1.4 (6.5)
34	phenyl	$(CH_2)_3NH_2$	H	CH=N	15	800	1600	1.5 (7.2)
35	3-indolyl	$(CH_2)_3NH_2$	H	CH=N	25	1600	330	nd

[a] Receptor binding affinities as defined in Table I, footnote *a*. [b] nd = not determined.

20 (Table III). Incorporation of a larger aromatic amino acid at position 2 provided an alternate means to enhance OT receptor affinity (21, Table III). This latter finding was an important advance in that it established a high potency series that utilizes only readily accessible amino acids.

By combining the potency- and solubility-enhancing substitutions, several analogs with the desired combination of properties were identified (Tables IV and V). For example, the D-Trp^2,D-His^6 combination analog 22 (Table IV) exhibited good OT receptor affinity and selectivity, as well as useful levels of aqueous solubility. Methylation of the indole nitrogen (23) had little effect on OT receptor affinity, indicating that the potency-enhancing effect of

Table VI. In Vitro and in Vivo Pharmacology of Optimized Cyclic Hexapeptides

	OT receptor affinity, K_i (nM)[a,b]			isolated rat uterus[a]			in situ rat uterus[e]
				OT-stimulated contractions[c]		OT-stimulated PI turnover[d]	
	rat	rhesus	human	pA_2	slope	IC_{50} (nM)	AD_{50} (μg/kg)
22	6.0 ± 0.26	17 ± 3.5	9.4 ● 2.2	8.60 ± 0.09	1.47 ± 0.47	67	103
25	0.70 ± 0.21	6.4 ± 0.49	5.1 ± 0.63	8.53 ± 0.08	2.02 ± 0.42	40	102
26	1.3 ± 0.15	2.3 ± 0.06	1.3 ± 0.11	8.82 ● 0.13	1.64 ± 0.23	42	90.3
33	3.4 ± 0.69	4.7 ± 0.60	4.5 ± 0.67	8.59 ± 0.22	1.09 ± 0.30		101
34	6.0 ± 0.34	33 ± 5.1	21 ± 5.4	8.47 ± 0.10	1.43 ± 0.18		71.6

[a] For complete experimental details, see Pettibone, et al.[25] [b] Displacement of [^{3}H]OT from binding sites in uterine tissue taken from pregnant rats (d22–23 gestation), near-term pregnant rhesus (d140–157 gestation), or nonlabor pregnant women (six at week 38–39, one at week 32 gestation). K_i values were calculated by the method of Cheng and Prusoff[24] and are group means ± SE ($n \geq 3$). [c] Isolated uterus from DES-pretreated rats. pA_2 and slope values (x ± 95% CL) were determined from regression analysis of log (dose ratio − 1) vs −log [antagonist] by the method of Schild.[26] Each antagonist was tested at three to four concentrations, with four to seven tissues per concentration. [d] Uterine slices from DES-pretreated rats were labeled with [^{3}H]myoinositol. IC_{50} values refer to the concentration of test compound that reduces by 50% the accumulation of total [^{3}H]inositol phosphates stimulated by exposure of the tissue to OT. [e] AD_{50} values refer to the dose of antagonist required for 50% reduction of the contractile response of the uterus to OT (1 μg/kg, iv bolus) in DES-pretreated rats. For complete experimental details, see Clineschmidt et al.[4e]

the indole ring is not the result of the indole N–H being involved in an intermolecular hydrogen bond with the receptor or an intramolecular hydrogen bond to stabilize a receptor-bound conformation. Consistent with this interpretation, the two naphthylalanine isomers **24** and **25** also exhibited high receptor affinity. The 2-naphthyl isomer **25** exhibited the highest affinity for the rat OT receptor seen in the series. By combining potency-enhancing substitutions at positions 2 and 4, only a modest improvement in OT receptor affinity was realized (compare **26** with **22**), whereas an additive effect was found when measuring receptor affinity using primate uterine tissue (vide infra). Incorporation of a methyl substituent on the amide bond at position 6 did not provide the ca. 2-fold improvement in OT receptor affinity seen in an earlier series (compare **27** with **22**). Changes in the 3-position amino acid proved to be detrimental (**28** and **29**), results which support the conclusion from the alanine substitution study on the importance of the 3-position isoleucine side chain.

In a related series that utilizes lysine at position 5 for aqueous solubility, a similar trend of increasing OT receptor affinity was found with the 2-position substitutions of D-Phe (**16**; Table III), D-Trp, D-1-Nal, and D-2-Nal (**30–32**; Table V). OT receptor affinity and selectivity in this series, however, was somewhat diminished relative to the D-His[6] series. The D-2-Nal[2],Lys[5] combination (**32**), in fact, produced an analog with moderately good AVP-V2 receptor affinity. Improved OT receptor affinity and selectivity could be achieved by replacing lysine at position 5 with the cyclic amino acid, piperazine-2(*S*)-carboxylic acid (**33**). A potent, selective, and water-soluble analog resulted by replacing L-Δ-Piz at position 5 in L-365,209 with ornithine (**34**). Combining potency-enhancing substitutions at positions 2 and 4 in this series unexpectedly produced a modest decrease in OT receptor affinity (**35**).

Pharmacology of Selected Analogs. Detailed in vitro and in vivo pharmacological studies of several of the more potent combination analogs have been reported.[4e,25,28] Comparison of rat, rhesus, and human uterine OT receptor affinities for several of the more potent compounds is given in Table VI. The rank order of affinity was the same for the two primate tissues, but in comparing the rat versus primate tissues the order was different due to the very high affinity of compound **25** for the rat tissue. In the primate tissues, the highest affinity was obtained with **26**, the compound that combines potency-enhancing substitutions at positions 2 and 4.

All of the compounds in Table VI proved to be potent antagonists of OT-induced contraction of the isolated rat uterus. From the Schild analysis, compounds **25**, **26**, and **34** gave slopes that differed significantly from unity, suggesting the lack of a strictly competitive interaction. With the exception of compound **25**, the range and rank order of antagonist potencies from the in vitro functional assay were reflective of the receptor affinities found in the rat uterus radioligand binding assay. Compounds **22**, **25**, and **26** did not contract the isolated rat uterus and inhibited OT-stimulated PI turnover in rat uterine slices, thus demonstrating their behavior as pure antagonists in vitro. At relatively high concentrations (>100 nM), however, compound **33** and to a lesser extent compound **34** exhibited agonist-like properties (e.g., uterine contractions, PI turnover). These effects were the result of activation of the uterine bradykinin receptor and not the OT receptor.[28] The weak affinity of **22** and **25** for AVP-V1a and AVP-V2 receptors as determined in the radioligand binding assays was followed up with in vitro functional assays in which both compounds were found to antagonize AVP-stimulated contraction of the isolated rat tail artery (AVP-V1a receptor; pA_2 = 6.06 ± 0.13 and 6.55 ± 0.30, respectively) and inhibit AVP stimulation of adenylate cyclase activity in the rat kidney medulla (AVP-V2 receptor; IC_{50} = 3100 ± 620 and 810 ± 200 nM, respectively).[25]

In agreement with the studies using the isolated rat uterus, the compounds in Table VI were found to be potent in vivo antagonists of OT-induced uterine contractions in the rat.[4e] AD_{50} values were estimated from experiments using anesthetized rats in which the inhibition of OT-stimulated uterine contractions was measured after iv administration of the test compound at several doses. The duration of action of compounds **22** and **25** in this assay exceeded 145 min when administered at a near maximal dose. In the near-term pregnant rhesus monkey, compounds **22** and **25** have additionally been characterized as effective antagonists of OT-induced uterine contractions, with no evidence of agonist properties.[4e]

Structural Comparison of Different Antagonist Classes. The only overt structural homology between OT and the cyclic hexapeptide antagonist class represented by L-365,209 is the occurrence of an aromatic amino acid followed by isoleucine at positions 2 and 3 (Figure 1). In

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the cyclic hexapeptide antagonist class these amino acids have been shown to be very important for modulating OT receptor affinity. From studies with OT analogs, the 2-position amino acid has been shown to be a key site where structural modifications can produce antagonists. For instance, many potent antagonist analogs of OT incorporate a D amino acid at position 2 having a substituted or otherwise enlarged aryl side chain (e.g., *O*-alkyltyrosine, 3,5-dihalotyrosine, *p*-alkylphenylalanine, tryptophan).[8c-e,29] Somewhat more latitude has been found for substitutions at position 3 in antagonist analogs of OT compared to the cyclic hexapeptide class, but incorporation of isoleucine is generally beneficial for minimizing binding to the AVP receptors.[8c-e,30] The potency-enhancing effect of an amino acid at position 4 that incorporates a side chain capable of hydrogen bonding is seen in both classes of antagonists, i.e., D-dehydropiperazic acid in the cyclic hexapeptide class and threonine in the OT analog class.[8c-e] Thus, circumstantial evidence suggests that there may be a structural correspondence between the amino acids at positions 2–4 in the two classes. This proposed structural homology coupled with a better understanding of the conformational features[8e,31] of the two classes may be helpful in designing smaller, perhaps non-peptide OT antagonists.[32]

Conclusion. Structure–activity studies with synthetic cyclic hexapeptides related to the natural product-derived OT antagonist, L-365,209, have been useful for identifying structural components that modulate OT receptor affinity, selectivity, and water solubility. By combining potency-enhancing substitutions at positions 2 and/or 4 with solubility-enhancing substitutions at positions 5 or 6, the desired combination of properties was achieved. Several optimized compounds (Table VI) have been characterized as potent antagonists of OT-stimulated uterine contractions in vitro and in vivo and are useful pharmacological tools that may aid in further defining the role of OT in both term and preterm labor.

Experimental Section

Biological Methods. Radioligand binding assays and in vitro and in vivo pharmacological studies were performed as described elsewhere.[4e,9,25,28]

Chemical Methods.[11] *N*-Boc and *N*-Fmoc amino acids were purchased from Bachem, Inc. D-2-Nal and D-1-Nal were purchased from Synthetek, Inc. and N-protected using the procedure of Bolin et al.[33] Pipecolic acid was resolved as the tartrate salt[34] and N-protected using the procedure of Bolin et al.[23] Boc-D-(*N*-Me)Phe was prepared by the method of Benoiton and Cheung.[35] *N*-Methyl Fmoc amino acids were prepared using the method of Freidinger and co-workers.[36] Boc-L-Pro-PAM-resin was purchased from Applied Biosystems Inc. and Bachem Inc. All solvents were reagent grade and stored over 4-Å molecular sieves. THF was distilled from CaH_2–$NaBH_4$ under inert atmosphere. Dioxane was dried and freed of peroxides by passage through a column of activity I neutral alumina.

The motorized shaker apparatus and reaction vessels used in the solid-phase syntheses were custom designed and produced in house with the following specifications. The reaction vessels for a 1-mmol scale measured 3 cm × 9 cm with a female ground glass joint on top to accommodate a drying tube and a fritted disk and stopcock on the bottom for draining by vacuum. The motorized shaker apparatus allows for a 90° shake of the reaction vessel at the rate of 32 shakes/min.

Uniplate silica gel GF TLC plates (10 × 20 cm, 250 μm) were purchased from Analtech, Inc. Determination of reaction pH was estimated by spotting an aliquot from the reaction mixture on wetted E. Merck pH sticks. ^{1}H NMR spectra were measured at 300 MHz on a Varian XL-300, at 400 MHz on a Varian XL-400, and at 360 MHz on a Nicolet NT-360 using $(CH_3)_4Si$ as an internal standard. Fast atom bombardment mass spectra (FAB MS) were obtained on a VG-ZAB-HF spectrometer using xenon as the reagent gas.

Analytical HPLC were run on a Spectra Physics SP4270/8800 instrument (methods 1–3) or a Hewlett-Packard 1084B instrument (method 4) using the following conditions. HPLC method 1: column, Vydac C_{18}, 0.21 × 15 cm; mobile phases, A = 0.1% TFA in H_2O, B = 0.1% TFA in acetonitrile; gradient, T = 0 min, 95% A, 5% B, T = 15 min, 0% A, 100% B; flow = 2.0 mL/min; UV detection at 215 nm. HPLC method 2: column, Vydac C_{18}, 0.21 × 15 cm; mobile phases, A = 0.1% H_3PO_4 in H_2O, B = acetonitrile; gradient, T = 0 min, 95% A, 5% B; T = 15 min, 5% A, 95% B; flow = 1.5 mL/min; UV detection at 210 nm. HPLC method 3: column, Vydac C_{18}, 0.21 × 15 cm; mobile phases, A = 0.1% H_3PO_4 in H_2O, B = acetonitrile; gradient, T = 0 min, 95% A, 5% B, T = 45 min, 5% A, 95% B; flow = 1.5 mL/min; UV detection at 210 nm. HPLC method 4: column, Waters MicroBondapak C_{18}, 0.39 × 30 cm; mobile phases, A = 0.1% H_3PO_4 in H_2O, B = acetonitrile; gradient, T = 0 min, 95% A, 5% B, T = 30 min, 5% A, 95% B; flow = 3.0 mL/min; temperature = 40 °C; UV detection at 210 nm.

Purification Methods. Method A. The crude cyclization product (1 mmol) was dissolved in DMF (60 mL) and H_2O (20 mL) and treated with Bio-Rad AG 501-X8 mixed ion exchange resin (20 mL dry volume) for 1 h at ambient temperature. The solution was filtered, and the filtrate was evaporated under reduced pressure. The residue was lyophilized from dioxane–H_2O.

Method B. The crude product (1 mmol) was dissolved in MeOH (8 mL) and purified by preparative HPLC using the following conditions: column, 5 × 30 cm C_{18} Waters DeltaPak Prep Cartridge; mobile phases, A = 0.1% TFA in H_2O, B = 0.1% TFA in acetonitrile; gradient, T = 0 min, 95% A, 5% B, T = 45 min, 0% A, 100% B; flow = 40 mL/min; UV detection at 220 nm. The fractions containing product were combined, and the solvents were removed by lyophilization.

Method C. Pressurized silica gel column chromatography using 230–400-mesh silica gel was performed according to the method of Still and co-workers.[37]

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Method D. The compound in MeOH was loaded onto preparative TLC plates (precoated E. Merck $60F_{254}$ silica gel plates; 20 cm × 20 cm; 0.25-mm, 0.50-mm, 1.0-mm, or 2.0-mm coating thickness). After elution, the product band was collected and the compound was obtained by washing with 7:3 DCM–MeOH.

General Synthetic Procedures. Procedure 1: Fmoc-AA-Cl/DIEA/DCM Coupling. A 1.1-mmol sample of Fmoc amino acid chloride was prepared from the Fmoc amino acid using the oxalyl chloride/catalytic DMF/DCM method of Carpino and coworkers.[14a] To a 0 °C solution of the amine component (1.0 mmol) in DCM (15 mL) was added a DCM solution of the Fmoc amino acid chloride, followed by DIEA (1.1 mmol). After 10 min, an aliquot was spotted on wetted E. Merck pH indicator sticks, and more DIEA was added as needed to obtain a reading of pH 8. After 2 h, the reaction was diluted with DCM and washed with 5% aqueous HCl, water, and saturated aqueous $NaHCO_3$. The DCM layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure.

Procedure 2: Fmoc-AA-Cl/AgCN/Toluene Coupling. A 1.1-mmol sample of Fmoc amino acid chloride was prepared as described in procedure 1, and to it was added a solution of the amine component (1.0 mmol) in dry toluene (6 mL). AgCN (2.2 mmol) was added, and the mixture was vigorously stirred in an oil bath maintained at 80–85 °C. After 30 min, the reaction was cooled, diluted with toluene, and filtered through Celite, and the solvent was evaporated under reduced pressure.

Procedure 3: BOP/DIEA/CH_3CN Coupling. To a solution of the acid component (1.0 mmol) and amine component (1.0 mmol) in acetonitrile (15 mL) at 0 °C were added BOP reagent[38] (1.1 mmol) and DIEA (1.0 mmol). The reaction was warmed to ambient temperature and stirred for 2–16 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and washed with 5% aqueous HCl, water, and saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure.

Procedure 4: BOP-Cl/DIEA/DCM Coupling. To a 0 °C solution of the acid component (1.0 mmol) in DCM (15 mL) was added DIEA (1.0 mmol) followed by BOP-Cl (1.0 mmol).[13] After 1.5 h, a DCM solution of the amine component (1.0 mmol) was added, followed by DIEA (2.2 mmol). The mixture was stirred at 0 °C for 16 h, diluted with DCM, and washed with 5% aqueous HCl, water, and saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure.

Procedure 5: DPPA/$NaHCO_3$/DMF Cyclization. To a 0 °C solution of the linear hexapeptide (1.0 mmol) in dry, degassed DMF (350 mL) was added solid $NaHCO_3$ (10 mmol) followed by DPPA (2.0 mmol).[17] The mixture was stirred at 0–5 °C for 48 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and washed with 5% aqueous HCl, water, and saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure.

Procedure 6: Deprotection with TFA/DCM. A 1-mmol sample of *N*-Boc-protected peptide was dissolved in 5 mL of 2:1 DCM–TFA at 0 °C. The solution was warmed to ambient temperature. When TLC or HPLC analysis indicated complete consumption of starting material, the solvents were removed under reduced pressure and the residue was partitioned between DCM and aqueous $NaHCO_3$ to obtain the free base.

Procedure 7: Deprotection with Et_2NH/CH_3CN. A solution of 1 mmol of the *N*-Fmoc-protected peptide was dissolved in 5 mL of acetonitrile at ambient temperature and treated with an equal volume of diethylamine. When TLC or HPLC analysis indicated complete consumption of starting material, the solvents were removed under reduced pressure. The residue was dissolved in acetonitrile and washed with hexane to remove dibenzofulvene. The acetonitrile layer was evaporated under reduced pressure.

Procedure 8: Hydrogenolysis with 10% Pd/C. A solution of 1 mmol of the *N*-Cbz-protected peptide in 15 mL of 99:1 EtOH–HOAc was flushed with argon for several minutes. To the solution was added 50 mg of 10% palladium on carbon, and the mixture was flushed with H_2 and stirred at ambient temperature under 1 atm of H_2 until TLC or HPLC analysis indicated complete consumption of starting material. The mixture was flushed with argon and filtered through Celite, and the filtrate was evaporated under reduced pressure.

Procedure 9: Hydrogenolysis with 20% $Pd(OH)_2/C$. To a solution of 1 mmol of the peptide in 20 mL of MeOH was added 75 mg of 20% palladium hydroxide on carbon, and the mixture was shaken on a Parr hydrogenation apparatus under 55 psi of H_2 for 16 h. The mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro] (1). Step a: Boc-D-(*N*-Me)Phe-Pro-O-(PAM)-resin. Boc-Pro-O-(PAM)-resin (1.32 g, 1.0 mmol) with a loading of 0.76 mequiv of nitrogen/g was placed in a shaker flask and swelled for 2 h in 20 mL of DCM. The resin was treated with 20 mL of the following: DCM wash (3×, 2 min each); 4 N HCl–dioxane deblocking (2×, 15 min each), DCM wash (3×, 2 min each), DMF wash (3×, 2 min each), 10% DIEA–DMF neutralization (2×, 2 min each), DMF wash (2×, 2 min each). Boc-D-(*N*-Me)Phe (0.558 g, 2.0 mmol) in 15 mL of 1:1 DCM–DMF was added followed by DIEA (0.350 mL, 2.0 mmol). The resin was shaken for 5 min when solid BOP reagent (0.884 g, 2.0 mmol) was added. The resin was shaken for 10 min, and more DIEA (0.04 mL) was added to bring the supernatant to pH 8. The reaction was shaken for an additional 15 h at ambient temperature. To complete the cycle, the supernatant was drained and the resin was washed for 2 min each with 20 mL of the following: DMF (2×), DCM, MeOH, DCM, MeOH, DCM (3×).

Step b: Fmoc-Pip-D-(*N*-Me)Phe-Pro-*O*-(PAM)-resin. The resin from the previous step was treated with 20 mL of the following: 4 N HCl–dioxane deblocking (2×, 15 min each) and DCM wash (6×, 2 min each). The resin was cooled to 5 °C in a cold room, when a 0 °C solution of Fmoc-Pip-Cl (2.0 mmol) in DCM (20 mL) was added, and the mixture was shaken for 5 min. DIEA (0.35 mL, 2.0 mmol) was added, the reaction mixture was shaken for 2 min, and the supernatant was adjusted to pH 8 by the addition of more DIEA (0.1 mL). The resin was shaken for 15 h at 5 °C. To complete the cycle, the supernatant was drained and the resin was washed for 2 min each with 20 mL of the following: DCM (3×), CH_3OH, DCM, CH_3OH, DCM (3×).

Steps c–e: Fmoc-D-Phe-Ile-D-Pip-Pip-D-(*N*-Me)Phe-Pro-O-(PAM)-resin. Coupling of the remaining three amino acids was accomplished by cycling the resin through the following protocol. The resin was treated with 20 mL of the following: DMF wash (3×, 2 min each), 20% piperidine–DMF deblocking (2×, 15 min each), DMF wash (3×, 2 min each), DCM wash (3×, 2 min each). The Fmoc amino acid chloride (2 mmol) in DCM (20 mL) was shaken with the resin at ambient temperature for 5 min, when DIEA (2 mmol) was added. The mixture was shaken for 10 min, and if necessary more DIEA was added to bring the supernatant to pH 8. The resin was shaken for 2 h at ambient temperature. The supernatant was drained and the resin was washed for 2 min each with 20 mL of the following: DCM (4×), MeOH, DCM, MeOH, DCM (3×).

Step f: H-D-Phe-Ile-D-Pip-Pip-D-(*N*-Me)Phe-Pro-$NHNH_2$. The resin was treated with 20 mL of the following: DMF wash (3×, 2 min each), 20% piperidine–DMF deblocking (2×, 15 min each). The resin was washed for 2 min each with 20 mL of the following: DMF (3×), DCM (4×), CH_3OH, DCM, CH_3OH. The resin was dried in vacuo for 24 h. The dried resin (1.5 g) had a nitrogen value of 4.97% from combustion analysis, indicating a peptide loading of 0.59 mmol/g. The resin was stirred with 1:1 MeOH–hydrazine (60 mL) under nitrogen at ambient temperature for 1 h and at 50 °C for 30 min. The volatiles were removed under reduced pressure at 50 °C, and MeOH (60 mL) was added and evaporated under reduced pressure three times. The residue was suspended in MeOH (60 mL) and filtered. The filtrate was evaporated under reduced pressure and the residue was dried in vacuo for 15 h. The residue was dissolved in BuOH (100 mL) and extracted with water (3 × 50 mL) to remove traces of hydrazine. The BuOH layer was evaporated under reduced pressure and MeOH (100 mL) was added and evaporated under reduced pressure. The hydrazide was dried in vacuo for 15 h

(38) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Peptide Coupling Reactions. IV. Benzotriazolyl-N-Oxy-tris(dimethylamino)phosphonium Hexafluorophosphate. *Tetrahedron Lett.* **1975**, *16*, 1219–1222.

(579 mg; 75% of theoretical; HPLC (method 1) retention time = 7.75 min, purity = 95%).

Step g: cyclo-(D-Phe-Ile-D-Pip-Pip-D-(*N*-Me)Phe-Pro) (1). The hydrazide (579 mg, 0.737 mmol) was dissolved in dry, degassed DMF (6 mL) under an atmosphere of nitrogen and cooled to −15 °C. To the stirred solution was added 5 N HCl–THF (0.69 mL, 3.5 mmol), and the reaction was further cooled to −25 °C. Isoamyl nitrite (0.105 mL, 0.774 mmol) was added in small portions over a period of 1 h, monitoring for the disappearance of excess isoamyl nitrite by spotting aliquots on acidified starch–KI paper. When a positive starch–KI test persisted for 30 min, addition of isoamyl nitrite was discontinued. HPLC analysis indicated complete conversion to the acyl azide (retention time = 9.29 min, method 1). The reaction was then diluted with dry, degassed, precooled (−25 °C) DMF (150 mL). DIEA (0.67 mL, 3.8 mmol) was added to obtain a pH 8 solution. The reaction temperature was maintained at −20 °C for 24 h. A single product was detected by HPLC analysis (retention time = 11.16 min, method 1). The solution was evaporated under reduced pressure, and the residue was purified (method A). Compound 1 was obtained as a white solid by lyophilization from dioxane (520 mg; 70% of theoretical). TLC: R_f = 0.33 (98:2:0.2 $CHCl_3$–MeOH–NH_4OH). HPLC (method 1): t_R = 11.14 min, purity = 99%. ^1H NMR (300 MHz, $CDCl_3$): δ 7.87 (d, J = 7 Hz, 1 H), 7.67 (d, J = 8.6 Hz, 1 H), 7.23 (m, 10 H), 5.48 (t, J = 7.6 Hz, 1 H), 5.30 (d, J = 4.4 Hz, 2 H), 4.53 (m, 3 H), 4.00 (m, 1 H), 3.83 (m, 1 H), 3.49 (m, 2 H), 2.83 (s, 3 H), 2.15 (m, 1 H), 0.81 (m, 5 H). FAB MS: m/z = 741 (M + H^+). Anal. ($C_{42}H_{56}N_6O_6 \cdot 1.35H_2O$) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pro-L-Pip-D-(*N*-Me)Phe-L-Pro] (2). H-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (obtained as described for compound 8) was coupled to Fmoc-D-Pro (procedure 4). Purification (method C, 28% acetone–hexane) gave Fmoc-D-Pro-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.30 in 30% acetone–hexane) in 91% yield, which was N-deprotected (procedure 7). Fmoc-D-Phe-L-Ile-OCH_2Ph was deprotected (procedure 8) and purified by crystallization from DCM–hexane to give Fmoc-D-Phe-L-Ile-OH (TLC R_f = 0.50 in 8:8:1.9:0.1 $CHCl_3$–hexane–MeOH–H_2O) in 80% yield. To a −20 °C $CHCl_3$ solution of the dipeptide was added 4-methylmorpholine (1.1 equiv) and pivaloyl chloride (1.1 equiv), and the solution was stirred for 5 h at −20 °C. A −20 °C $CHCl_3$ solution of the tetrapeptide from above was added dropwise, and the resulting mixture was stirred at −20 °C for 18 h. After aqueous extractive workup, the product was purified (method D, 4% MeOH–DCM) to give Fmoc-D-Phe-L-Ile-D-Pro-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.45 in 4% MeOH–DCM) in 62% yield. The hexapeptide was N-deprotected (procedure 7), purified (method C, 0–7% MeOH–DCM gradient elution, 85% yield), C-deprotected (procedure 7), cyclized (procedure 5), and purified (method D, 95:5:0.5 DCM–MeOH–NH_4OH) to give the title compound as a solid in 60% yield. TLC: R_f = 0.55 (95:5:0.5 $CHCl_3$–MeOH–H_2O). HPLC (method 3): t_R = 21.17 min; 98.4% purity. FAB MS: m/z = 727 (M^+ + H). ^1H NMR (300 MHz, DMSO-d_6): δ 7.2–7.4 (m, 10 H), 7.07 (d, J = 7 Hz, 1 H), 6.95 (d, J = 6 Hz, 1 H), 5.46 (dd, J = 5, 6 Hz, 1 H), 5.15 (d, J = 3 Hz, 1 H), 4.81 (dd, J = 2, 6 Hz, 1 H), 4.52 (q, J = 5 Hz, 1 H), 4.45 (dd, J = 2, 6 Hz, 1 H), 4.31 (dd, J = 5, 6 Hz, 1 H), 2.71 (s, 3 H), 0.80 (overlapping d and t, 6 H). Anal. ($C_{41}H_{54}N_6O_6 \cdot 0.1CHCl_3$) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pro-D-(*N*-Me)Phe-L-Pro] (3). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Fmoc-L-Pro was used in step b. Workup and purification (method A) gave the title compound in 39% yield. HPLC (method 1): t_R = 9.98 min; purity 98%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.35 (m, 10 H), 5.40 (t, J = 5 Hz, 1 H), 5.10–5.20 (overlapping m, 2 H), 4.55–4.65 (overlapping m, 4 H), 4.35–4.45 (overlapping m, 3 H), 2.86 (s, 3 H), 0.80 (overlapping d and t, 6 H). FAB MS: m/z = 727 (M + H^+). Anal. ($C_{41}H_{54}N_6O_6 \cdot 0.8H_2O$) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pip] (5). Boc-L-Pip was coupled to H-D-(*N*-Me)Phe-OCH_2Ph (procedure 4). Purification (method C, 7:1 hexane–acetone) gave Boc-L-Pip-D-(*N*-Me)Phe-OCH_2Ph in 62% yield as a colorless oil. The dipeptide was N-deprotected by treatment with HCl gas in EtOAc, and the HCl salt was coupled to Fmoc-D-Pip (procedure 1). Purification (method C, 1:3 acetone–hexane) gave Fmoc-D-Pip-L-Pip-D-(*N*-Me)Phe-OCH_2Ph in 92% yield (TLC R_f = 0.50, 30% acetone–hexane). The tripeptide was N-deprotected (procedure 7) and coupled to Fmoc-L-Ile (procedure 2). Purification (method C, 1:3 acetone–hexane) gave Fmoc-L-Ile-D-Pip-L-Pip-D-(*N*-Me)-Phe-OCH_2Ph (TLC R_f = 0.40 in 30% acetone–hexane) in 82% yield. The tetrapeptide was N- and C-deprotected (procedures 7 and 9). The deprotected tetrapeptide (400 mg, 0.655 mmol) in 10 mL of DCM was treated with chlorotrimethylsilane (0.70 mmol) and stirred at ambient temperature for 30 min to form the trimethylsilyl ester. The solution was treated with DIEA (0.72 mmol) and cooled to −17 °C. A solution of Cbz-D-Phe (0.82 mmol) and 4-methylmorpholine (0.85 mmol) in 6 mL of EtOAc was cooled to −17 °C and treated with isobutyl chloroformate (0.82 mmol). The mixture was stirred for 10 min, and to it was added the solution of the tetrapeptide trimethylsilyl ester. The resulting mixture was stirred for 5 min and then warmed to ambient temperature for 15 min. The reaction mixture was diluted with EtOAc, partially concentrated to remove DCM, and washed with water, 5:1 water/saturated aqueous $NaHCO_3$, water, and 10% aqueous $KHSO_4$. The residue was purified (method C, gradient elution of 5–10% MeOH–DCM) to yield Cbz-D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-OH as a foam (TLC R_f = 0.18 in 7% MeOH–DCM) in 80% yield. A solution of 151 mg (0.19 mmol) of the pentapeptide in 0.7 mL of $CHCl_3$ was cooled to −22 °C and treated with 0.044 mL (0.040 mmol) of 4-methylmorpholine. After the solution had been stirred for 5 min, 0.023 mL (0.019 mmol) of pivaloyl chloride was added dropwise. The mixture was stirred for 8 h at −22 °C, and then to it was added a −22 °C solution of H-L-Pip-OCH_2Ph in 0.5 mL of $CHCl_3$. The mixture was stirred for 2 days at −22 °C and then was extracted with water (2×), 10% aqueous $KHSO_4$, and brine. The crude product was purified (method D, 5% MeOH–DCM) to yield 110 mg of a white foam (TLC R_f = 0.30 in 30% acetone–hexane). The hexapeptide was simultaneously N- and C-deprotected (procedure 9) and then cyclized (procedure 5). Purification (method D, 1% MeOH–DCM) gave the title compound as a solid in 62% yield. TLC: R_f = 0.59 (8:8:1.9:0.1 $CHCl_3$–hexane–MeOH–H_2O). HPLC (method 3): t_R = 21.34 min; 98.3% purity. FAB MS: m/z = 755 (M + H^+). ^1H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, J = 8.2 Hz, 1 H), 7.53 (d, J = 7.2 Hz, 1 H), 7.2–7.3 (m, 10 H), 5.72 (t, J = 5.1 Hz, 1 H), 5.30 (d, J = 4.8 Hz, 1 H), 5.07 (d, J = 4.8 Hz, 1 H), 5.03 (br s, 1 H), 4.45 (q, J = 5.2 Hz, 1 H), 4.38 (t, J = 5.9 Hz, 1 H), 2.67 (s, 3 H), 0.75 (d, J = 6.4 Hz, 3 H), 0.70 (t, J = 7.3 Hz). Anal. ($C_{43}H_{58}N_6O_6 \cdot 0.25$hexane$\cdot 0.25$MeOH) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-Phe-L-Pro] (6). Fmoc-L-Pip was coupled to the HCl salt of H-D-Phe-L-Pro-OCH_2Ph (procedure 1) to give Fmoc-L-Pip-D-Phe-L-Pro-OCH_2Ph (TLC R_f = 0.25 in 2.5% MeOH–DCM) in 85% yield after purification (method C, 25% acetone–hexane). The tripeptide was N-deprotected (procedure 7), coupled to Fmoc-D-Pip (procedure 1), and purified (method C, 28% acetone–hexane) to give Fmoc-D-Pip-L-Pip-D-Phe-L-Pro-OCH_2Ph (TLC R_f = 0.35 in 30% acetone–hexane) in 89% yield after purification (method C, 25% acetone–hexane). The tetrapeptide was N-deprotected (procedure 7), coupled to Fmoc-L-Ile (procedure 2), and purified (method C, 28% acetone–hexane) to give Fmoc-L-Ile-D-Pip-L-Pip-D-Phe-L-Pro-OCH_2Ph in 78% yield. The pentapeptide was N-deprotected (procedure 7), coupled to Cbz-D-Phe (procedure 3), and purified (method C, 32% acetone–hexane) to give Cbz-D-Phe-L-Ile-D-Pip-L-Pip-D-Phe-L-Pro-OCH_2Ph in 90% yield. The hexapeptide was simultaneously N- and C-deprotected (procedure 9) and cyclized (procedure 5). The crude product was purified (method A and then method D, 6% MeOH–DCM) to give the title compound as a white solid (42% for two steps). TLC: R_f = 0.37 (6% MeOH–DCM). HPLC (method 4): t_R = 16.80 min; 95.8% purity. FAB MS: m/z = 727 (M + H^+). ^1H NMR (360 MHz, DMSO-d_6): δ 8.28 (d, J = 8 Hz, 1 H), 8.11 (d, J = 9 Hz, 1 H), 7.2–7.3 (m, 10 H), 6.94 (d, J = 7 Hz, 1 H), 5.27 (d, J = 5 Hz, 1 H), 5.02 (d, J = 4 Hz, 1 H), 4.68 (q, J = 7 Hz, 1 H), 4.45–4.55 (overlapping m, 2 H), 4.17 (m, 1 H), 0.73 (overlapping d and t, 6 H). Anal. ($C_{41}H_{54}N_6O_6 \cdot 0.65$hexane$\cdot 0.6H_2O$) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-allyl)Phe-L-Pro] (7). Fmoc-L-Pip-D-Phe-L-Pro-OCH_2Ph (obtained as described for compound 6) was N-deprotected (procedure 7) and coupled to Boc-D-Pip (procedure 4). Purification (method C, 25% acetone/hexane) gave Boc-D-Pip-L-Pip-D-Phe-Pro-OCH_2Ph (TLC R_f =

0.24 in 25% acetone–hexane) in 90% yield. A solution of 382 mg (0.566 mmol) of the tetrapeptide was dissolved in 3 mL of 1:1 DMF–allyl bromide. The solution was cooled to 0 °C, and to it was added 54 mg (1.4 mmol) of 60% NaH in mineral oil. The mixture was stirred at 0 °C for 45 min, quenched with 0.30 mL of acetic acid, and concentrated under reduced pressure. The residue was dissolved in EtOAc and after extractive workup with aqueous $NaHCO_3$, water, and brine was purified (method C, 20% acetone–hexane) to give Boc-D-Pip-L-Pip-D-(*N*-allyl)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.44 in 30% acetone–hexane) in 52% yield. The N-allylated tetrapeptide was N-deprotected using 98% formic acid at ambient temperature for 2 h. The solvent was removed under reduced pressure. After extractive aqueous workup to remove formic acid, the tetrapeptide free base was coupled to Fmoc-L-Ile (procedure 2) and purified (method D, 25% acetone–hexane) to give Fmoc-L-Ile-D-Pip-L-Pip-D-(*N*-allyl)-Phe-L-Pro-OCH_2Ph in 67% yield. The pentapeptide was N-deprotected (procedure 7) and coupled to Fmoc-D-Phe (procedure 3) to yield the protected hexapeptide in 94% yield, which was used without purification. The hexapeptide was N-deprotected (procedure 7) and then the benzyl ester was removed by saponification of with aqueous NaOH in MeOH at ambient temperature. The crude linear hexapeptide was dried in vacuo (94% yield), cyclized (procedure 5), and purified (method A, followed by method D, 8:8:1.9:0.1 $CHCl_3$–hexane–MeOH–H_2O) to give the title compound as a solid in 49% yield. TLC: R_f = 0.46 (8:8:1.9:0.1 $CHCl_3$–hexane–MeOH–H_2O). HPLC (method 4): t_R = 18.54 min; 96.0% purity. FAB MS: m/z = 767 (M + H^+). ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 6.9 Hz, 1 H), 7.15–7.35 (m, 10 H), 6.87 (d, J = 9.0 Hz, 1 H), 5.88 (m, 1 H), 5.56 (dd, J = 4.0, 10.4 Hz, 1 H), 5.42 (d, J = 5.7 Hz, 1 H), 5.26 (dd, J = 0.5, 17.1 Hz, 1 H), 5.22 (dd, J = 1.0, 10.4 Hz, 1 H), 5.03 (m, 1 H), 4.65 (dd, J = 5.3, 9.1 Hz, 1 H), 4.60 (m, 1 H), 4.42 (d, J = 7.2 Hz, 1 H), 0.85 (overlapping d and t, 6 H). Anal. ($C_{44}H_{58}N_6O_6$·0.25hexane) C, H, N.

cyclo-[D-Phe-L-(*N*-Me)Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro] (8). Fmoc-L-Pip was coupled to the HCl salt of H-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (procedure 1) to give Fmoc-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.35 in 2.5% MeOH–DCM) in 74% yield after crystallization from $CHCl_3$–hexane. The tripeptide was N-deprotected (procedure 7), coupled to Fmoc-D-Pip (procedure 2), and purified (method C, 28% acetone–hexane) to give Fmoc-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.40 in 30% acetone–hexane) in 90% yield after purification (method C, 25% acetone–hexane). The tetrapeptide was N-deprotected (procedure 7), coupled to Fmoc-L-Ile (procedure 2), and purified (method C, 28% acetone–hexane) to give Fmoc-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 90% yield. The pentapeptide was N-deprotected (procedure 7), coupled to Cbz-D-Phe (procedure 3), and purified (method C, 32% acetone–hexane) to give Cbz-D-Phe-L-Ile-D-Pip-D-(*N*-Me)-Phe-L-Pro-OCH_2Ph in 90% yield. The hexapeptide was simultaneously N- and C-deprotected (procedure 9) and cyclized (procedure 5). The crude product was purified (method A, followed by method D, 6% MeOH–DCM) to give the title compound as a white solid (45% for two steps). TLC: R_f = 0.48 (8% MeOH–DCM). HPLC (method 4): t_R = 18.33 min; 98.4% purity. FAB MS: m/z = 755 (M + H^+). ^1H NMR (400 MHz, DMSO-d_6): δ 8.37 (d, J = 7 Hz, 1 H), 7.2–7.3 (m, 10 H), 5.32 (t, J = 7 Hz, 1 H), 5.28 (m, 2 H), 4.97 (d, J = 8 Hz, 1 H), 4.83 (q, J = 7 Hz, 1 H), 4.32 (dd, J = 3, 8 Hz, 1 H), 2.89 (s, 3 H), 2.88 (s, 3 H), 0.75 (d, J = 7 Hz, 3 H), 0.68 (t, J = 7 Hz, 3 H). Anal. ($C_{41}H_{56}N_6O_6$·0.3DCM) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-(*N*-Me)-Ala] (9). The title compound was prepared according to the procedure used for compound **5**, except that H-L-(*N*-Me)Ala-OCH_2Ph was used in place of H-L-Pip-OCH_2Ph to form the linear hexapeptide. The title compound was obtained as a solid after purification (method D, 95:5:0.5 $CHCl_3$–MeOH–NH_4OH). TLC: R_f = 0.52 (95:5:0.5 $CHCl_3$–MeOH–NH_4OH). HPLC (method 1): t_R = 8.72 min, 95.1% purity. FAB MS: m/z = 729 (M + H^+). ^1H NMR (400 MHz, DMSO-d_6): δ 8.31 (d, J = 9 Hz, 1 H), 7.89 (d, J = 8 Hz, 1 H), 5.64 (t, J = 7 Hz, 1 H), 5.15 (d, J = 5 Hz, 1 H), 5.05 (overlapping m, 2 H), 4.56 (q, J = 7 Hz, 1 H), 4.43 (t, J = 8 Hz, 1 H), 2.75 (s, 3 H), 2.73 (s, 3 H), 0.99 (d, J = 7 Hz, 3 H), 0.75 (d, J = 7 Hz, 3 H), 0.70 (t, J = 7 Hz, 3 H). Anal. ($C_{41}H_{56}N_6O_6$·1.4H_2O) C, H, N.

cyclo-[D-Ala-L-Ile-D-Pip-L-Pip-D-Phe-L-Pro] (10). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exceptions: Boc-D-Phe was used in step a and Fmoc-D-Ala was used in step e. Workup and purification (method A) gave the title compound in 70% yield. HPLC (method 1): t_R = 8.80 min; purity 99%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.30 (m, 5 H), 5.18 (br d, J = 3 Hz, 1 H), 5.09 (br s, 1 H), 4.75–4.85 (overlapping m, 2 H), 4.34 (q, J = 7 Hz, 1 H), 4.19 (br t, J = 3 Hz, 1 H), 1.32 (d, J = 7 Hz, 3 H), 0.92 (d, J = 7 Hz, 3 H), 0.88 (t, J = 7 Hz, 3 H). FAB MS: m/z = 651 (M + H^+). Anal. ($C_{35}H_{50}N_6O_6$) C, H, N.

cyclo-[D-Phe-L-Ala-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro] (11). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Fmoc-L-Ala was used in step d. Workup and purification (method A) gave the title compound in 28% yield. HPLC (method 1): t_R = 9.66 min; purity 98.5%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.35 (m, 10 H), 5.45 (t, J = 5 Hz, 1 H), 5.41 (d, J = 3 Hz, 1 H), 5.22 (m, 1 H), 4.61 (m, 1 H), 4.45–4.55 (overlapping m, 2 H), 2.75 (s, 3 H), 1.18 (d, J = 7 Hz, 3 H). FAB MS: m/z = 699 (M + H^+). Anal. ($C_{39}H_{50}N_6O_6$·1.5H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-(*N*-Me)Ala-L-Pip-D-(*N*-Me)Phe-L-Pro] (12). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Fmoc-D-(*N*-Me)Ala was used in step c. Workup and purification (method B) gave the title compound in 48% yield. HPLC (method 1): t_R = 9.95 min; purity 99%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.35 (m, 10 H), 5.55 (overlapping m, 2 H), 5.28 (d, J = 3 Hz, 1 H), 4.40–4.55 (overlapping m, 3 H), 3.05 (s, 3 H), 2.81 (s, 3 H), 1.25 (d, J = 7 Hz, 3 H), 0.78 (overlapping d and t, 6 H). FAB MS: m/z = 715 (M + H^+). Anal. ($C_{40}H_{54}N_6O_6$·0.45H_2O·0.85TFA) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-(*N*-Me)Ala-D-(*N*-Me)Phe-L-Pro] (13). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Fmoc-D-(*N*-Me)Ala was used in step b. Workup and purification (method A) gave the title compound in 64% yield. HPLC (method 1): t_R = 10.17 min; purity 99%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.35 (m, 10 H), 5.55 (t, J = 7 Hz, 1 H), 5.32 (q, J = 7 Hz, 1 H), 5.28 (d, J = 5 Hz, 1 H), 4.50–4.60 (overlapping m, 2 H), 4.28 (m, 1 H), 2.90 (s, 3 H), 2.70 (2, 3 H), 1.05 (d, J = 7 Hz, 3 H), 0.85 (d, J = 7 Hz, 3 H), 0.80 (t, J = 7 Hz, 3 H). FAB MS: m/z = 715 (M + H^+). Anal. ($C_{40}H_{54}N_6O_6$·H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Ala-L-Pro] (14). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Boc-D-(*N*-Me)Ala was used in step a. Workup and purification (method B) gave the title compound in 44% yield. HPLC (method 1): t_R = 9.41 min; purity 99%. ^1H NMR (300 MHz, CD_3OD): δ 7.15–7.30 (m, 5 H), 5.46 (d, J = 4 Hz, 1 H), 5.30 (br d, J = 4 Hz, 1 H), 5.20 (q, J = 7 Hz, 1 H), 4.43–4.51 (overlapping m, 3 H), 2.66 (s, 3 H), 1.23 (d, J = 7 Hz, 3 H), 0.88 (d, J = 7 Hz, 3 H), 0.82 (t, J = 7 Hz, 3 H). FAB MS: m/z = 665 (M + H^+). Anal. ($C_{36}H_{52}N_6O_6$·TFA) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Asp-D-(*N*-Me)Phe-L-Pro] (15). The hydrochloride salt of H-D-(*N*-Me)Phe-L-Pro-OCH_2Ph was coupled to Fmoc-L-(*O*-*tert*-butyl)Asp (procedure 4). The product was purified (method C, 25% acetone–hexane) to give Fmoc-L-(*O*-*tert*-butyl)Asp-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 76% yield. The tripeptide was N-deprotected (procedure 7), coupled to Fmoc-D-Pip (procedure 2), and purified (method C, 25% acetone–hexane) to give Fmoc-D-Pip-L-(*O*-*tert*-butyl)Asp-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 74% yield. The tetrapeptide was N-deprotected (procedure 7), coupled to Fmoc-L-Ile (procedure 2), N-deprotected (procedure 7), and purified (method C, 5% MeOH–DCM) to give H-L-Ile-D-Pip-L-(*O*-*tert*-butyl)Asp-D-(N-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.50 in 8% MeOH–DCM) in 70% yield. The pentapeptide was coupled to Cbz-D-Phe (procedure 3) and purified (method C, 30% acetone–hexane) to give Cbz-D-Phe-L-Ile-D-Pip-L-(*O*-*tert*-butyl)Asp-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (TLC R_f = 0.30 in 30% acetone–hexane) in 90% yield. The hexapeptide was simultaneously N- and C-deprotected

(procedure 9), cyclized (procedure 5), and purified (method C, 96:2.5:0.25 DCM–MeOH–NH_4OH) to give the *tert*-butyl ester of the title compound (TLC R_f = 0.57 in 95:5:0.5 $CHCl_3$–MeOH–H_2O; HPLC (method 4) t_R = 20.30 min) in 80% yield. The *tert*-butyl ester was cleaved by treatment with neat TFA at 0 °C for 45 min. After aqueous extractive workup and purification (method D, 90:10:1 DCM–MeOH–H_2O), the title compound was obtained as a solid in 50% yield. TLC: R_f = 0.20 (92:8:0.8 $CHCl_3$–MeOH–H_2O). HPLC (method I): t_R = 16.17 min; 90.4% purity. FAB MS: m/z = 745 (M + H^+). ^{1}Hz NMR (400 MHz, DMSO-d_6): δ 12.2 (br s, 1 H), 8.24 (d, J = 9.1 Hz, 1 H), 7.53 (d, J = 7.7 Hz, 1 H), 7.44 (d, J = 7.2 Hz, 1 H), 7.15–7.30 (m, 10 H), 5.32 (t, J = 7.1 Hz, 1 H), 5.04 (br q, J = 7 Hz, 1 H), 4.75 (br d, J = 5 Hz, 1 H), 4.35–4.48 (overlapping m, 3 H), 2.83 (s, 3 H), 0.80 (d, J = 7 Hz, 3 H), 0.76 (t, J = 7 Hz, 3 H). Anal. ($C_{40}H_{52}N_8O_8$·0.5H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Lys-D-(*N*-Me)Phe-L-Pro] (16). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: D-(N^{α}-Fmoc,N^{ϵ}-Boc)-Lys (2 mmol) was employed in step b using an active ester coupling with BOP reagent (2 mmol) and DIEA (2 mmol) in DMF. Workup and purification (method B) gave the D-(N^{ϵ}-Boc)-Lys[5] derivative of the title compound (HPLC (method 1) t_R = 11.72 min; purity 99%) in 48% yield. N-Deprotection (procedure 6), purification (method B), and lyophilization from dioxane gave the TFA salt of title compound as a white powder in 90% yield. HPLC (method 1): t_R = 8.14 min; purity 99%. ^{1}H NMR (400 MHz, CD_3OD): δ 7.20–7.30 (m, 10 H), 5.31 (dd, J = 6, 9 Hz, 1 H), 4.68 (t, J = 7 Hz, 1 H), 4.50–4.60 (m, 3 H), 2.89 (s, 3 H), 0.70–0.80 (overlapping d and t, 6 H). FAB MS: m/z = 758 (M + H^+). Anal. ($C_{42}H_{59}N_7O_6$·TFA) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-His-D-(*N*-Me)Phe-L-Pro] (17). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Fmoc-L-(DNP)His (2 mmol) was used in a modified version of step b. Due to the insolubility of the amino acid in DCM, an active ester coupling was used. The amino acid was dissolved in DMF (15 mL) and cooled to 0 °C and then added to the resin at 5 °C. The BOP reagent (2 mmol) was added and the reaction was adjusted to pH 8 with DIEA (3 mmol) and the resin was shaken for 15 h at 5 °C. The remaining steps proceeded as previously described. The DNP protecting group was labile to the piperidine–DMF treatments used to remove the Fmoc protecting groups. Workup and purification (method B) gave the TFA salt of the title compound in 15% yield. HPLC (method 1): t_R = 8.06 min; purity 98.5%. ^{1}H NMR (400 MHz, CD_3OD): δ 9.76 (s, 1 H), 7.2–7.3 (m, 10 H), 7.20 (s, 1 H), 5.43 (t, J = 7 Hz, 1 H), 5.08 (t, J = 7 Hz, 1 H), 4.79 (br d, J = 5 Hz, 1 H), 4.45–4.60 (overlapping m, 3 H), 2.90 (s, 3 H), 0.82 (overlapping d and t, 6 H). FAB MS: m/z = 767 (M + H^+). Anal. ($C_{42}H_{54}N_8O_6$·0.85TFA·2.05H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-Lys-L-Pro] (18). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: D-(N^{α}-Boc,N^{ϵ}-2-Cl-Cbz)-Lys was used in step 1. Workup and purification (method B) gave the D-(N^{ϵ}-2-Cl-Cbz)-Lys[6] derivative of the title compound (HPLC (method 1) t_R = 11.10 min; purity 97%) in 60% yield. The compound was N-deprotected (procedure 8), and the free base was isolated by partitioning between EtOAc and aqueous Na_2CO_3. The title compound was obtained as a white powder by lyophilization from dioxane. HPLC (method 1): t_R = 7.71 min; purity 99%. ^{1}H NMR (400 MHz, CD_3OD): δ 7.20–7.30 (m, 5 H), 5.36 (d, J = 5 Hz, 1 H), 5.15 (br d, J = 5 Hz, 1 H), 5.60–5.70 (m, 3 H), 4.22 (br dd, 1 H), 0.70 (overlapping d and t, 6 H). FAB MS: m/z = 708 (M + H^+). Anal. ($C_{38}H_{57}N_7O_6$·0.65H_2O·0.25dioxane) C, H, N.

cyclo-[D-Phe-L-Ile-D-Pip-L-Pip-D-His-L-Pro] (19). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception: Boc-D-(DNP)-His was used in step a. The DNP protecting group was labile to the piperidine–DMF treatments used to remove the Fmoc protecting groups. Workup and purification (method B) gave the TFA salt of the title compound in 28% yield. HPLC (method 1): t_R = 7.49 min; purity 99%. ^{1}H NMR (300 MHz, CD_3OD): δ 9.80 (d, J = 1 Hz, 1 H), 7.33 (s, 1 H), 7.15–7.30 (m, 5 H), 5.30 (d, J = 4 Hz, 1 H), 5.07 (d, J = 4 Hz, 1 H), 4.92 (m, 1 H), 4.60–4.70 (overlapping m, 2 H), 4.28 (dd, J = 5, 12 Hz, 1 H), 0.80 (overlapping d and t, 6 H). FAB MS: m/z = 717 (M + H^+). Anal. ($C_{38}H_{52}N_8O_6$·1.85TFA·0.15H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-Δ-Piz-L-Pip-D-(*N*-Me)Phe-L-Pro] (20). H-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph (obtained as described for compound 8) was coupled to D-(N^{α}-Fmoc,N^{3}-Cbz)Piz (procedure 1 using aqueous Na_2CO_3 instead of DIEA) and purified (method C, 25% acetone–hexane) to give Fmoc-D-(N^{3}-Cbz)Piz-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 84% yield. The tetrapeptide was N-deprotected (procedure 7), coupled to Fmoc-L-Ile (procedure 2), and purified (method C, 25% acetone–hexane) to give Fmoc-L-Ile-D-(N^{3}-Cbz)Piz-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 55% yield. The pentapeptide was N-deprotected (procedure 7), coupled to Cbz-D-Phe (procedure 3), and purified (method C, 30% acetone–hexane) to give Cbz-D-Phe-L-Ile-D-(N^{3}-Cbz)Piz-L-Pip-D-(*N*-Me)Phe-L-Pro-OCH_2Ph in 76% yield. The hexapeptide was simultaneously N- and C-deprotected (procedure 9), cyclized (procedure 5), and purified (method D, 95:5:0.5 $CHCl_3$–MeOH–NH_4OH) to give cyclo-(D-Phe-L-Ile-D-Piz-L-Pip-D-(*N*-Me)Phe-L-Pro) (TLC R_f = 0.40 in 95:5:0.5 $CHCl_3$–MeOH–NH_4OH) in 50% yield. A solution of 39 mg (0.053 mmol) of the cyclic hexapeptide in 0.5 mL of pyridine was cooled to 0 °C, and to it was added 0.0063 mL (0.053 mmol) of *tert*-butyl hypochlorite. The mixture was treated with additional portions of *tert*-butyl hypochlorite (0.048 mL total) during the next 4 h. After the mixture was stirred for an additional 1.5 h, the solvents were removed under reduced pressure and the residue was dissolved in DCM and washed with 10% aqueous $KHSO_4$, aqueous $NaHCO_3$, and brine. The crude product was purified (method D, 95:5:0.5 $CHCl_3$–MeOH–H_2O) to yield the title compound as a solid in 26% yield. TLC: R_f = 0.48 (95:5:0.5 $CHCl_3$–MeOH–H_2O). HPLC (method 1): t_R = 10.67 min; 97.4% purity. FAB MS: m/z 740 (M + H^+). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41 (overlapping d, J = 6.7, 10 Hz, 2 H), 7.2–7.3 (m, 10 H), 6.99 (br s, 1 H), 5.33 (m, 2 H), 5.24 (d, J = 5.1 Hz, 1 H), 4.96 (dd, J = 4.4, 9.2 Hz, 1 H), 4.57 (q, J = 7.0 Hz, 1 H), 4.47 (dd, J = 2.6, 8.1 Hz, 1 H), 2.61 (s, 3 H), 0.74 (overlapping d and t, 6 H). Anal. ($C_{41}H_{53}N_7O_6$·0.1hexane·0.2DCM) C, H, N.

cyclo-[D-Trp-L-Ile-D-Pip-L-Pip-D-(*N*-Me)Phe-L-Pro] (21). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 1, with the following exception. Due to the insolubility of Fmoc-D-Trp in DCM to form the acid chloride, an active ester coupling was used in step e. Fmoc-D-Trp (2 mmol) in DMF (20 mL) was added to the resin. The BOP reagent (2 mmol) was added, the reaction was brought to pH 8 with the addition of DIEA (2 mmol), and the resin was shaken for 15 h. Workup and purification (method A) followed by lyophilization from dioxane gave the title compound in 52% yield. HPLC (method 1): t_R = 10.64 min; purity 99%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12 (br s, 1 H), 7.70 (m, 2 H), 7.37 (d, J = 8 Hz, 1 H), 7.15–7.3 (m, 8 H), 6.86 (d, J = 9 Hz, 1 H), 5.40 (t, J = 7 Hz, 1 H), 5.32 (br s, 1 H), 5.18 (br d, J = 5 Hz, 1 H), 4.68–4.76 (overlapping m, 2 H), 4.60 (d, J = 7 Hz, 1 H), 2.36 (s, 3 H), 0.89 (d, J = 7 Hz, 3 H), 0.84 (t, J = 7 Hz, 3 H). FAB MS: m/z = 780 (M + H^+). Anal. ($C_{44}H_{57}N_7O_6$·0.2dioxane·0.7H_2O) C, H, N.

cyclo-[D-Trp-L-Ile-D-Pip-L-Pip-D-His-L-Pro] (22). The title compound was prepared on a 1-mmol scale according to the solid-phase procedures given for compounds 19 and 21. Workup and purification (method C, 85:15:1 DCM–MeOH–NH_4OH) gave a product which was lyophilized from HOAc–H_2O and then crystallized from MeOH to give the title compound in 33% yield. HPLC (method 1): t_R = 7.45 min; purity 99%. ^{1}H NMR (DMSO-d_6): δ 8.19 (m, 2 H), 7.57 (d, J = 7.8 Hz, 1 H), 7.50 (s, 1 H), 7.30 (d, J = 8.0 Hz, 1 H), 7.14 (d, J = 1.3 Hz, 1 H), 6.98 (m, 4 H), 6.72 (s, 1 H), 5.26 (d, J = 4.1 Hz, 1 H), 5.03 (d, J = 1.3 Hz, 1 H), 4.75 (m, 1 H), 4.54 (m, 1 H), 4.22 (m, 1 H), 3.97 (m, 1 H), 3.70 (m, 1 H), 3.36 (m, 1 H), 0.74 (m, 5 H). FAB MS: m/z = 756 (M + H^+). Anal. ($C_{40}H_{53}N_9O_6$·2H_2O) C, H, N.

cyclo-[D-(N^{indole}-Me)Trp-L-Ile-D-Pip-L-Pip-D-His-L-Pro] (23). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound 22, with the following exception: Fmoc-D,L-(N^{indole}-methyl)Trp was used in step e. Workup and purification (method B) gave a mixture of

diastereomers which were separated (method D, 90:10:1 $CHCl_3$–MeOH–NH_4OH). Each isomer was lyophilized from H_2O–dioxane containing 0.1% TFA. The higher R_f isomer had a circular dichroism spectrum which was essentially superimposable on that of compound **22** and thus was assigned as the D-(N^{indole}-methyl)Trp^2 isomer. HPLC (method 1): t_R = 8.15 min; purity 100%. TLC R_f = 0.35 (90:10:1 $CHCl_3$–MeOH–NH_4OH). ^1H NMR (360 MHz, DMSO-d_6) (TFA salt) δ 9.0 (s, 1 H), 8.27 (d, J = 10 Hz, 1 H), 8.06 (d, J = 12 Hz, 1 H), 7.59 (d, J = 11 Hz, 1 H), 7.38 (s, 1 H), 7.35 (overlapping signals, 2 H), 7.11 (s, 1 H), 7.05–7.15 (m, 2 H), 6.96–7.02 (m, 2 H), 5.26 (d, J = 4 Hz, 1 H), 5.02 (br s, 1 H), 4:79 (m, 1 H), 4.50 (m, 2 H), 4.29 (dd, J = 4, 8 Hz, 1 H), 3.56 (s, 3 H), 0.73 (d, J = 7 Hz, 3 H), 0.67 (t, J = 7 Hz, 3 H). FAB MS: m/z = 770 (M + H$^+$). Anal. ($C_{41}H_{55}N_9O_6$·0.25H_2O·0.75TFA) C, H, N.

cyclo-[D-1-Nal-L-Ile-D-Pip-L-Pip-D-His-L-Pro] (24). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **22**, with the following exception: Fmoc-D-1-Nal was used in step e. Workup and purification (method B) gave the TFA salt of the title compound in 14% yield. HPLC (method 1): t_R = 8.65 min; purity 99%. ^1H NMR (DMSO-d_6): δ 9.00 (8.33 (d, J = 8 Hz, 1 H), 8.18 (t, J = 8 Hz, 2 H), 7.91 (d, J = 7 Hz, 1 H), 7.79 (t, J = 5 Hz, 1 H), 7.55–7.60 (m, 3 H), 7.39 (overlapping s and d, 2 H), 7.03 (d, J = 7 Hz, 1 H), 5.25 (d, J = 4 Hz, 1 H), 5.04 (d, J = 4 Hz, 1 H), 4.77 (q, J = 7 Hz, 1 H), 4.65 (q, J = 7 Hz, 1 H), 5.54 (t, J = 7 Hz, 1 H), 0.73 (overlapping d and t, 6 H). FAB MS: m/z = 729 (M + H$^+$). Anal. ($C_{42}H_{54}N_8O_6$·2TFA·0.45H_2O) C, H, N.

cyclo-[D-2-Nal-L-Ile-D-Pip-L-Pip-D-His-L-Pro] (25). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **22**, with the following exception: Fmoc-D-2-Nal was used in step e. Workup and purification (method B) gave the TFA salt of the title compound in 12% yield. The free base was obtained by partitioning between EtOAc and aqueous Na_2CO_3 and was crystallized from EtOAc–MeOH. HPLC (method 1): t_R = 8.48 min; purity 99%. ^1H NMR (DMSO-d_6): δ 8.35 (d, J = 8.0 Hz, 1 H), 8.18 (d, J = 8.6 Hz, 1 H), 7.85 (d, J = 8.0 Hz, 1 H), 7.80 (d, J = 8.6 Hz, 2 H), 7.72 (s, 1 H), 7.46 (m, 5 H), 6.92 (d, J = 8.0 Hz, 1 H), 5.27 (d, J = 4.6 Hz, 1 H), 5.03 (d, J = 4.0 Hz, 1 H), 4.74 (dd, J = 5.7 Hz, J = 4.3 Hz, 1 H), 4.62 (dd, J = 5.6 Hz, J = 4.3 Hz, 1 H), 4.50 (t, J = 8.6 Hz, 1 H), 4.21 (m, 1 H), 3.94 (d, J = 13.4 Hz, 1 H), 3.72 (d, J = 8.57 Hz, 1 H), 3.05 (m, 2 H), 2.78 (m, 3 H), 0.68 (d, J = 6.4 Hz, 2 H), 0.49 (t, J = 7.7 Hz, 3 H). FAB MS: m/z = 767 (M + H$^+$). Anal. ($C_{42}H_{54}N_8O_6$·EtOAc·2.5H_2O) C, H, N.

cyclo-[D-Trp-L-Ile-D-Δ-Piz-L-Pip-D-His-L-Pro] (26). Fmoc-L-Ile-D-Δ-Piz-OH was prepared as follows. A suspension of 1.55 g (5.86 mmol) of (N^3-Cbz)-D-piperazic acid in 30 mL of DCM was treated with 0.82 mL (6.45 mmol) of chlorotrimethylsilane, and the mixture was stirred for 30 min at ambient temperature. The resulting solution was cooled to 0 °C and treated with 3.05 mL (14.0 mmol) of DIEA. After 5 min, a 0 °C solution of Fmoc-L-Ile-Cl (7.62 mmol) in 10 mL of DCM was added via cannula. After being stirred for 1.5 h, the mixture was quenched by addition of ca. 5 mL of dry MeOH. The mixture was stirred for 10 min and was neutralized by addition of DIEA to pH 8. The reaction mixture was evaporated, and the residue was partitioned between ether and 1 N aqueous HCl. The ether layer was treated with $^1/_2$ volume of hexane and extracted with 5% aqueous $NaHCO_3$ until no more product was observed in the organic phase by TLC. The combined aqueous layers were made acidic (pH 1) with 6 N aqueous HCl and extracted with 3 portions of ether. The organic extracts were washed with water and brine, dried, and concentrated to yield Fmoc-L-Ile-D-(N^3-Cbz)Piz-OH as a foam in 85% yield (TLC, R_f = 0.53, 2:2:1:0.1 $CHCl_3$–hexane–MeOH–H_2O). A solution of 22.1 g (36.8 mmol) of the above dipeptide was taken up in 75 mL of 95% aqueous ethanol, treated with 2.0 g of 10% Pd/C, and hydrogenated in a Parr shaker for 11 h under 25 psig of H_2. The reaction mixture was filtered and concentrated, and the residue was purified (method C, gradient elution from 8:8:1.9:0.1 to 2:2:1:0.1 $CHCl_3$–hexane–MeOH–H_2O) to give Fmoc-L-Ile-D-Piz-OH as a foam in 88% yield (TLC, R_f = 0.39, 2:2:1:0.1 $CHCl_3$–hexane–MeOH–H_2O). A solution of 2.64 g (5.67 mmol) of this dipeptide in 20 mL of DCM was treated with 0.69 mL (8.5 mmol) of pyridine followed by 0.79 mL (6.2 mmol) of chlorotrimethylsilane. The mixture was stirred for 25 min at ambient temperature, cooled to −24 °C when 2.06 mL (25.3 mmol) of pyridine was added, followed by 0.87 mL (7.3 mmol) of *tert*-butyl hypochlorite in two portions. The mixture was stirred for 1 h, poured into 50 mL of ether and washed with 5% aqueous citric acid (4×), water, and brine. In addition to the desired dipeptide, the crude product contained ca. 25% of Fmoc-L-(N^α-Cl)Ile-D-Δ-Piz-OH, which was converted to the desired compound as follows. The crude product mixture was taken up in 15 mL of DCM and added dropwise during 20 min to a well stirred mixture of 3 g of NaI, 5 g of $Na_2S_2O_3$, and 0.25 g of tetrabutylammonium iodide dissolved in 20 mL of 1:1 H_2O–DCM. The mixture was stirred for 1 h and poured into 50 mL of ether. The layers were separated. The organic layer was washed with water, 5% aqueous $Na_2S_2O_3$, water, and brine, dried, and concentrated to a foam. This material was purified (method C, 70:30:1 toluene–EtOAc–formic acid) to yield Fmoc-L-Ile-D-Δ-Piz-OH as a solid in 74% yield (TLC, R_f = 0.23, 70:30:1 toluene–EtOAc–formic acid; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7 Hz, 2 H), 7.60 (d, J = 7 Hz, 2 H), 7.3–7.4 (m, 4 H), 6.98 (br s, 1 H), 5.62 (d, J = 7 Hz, 1 H), 5.1–5.3 (overlapping m, 2 H), 4.38 (m, 2 H), 4.22 (t, J = 7 Hz, 1 H), 0.90 (overlapping d and t, 6 H)). The HCl salt of H-D-(DNP)His-L-Pro-OCH_2Ph was coupled to Fmoc-L-Pip (procedure 1). Purification (method C, 9:1 DCM–MeOH) gave Fmoc-L-Pip-D-(DNP)His-L-Pro-OCH_2Ph in 85% yield. The tripeptide was N-deprotected (procedure 7) and coupled to Fmoc-L-Ile-D-Δ-Piz (procedure 3) to give Fmoc-L-Ile-D-Δ-Piz-L-Pip-D-(DNP)His-L-Pro-OCH_2Ph in 60% yield. The pentapeptide was N-deprotected by treatment with 1:1 piperidine–DMF at ambient temperature for 1 h. This treatment also removed the 2,4-dinitrophenyl protecting group on the imidazole ring of histidine. The solvents were removed under reduced pressure, and the residue was partitioned between acetonitrile and hexane to remove dibenzofulvene. The acetonitrile layer was evaporated under reduced pressure and the pentapeptide was coupled to Fmoc-D-Trp (procedure 3). Purification (method C, 90:10:0.1 DCM–MeOH–NH_4OH) gave Fmoc-D-Trp-L-Ile-D-Δ-Piz-L-Pip-D-His-L-Pro-OCH_2Ph in 65% yield. The hexapeptide was N-deprotected (procedure 7) and treated with 5:1 MeOH–hydrazine at ambient temperature for 16 h. The solvents were removed under reduced pressure, and the residue was partitioned between 1-butanol and water to remove excess hydrazine. The cyclization was accomplished as described for compound 1. The title compound (**26**) was obtained as the acetic acid salt in 60% yield after purification (method C, 90:10:2:1 DCM–MeOH–H_2O–HOAc) and lyophilization from acetonitrile–H_2O–HOAc. HPLC (method 4): t_R = 11.59 min; purity >97%. ^1H NMR (360 MHz, CD_3OD) (TFA salt): δ 8.8 (s, 1 H), 7.3 (s, 1 H), 7.0–7.2 (m, 4 H), 7.08 (s, 1 H), 6.93 (br d, 1 H), 5.43 (br s, 1 H), 5.32 (d, J = 5 Hz, 1 H), 4.98 (br d, 1 H), 4.7–4.8 (overlapping m, 2 H), 4.27 (dd, J = 4, 11 Hz, 1 H), 0.70 (t, J = 7 Hz, 3 H), 0.64 (d, J = 7 Hz, 3 H). FAB MS: m/z = 755 (M + H$^+$). Anal. ($C_{39}H_{50}N_{10}O_6$·1.1H_2O·1.25HOAc) C, H, N.

cyclo-[D-Trp-L-Ile-D-Pip-L-Pip-D-(N^α-Me)His-L-Pro] (27). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **22**, with the following exception: Boc-D-(N^{im}-tosyl,N^α-Me)His (2 mmol) was used in step a. The tosyl group was labile to the piperidine–DMF treatments used to remove the Fmoc protecting groups. Workup and purification (method B) gave the TFA salt of the title compound in 12% yield. HPLC (method 1) t_R = 7.92 min; purity 99%. ^1H NMR (300 MHz, CD_3OD) δ 8.78 (d, J = 2 Hz, 1 H), 7.70 (d, J = 7 Hz, 1 H), 7.60 (overlapping d, 2 H), 7.32 (overlapping d and s, 2 H), 7.12 (s, 1 H), 7.09 (t, J = 7 Hz, 1 H), 7.02 (t, J = 7 Hz, 1 H), 5.37 (d, J = 5 Hz, 1 H), 5.27 (d, J = 5 Hz, 1 H), 5.20 (t, J = 7 Hz, 1 H), 4.55–4.70 (overlapping m, 3 H), 2.54 (s, 3 H), 0.82 (overlapping d and t, 6 H). FAB MS: m/z = 770 (M + H$^+$). Anal. ($C_{41}H_{55}N_9O_6$·1.45TFA·0.75H_2O) C, H, N.

cyclo-[D-Trp-L-Leu-D-Pip-L-Pip-D-His-L-Pro] (28). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **22**, with the following exception: Fmoc-L-Leu-Cl was used in step d. Workup and purification (method B) gave the TFA salt of the title compound in 16% yield. HPLC (method 1) t_R = 7.66 min; purity 99%. ^1H NMR (300 MHz, CD_3OD) δ 8.78 (s, 1 H), 7.57 (d, J = 7 Hz, 1 H), 7.31 (overlapping d and s, 2 H), 7.10 (s, 1 H), 7.08 (t, J = 7 Hz, 1 H), 7.00 (t, J = 7 Hz, 1 H), 5.21 (d, J = 4 Hz, 1 H), 5.07 (br s,

1 H), 4.62 (dd, J = 7, 8 Hz, 1 H), 4.32 (dd, J = 5, 7 Hz, 1 H), 0.75 (d, J = 7 Hz, 3 H), 0.70 (d, J = 7 Hz, 3 H). FAB MS: m/z = 756 (M + H$^+$). Anal. ($C_{40}H_{53}N_9O_6$·1.9TFA·0.4H_2O) C, H, N.

cyclo-[D-Trp-L-Nle-D-Pip-D-His-L-Pro] (29). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **22**, with the following exception: Fmoc-L-Nle-Cl was used in step d. Workup and purification (method B) gave the TFA salt of the title compound in 29% yield. HPLC (method 1): t_R = 7.76 min; purity 99%. ^{1}H NMR (300 MHz, CD_3OD) δ 8.78 (d, J = 2 Hz, 1 H), 7.56 (d, J = 7 Hz, 1 H), 7.31 (overlapping s and d, 2 H), 7.12 (s, 1 H), 7.08 (t, J = 7 Hz, 1 H), 6.99 (t, J = 7 Hz, 1 H), 5.08 (m, 2 H), 4.60–4.75 (overlapping m, 2 H), 4.32 (dd, J = 4, 7 Ha, 1 H), 0.80 (t, J = 7 Hz, 3 H). FAB MS: m/z = 756 (M + H$^+$). Anal. ($C_{40}H_{53}N_9O_6$·2TFA) C, H, N.

cyclo-[D-Trp-L-Ile-D-Pip-L-Lys-D-(*N*-Me)Phe-L-Pro] (30). The title compound was prepared on a 1-mmol scale according to the solid-phase procedures given for compounds **16** and **21**. Workup and purification (method B) gave the N^{ϵ}-Boc-Lys^5 derivative of the title compound in 35% yield (HPLC (method 1) t_R = 10.90 min; purity 99%). N-Deprotection (procedure 6), purification (method B), and lyophilization gave the TFA salt of the title compound. HPLC (method 1) t_R = 7.80 min; purity 99%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (d, J = 8 Hz, 1 H), 7.1–7.4 (m, 7 H), 4.85 (m, 2 H), 4.68 (m, 2 H), 4.59 (d, J = 7 Hz, 1 H), 4.43 (d, J = 7 Hz, 1 H), 2.87 (s, 3 H), 0.75 (m, 6 H). FAB MS: m/z = 797 (M + H$^+$). Anal. ($C_{44}H_{60}N_8O_6$·1.65TFA·1.45H_2O) C, H, N.

cyclo-[D-1-Nal-L-Ile-D-Pip-L-Lys-D-(*N*-Me)Phe-L-Pro] (31). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **30**, with the following exception: D-Fmoc-1-Nal was used in step e. Workup and purification (method B) gave the N^{ϵ}-Boc-Lys^5 derivative of the title compound (HPLC (method 1) t_R = 12.15 min; purity 99%) in 40% yield. N-Deprotection (procedure 6), purification (method B), and lyophilization gave the TFA salt of the title compound. HPLC (method 1): t_R = 8.88 min; purity 99%. ^{1}H NMR (400 MHz, CD_3OD): δ 7.88 (d, J = 7 Hz, 1 H), 7.78 (t, J = 6 Hz, 1 H), 7.58 (t, J = 7 Hz, 1 H), 7.50 (t, J = 7 Hz, 1 H), 7.41 (d, J = 7 Hz, 1 H), 7.2–7.3 (m, 7 H), 5.22 (br t, J = 7 Hz, 1 H), 4.89 (br d, J = 5 Hz, 1 H), 4.71 (overlapping m, 2 H), 4.55 (dd, J = 4, 8 Hz, 1 H), 4.41 (d, J = 8 Hz, 1 H), 2.99 (s, 3 H), 0.75 (d, J = 7 Hz, 3 H), 0.70 (m, 3 H). FAB MS: m/z = 808 (M + H$^+$). Anal. ($C_{46}H_{61}N_7O_6$·1.95TFA·1.95H_2O) C, H, N.

cyclo-[D-2-Nal-L-Ile-D-Pip-L-Lys-D-(*N*-Me)Phe-L-Pro] (32). The title compound was prepared on a 1-mmol scale according to the solid-phase procedure used for compound **30**, with the following exception: D-Fmoc-2-Nal was used in step e. Workup and purification (method B) gave the N^{ϵ}-Boc-Lys^5 derivative of the title compound (HPLC (method 1) t_R = 12.02 min; purity 99%) in 31% yield. N-Deprotection (procedure 6), purification (method B), and lyophilization gave the TFA salt of the title compound. HPLC (method G): t_R = 8.93 min; purity 99%. ^{1}H NMR (360 MHz, CD_3OD): δ 7.75–7.85 (m, 3 H), 7.40–7.50 (m, 2 H), 7.15–7.30 (m, 7 H), 5.28 (t, J = 8 Hz, 1 H), 4.80 (br d, J = 5 Hz, 1 H), 4.68 (t, J = 7 Hz, 1 H), 4.50–4.60 (m, 2 H), 2.66 (s, 3 H), 0.79 (d, J = 7 Hz, 3 H), 0.66 (t, J = 7 Hz, 3 H). FAB MS: m/z = 808 (M + H$^+$). Anal. ($C_{46}H_{61}N_7O_6$·1.85TFA·1.45H_2O) C, H, N.

cyclo-[D-Trp-L-Ile-D-Pip-L-Ppz-D-(*N*-Me)Phe-L-Pro] (33). The (N^{β}-Cbz)Ppz^5 derivative of the title compound was prepared according to the solid-phase protocol used for compound **1**, with the following exceptions: L-(N^{α}-Fmoc,N^{β}-Cbz)Ppz-Cl (2 mmol) was used in step b, and Fmoc-D-Trp was coupled using the BOP reagent with DIEA in DMF in step e. The Cbz group was removed (procedure 8) and after purification (method B) and lyophilization, the TFA salt of the title compound was obtained as a white powder in 10% yield. HPLC (method 3) t_R = 19.23 min; >97% purity. ^{1}H NMR (360 MHz, DMSO-d_6): δ 8.28 (d, J = 9 Hz, 1 H), 7.94 (d, J = 7 Hz, 1 H), 7.61 (d, J = 8 Hz, 1 H), 7.15–7.35 (m, 6 H), 7.05 (t, J = 7 Hz, 1 H), 6.96 (t, J = 7 Hz, 1 H), 5.58 (br s, 1 H), 5.40 (t, J = 7 Hz, 1 H), 5.30 (br d, J = 5 Hz, 1 H), 4.48 (t, J = 8 Hz, 1 H), 4.41 (q, J = 7 Hz, 1 H), 4.33 (t, J = 6 Hz, 1 H), 2.86 (s, 3 H), 0.78 (d, J = 7 Hz, 3 H), 0.73 (t, J = 7 Hz, 3 H). MS FAB: m/z = 781 (M + H$^+$). Anal. ($C_{43}H_{56}N_8O_6$·2.3TFA·0.5H_2O) C, H, N.

cyclo-[D-Phe-L-Ile-D-Δ-Piz-L-Orn-D-(*N*-Me)Phe-L-Pro] (34). The HCl salt of H-D-(*N*-Me)Phe-L-Pro-OCH$_2$Ph was coupled to L-(N^{α}-Fmoc,N^{δ}-Boc)-Orn (procedure 4). Purification (method C, 30% acetone–hexane) gave Fmoc-L-(N^{δ}-Boc)Orn-D-(*N*-Me)-Phe-L-Pro-OCH$_2$Ph in 64% yield. The tripeptide was N-deprotected (procedure 7), coupled using procedure 3 to Fmoc-L-Ile-D-Δ-Piz (obtained as described for compound **26**), and purified (method C, 0–5% MeOH–DCM gradient elution) to give Fmoc-L-Ile-D-Δ-Piz-L-(N^{δ}-Boc)Orn-D-(*N*-Me)Phe-L-Pro-OCH$_2$Ph (TLC R_f = 0.25 in 5% MeOH–DCM) in 86% yield. The pentapeptide was N-deprotected (procedure 7), coupled to Fmoc-D-Phe (procedure 3), N-deprotected (procedure 7), and purified (method C, 0–6% MeOH–DCM) to give H-D-Phe-L-Ile-D-Δ-Piz-L-(N^{δ}-Boc)Orn-D-(*N*-Me)Phe-L-Pro-OCH$_2$Ph (TLC R_f = 0.45 in 7.5% MeOH–DCM) in 85% yield. A solution of the linear hexapeptide (1.73 g, 1.80 mmol) in 2.3 mL of MeOH was treated with 1.0 mL of anhydrous hydrazine, and the mixture was allowed to stand for 2 h at ambient temperature. The volatiles were removed in vacuo, the residue was partitioned between BuOH and water, and the organic layer was washed with another two portions of water. Concentration of the BuOH layer yielded a foam which was used without further purification. Cyclization was accomplished using the procedure in step g for compound **1**. Purification (method C, gradient of 0–6% MeOH–DCM) gave the L-(N^{δ}-Boc-(Orn^5 derivative of the title compound (TLC R_f = 0.19 in 95:5:0.5 $CHCl_3$–MeOH–H_2O; HPLC (method 2) t_R = 19.22 min) in 55% yield. The cyclic hexapeptide was N-deprotected by treatment with 98% formic acid at ambient temperature for 1 h. Purification (method C, gradient elution using 1:1 A:B, 2:1 A:B, and 3:1 A:B, where A = 2:2:1:0.1 DCM–hexane–MeOH–NH_4OH and B = DCM) gave the title compound in 55% yield and recovered starting material in 28% yield. The acetic acid salt of the title compound was obtained as a white powder by lyophilization from 1:10 HOAc–H_2O and then from H_2O–acetonitrile. TLC: R_f = 0.14 (90:10:1 $CHCl_3$–MeOH–NH_4OH). HPLC (method 4): t_R = 13.2 min; 98.2% purity. FAB MS: m/z = 743 (M + H$^+$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15–7.35 (m, 10 H), 6.97 (d, J = 3 Hz, 1 H), 5.20–5.35 (m, 2 H), 4.88 (br d, J = 4 Hz, 1 H), 4.55–4.65 (m, 3 H), 3.61 (t, J = 8 Hz, 1 H), 2.89 (s, 3 H), 0.79 (overlapping d and t, 6 H). Anal. ($C_{40}H_{54}N_8O_6$·1.25HOAc·2H_2O) C, H, N.

cyclo-[D-Trp-L-Ile-D-Δ-Piz-L-Orn-D-(*N*-Me)Phe-L-Pro] (35). The title compound was prepared in a manner similar to that for **34**, except that Fmoc-D-Trp was substituted for Fmoc-D-Phe in the penta- to hexapeptide step. The title compound was purified (method D, 85:15:1.5 DCM–MeOH–NH_4OH) to yield a solid. TLC: R_f = 0.57 (80:20:2 DCM–MeOH–NH_4OH). HPLC (method 2): t_R = 9.23 min; 94.8% purity. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.52 (d, J = 10 Hz, 1 H), 7.62 (d, J = 7 Hz, 1 H), 6.95–7.40 (overlapping m, 12 H), 5.46 (dd, J = 6, 8 Hz, 1 H), 4.99 (dd, J = 4, 7 Hz, 1 H), 4.74 (br d, J = 4 Hz, 1 H), 4.40–4.60 (m, 3 H), 2.71 (s, 3 H), 0.78 (overlapping d and t, 6 H). FAB MS: m/z = 743 (M + H$^+$). Anal. ($C_{42}H_{55}N_9O_6$·1.25$CHCl_3$·0.2H_2O) C, H, N.

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