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PEPTIDE AND PSEUDOPEPTIDE ANALOGUES OF CHOLECYSTOKININ. CHEMICAL MODIFICATIONS OF THE MET^{28}-GLY^{29} REGION.

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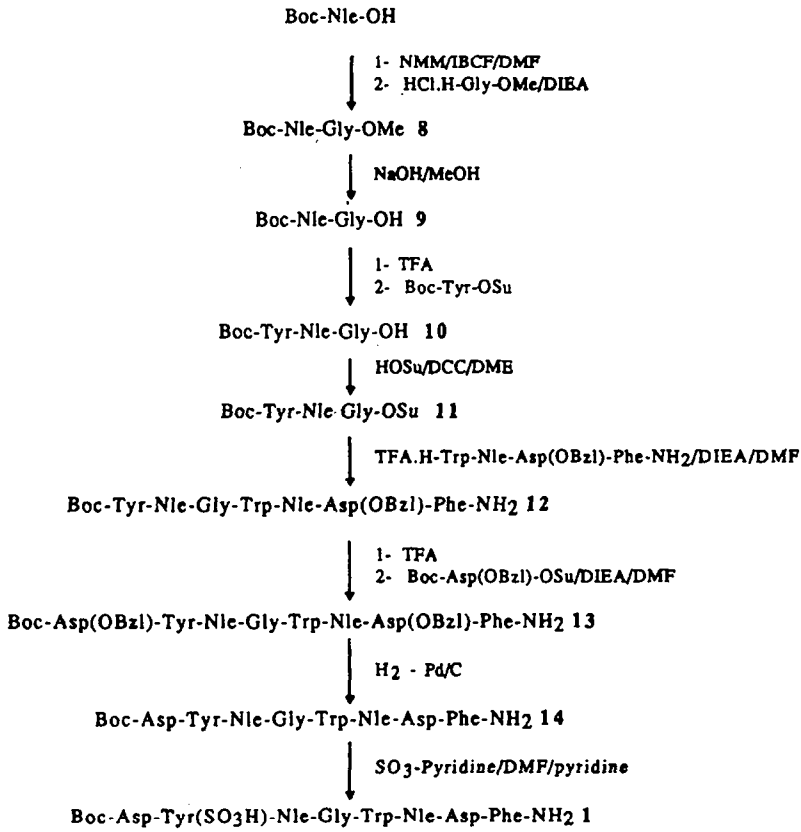
Abstract : The syntheses of a series of analogues of the C-terminal hepta- and octa-peptide of cholecystokinin are described in detail. These peptide and pseudopeptide analogues were obtained by modifying the Nle^{28}-Gly^{29} region, either by replacing the Gly^{29} residue by D-Alanine, Sarcosine, or by replacing the peptide bond between Nle^{28} and Gly ^{29} by a "ketomethylene bond" ($COCH_2$), by a "carba bond" (CH_2CH_2) or by a "retro-inverso modification" (NHCO).

Cholecystokinin (CCK) is a peptide hormone of 33 aminoacid residues first isolated from hog intestine[1], which stimulates pancreatic amylase release, gall bladder contraction, and gastrointestinal motility[2]. CCK was also found in the brain[3] where it functions as a neuromodulator and a neurotransmitter[4]. It has been shown that the C-terminal heptapeptide Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 reproduced the whole range of biological activities of CCK[5]. Addition of the next aspartic acid N-terminal residue slightly modulates the potency of the hormone. In the course of our structure-activity relationships studies on CCK, we recently developped a series of analogues[6] in which each peptide bond was replaced by a "reduced bond" (CH_2-NH), in order to increase stability towards enzymatic degradation. We thus obtained a set of molecules that all behaved as CCK full agonists with various potencies. Among them, Z-Tyr(SO_3H)-Nle-$\Psi(CH_2$-NH)-Gly-Trp-Nle-Asp-Phe-NH_2 was the most potent in inhibiting the binding of labeled ^{125}I-BH-CCK-9 to rat pancreatic acini (IC_{50} = 0.01 mM), in inhibiting the binding of labeled ^{125}I-BH-CCK-9 to guinea pig brain membranes (IC_{50} = 0.01 mM) and in stimulating amylase release from rat pancreatic acini (maximum stimulation was obtained at a concentration of approximately 3 nM). Moreover, it has been shown that enzymatic cleavage between residues Met^{28} and Gly^{29} was the major degradation pattern of CCK in rat hypotalamic synaptosomes[7]. From these results, we decided to investigate more in detail the region Met^{28}-Gly^{29} in order to prevent its enzymatic cleavage, by synthesizing compounds 2 to 6. We also synthesized the potent CCK agonist[8] Boc-[Nle^{28}, Nle^{31}]-CCK-8 (1).

In compounds 2 and 3, the glycine residue was replaced by a sarcosine (N-methyl-glycine) and a D-alanine respectively[9]; in compounds 4, 5 and 6, the peptide bond between positions 28 and 29 was replaced by a ketomethylene, retro-inverso, and carba bond respectively. For easier syntheses, norleucines were used in place of the two methionine residues, because this change has been shown not to influence the biological activities of the analogues[10,11]. The N-terminal end of the derivatives was either a tyrosine sulfate or an aspartyl-tyrosine sulfate moiety and was protected against aminopeptidase degradation by a benzyloxycarbonyl (Z) or a tert-butyloxycarbonyl (Boc) group, because it has been already demonstrated that analogues of the C-terminal part of CCK bearing an Nα-protecting group were somewhat more stable and more potent than the analogues with a free amino group[12].

Boc-Asp-Tyr(SO3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 1
Z-Asp-Tyr(SO3H)-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 2
Boc-Asp-Tyr(SO3H)-Nle-D-Ala-Trp-Nle-Asp-Phe-NH_2 3
Boc-Tyr(SO3H)-Nle-Ψ(CO-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 4
Boc-Asp-Tyr(SO3H)-Nle-Ψ(NH-CO)-Gly-Trp-Nle-Asp-Phe-NH_2 5
Boc-Tyr(SO3H)-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 6

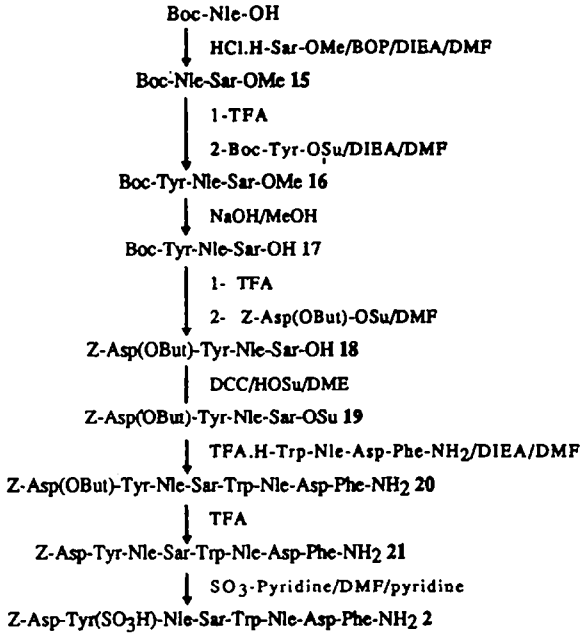
Synthesis of compound 1 was carried out according to the classical procedures of solution peptide synthesis (Scheme I). We took advantage of the presence of a non-racemizable residue (glycine) to build separately fragments Boc-Trp-Nle-Asp(OBzl)-Phe-NH_2[13] (7) and Boc-Tyr-Nle-Gly-OH (10). Compound 10 was converted into its N-hydroxysuccinimide ester[14] (11) which was allowed to react with the TFA salt of the partially deprotected peptide H-Trp-Nle-Asp(OBzl)-Phe-NH_2 to lead to the protected unsulfated CCK analogue Boc-Tyr-Nle-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 (12). Partial deprotection, adding of aspartic acid, subsequent deprotection and sulfation of tyrosine afforded Boc-Asp-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (1).



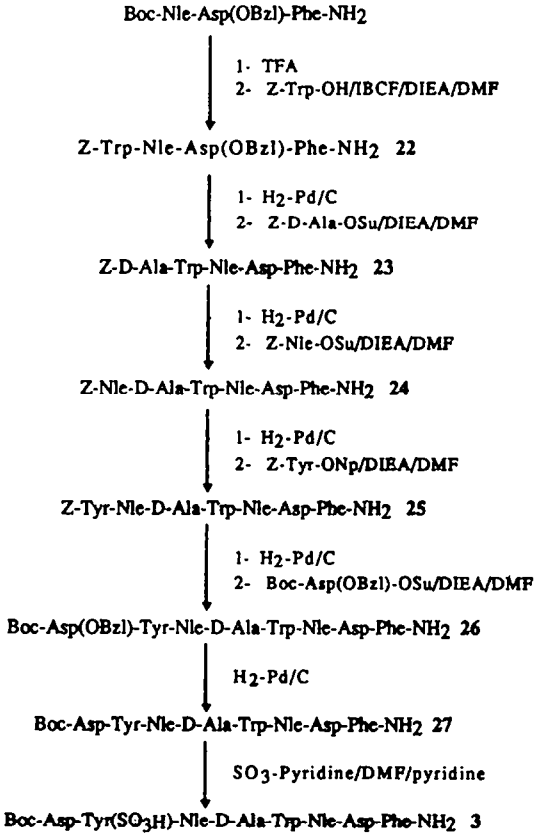
Scheme I

A similar strategy was used in the synthesis of Z-Asp-Tyr(SO_3H)-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 2 (Scheme II). Fragments Z-Asp(OBut)-Tyr-Nle-Sar-OSu 19 and TFA . H-Trp-Nle-Asp-Phe-NH_2 were assembled separately and coupled. Deprotection and sulfation of tyrosine afforded Z-Asp-Tyr(SO_3H)-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 (2). ^1H NMR study of this compound showed a set of two signals (in a ratio of approximatly 1:1) for each residue, due to the cis/trans isomerism of Nle-Sar amide bond.

Synthesis of compound 3 was carried out step by step according to Scheme III, starting from Boc-Nle-Asp(OBzl)-Phe-NH_2[15]. Classical peptide synthesis led to the final peptide Boc-Asp-Tyr(SO_3H)-Nle-D-Ala-Trp-Nle-Asp(OBzl)-Phe-NH2 (3).

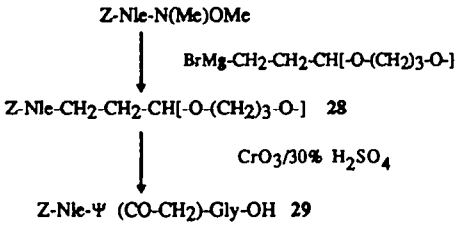


Scheme II

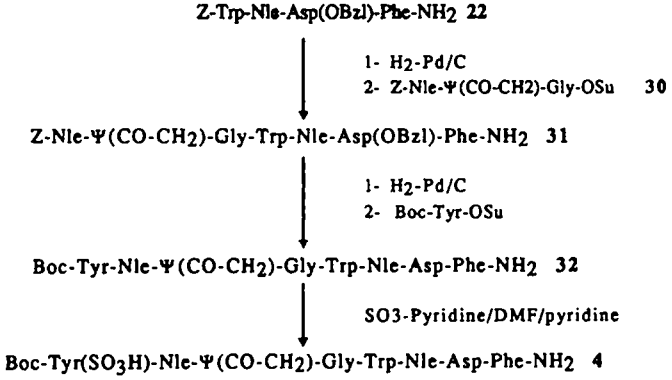


Scheme III

The key step in the synthesis of compound 4 was the obtention of the N-protected dipeptide isostere Z-Nle-Ψ(CO-CH_2)-Gly-OH (29). It was prepared through the reaction of the Grignard reagent generated from 2-(2-bromoethyl)-1,3-dioxane and magnesium, as described by Johnson and Miller[16], and Z-Nle-N(Me)OMe[6] according to Dufour et al.[17] (Scheme IV), and subsequent oxydation by the Jones reagent[16]. The next steps of the synthesis are described in Scheme V. Formation of the N-hydroxysuccinimide ester (30) of Z-Nle-Ψ(CO-CH_2)-Gly-OH (29), coupling to the C-terminal tetrapeptide, deprotection and sulfation of the tyrosine residue afforded Boc-Tyr(SO_3H)-Nle-Ψ(CO-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (4).



Scheme IV



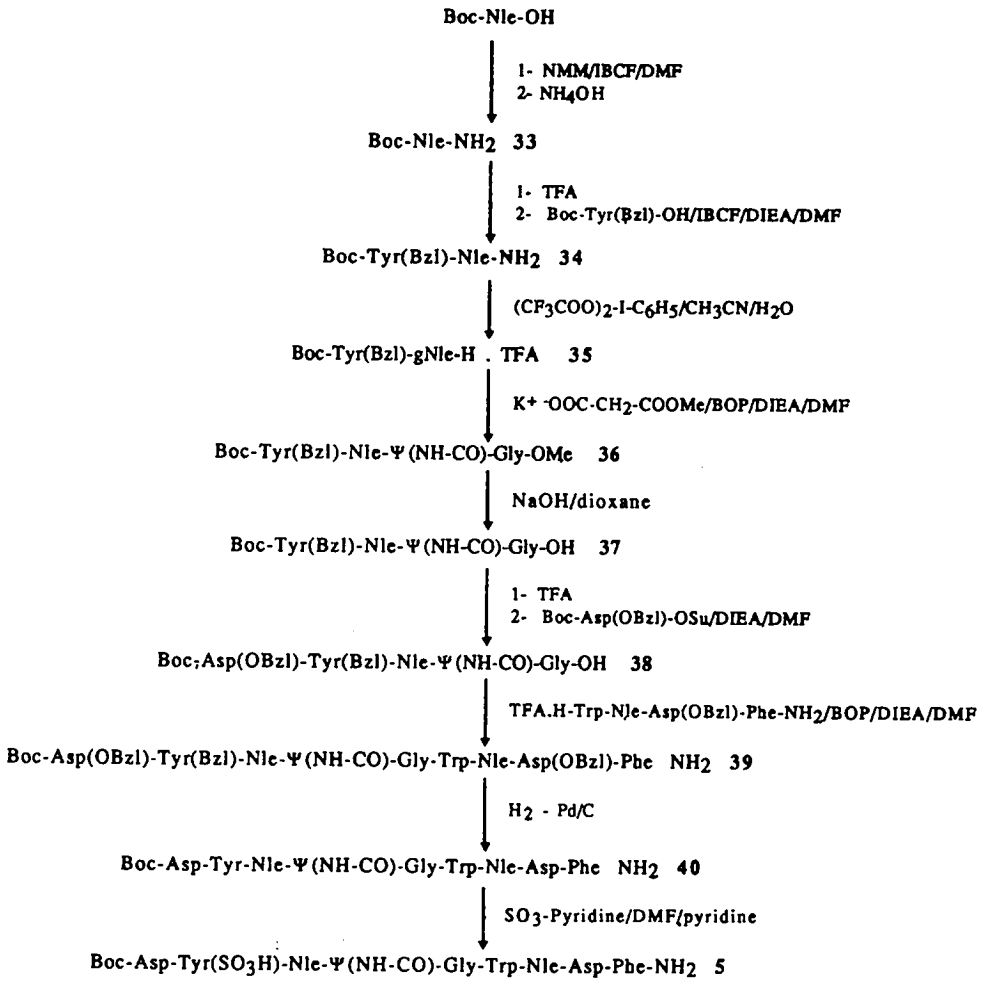
Scheme V

Partial retro-inverso modification, first introduced by Shemyakin et al.[18], involves the reversal of the direction of a single peptide bond. It was shown to lead to peptide isomers retaining, in some cases, biological activity[19]. On the contrary, we have demonstrated that retro-inverso modification could produce antagonists in the gastrin series[20]. This modification can be accomplished by inclusion of a 1,1-diaminoalkane (gem-diamino residue) followed by a malonyl residue. The gem-diamino residue is easily obtained from an amide by reaction with [bis(trifluoroacetoxy)-iodo]benzene with retention of the spatial

The use of a side chain protection of tyrosine was mandatory, in order to prevent side reactions during gem-diaminoalkane formation[21]. This reaction was carried out from the dipeptide amide Boc-Tyr(Bzl)-Nle-NH_2 (34) to facilitate monitoring (UV absorption) and handling of the desired product.

Coupling of the modified glycine was easily performed from the potassium salt of monomethyl malonate using BOP[22]. Pseudo-peptide Boc-Asp-Tyr(SO_3H)-Nle-Ψ(NH-CO)-Gly-Trp-Nle-Asp-Phe-NH_2* 5 was then obtained from Boc-Tyr(Bzl)-Nle-Ψ(NH-CO)-Gly-OMe (36) according to the classical methods of solution peptide synthesis.

The key step in the synthesis of Boc-Tyr(SO_3H)-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (6) was the obtention of Boc-Nle-Ψ(CH_2-CH_2)-Gly-OH (44). The first synthetic pathway was the condensation of Boc-L-norleucinal[23] and the Wittig reagent Φ_3P=CH_2-CH_2-COOMe, but we would have had to face two important problems: instability of the Wittig reagent and possible racemization of the aldehyde.



Scheme VI

We thus first homologated the norleucine residue according to Wakamiya[24]. The corresponding aldehyde (Scheme VII) was allowed to react with the stable, commercially available methyl (triphenylphosphoranylidene) acetate to obtain, after saponification, 5(S)-(N-tert-Butyloxycarbonyl) amino-non-2-enoic acid (43), which was reduced by catalytic hydrogenation to Boc-Nle-Ψ(CH_2-CH_2)-Gly-OH (44). It was coupled to H-Trp-Nle-Asp(OBzl)-Phe-NH_2 (obtained from Fmoc-Trp-Nle-Asp(OBzl)-Phe-NH_2[6] by cleavage with diethylamine in DMF) using BOP[22]. We did not observe any substitution or ring closure of the β-aspartyl benzyl ester. The end of the synthesis was carried out as usual to lead to the pseudo-peptide Boc-Tyr(SO_3H)-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (6).

All the final products were caracterized by 2D ^{1}H-NMR spectroscopy (see Tables I to VI). Biological activities of the compounds obtained herein will be reported elsewhere.

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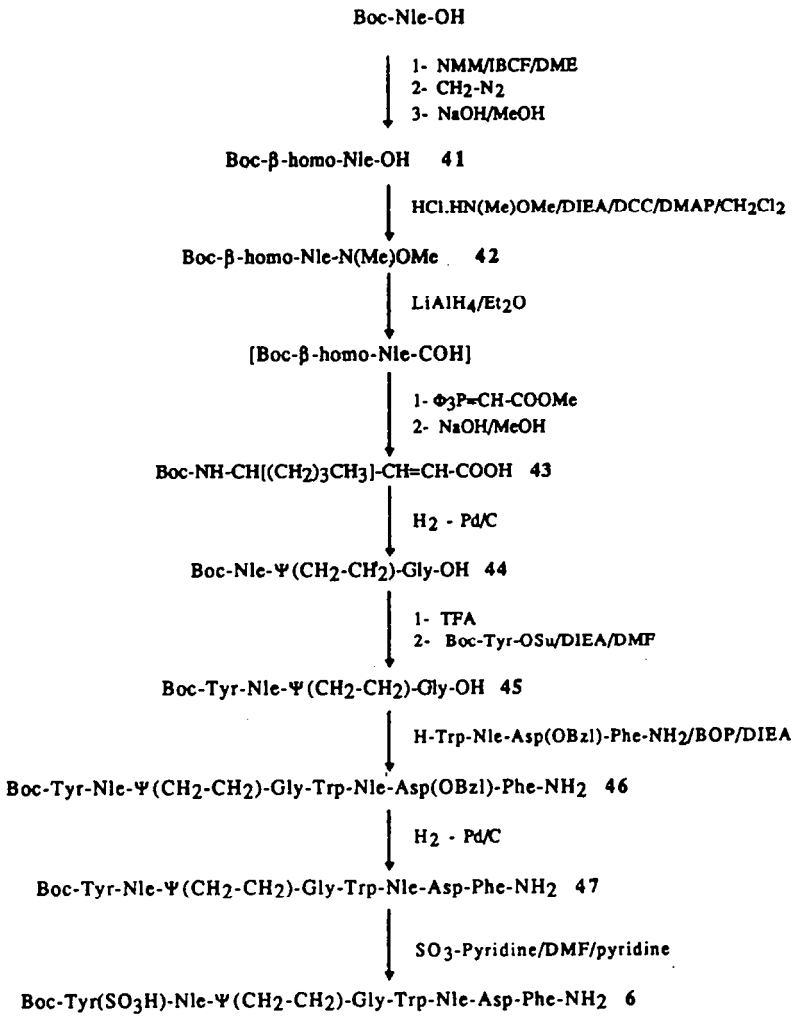
* The retro-inverso modification Nleψ(NHCO)Gly can also be written gNle-mGly, thus referring to the gem-diamino alkane and the malonyl residue introduced in the peptide sequence.


Boc-Nle-OH
1- NMM/IBCF/DME
2- CH_2-N_2
3- NaOH/MeOH
Boc-β-homo-Nle-OH 41
HCl.HN(Me)OMe/DIEA/DCC/DMAP/CH_2Cl_2
Boc-β-homo-Nle-N(Me)OMe 42
$LiAlH_4/Et_2O$
[Boc-β-homo-Nle-COH]
1- Φ_3P=CH-COOMe
2- NaOH/MeOH
Boc-NH-CH[$(CH_2)_3CH_3$]-CH=CH-COOH 43
H_2 - Pd/C
Boc-Nle-Ψ(CH_2-CH_2)-Gly-OH 44
1- TFA
2- Boc-Tyr-OSu/DIEA/DMF
Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-OH 45
H-Trp-Nle-Asp(OBzl)-Phe-NH_2/BOP/DIEA
Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 46
H_2 - Pd/C
Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 47
SO_3-Pyridine/DMF/pyridine
Boc-Tyr(SO_3H)-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 6

Scheme VII

	NH		Hα		Hβ		Others	
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)
Asp	6.85	8.2	4.25		2.44			
Tyr	8.47	bd	4.27	3.3-9.8	3.03		o 7.02	
					2.89		m 7.09	
					^2J	13.9		
Nle	8.20	8.6	4.16		1.52		δ,γ 1.4 to 1.1	
							Me 0.84 (0.82)	6.9
Gly	8.37	6.0-4.9	3.82					
			3.51					
Trp	8.73	6.0	4.44		3.20		NHin d 10.80	2.0
					3.03		s 7.10	
					^2J	15.0	d 7.49	
							d 7.29	
							t 6.90	
							t 7.02	
Nle	8.80	bd	3.98		1.58		δ,γ 1.4 to 1.1	
							Me 0.82(0.84)	6.9
Asp	7.72	6.7	4.33		2.40			
Phe	7.68	8.6	4.25		3.08		Ar 7.25 to 7.0	
					2.80			
					^2J	13.7		

Table I: NMR data of Boc-Asp-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (1) in DMSO-d_6.

	NH		Hα		Hβ		Others
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)
Asp	7.23		4.20		2.34		
Tyr	8.63-8.57	8.2-8.2	4.32-4.33		3.04		o 7.03
					2.78		m 7.10
Nle	8.39-8.46	8.7	4.64-4.41		1.53-1.50		δ,γ 1.4 to 1.1
							Me 0.87-0.75
Sar			3.72-3.67				NMe 2.57-2.88
			4.34-4.14				
			^2J	18-16			
Trp	8.18-8.79	8.72-bd	4.52-4.60	4.7	3.17-3.17		NHin 11.24-11.10
					2.99-2.99		s 7.12-7.14
					^2J	14.6	d 7.56-7.61
							d 7.31
							t 6.96
							t 7.03
Nle	8.11-8.42	7.6	4.15-4.18		1.46		δ,γ 1 4 to 1.1
							Me 0.87-0.75
Asp	8.11-8.18	7.6-7.8	4.33-4.34		2.26		
Phe	8.43-8.48	8.6	4.27-4.27		3.09		Ar 7.4 to 6.9
					2.82		

Table II: NMR data of Z-Asp-Tyr(SO_3H)-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 (2) in DMSO-d_6. bd: broad; s: singlet; d: doublet; t: triplet.

	NH		Hα		Hβ		Others	
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)
Asp	7.1		4.20	4.4-8.9	2.54			
					2.38			
					^2J	16.5		
Tyr	7.69	7.7	4.47	4.7-8.8	2.96		o 7.03	
					2.74		m 7.10	
					^2J	13.5		
Nle	8.01	7.7	4.18		1.58		Me 0.82(0.81)	6.8
					1.49			
D-Ala	7.85	7.4	4.25		0.95	6.9		
Trp	8.07	8.4	4.59	4.0-9.8	3.17		NHin 10.71	2.0
					2.94		s 7.08	
					^2J	14.7	d 7.58	7.7
							d 7.29	8.1
							t 7.03	
							t 6.93	
Nle	8.05	8.2	4.18		1.58		Me 0.81(0.82)	6.8
					1.49			
Asp	8.17	7.7	4.51	6.5-7.2	2.67			
					2.49			
					^2J	16.8		
Phe	7.77	8.3	4.36	5.1-8.5	3.04		Ar 7.3 to 7.0	
					2.85			
					^2J	13.9		

Table III: NMR data of Boc-Asp-Tyr(SO_3H)-Nle-D-Ala-Trp-Nle-Asp-Phe-NH_2 (3) in DMSO-d_6.

	NH		Hα		Hβ		Others	
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)
Tyr	7.02		4.12	3.5-10	2.90		o 7.14	8.7
					2.71		m 7.05	
					^2J	13.7		
Nle	8.38	7.5	4.21		1.68		δ,γ 1.4 to 1.1	
					1.44		Me 0.84(0.82)	7.2
ψ(CO-CH_2)							2.63 and 2.51	
Gly			3.82	4.5				
			3.51	8.8				
			^2J	15.8				
Trp	8.06	7.4	4.48		3.12		NHin d 10.78	2.1
					2.95		s 7.15	
					^2J	15.0	d 7.54	7.9
							d 7.30	8.1
							t 7.03	
							t 6.93	
Nle	7.84	7.2	4.10		1.56		δ,γ 1.4 to 1.2	
					1.47		Me 0.82(0.84)	7.2
Asp	8.02	7.4	4.43	7.0	2.40			
					^2J	16.3		
Phe	7.84	8.6	4.32	4.7-8.8	3.06		Ar 7.3 to 7.0	
					2.85			
					^2J	13.9		

Table IV: NMR data of Boc-Tyr(SO_3H)-Nle-ψ(CO-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (4) in DMSO-d_6.

	NH		Hα		Hβ		Others	
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)
Asp	6.89	7.0	4.23		2.51			
					2.37			
					^2J	15.6		
Tyr	7.95	bd	4.30	4.2-9.2	2.95		o 7.02	8.6
					2.79		m 7.09	
					^2J	14.0		
*gNle	8.53	6.7	5.41		1.51		δ,γ 1.4 to 1.1	
	8.25	8.0					Me 0.82	6.8
*mGly			3.11					
Trp	8.72		4.50	4.6-9.2	3.19		NHin d 10.82	2.2
					3.00		s 7.22	
					^2J	14.8	d 7.52	7.8
							d 7.31	8.1
							t 7.03	
							t 6.92	
Nle	8.16	7.2	4.07		1.57		δ,γ 1.4 to 1.1	
					1.48		Me 0.82	6.8
Asp	7.90	7.4	4.43	7.0-6.3	2.42			
					2.54			
					^2J	16.6		
Phe	7.76	8.0	4.32	4.7-9.2	3.07		Ar 7.3 to 7.0	
					2.85			
					^2J	14.0		

Table V: NMR data of Boc-Asp-Tyr(SO_3H)-ψ(NH-CO)-Trp-Nle-Asp-Phe-NH_2 (5) in DMSO-d_6. *gNle-mGly is an other usual notation for Nle-ψ(NH-CO)-Gly.

	NH		Hα		Hβ		Others	
	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)	δ(ppm)	^3J(Hz)
Tyr	6.74	8.5	4.05	4.0-10.0	2.82		o 7.05	
					2.70		m 7.12	
					^2J	13.8		
Nle	7.43		3.61		1.22		Me 0.83(0.82)	6.8
ψ(CH_2-CH_2)							1.20 and 1.33	
Gly		2.02						
Trp	7.89	8.1	4.55	4.7-9.4	3.12		NHin d 10.79	2.2
					2.93		s 7.11	
					^2J	14.9	d 7.58	7.8
							d 7.30	8.0
							t 7.02	
							t 6.94	
Nle	7.92	7.8	4.19		1.57		Me 0.82(0.83)	6.3
					1.45			
Asp	8.10	7.7	4.45	6.2-7.2	2.39			
					2.53			
					^2J	16.2		
Phe	8.05	8.3	4.32	4.5-9.1	3.06		Ar 7.3 to 7.0	
					2.84			
					^2J	13.9		

Table VI: NMR data of Boc-Tyr(SO_3H)-ψ(CH_2-CH_2)-Trp-Nle-Asp-Phe-NH_2 (6) in DMSO-d_6.

EXPERIMENTAL

Melting points were taken on a Büchi apparatus in open capillary tubes. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by "Le Service de Microanalyses de l'ENSCM" (Montpellier, France). Ascending TLC was performed on precoated plates of silica gel 60 F 254 (Merck) using the following solvent systems (by volume): A, AcOEt/hexane, 5:5; B, AcOEt/hexane, 7:3; C, AcOEt ; D, chloroform/methanol/acetic acid, 85:10:5; E, AcOEt/pyridine/acetic acid/water, 80:20:5:10; F, AcOEt/pyridine/acetic acid/water, 60:20:5:10; G, AcOEt/hexane/acetic acid 20:80:1; H, AcOEt/hexane/acetic acid 40:60:1. Peptide derivatives were located with UV light (254 nm), charring reagent or ninhydrin. Column chromatographies were performed with silica gel 60, 60-229 mesh, ASTM (Merck). HPLC purifications were run on a Merck/Hitachi instrument on a Beckman Ultrasphere ODS (0.5 µm) 10x250 mm column, with an UV detection at 279 nm, at a flow rate of 3 ml/min of a mixture of A: ammonium acetate 0.01 M, pH 6.5, and B: methanol. ^1H NMR spectra were recorded on a Brücker WM 360 WB spectrometer equipped with an aspect 2000 computer operating in the Fourier Transform mode with quadrature detection at 360 MHz. Spectra were recorded at 293°K. Amino acids and derivatives were purchased from Bachem (Switzerland). All reagents and solvents were of analytical grade. BOP was recrystallized from acetone and ether. The following abbreviations were used: DMF, dimethylformamide; DME, 1,2-dimethoxyethane; HOBT, 1-hydroxybenzotriazole; HOSu, N-hydroxysuccinimide; DIEA, N,N-diisopropylethylamine; DMAP, 4-(N,N-dimethyl)-amino-pyridine; BOP, benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate; NMM, N-methylmorpholine; IBCF, isobutyl-chloroformiate; DCC, N,N'-dicyclohexylcarbodiimide. Other abbreviations used were those recommended by the IUPAC-IUB Commission (Eur. J. Biochem. 1984, 138, 9-37).

Boc-Trp-Nle-Asp(OBzl)-Phe-NH_2 (7) : Boc-Nle-Asp(OBzl)-Phe-NH_2[15] (5.03 g, 8.62 mmol) was treated with TFA (15 ml) for 30 min at room temperature. The partially deprotected peptide precipitated upon addition of ether as its trifluoroacete salt. It was collected by filtration, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of Boc-Trp-OSu[14] (3.01 g, 7.49 mmol) in DMF (15 ml), followed by DIEA (1.48 ml, 8.62 mmol). After 2h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (200 ml). It was collected, washed with water, saturated aqueous $NaHCO_3$, water, ether, dried in vacuo (5.66 g, 98%); mp 165-168°C; Rf(C) = 0.50; $[\alpha]_D^{20}$ = -31.0° (c 1; DMF).

Boc-Nle-Gly-OMe (8) : To a cold (-20°C) solution of Boc-Nle-OH (2.84 g, 12.3 mmol) in DMF (10 ml) were successively added NMM (1.38 ml, 12.3 mmol) and IBCF (1.67 ml, 12.3 mmol). After 5 min, HCl.H-Gly-OMe was then added, followed by DIEA (2.4 ml, 14.6 mmol), and the mixture stirred for 1h at room temperature. The solvent was concentrated in vacuo to leave a residue which was dissolved in AcOEt (200 ml). The solution was washed with 1M aqueous $KHSO_4$ (3x100 ml), water, saturated aqueous $NaHCO_3$ (3x100 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane 7:3) to afford 8 as a white solid which was recrystallized in a mixture of AcOEt and hexane (2.57 g, 69%); mp 98-100°C; Rf(B) = 0.68; $[\alpha]_D^{20}$ = -11.5° (c 1; DMF).

Boc-Nle-Gly-OH (9): To a solution of 8 (2.42 g, 8.02 mmol) in methanol (10 ml), was added 1N aqueous NaOH (9.6 ml, 9.6 mmol). After 10 min no more starting material could be detected by TLC. The methanol was concentrated in vacuo, and the residue dissolved in water (40 ml). This solution was acidified with 1N aqueous $KHSO_4$ and extracted with AcOEt (3x40 ml). The organic extracts were washed with 1N aqueous $KHSO_4$ (2x40 ml), brine, dried over $MgSO_4$ and concentrated in vacuo to leave 9 as an oil 2.35 g, 99%; Rf(D) = 0.64.

Boc-Tyr-Nle-Gly-OH (10) : Compound 9 (2.21 g, 8 mmol) was deprotected in TFA as already described

precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (50 ml). It was collected, washed with water, dried in vacuo (2.98 g, 85%); mp 90°C (dec); Rf(D) = 0.39; $[\alpha]_D^{20}$ = -9.7° (c 1; DMF).

Boc-Tyr-Nle-Gly-OSu (11) : To a cold (0 °C) solution of 10 (2.92 g, 6.5 mmol) in DME (20 ml), were successively added HOSu (1.12 g, 9.75 mmol), DCC (1.34 g, 6.5 mmol). After 4h stirring at room temperature, the precipitated DCU was filtered off, the solvent was concentrated in vacuo to leave a residue which was dissolved in AcOEt (50 ml). The solution was washed with cold 2% aqueous $NaHCO_3$ (3x40 ml), water, 1M aqueous $KHSO_4$ (3x40 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue gave a white solid upon trituration with ether (2.47 g, 70%); mp 110-115°C; Rf(C) = 0.62; $[\alpha]_D^{20}$ = -7.1° (c 1; DMF).

Boc-Tyr-Nle-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 (12) : Compound 7 (1.5 g, 1.95 mmol) was deprotected in TFA as already described. The deprotected peptide was added to a solution of 11 (0.952 g, 1.74 mmol) in DMF (10 ml), followed by DIEA (0.34 ml, 1.95 mmol). After 2h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, saturated aqueous $NaHCO_3$, water, ether, dried in vacuo (1.9 g, 98%); mp 190°C (dec); Rf(D) = 0.50; $[\alpha]_D^{20}$ = -17.0° (c 1; DMF).

Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 (13): Compound **12** (0.60 g, 0.544 mmol) was deprotected in TFA as already described. The deprotected peptide was added to a solution of Boc-Asp(OBzl)-OSu[14] (0.190 g, 0.45 mmol) in DMF (5 ml), followed by DIEA (0.09 ml, 0.544 mmol). After 2h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, saturated aqueous $NaHCO_3$, water, ether, dried in vacuo (0.500 g, 85%); mp 220°C (dec); Rf(E) = 0.85; $[\alpha]_D^{20}$ = -12.4° (c 1; DMF).

Boc-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (14): Compound **13** (0.300 g, 0.23 mmol) was hydrogenated overnight in a mixture of DMF (10 ml) and ethanol (150 ml) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, the filtrate concentrated in vacuo, and the residue triturated in ether to afford the deprotected peptide (0.210 g, 81%); mp 205°C (dec); Rf(F) = 0.85; $[\alpha]_D^{20}$ = -24.4° (c 1; DMF).

Boc-Asp-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (1): To a solution of compound **14** (0.200 g, 0.177 mmol) in a mixture of DMF (2 ml) and pyridine (2 ml) was added SO_3-pyridine complex (1.2 g). After overnight stirring at room temperature, the solvents were concentrated in vacuo and the excess of complex was hydrolysed with water (10 ml) for 30 min, while the pH was maintained around 7-8 by addition of 10% aqueous sodium carbonate. The solution was then acidified to pH 5 by addition of 1M aqueous $KHSO_4$ and extracted with n-BuOH (3x20 ml). The organic phases were washed with water and concentrated in vacuo to leave a solid residue which was triturated with ether, collected and dried in vacuo. HPLC purification (Rt= 4.9 min, A/B 35:65) and lyophilisation afforded compound **1**(103 mg, 48%); mp 194°C (dec.); Rf(F) = 0.35; $[\alpha]_D^{20}$ = -22.0° (c 1; DMF). NMR data are given in table I.

Boc-Nle-Sar-OMe (15) : To a cold (0 °C) solution of Boc-Nle-OH (1.5 g, 6.48 mmol) in dichloromethane (10 ml) were successively added HCl.H-Sar-OMe (1.09 g, 7.77 mmol), BOP[22] (2.87 g, 6.48 mmol), and DIEA (2.23 ml, 12.96 mmol). After 2h stirring at room temperature, the solvent was concentrated in vacuo to leave a residue which was dissolved in AcOEt (200 ml). The solution was washed with 1M aqueous $KHSO_4$ (3x100 ml), water, saturated aqueous $NaHCO_3$ (3x100 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane 5:5) to leave **15** as a colorless oil (1.21 g, 59%); Rf(A) = 0.60.

Boc-Tyr-Nle-Sar-OMe (16) : Compound **15** (1.11 g, 3.51 mmol) was partially deprotected with TFA as previously described. It was added to a solution of Boc-Tyr-OSu[14] (1.06 g, 2.81 mmol) in DMF (15 ml), followed by DIEA (0.6 ml, 3.51 mmol). After 5h stirring at room temperature the reaction mixture was treated as described above. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane 7:3) to leave compound **16** as a white solid (0.88 g, 66%); mp 68-70°C; Rf(B) = 0.42; $[\alpha]_D^{20}$ = -16.5° (c 1; DMF).

Boc-Tyr-Nle-Sar-OH (17) : To a solution of compound **16** (0.84 g, 1.75 mmol) in methanol (5 ml), was added 1N aqueous NaOH (3.85 ml, 3.85 mmol). After 20 min no more starting material could be detected by TLC. The methanol was concentrated in vacuo, and the residue dissolved in water (40 ml). This solution was acidified with 1N aqueous $KHSO_4$ and extracted with AcOEt (3x40 ml). The organic extracts were washed with 1N aqueous $KHSO_4$ (2x40 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue gave a white solid upon trituration with hexane (0.8 g, 99%); mp 95-98°C; Rf(D) = 0.54°; $[\alpha]_D^{20}$ = -15.6 (c 1; DMF).

Z-Asp(OBut)-Tyr-Nle-Sar-OH (18) : Compound **17** (0.64 g, 1.35 mmol) was deprotected in TFA as described above. The deprotected peptide was added to a solution of Z-Asp(OBut)-OSu[14] (0.46 g, 1.08 mmol) in DMF (5 ml), followed by DIEA (0.24 ml, 1.35 mmol). After 5h stirring at room temperature the reaction mixture was treated as described for **16**. The residue was purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH/AcOH 85:7:3) to leave a white solid (0.54 g, 74%); mp 91-94°C; Rf(D) = 0.65; $[\alpha]_D^{20}$ = -20.9° (c 1; DMF).

Z-Asp(OBut)-Tyr-Nle-Sar-OSu (19) : To a cold (0 °C) solution of compound **18** (0.49 g, 0.73 mmol) in DME (10 ml), were successively added HOSu (0.10 g, 0.88 mmol), and DCC (0.151 g, 0.73 mmol). After 4h stirring at room temperature, the precipitated DCU was filtered off, the solvent was concentrated in vacuo to leave a residue which was dissolved in AcOEt (50 ml). The solution was washed with cold 2% aqueous $NaHCO_3$ (3x40 ml), water, 1M aqueous $KHSO_4$ (3x40 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue gave a white solid upon trituration with ether (0.376 g, 67%); mp 80-82°C; Rf(A) = 0.69; $[\alpha]_D^{20}$ = -24.8° (c 1; DMF).

Z-Asp(OBut)-Tyr-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 (20) : Boc-Trp-Nle-Asp-Phe-NH_2[13] (0.450 g, 0.51 mmol) was treated with TFA for 30 min at room temperature. The deprotected peptide precipitated upon addition of ether as its trifluoroacete salt. It was collected by filtration, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of **19** (0.326 g, 0.426 mmol) in DMF (5 ml), followed by DIEA (0.09 ml, 0.51 mmol). After 5h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (50 ml). It was collected, washed with water, AcOEt, ether, dried in vacuo (0.52 g, 99%); mp 190°C (dec.); Rf(D) = 0.44; $[\alpha]_D^{20}$ = -21.6° (c 1; DMF).

Z-Asp-Tyr-Nle-Sar-Trp-Nle-Asp-Phe-NH_2 (21) : Compound **20** (0.50 g, 0.41 mmol) was treated with TFA for 45 min at room temperature. The partially deprotected peptide precipitated upon addition of

ether. It was collected by filtration, washed with ether, and dried in vacuo over KOH pellets. It was purified by silica gel column chromatography (eluent AcOEt/pyridine/acetic acid/water 80:20:5:10)(0.19 g, 38%); mp 177°C (dec.); Rf(E) = 0.45; $[\alpha]D^{20}$ = -36.3° (c 1; DMF).

Z-Asp-Tyr(SO3H)-Nle-Sar-Trp-Nle-Asp-Phe-NH2 (2) : Synthesized as previously described for compound 1 from compound 21 (610 mg, 0.54 mmol). HPLC purification (Rt= 5.06 min, A/B 35:65) and lyophilisation afforded compound 2 (50 mg, 31%); mp 210°C (dec.); Rf(E) = 0.17; $[\alpha]D^{20}$ = -17.2° (c 0.18; DMF). NMR data are given in table II.

Z-Trp-Nle-Asp(OBzl)-Phe-NH2 (22) : Boc-Nle-Asp(OBzl)-Phe-NH_2[15] (2.3 g, 4.02 mmol) was partially deprotected in TFA as described above. To a cold (-20°C) solution of Z-Trp-OH (1.13 g, 3.35 mmol) in DMF (15 ml) were successively added NMM (0.37 ml, 3.35 mmol) and IBCF (0.45 ml, 3.35 mmol). After 5 min, the partially deprotected peptide was then added as its trifluoroacetate salt, followed by DIEA (0.69 ml, 4.02 mmol), and the mixture stirred for 1h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (200 ml). It was collected, washed with water, saturated aqueous $NaHCO_3$, water, dried in vacuo (2.80 g, 99%); mp 234°C; Rf(C)= 0.34; $[\alpha]D^{20}$ = -28.3° (c 1; DMF).

Z-D-Ala-Trp-Nle-Asp-Phe-NH2 (23) : Compound 22 (1.72 g, 2.41 mmol) was hydrogenated overnight in a mixture of DMF/AcOH/H_2O 60:10:5 (75 ml) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, the filtrate concentrated in vacuo, and the residue triturated in ether to afford the acetate of the deprotected peptide. It was added to a mixture of Z-D-Ala-OSu[14] (0.736 g, 2.30 mmol) in DMF (10 ml), followed by DIEA (0.42 ml, 2.41 mmol), and the mixture was stirred for 3h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (50 ml). It was collected, washed with water, ether, dried in vacuo (1.69 g, 94%); mp 215°C; Rf(D) = 0.30; Rf(E) = 0.61; $[\alpha]D^{20}$ = -16.5° (c 1; DMF).

Z-Nle-D-Ala-Trp-Nle-Asp-Phe-NH2 (24) : Compound 23 (1.59 g, 2.03 mmol) was deprotected by hydrogenation as described above. The deprotected peptide was added to a mixture of Z-Nle-OSu[14] (0.669 g, 1.85 mmol) in DMF (10 ml), followed by DIEA (0.35 ml, 2.03 mmol), and the mixture was stirred for 3h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (200 ml). It was collected, washed with water, ether, dried in vacuo (1.38 g, 83%); mp 225°C (dec); Rf(D) = 0.36; Rf(E) = 0.62; $[\alpha]D^{20}$ = -19.6° (c 1; DMF).

Z-Tyr-Nle-D-Ala-Trp-Nle-Asp-Phe-NH2 (25) : Compound 24 (1.18 g, 1.31 mmol) was deprotected by hydrogenation as described above. The deprotected peptide was added to a mixture of Z-Tyr-ONp[25] (0.494 g, 1.13 mmol) in DMF (10 ml), followed by DIEA (0.35 ml, 2.03 mmol), and the mixture was stirred overnight at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (200 ml). It was collected, washed with water, ether, dried in vacuo (1.04 g, 95%); mp 225°C (dec); Rf(D) = 0.26; $[\alpha]D^{20}$ = -14.5° (c 1; DMF).

Boc-Asp(OBzl)-Tyr-Nle-D-Ala-Trp-Nle-Asp-Phe-NH2 (26) : Compound 25 (0.940 g, 0.89 mmol) was deprotected by hydrogenation as previously described. The deprotected peptide was added to a mixture of Boc-Asp(OBzl)-OSu[14] (0.315 g, 0.75 mmol) in DMF (10 ml), followed by DIEA (0.15 ml, 0.89 mmol), and the mixture was stirred for 3h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (200 ml). It was collected, washed with water, ether, dried in vacuo (0.740 g, 80%); mp 225°C (dec); Rf(D) = 0.53; Rf(E) = 0.64; $[\alpha]D^{20}$ = -18.5° (c 1; DMF).

Boc-Asp-Tyr-Nle-D-Ala-Trp-Nle-Asp-Phe-NH2 (27) : Compound 26 (0.64 g, 0.52 mmol) was hydrogenated overnight in a mixture of DMF/AcOH/H_2O 60:10:5 (75 ml) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, the filtrate concentrated in vacuo, and the residue triturated in ether to afford the partially deprotected peptide. It was collected, washed with ether, dried in vacuo (0.560 g, 94%); mp 225°C ; Rf(D) = 0.16; Rf(E) = 0.42; $[\alpha]D^{20}$ = -29.5° (c 1; DMF).

Boc-Asp-Tyr(SO3H)-Nle-D-Ala-Trp-Nle-Asp-Phe-NH2 (3) : Synthesized as described for compound 1 from compound 27 (200 mg, 0.17 mmol). HPLC purification (Rt= 7.85 min, A/B 38:62) and lyophilisation afforded compound 3 (104 mg, 50%); mp 200°C (dec.); Rf(E) = 0.15; Rf(F) = 0.36; $[\alpha]D^{20}$ = -25.0° (c 1; DMF). NMR data are given in table III.

2[3-oxo-4(S)-N-(benzyloxycarbonyl)-aminooctyl]-1,3-dioxane (28) : A solution of Z-Nle-N(Me)OMe[6] (1.58 g, 5.13 mmol) in ether (100 ml) was added dropwise at room temperature under argon to a stirred solution of the Grignard reagent generated from 2-(2-bromoethyl)-1,3-dioxane (3.49 ml, 25.6 mmol) and magnesium (0.66g, 27 mmol) in 100 ml of ether. After 1h stirring the solution/suspension was cooled down to 0°C and treated with 1M aqueous $KHSO_4$ (200 ml). The organic phase was decanted, washed with 1M aqueous $KHSO_4$ (3x100 ml), water, saturated aqueous $NaHCO_3$ (3x100 ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane 1:3) to leave 28 as a white solid from a mixture of ether and hexane (1,51 g, 81%); mp 63-66°C (dec.); Rf(A) = 0.67; $[\alpha]D^{20}$ = -14.6° (c 1; DMF); NMR (DMSO d_6) δ ppm 7.63 (d, 1H, NH, $J_{NH\text{-}CH}$ 5' = 7.6 Hz), 7.35 (m, 5H, Ar), 5.04 (s, 2H, CH_2 Z), 4.47 (m, 1H, H *1*), 3.95 (m, 1H, H 5'), 3.95 (m, 2H, H *3eq*, H *5eq*), 3.65 (m, 2H, H

3ax, H *5ax*), 2.50 (m, 2H, CH_2 *3'*), 1.83 (m, 1H, H *4ax*), 1.66 (m, 2H, H *1'*), 1.65 and 1.45 (m, 2H, H *6'*), 1.30 (m, 1H, H *4eq*), 1.27 (m, 4H, CH_2 *7'* and *8'*), 0.84 (t, 3H, CH_3 *9'*); Anal. $C_{20}H_{29}NO_5$ calc: C 65.94, H 7.99, N 3.59, found C 65,71, H 8.10, N 3.51.

Z-Nle-Ψ(CO-CH2)-Gly-OH (29) : A solution of compound **28** (0.86 g, 2.37 mmol) in acetone (15 ml) was treated at 0°C with Jones reagent (2.37 g, 23.7 mmol CrO_3 in 15 ml H_2SO_4 30%).The solution was stirred for 1h at room temperature, and the excess of oxydizing reagent was neutralized by addition of iPrOH at 0°C. The resulting green solution was poured into a mixture of dichloromethane (50 ml) and water (50 ml). The aqueous phase was extracted with dichloromethane (2x50 ml), and the combined organic layers were washed with water (3x50ml), brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized in a mixture of AcOEt and hexane (0.496 g, 65%); mp 84-87°C; Rf(D) = 0.89; $[\alpha]D^{20}$ = -21.5° (c 1; DMF); NMR (DMSO d6) δ ppm 7.66 (d, 1H, NH, J_{NH-CH}= 7,8 Hz), 7.36 (m, 5H, Ar), 5.05 (s, 2H, CH_2 Z), 3.99 (m, 1H, CH α Nle), 2.69, 2.39 (m, 4H, CH_2), 1.66, 1.48 (m, 2H, CH_2 β Nle), 1.30 (m, 4H, CH_2 γ and δ Nle), 0.85 (t, 3H, CH_3); Anal. $C_{17}H_{23}NO_5$ calc: C 62.87, H 7.23, N 4.25, found C 63,06, H 7.17, N 4.21.

Z-Nle-Ψ(CO-CH2)-Gly-OSu (30) : Synthesized as described for compound **11** from compound **29** (0.62 g, 1.92 mmol) and recrystallised in a mixture of AcOEt and hexane (0.684 g, 85%); mp 84-87°C; Rf(A) = 0.42; $[\alpha]D^{20}$ = -25.4° (c1; DMF); NMR (DMSO d_6) δ ppm 7.71 (d, 1H, NH, J_{NH-CH}= 7,7 Hz), 7.4-7.3 (m, 5H, Ar), 5.04 (s, 2H, CH_2 Z), 4.02 (m, 1H, CH α Nle), 2.85-2.79 (m, 6H, CH_2), 1.66, 1.48 (m, 2H, CH_2 β Nle), 1.26 (m, 4H, CH_2 γ and δ Nle), 0.84 (t, 3H, CH_3).

Z-Nle-Ψ(CO-CH2)-Gly-Trp-Nle-Asp-Phe-NH2 (31) : Compound **22** (1.19 g, 1.49 mmol) was deprotected by hydrogenation as described for compound **23**. The deprotected peptide was added to a mixture of **30** (0.520 g, 1.24 mmol) in DMF (10 ml), followed by DIEA (0.26 ml, 1.49 mmol), and the mixture was stirred for 3h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, ether, dried in vacuo (1.09 g, 99%); mp 215°C (dec); Rf(D) = 0.55; $[\alpha]D^{20}$ = -22.5° (c 1; DMF); Anal. $C_{47}H_{59}N_7O_{10}$ calc: C 61.40, H 6.45, N 11.04, found C 60,94, H 6.59, N 10.98.

Boc-Tyr-Nle-Ψ(CO-CH2)-Gly-Trp-Nle-Asp-Phe-NH2 (32) : Compound **31** (0.50 g, 0.57 mmol) was deprotected by hydrogenation for 2h as described above. The deprotected peptide was added to a mixture of Boc-Tyr-OSu (0.189 g, 0.50 mmol) in DMF (10 ml), followed by DIEA (0.10 ml, 0.57 mmol), and the mixture was stirred for 3h at room temperature. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, ether, dried in vacuo (0.423 g, 85%); mp 200°C (dec); Rf(E) = 0.82; $[\alpha]D^{20}$ = -24.5° (c 1; DMF); Anal. $C_{53}H_{70}N_8O_{12}$ calc: C 60.97, H 7.05, N 10.91, found C 60,80, H 7.24, N 11.27.

Boc-Tyr(SO3H)-Nle-Ψ(CO-CH2)-Gly-Trp-Nle-Asp-Phe-NH2 (4) : Synthesized as described for compound **1** from compound **32** (150 mg, 0.15 mmol). HPLC purification (Rt= 7.92 min, A/B 34:66) and lyophilisation afforded compound **4** (76 mg, 46%); mp 210°C (dec.); Rf(F) = 0.50; $[\alpha]D^{20}$ = -31.1° (c1; DMF). NMR data are given in table IV.

Boc-Nle-NH2 (33) : To a cold (-20°C) solution in THF (20 ml) of Boc-Nle-OH (2.64 g, 11.41 mmol) were successively added NMM (1.28 ml, 11.41 mmol) and IBCF (1.55 ml, 11.41 mmol). After 5 min, 37% aqueous NH_4OH (2 ml) was added, and the mixture stirred for 10 min at room temperature. The solvent was then concentrated in vacuo to leave a residue which was dissolved in AcOEt (200 ml). The solution was washed with 1M aqueous $KHSO_4$ (3x100 ml), water, saturated aqueous $NaHCO_3$ (3x100 ml), brine, dried over $MgSO_4$ and concentrated in vacuo to afford a solid residue which was recrystallised in a mixture of AcOEt and hexane (1.98 g, 75%); mp 122-124°C; Rf(B) = 0.46; $[\alpha]D^{20}$ = +3.6° (c 0.66; DMF).

BocTyr(Bzl)-Nle-NH2 (34) : Compound **33** (1.97 g, 8.55 mmol) was deprotected in TFA as previously described. To a cold (-20°C) solution in DMF (10 ml) of BocTyr(Bzl)-OH (2.89 g, 7.78 mmol) were successively added NMM (0.87 ml, 7.78 mmol) and IBCF (1.06 ml, 7.78 mmol). After 5 min, the deprotected compound was added, followed by DIEA (1.47 ml, 8.55 mmol). After 1h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with 1M aqueous $KHSO_4$),water, saturated aqueous $NaHCO_3$, water, dried in vacuo (2.87 g, 76%); mp 178-181°C; Rf(C) = 0.56; $[\alpha]D^{20}$ = -3.5° (c 1; DMF).

BocTyr(Bzl)-gNle-H.TFA (35) : To a solution of compound **34** (1.84 g, 3.8 mmol) in a mixture of acetonitrile and water (v/v) (20 ml), was added [bis(trifluoroacetoxy)iodo]benzene[21] (2.58 g, 6 mmol). The reaction mixture was stirred for 2h at room temperature, and the solvents were concentrated in vacuo. Upon trituration with ether,the residue afforded compound **26** as white solid which was used in the next step without further purification.

BocTyr(Bzl)-Nle-Ψ(NH-CO)-Gly-OMe (36) : To a cold (0 °C) solution of potassium monomethylmalonate (2.24 g, 5.3 mmol) in DMF (10 ml) were successively added compound **35** (1.5 g, 2.65 mmol), BOP[22] (2.34 g, 5.3 mmol), and DIEA (0.46 ml, 2.65 mmol). After overnighth stirring at room temperature the reaction product precipitated as a white solid upon addition of saturated aqueous $NaHCO_3$ (100 ml). It was collected, washed with saturated aqueous $NaHCO_3$, water, 1M aqueous $KHSO_4$, water, ether,

dried in vacuo (1.09 g, 74%); mp 167-168°C ; Rf(B) = 0.55; $[\alpha]_D^{20}$ = +8.4° (c 1; DMF).

BocTyr(Bzl)-Nle-Ψ(NH-CO)-Gly-OH (37) : To a solution of compound 36 (0.985 g, 1.77 mmol) in dioxane (10 ml), was added 2N aqueous NaOH (1.1 ml, 2.2 mmol). After 1h no more starting material could be detected by TLC. The reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, ether, dried in vacuo (0.934 g, 97%); mp 195°C (dec); Rf(D) = 0.66; $[\alpha]_D^{20}$ = +6.8° (c 1; DMF).

Boc-Asp(OBzl)-Tyr(Bzl)-Nle-Ψ(NH-CO)-Gly-OH (38) : Compound 37 (0.90 g, 1.66 mmol) was partially deprotected in TFA as described above. The partially deprotected peptide was added to a solution of Boc-Asp(OBzl)-OSu[14] (0.631 g, 1.5 mmol) in DMF (10 ml), followed by DIEA (0.29 ml, 1.66 mmol). After 2h stirring at room temperature the reaction product precipitated as a white solid upon addition of 1M aqueous $KHSO_4$ (100 ml). It was collected, washed with water, ether, dried in vacuo (0.675 g, 60%); mp 190-193°C; Rf(E) = 0.74; $[\alpha]_D^{20}$ = -8.2° (c 1; DMF).

Boc-Asp(OBzl)-Tyr(Bzl)-Nle-Ψ(NH-CO)-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 (39) : Boc-Trp-Nle-Asp(OBzl)-Phe-NH_2 (7) (0.607 g, 0.79 mmol) was partially deprotected in TFA as previously described. The partially deprotected peptide was added to a solution of compound 38 in DMF (10 ml), followed by BOP[22] (0.32 g, 0.72 mmol), and DIEA (0.26 ml, 1.51 mmol). After overnight stirring at room temperature the reaction product precipitated as a white solid upon addition of saturated aqueous $NaHCO_3$ (100 ml). It was collected, washed with saturated aqueous $NaHCO_3$, water, 1M aqueous $KHSO_4$, water, ether, dried in vacuo (0.970 g, 97%); mp 225°C (dec); Rf(E) = 0.44; $[\alpha]_D^{20}$ = -10.4° (c 1; DMF).

Boc-Asp-Tyr-Nle-Ψ(NH-CO)-Gly-Trp-Nle-Asp-Phe-NH_2 (40) : Compound 39 (0.870 g, 0.62 mmol) was deprotected by hydrogenation as described for compound 27, for 48h at 30°C. It was purified by silica gel column chromatography (eluent AcOEt/pyridine/AcOH/H_2O 120:20:5:10) to afford a white solid (0.640 g, 72%); mp 190°C (dec); Rf(E) = 0.30; $[\alpha]_D^{20}$ = -13.5° (c 1; DMF).

Boc-Asp-Tyr(SO_3H)-Nle-Ψ(NH-CO)-Gly-Trp-Nle-Asp-Phe-NH_2 (5) : Synthesized as previously described for compound 1 from compound 40 (610 mg, 0.54 mmol). HPLC purification (Rt= 7.98 min, A/B 40:60) and lyophilisation afforded compound 5 (370 mg, 57%); mp 190°C (dec.); Rf(E) = 0.27; Rf(F) = 0.39; $[\alpha]_D^{20}$ = -32.5° (c 1; DMF). NMR data are given in table V.

Boc-β-homo-Nle-OH (41) : To a cold (-20°C) solution of Boc-Nle-OH (9.4 g, 40.6 mmol) in ethylene glycol dimethylether (50 ml) were added under vigourous stirring NMM (4.55 ml, 40.6mmol) and IBCF (5.53 ml, 40.6 mmol). After 5 min , the precipitated salt was filtered off, and the mixture was treated with diazomethane in ether (70 mmol), and the mixture stirred at 0°C for 20 min. It was then concentrated in vacuo, the residue dissolved in methanol (200 mL), and treated with silver benzoate (2 g, 8 mmol) in triethylamine (20 mL). After 30 min stirring at room temperature, the solvent was concentated in vacuo, the residue dissolved in AcOEt (300 mL) and the insoluble material was removed by filtration. The filtrate was washed with saturated sodium bicarbonate (3 x 100 mL), water (100 mL), 1M aqueous $KHSO_4$ (3 x 100 mL), brine, dried over magnesium sulfate, and concentated in vacuo. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane 1:9) to afford the methyl ester as an oil (7 g, 27 mmol, 66%). It was dissolved in methanol (20 mL) and treated with 2 N NaOH (15 ml, 30 mmol) at 20°C for 30 min. Upon acidification with 1M aqueous $KHSO_4$, the expected compound precipitated. It was collected by filtration, washed thoroughly with water and dried in vacuo (4.8 g, 72%); mp 84-87°C ; Rf(D) = 0.86; $[\alpha]_D^{20}$ = -1.2° (c 0.5; DMF).

Boc-β-homo-Nle-N(Me)OMe (42) : To a cold (0°C) solution of compound 41 (4.55 g, 18.55 mmol) in dichloromethane (100 ml) were successively added N,O-dimethyhydroxylamine hydrochloride (2.17 g, 22.26 mmol), DIEA (3.83 ml, 22.36 mmol), DMAP (50 mg) and DCC (3.83 g, 18.55 mmol). After 4h stirring at room temperature, the precipitated DCU was filtered off, washed with dichloromethane (2x100 ml). The combined organic layers were washed with 1M aqueous $KHSO_4$ (3x100 ml), water, saturated aqueous $NaHCO_3$ (3x100 ml), brine, dried over $MgSO_4$ and concentrated in vacuo to leave 42 as a colorless oil (5.08 g, 95%); Rf(B) = 0.77.

5(S)-(N-tert-Butyloxycarbonyl)amino-non-2-enoic acid (43) : To a cold (0°C) solution of compound 42 (5.08 g, 17.62 mmol) in anhydrous ether (100 ml), was added under vigourous stirring, in portions, over a period of 15 min, $LiAlH_4$ (2 g, 54 mmol). After 15 min more, AcOEt (300 ml) was carefully added, followed by 1M aqueous $KHSO_4$ (500 ml), and the mixture was stirred at room temperature for 30 min. The organic layer was collected, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to leave the corresponding aldehyde which was quickly used in the next step without any further purification. To a solution of the aldehyde in benzene (80 ml), was added methyl (triphenylphosphoranylidene)-acetate (5.89 g, 17.62 mmol). After 1h stirring at room temperature, the solvent was removed in vacuo and the residue applied to a silica gel column (eluent AcOEt/hexane 2:8) to afford the corresponding methyl ester as a clean oil (4.47 g, 15.66 mmol). It was dissolved in methanol and treated with 2N aqueous NaOH (25 ml, 17 mmol). After 2h at 30°C, 43 was precipitated upon addition of 1M aqueous $KHSO_4$ (300 ml), washed with water and dried in vacuo (4.04 g, 83%); mp 81-83°C ; Rf(D) = 0.86; $[\alpha]_D^{20}$ = -23.5° (c 1; DMF); NMR (DMSO d6) δ ppm 12.01 (s, 1H, COOH), 6.67 (d, 1H, NH, J_{NH-H5} = 8.9 Hz), 6.75(m, 1H, CH 3, J_{CH-CH_2} =7.3 Hz, J_{CH-CH} =15.4 Hz trans), 5.75 (d,

1H, CH 2), 3.46 (m, 1H, CH 5), 2.31 and 2.19 (m, 2H, CH_2 *4*, ^2J = 3Hz), 1.4-1.2 (m, 6H, CH_2 *6, 7, 8*), 0.85 (d,3H, CH_3).

Boc-Nle-Ψ(CH_2-CH_2)-Gly-OH (44) : Compound 43 (3.73 g, 13.7 mmol) was hydrogenated for 1h in AcOEt (200 ml) in the presence of a 10% Pd/C catalyst under 5 atm and at room temperature. The catalyst was filtered off, the solvent concentrated under reduced pressure, and the resitue crystallized upon trituration with ether (2.74 g, 73%); mp 72-74°C ; Rf(G) = 0.44; $[\alpha]_D^{20}$ = +5.4° (c 1; DMF).

Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-OH (45): Compound 44 (2.30 g, 8.41 mmol) was deprotected in TFA in the usual manner. The deprotected pseudo-peptide was added to a solution of Boc-Tyr-OSu[14] (2.88 g, 7.6 mmol) in DMF (20 ml), followed by DIEA (1.45 ml, 8.41 mmol) and the mixture stirred for 2h at room temperature. The solvent was then concentated in vacuo, the residue dissolved in AcOEt (100 mL) and the solution was washed with saturated sodium bicarbonate (3 x 100 mL), water (100 mL), 1M aqueous $KHSO_4$ (3 x 100 mL), brine, dried over magnesium sulfate, and concentated in vacuo. The residue was purified by silica gel column chromatography (eluent: AcOEt/hexane/acetic acid 60:40:1) to afford compound 45, which was recrystallized in a mixture of ethanol and ether (2.01 g, 60%); mp 169-174°C ; Rf(H) = 0.45; $[\alpha]_D^{20}$ = -4.8° (c 0.5; DMF).

Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp(OBzl)-Phe-NH_2 (46) : Fmoc-Trp-Nle-Asp(OBzl)-Phe-NH_2[6] (0.90 g, 1.01 mmol) was treated with a mixture of diethylamine (1 ml) in DMF (9 ml) for 20 min at room temperature. Evaporation of the solvent in vacuo and trituration of the residue in ether afforded pure partially deprotected peptide. It was added to a solution of compound 45 (0.410 g, 0.94 mmol) and BOP[22] (0.397 g, 0.9 mmol) in DMF (5 ml), followed by DIEA (0.106 ml, 0.94 mmol). After 4h stirring at room temperature, compound 46 precipitated upon addition of saturated aqueous $NaHCO_3$ (200 ml). It was collected washed with water, 1M aqueous $KHSO_4$ (3x100 ml), water, dried in vacuo (0.75 g, 77%); mp 210-212°C ; Rf(D) = 0.86; $[\alpha]_D^{20}$ = -18.4° (c 1; DMF).

Boc-Tyr-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (47) : Compound 46 (0.65 g, 0.598 mmol) was hydrogenated in the usual manner to affordcompound 47 as a white solid (0.506 g, 85%); mp 210-212°C (dec); Rf(E) = 0.81; $[\alpha]_D^{20}$ = -21.1° (c 1; DMF).

Boc-Tyr(SO_3H)-Nle-Ψ(CH_2-CH_2)-Gly-Trp-Nle-Asp-Phe-NH_2 (6) : Synthesized as described for compound 1 from compound 47 (250 mg, 0.251 mmol). HPLC purification (Rt= 10.29 min, A/B 32:68) and lyophilisation afforded compound 6 (135 mg, 50%); mp 190°C (dec.); Rf(E) = 0.31; Rf(F) = 0.52; $[\alpha]_D^{20}$ = -17.1° (c1; DMF). NMR data are given in table VI.

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