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Syntheses and biological activities of bombesin analogs modified in the C-terminal dipeptide part

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Summary — Bombesin receptor antagonists are possible therapeutic agents due to their ability to act as inhibitors of cellular proliferation. On the basis of our hypothesis on the mechanism of action of gastrin associating an activating enzyme system to the receptor and on the results reported in the litterature, we have synthesized bombesin analogues which have been modified in the C-terminal Leu^{13}–Leu^{14} amide part. We have shown that modification in the C-terminal part of the bombesin strongly affected the biological activity in rat pancreatic acini. The most potent compound which is described here, H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2)Leu-NH_2, was able to recognize the bombesin receptor on rat pancreatic acini (Ki 4.3 nM) and antagonized the bombesin stimulated amylase secretion (Ki 7.7 nM).

bombesin / agonist / antagonist / partial agonist / pseudopeptide

Introduction

Bombesin (BN) is a 14-amino acid peptide that was originally isolated from amphibian skin [1]. Gastrin releasing peptide (GRP) is a mammalian 27-amino acid peptide which has very similar biological properties to that of bombesin. Small-cell lung carcinoma cell lines (SCLC) express high-affinity receptors for GRP and BN, and exhibit a mitogenic response to these peptides, suggesting that they can act as autocrine factors for these cells. On this basis, it has been suggested that bombesin receptor antagonists should be able to suppress the growth of these cells. On the other hand, bombesin receptor antagonists may have an interesting effect on prostate cancer treatment in addition with LH-RH [2]. As a result, there has been considerable interest in the design and development of competitive BN or GRP receptor antagonists as possible therapeutic agents [3].

We have previously reported a strategy for the design of gastrin receptor antagonists, either by suppressing the C-terminal amino acid residue [4], the C-terminal amide function [5] or by chemically modifying a specific peptide bond within the peptide chain [6]. The rational synthesis of gastrin receptor antagonists was explained by the existence of an activating enzyme system 'associated' with the receptor, responsible for the release of the 'active part' of the peptide hormone within the receptor [7–9]. This strategy was successfully applied to the design and synthesis of various peptide hormone receptor antagonists, particularly amidated peptide hormones (ie, secretin, substance P, etc). Especially, peptide backbone modification through carbonyl reduction led Coy et al to the synthesis of a bombesin receptor antagonist, [Leu^{13}-ψ(CH_2-NH)Leu^{14}]-bombesin [10–13]. This pseudopeptide showed selectivity for the bombesin receptor and was potent in antagonizing murine 3T3 cell growth in response to bombesin. On the basis of our hypothesis and to verify its significance in the case of bombesin, we describe in this report several derivatives of the potent bombesin analog [D-Phe^6, Leu^{13}, Leu^{14}] bombesin [6–14] in which: (i) the Leu residue in position 14 has been replaced by non-natural amino acids, (ii) the peptide bond between Leu^{13} and Leu^{14} has been modified.

Chemistry

All the peptides and pseudopeptides were synthesized in solution by condensation of two fragments, the hexapeptide Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'**

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with the different tripeptides and pseudotripeptides modified at the C-terminal dipeptide. Amino acids were coupled as their Nα-Boc, -Z or -Fmoc derivatives, with BOP [14] as activating reagent. The synthesis of 2-amino-indane carboxylic acid (Aic) was performed by the Bucherer–Lieb method [15]; 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) was prepared following the workup of Hayashi and coll [16] from phenylalanine; homologation of the leucine residue was performed by an Arndt–Eistert reaction as previously described [17].

Synthesis of compound **8**: H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2NH)Leu-NH_2 was performed by segment condensation of Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH with the corresponding pseudotripeptide H-His-LeuΨ(CH_2NH)Leu-NH_2; H-LeuΨ(CH_2NH)-Leu-NH_2 was prepared from Z-Leucinal and H-Leu-NH_2 as previously described [18, 19] in the presence of sodium cyanoborohydride. In the synthesis of gastrin and cholecystokinin analogs, the introduction of a 'carba' bond led us to potent agonists and antagonists: it was therefore decided to apply this strategy in the case of bombesin analogs. Dipeptides containing a 'carba' bond were first developed in our laboratory [20, 21]. The condensation of diethyl phosphonoacetate with Boc β-homoLeucinal was performed according to scheme 1. After hydrogenolysis, the two diastereoisomers **5d** were cyclized into corresponding lactams **5e'** and **5e''** which were easily separated by silica gel chromatography. The absolute configuration of these two lactams was determinated by ^{1}H-NMR NOE experiments. Acid hydrolysis and protection of the amine function with a Boc group allowed us to obtain Boc-L-Leu-ψ(CH_2-CH_2)-L-Leu-OH **5f** and Boc-L-Leu-ψ(CH_2-CH_2)-D-Leu-OH **6a** which were converted into their corresponding amide **5g** and **6b.**

β-homologated amino-acids were introduced in positions 13 or 14 because this kind of modification was successful in the synthesis of CCK-4 antagonists [17].

The pseudodipeptide Boc-LeuΨ(CH_2)Leu-$CONH_2$ was prepared by condensation of the phosphonoacetate with Boc-Leucinal as described in scheme 2. The cyclization into lactams **7c** did not allow the separation of the two diastereoisomers. The mixture was therefore used for the synthesis of compounds **7** and **7'** (H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2)-Leu-$CONH_2$ and H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2)(D)Leu-$CONH_2$) which were separated by HPLC but the absolute configuration of the C-terminus residue was not assigned.

The substitution of the peptide bond between Leu^{13} and Leu^{14} by an oxymethylene bond was performed following the method developed by Tenbrink [22] and led to the synthesis of the pseudodipeptide Boc-Leu-ψ(CH_2O)Nle-OH **9e** (scheme 3). It was converted onto its amide derivative **9f** and after removal of the Boc protecting group and condensation with Z-His(Z)-OH yielded the pseudotripeptide Z-His(Z)-LeuΨ(CH_2O)Nle-$CONH_2$ **9g**. Removal of the Z protecting groups by hydrogenolysis followed with condensation with the hexapeptide **1g'** yielded compound **9**.

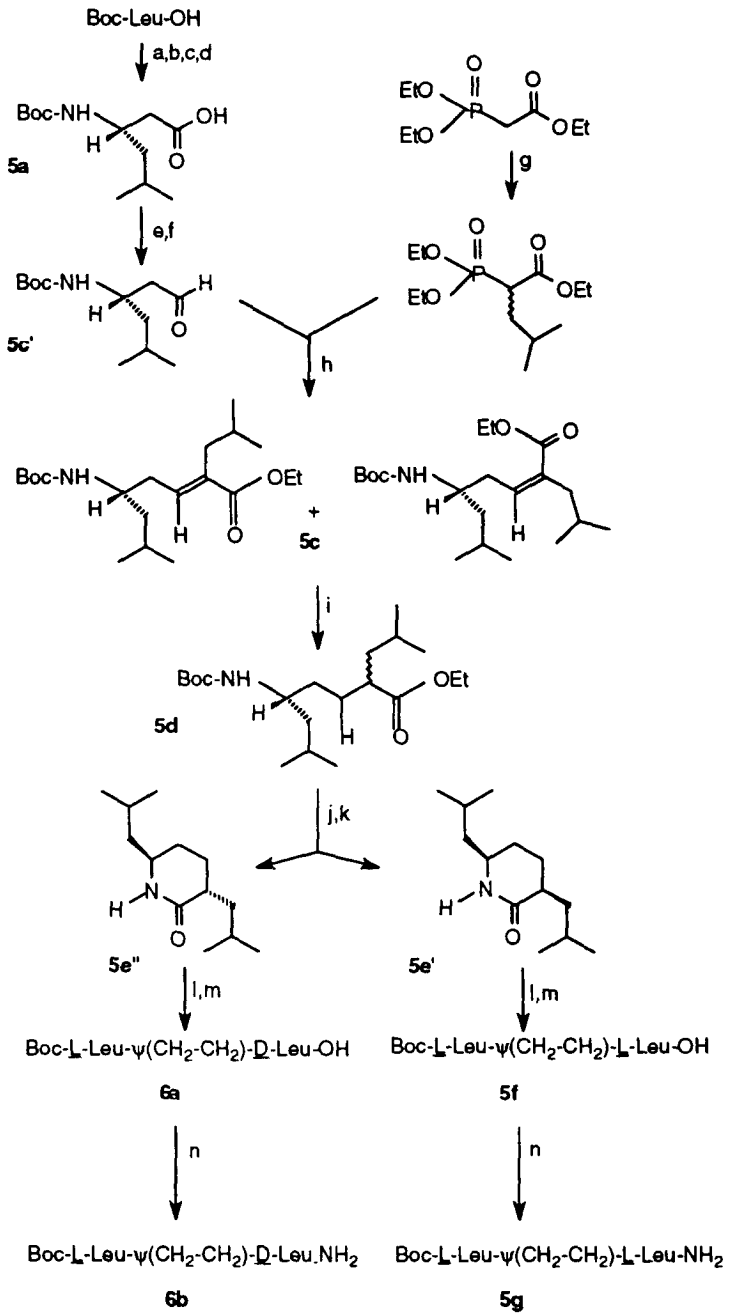
Biological results and discussion

All the peptides were tested for their ability to inhibit binding of 3-[^{125}I] (Tyr^{15})Gastrin releasing peptide from rat pancreatic acini or to inhibit bombesin stimulation of amylase release.

The biological results are summarized in table I: all tested bombesin derivatives were compared with bombesin and the previously reported [D-Phe^6, Leu$^{13}\Psi$(CH_2NH)Leu^{14}] bombesin [6–14] referred to as compound **8**.

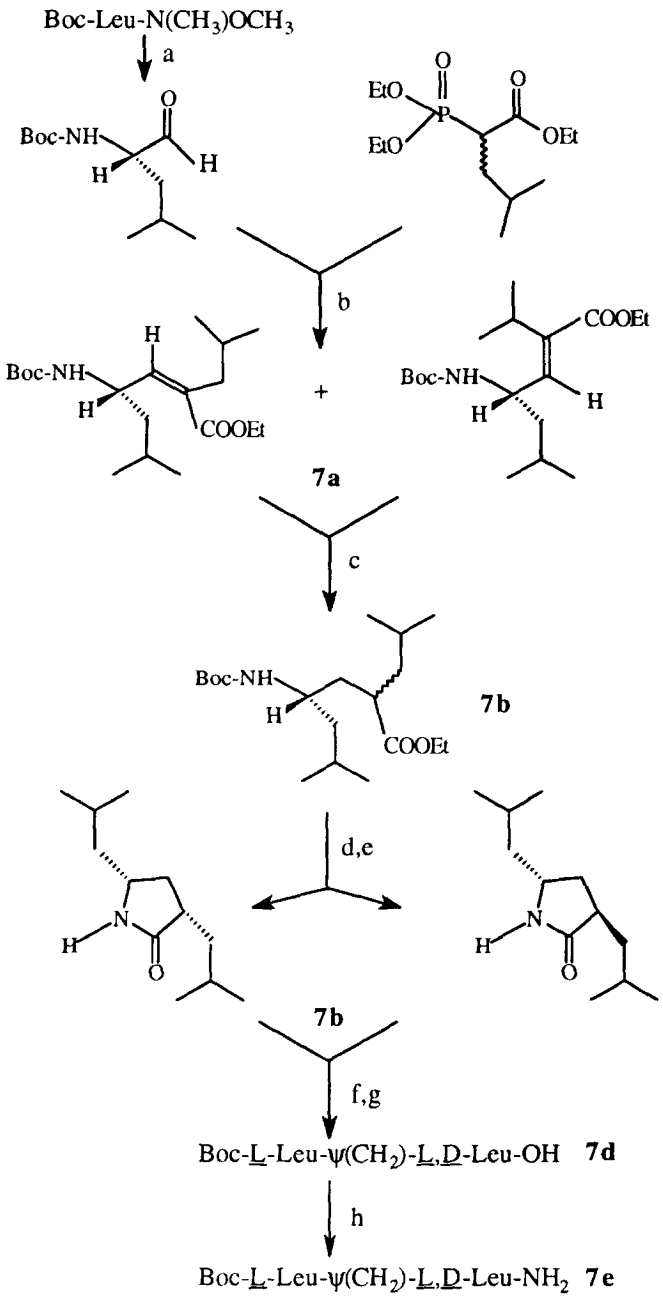
Replacement of Leu^{14} residue in the potent bombesin analog [D-Phe^6, Leu^{13}, Leu^{14}]-bombesin [6–14] by a β-homo-Leu-NH_2 (compound **1**) or a β-homo-Phe-NH_2 (compound **3**) yielded two analogs able to recognize the bombesin receptor on rat pancreatic acini with high potency (Ki 5.8 nM and 9.7 nM respectively). These two derivatives behaved as full agonists in stimulating amylase release from rat pancreatic acini. Bombesin analogues containing an aromatic or a conformationally constrained secondary amine at the C-terminal have been described [23, 24]. These derivatives exhibited antagonistic activities in the nanomolar range on Swiss 3T3 cell growth. To investigate the influence of 2-amino-indane carboxylic acid (Aic) and 1,2,3,4-tetrahydroisoquinoline carboxylic acid (Tic) at the C-terminal position, these non natural amino acid residues were introduced to replace Leu^{14}. The bombesin derivatives containing Aic, Tic and D-Tic (**10**, **11** and **12** respectively) showed low binding affinity for the BN receptor in rat pancreatic acini (Ki in the range 150–250 nM). These compounds presented an agonistic activity in the range 10–40 nM except for compound **11** which possessed partial agonist activity, exibiting 67% of the maximal response induced by bombesin at 3×10^{-6} M. Replacement of the residue Leu^{14} by β-homo-D-Phe-NH_2 (compound **4**) produced a less potent analogue. Interestingly, compound **4** behaved as a weak antagonist (Ki 483 nM). When the C-terminal dipeptide was replaced by β-homo-Leu-D-Nle-NH_2, the resulting analogue was less potent in recognizing the bombesin receptor on rat pancreatic acini (Ki 43.3 nM) and behaved as a bombesin receptor antagonist (Ki 393 nM). Taken alltogether, these results confirm the significance of the C-terminal dipeptide of bombesin.

Compounds **5** and **6** containing the carba modification between Leu^{13} and Leu^{14} presented lower binding



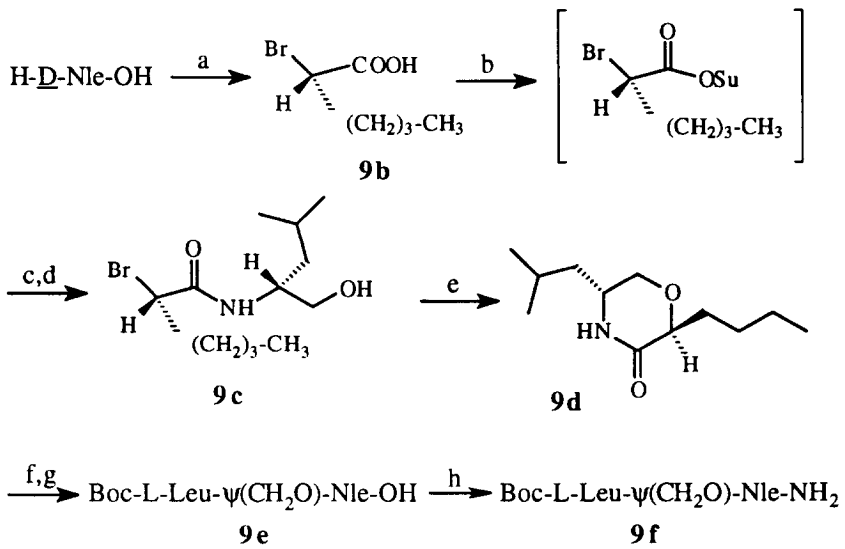
Scheme 1. Synthesis of the 'carba' bond. a: NMM, IBCF; b: CH_2N_2; c: C_6H_5COOAg, TEA, MeOH; d: NaOH; e: HCl, $HN(CH_3)OCH_3$, BOP, NMM; f: $AlLiH_4$; g: NaH, $Br–CH_2–CH(CH_3)_2$; h: NaH, DME; i: H_2, Pd/c; j: TFA; k: Δ, pyridine; l: HCl, 6N; m: Boc_2O; n: DMF, –20 °C, IBCF, NH_4OH.

affinities than Coy's compound (compound **8**) having a reduced bond between Leu[13] and Leu[14]. These two compounds exhibited partial agonist activity, the L-leucine diastereoisomer containing analog (compound **5**) being more potent than its parent D isomer (compound **6**).



Scheme 2. Synthesis of the 'methylene' bond. a: $AlLiH_4$; b: NaH, DME; c: H_2, Pd/c; d: TFA; e: Δ, pyridine; f: HCl, 6N; g: Boc_2O; h: DMF, –20 °C, IBCF, NH_4OH.

The analogues containing the methylene bond between Leu[13] and Leu[14] (compounds **7** and **7'**) exhibited interesting properties. One of them, compound **7**, exhibited a reasonable binding affinity for the BN receptor (Ki = 37.3 nM) and a weak and partial agonist activity in the amylase secretion (EC_{50} = 64% at 10^{-5} M). The other diastereoisomer (**7'**) exhibited a higher binding affinity (Ki = 4.3 nM) and was five times more potent than compound **8** in antagonizing bombesin-induced amylase secretion (IC_{50} = 23 nM).



Scheme 3. Synthesis of the 'oxymethylene' bond. a: $NaNO_2/KBr/H_2SO_4$; b: HOSu/DCC/DME; c: TFA; d: H-Leucinol; e: NaH, −15 °C; f: Boc_2O/DMAP; g: NaOH, 2N; h: DMF, −20 °C, IBCF, NH_4OH.

Table I. Biological activities of the bombesin analogs on rat pancreatic acini. NA, no activity up to 10 μM. Results of at least four separate experiments in duplicate.

Compounds	*Binding Acini Ki (nM)*	*Amylase*	
		EC_{50} (nM)	*K_i (nM)*
Bombesin	1.8	0.07	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2NH)Leu-NH_2 **8**	22.7	NA	34
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-Leu-NH_2 **1**	5.8	0.3	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-β-h-Leu-D-Nle-NH_2 **2**	43.3	NA	393
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-Phe-NH_2 **3**	9.7	2.1	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-D-Phe-NH_2 **4**	243.3	NA	483
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2 **10**	158.3	17.6	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2 **11**	206.7	67% (3.10^{-6} M)	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH_2 **12**	242.5	41.3	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2CH_2)D-Leu-NH_2 **6**	65.0	33% (3.10^{-6} M)	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2CH_2)Leu-NH_2 **5**	39.3	52% (1.10^{-7} M)	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2)Leu-NH_2 (D or L) **7**	37.3	64% (1.10^{-5} M)	
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2)Leu-NH_2 (D or L) **7'**	4.3	NA	7.7
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ψ(CH_2O)Nle-NH_2 **9**	6.9	NA	20.8

Compound **9** with an oxymethylene bond between Leu[13] and Leu[14] was more potent than compound **8** (Ki of 6.9 nM) and presented an antagonist activity on amylase secretion (IC_{50} = 62.5 nM), in accordance with previously reported results [25].

We have recently presented an hypothesis on the mechanism of action of gastrin associating an 'activating enzyme' associated to the receptor to promote the biological activity of the hormone [7–9]. Briefly, after gastrin binds to the receptor, an enzyme system identified as 'angiotensin-like converting enzyme activity' cleaves the peptide bond between methionine and aspartic acid to release within the receptor the C-terminal dipeptide H-Asp-Phe-NH_2. This dipeptide is believed to act as the active moiety responsible for the biological activity. We believe this mechanism of action could apply to other amidated peptide hormones, particularly to bombesin. We have tried to apply this hypothesis to bombesin to rationally design and synthesize bombesin receptor antagonists. From the results described in the literature, particularly those of Coy and his colleagues [10], we assumed that the activating enzyme system, if any, would hydrolyze the bond between the two last aminoacid residues, and will release within the receptor H-Leu-NH_2, supposed to be the active moiety responsible for the biological activity. In this respect, replacement of the peptide bond between the two last aminoacid residues in bombesin by a non-hydrolyzable bond (eg, a pseudopeptide bond) would produce bombesin receptor antagonists.

The results reported in this paper showed that nor modification of the peptide bond between Leu[13] and Leu[14] in the potent nonapeptide bombesin analog, neither replacement producing enhanced stability of the bond between the two last aminoacid residues, is sufficient to produce full bombesin receptor antagonists. The agonist activity or the partial agonist activity that was observed in rat pancreatic acini with compounds **5**, **6**, **7** and **12** do not fully support our hypothesis concerning the mechanism of action of bombesin associating an activating enzyme cleaving the bond between the two C-terminal residues, with the receptor suggesting that the mechanism of action of bombesin on rat pancreatic acini is more complicated than hypothesized. However, we have shown and confirmed that it was possible to obtain potent bombesin receptor antagonists using this strategy.

Experimental protocols

Chemistry

Melting points were taken on a Buchi apparatus in open capillary tubes. Optical rotation were determined with a Perkin-Elmer 141 polarimeter at 20 °C. Ascending TLC was performed on precoated plates of silica gel 60 F_{254} (Merck) with the following solvent systems (by volume): AcOEt/hexane: A, (1:5); B, (3:7); C, (5:5); D, (7:3); AcOEt: E; $CHCl_3$/MeOH/AcOH: F, (180:10:5); G, (120:10:5); H, (85:10:5); I, (60:10:5); J, (40:10:5); AcOEt/Pyridine/AcOH/H_2O: K, (120:20:5:10); L, (80:20:5:10); M, (60:20:5:10); N, (50:20:5:10); O, (40:20:5:10). Peptide derivatives were located with charring reagent or ninhydrine. Column chromatography was performed with silica gel Kieselguhr Merck® G 0.05–0.2 mm. HPLC purifications were run on a Merck-Hitachi instrument on a C18 Bondapack® (10 µm), 250 x 10 mm, with an UV detection at 280 nm, at a flow rate of 7 mL/min of a mixture of A: ammonium acetate 0.05 M, pH 6.5, and B: methanol, in isocratic mode. Capillary Zone Electrophoresis (CZE) were performed on a PACE 5000 Beckman instrument, using an uncoated fused silica capillary. Mass spectra were recorded on a JEOL JMS DX 100 and DX 300 spectrometer in FAB positive mode. L, D aminoacids and derivatives were from Bachem, Novabiochem or Propeptide. All reagents were of analytical grade. The following abbreviations were used: BOP, [(benzotriazolyl)-oxy]tris(dimethylaminophosphonium)hexafluoro-phosphate; DME, ethylene glycol dimethyl ether; DMF, dimethylformamide; NMM, *N*-methyl-morpholine; IBCF, *iso*butyl-chloroformate. Other abbreviations used were those recommended by IUPAC-IUB Commission [*Eur J Biochem* (1984) 138, 9–37].

All final compounds were purified by reversed phase HPLC; the purity was assessed by analytical reversed phase C 18 HPLC and by capillary zone electrophoresis (CZE). The purification conditions and the physical and analytical data are reported in Table II.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-Leu-NH_2 ***1***

Z-Leu-β-homo-Leu-NH_2 ***1a***. Z-Leu-OH (1.54 g, 5.8 mmol) was dissolved in DMF (10 mL) containing HCl, H-β-homo-Leu-NH_2 [17] (1 g, 5.53 mmol) and BOP (2.56 g, 5.8 mmol). NMM (1.26 mL, 11.33 mmol) was added to this solution. The reaction mixture was stirred for 2 h at room temperature. Then, a saturated aqueous sodium bicarbonate solution (100 mL) was added and the precipitate which formed was filtered. It was washed with water, 1 N aqueous potassium hydrogen sulfate, water, hexane, ether and dried in vacuo over KOH. Yield 98% (2.12 g); TLC R_f(G) 0.7, (E) 0.52; mp 173–175 °C; $[\alpha]_D$ –26 (*c* 0.9, DMF).

Z-His(Z)-Leu-β-homo-Leu-NH_2 ***1b***. Compound **1a** (2.08 g, 5.31 mmol) was hydrogenated at room temperature in DMF/EtOH/water: 10:1:1 (100 mL) containing 11 N hydrochloric acid (0.48 mL) in the presence of a 10% Pd/C catalyst. After 4 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue which solidified upon trituration in ether. It was collected, washed with ether, and dried in vacuo over KOH pellets. Yield 98%. It was dissolved in DMF (10 mL) containing Z-His(Z)-OH,EtOH (2.56 g, 5.46 mmol), BOP (2.41 g, 5.46 mmol). NMM (1.18 mL, 10.66 mmol) was added to this solution and the reaction mixture was stirred for 2 h at room temperature. Then a saturated aqueous sodium bicarbonate solution (100 mL) was added and the precipitate which formed was filtered. It was collected, washed with water, 1 N aqueous potassium hydrogen sulfate, water, hexane, ether and dried in vacuo over KOH. Yield 90% (3.16 g); TLC R_f(G) 0.63, (E) 0.17; mp 122–134 °C (dec); $[\alpha]_D$ –13.5 (*c* 1, DMF).

Boc-Val-Gly-OBzl ***1c***. To a solution of Boc-Val-OH (7.78 g, 35.8 mmol) in DMF (20 mL) containing TFA, H-Gly-OBzl (10.42 g, 37.31 mmol) and BOP (15.84 g, 35.8 mmol), NMM

Table II. Physical characteristics of compounds **1** to **12**.

Compounds	*$[\alpha]_D$ (c, DMF)*	*HPLC Rt [A:B]*	*CZE Rt*	*Formula*	*Mass m/z [M + H⁺]*
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-Leu-NH_2 **1**	–4 (1)	19', [35:65]	5.44	$C_{54}H_{78}N_{14}O_{10}$	1083
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-β-h-Leu-D-Nle-N_2 **2**	–4.5 (0.9)	15', [28:72]	5.69	$C_{54}H_{78}N_{14}O_{10}$	1083
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-Phe-NH_2 **3**	–1.2 (0.7)	22', [33:67]	5.48	$C_{57}H_{76}N_{14}O_{10}$	1117
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-h-D-Phe-NH_2 **4**	–1.1 (1)	20', [35:65]	5.48	$C_{57}H_{76}N_{14}O_{10}$	1117
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2 **10**	–6 (0.9)	25', [25:75]	5.50	$C_{57}H_{74}N_{14}O_{10}$	1115
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2 **11**	–1.6 (0.9)	24', [34:66]	5.48	$C_{57}H_{74}N_{14}O_{10}$	1115
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH_2 **12**	–0.7 (1)	22', [31:69]	5.50	$C_{57}H_{74}N_{14}O_{10}$	1115
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2NH)Leu-NH_2 **8**	+2.7 (1.4)	26', [25:65]	5.51	$C_{53}H_{78}N_{14}O_9$	1055
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2CH_2)Leu-NH_2 **6**	–8.3 (1.2)	29', [30:70]	5.55	$C_{54}H_{79}N_{13}O_9$	1054
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ(CH_2CH_2)Leu-NH_2 **5**	–5.3 (1)	27', [33:67]	5.96	$C_{54}H_{79}N_{13}O_9$	1054
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2)(L or D)Leu-NH_2 **7** and **7'**	–	25', 28' [30:70]	5.50, 5.49	$C_{53}H_{77}N_{13}O_9$	1040
H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2O)Nle-NH_2 **9**	–18 (1.2)	22', [34:66]	5.51	$C_{53}H_{77}N_{13}O_{10}$	1056

HPLC purifications were run with a Merck-Hitachi instrument on a C18 Lichrospher Hibar Pre-Packed column 10 μm, 250 x 25 mm, with a UV detection of 280 nm, at a flow rate of 7 mL/mn of a mixture of A: ammonium acetate 0.05 M, pH 6.5, and B: methanol in isocratic mode. [A:B] corresponds to the preparative eluents system, Rt: retention time. CZE was performed on a PACE 5000 Beckman using an uncoated fused silica capillary (75 μm x 50 cm, 100 x 800 μm aperture), pressure injection, run conditions 15 min, 20 °C, 15 kV, 65 mM sodium tetraborate buffer, pH 8.4.

(8.10 mL, 73 mmol) was added. After 2 h at room temperature, the expected compound **1c** was precipitated upon addition of a saturated aqueous sodium bicarbonate solution (100 mL). It was collected, washed with water, 1 N aqueous potassium hydrogen sulfate, water, hexane, ether and dried in vacuo over KOH. Yield 98% (12.8 g); TLC R_f(C) 0.89, (B) 0.35; mp 72–74; $[\alpha]_D$ –9.5 (*c* 1.1, DMF).

Boc-Ala-Val-Gly-OBzl ***1d***. Compound **1c** (11 g, 30.2 mmol) was partially deprotected with trifluoroacetic acid (20 mL). After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, co-evaporated with hexane and rinsed with ether. The expected TFA salt was dissolved in DMF (50 mL) containing Boc-Ala-OH (5.72 g, 30.24 mmol), BOP (13.37 g, 30.24 mmol) and NMM (6.77 mL, 60.48 mmol). The mixture was stirred for 1 h at room temperature and treated as described for compound **1a**. Yield 75% (9.9 g); TLC R_f(D) 0.72, (C) 0.48; mp 144–146 °C; $[\alpha]_D$ –23 (*c* 1.1, DMF).

Fmoc-Trp-Ala-Val-Gly-OBzl ***1e***. Compound **1d** (4.8 g, 10.65 mmol) was partially deprotected with trifluoroacetic acid (10 mL). After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, co-evaporated with hexane and rinsed with ether. The expected TFA salt was dissolved in DMF (20 mL) containing Fmoc-Trp-OH (4.8 g, 11.2 mmol), BOP (4.95 g, 11.2 mmol) and NMM (2.44 mL, 22 mmol). The reaction mixture was stirred for 1 h at room temperature and treated as described for compound **1a**. Yield 97% (7.7 g); TLC R_f(E) 0.63, (D) 0.22; mp 191–193 °C; $[\alpha]_D$ –20 (*c* 1, DMF).

Fmoc-Gln-Trp-Ala-Val-Gly-OBzl ***1f***. Compound **1e** (7 g, 9.4 mmol) was partially deprotected in a mixture of DMF (94 mL) and diethylamine (9.4 mL) for 30 min at room temperature. The mixture was concentrated in vacuo to leave a residue that crystallized upon trituration in ether (yield 97%). It was dissolved in DMF (20 mL) containing Fmoc-Gln-OH (3.6 g, 9.7 mmol), BOP (4.3 g, 9.7 mmol) and NMM (1.07 mL, 9.7 mmol). The reaction mixture was stirred for 2 h at room temperature and treated as described for compound **1a**. Yield 85% (6.8 g); TLC R_f(I) 0.56, (H) 0.3; mp 229–230 °C; $[\alpha]_D$ –21 (*c* 1, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OBzl ***1g***. Compound **1f** (6.13 g, 7.03 mmol) was partially deprotected in a mixture of DMF (70 mL) and diethylamine (7 mL) for 30 min at room temperature. The mixture was concentrated in vacuo to leave a residue that crystallized upon trituration in ether: yield 91%. It was dissolved in DMF containing Boc-D-Phe-OH (1.86 g,

7.03 mmol), BOP (3.11 g, 7.03 mmol) and NMM (0.77 mL, 6.7 mmol). The mixture was stirred for 1 h at room temperature and treated as described for compound **1a**. Yield 89% (5.1 g); TLC R_f(H) 0.51, (G) 0.26; mp 230 °C dec; $[\alpha]_D$ –28 (*c* 1.1, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-Leu-NH$_2$ ***1h***. Compound **1g** (2 g, 2.23 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH to produce the free acid Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'**: yield 96%.

Compound **1b** (3.08 g, 4.65 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.84 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH to yield H-His-Leu-β-homo-Leu-NH$_2$ **1b'**. Yield 98%.

Compound **1b'** (0.2 g, 0.428 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.345 g, 0.428 mmol), BOP (0.35 g, 0.428 mmol) and NMM (0.142 mL, 1.28 mmol). After 2 h at room temperature, the expected compound **1h** was precipitated upon addition of a saturated aqueous sodium bicarbonate solution. It was collected, washed with water, a 1 N aqueous potassium hydrogen sulfate solution, water, hexane, ether and dried in vacuo over KOH. Yield 70% (0.35 g); TLC R_f(M) 0.4, (L) 0.21; mp 205–220 °C dec; $[\alpha]_D$ –12.8 (*c* 1.4, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-Leu-NH$_2$ ***1***. Compound **1h** (0.2 mg, 0.17 mmol) was added to a cold (0 °C) solution of 2-methyl-indole (222 mg, 1.7 mmol) in TFA (10 mL) and the mixture was stirred under argon atmosphere for 30 min at room temperature. The expected compound precipitated upon addition of ether (100 mL). It was collected, thoroughly washed with ether, and dried in vacuo over KOH pellets: yield 89% (0.18 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-β-homo-Leu-D-Nle-NH$_2$ ***2***

Z-β-homo-Leu-D-Nle-NH$_2$ ***2a*** was prepared from Z-β-homo-Leu-OH (2 g, 7.2 mmol) as described for compound **1a**. Yield 98% (2.61 g); TLC R_f(G) 0.63, (E) 0.46; mp 184–187 °C; $[\alpha]_D$ –12.8 (*c* 1.1, DMF).

Z-His(Z)-β-homo-Leu-D-Nle-NH$_2$ ***2b*** was prepared from compound **2a** (2 g, 5.1 mmol) as described for compound **1b**. Yield 91% (2.9 g); TLC R_f(G) 0.51, (E) 0.12; mp 146–150 °C; $[\alpha]_D$ –3 (*c* 0.9, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-β-homo-Leu-D-Nle-NH$_2$ ***2c***. Compound **2b** (2 g, 3 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH to yield H-His-β-homo-Leu-D-Nle-NH$_2$ **2b'** (96%). Compound **2b'** (0.2 g, 0.428 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.345 g, 0.428 mmol), BOP (0.35 g, 0.43 mmol) and NMM (0.142 mL, 1.28 mmol). After 2 h at room temperature, the expected compound was precipitated upon addition of a saturated aqueous sodium bicarbonate solution. It was collected, washed with water, 1 N aqueous potassium hydrogen sulfate solution, water, hexane, ether and dried in vacuo over KOH. It was treated as described for compound **1h**. Yield 65% (2.25 g); TLC R_f(M) 0.38, (L) 0.15; mp 230 °C dec; $[\alpha]_D$ –11 (*c* 0.9, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-β-homoLeu-D-Nle-NH$_2$ ***2***. Prepared from compound **2c** (0.2 g, 0.17 mmol) as described for compound **1**. Yield 98% (0.2 g). It was purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-Phe-NH$_2$ ***3***

Boc-Leu-β-homo-Phe-NH$_2$ ***3a***. Boc-Leu-OH, H_2O (1.37 g, 5.49 mmol) was dissolved in DMF containing TFA, H-β-homo-Phe-NH$_2$ (1.53 g, 5.23 mmol), BOP (2.43 g, 5.49 mmol) and NMM (1.2 mL, 10.7 mmol). The reaction mixture was stirred 1 h at room temperature and treated as described for compound **1a**. Yield 95% (1.94 g); TLC R_f(G) 0.75, (E) 0.42; mp 199–203 °C; $[\alpha]_D$ –28 (*c* 0.45, DMF).

Z-His(Z)-Leu-β-homo-Phe-NH$_2$ ***3b***. Compound **3a** (1.9 g, 4.85 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 93%. It was dissolved in DMF containing Z-His(Z)-OH, EtOH (2.22 g, 4.73 mmol), BOP (2 g, 4.73 mmol) and NMM (1.02 mL, 9.24 mmol). The reaction mixture was stirred 2 h at room temperature and treated as described for compound **1a**. Yield 98% (3 g); TLC R_f(G) 0.72, (F) 0.39; mp 94–108 °C dec; $[\alpha]_D$ –7 (*c* 1.1, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-Phe-NH$_2$ ***3c***. Compound **3b** (1 g, 1.43 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.26 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH to yield H-His-Leu-β-homo-Phe-NH$_2$ (98%). This compound (0.7 g, 1.4 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (1.13 g, 1.4 mmol), BOP (0.62 g, 1.4 mmol) and NMM (0.46 mL, 4.2 mmol). After 2 h at room temperature, the expected compound was precipitated upon addition of a saturated aqueous sodium bicarbonate solution. It was collected, washed with water, 1 N aqueous potassium hydrogen sulfate solution, water, hexane, ether and dried in vacuo over KOH. Yield 60% (1 g); TLC R_f(M) 0.42, (L) 0.37; mp 230 °C dec; $[\alpha]_D$ –7 (*c* 1.5, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-Phe-NH$_2$ ***3***. Prepared from compound **3c** (0.2 g, 0.164 mmol) as described for compound **1**. Yield 98% (0.198 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-D-Phe-NH$_2$ ***4***

Boc-β-homo-D-Phe-OH ***4a***. Mesylchloride (2.5 mL, 32 mmol) was added dropwise to a solution of Boc-D-Phe-ol (7.1 g, 28.3 mmol) in pyridine (20 mL). After 1 h, a precipitate was formed by addition of a mixture of 1 N HCl (50 mL) in

water (800 mL). It was collected by filtration, washed with water, 1 M $KHSO_4$ and dissolved in DMSO (80 mL). After addition of KCN (5.5 g, 84.9 mmol), the reaction mixture was stirred over 24 h at 50 °C. The product was precipitated by addition of water, collected by filtration and washed with water. It was dissolved in methanol (100 mL) and 30% KOH (100 mL). The reaction mixture was stirred for 12 h in refluxing solvent, methanol was removed in vacuo and the residue dissolved in ethyl acetate. The organic layer was washed with 1 M $KHSO_4$, dried over Na_2SO_4 and concentrated in vacuo. The expected product was precipitated in a mixture of ether/hexane (20:80), collected by filtration and washed with hexane. Yield 35% (2.77 g); TLC R_f(G) 0.72, (F) 0.38; mp 62–65 °C; $[\alpha]_D$ +14.5 (*c* 1, DMF).

Boc-β-homo-D-Phe-NH_2 ***4b***. To a solution of compound **4a** (2 g, 7.2 mmol) in DMF cooled at –20 °C, NMM (1.07 mL, 9.7 mmol) and IBCF (1.31 mL, 9.7 mmol) were added. After 5 min under stirring, 3.45 mL of NH_4OH (48.3 mmol) was added. The reaction mixture was stirred for 10 min. The product was precipitated with water, collected by filtration, washed with water and hexane. It was dried over KOH pellets. Yield 70% (1.88 g); TLC R_f(E) 0.33, (D) 0.11; mp 165–167 °C; $[\alpha]_D$ +17 (*c* 0.8, DMF).

Boc-Leu-β-homo-D-Phe-NH_2 ***4c***. This was prepared from compound **4b** (1.7 g, 5.96 mmol) as described for compound **1d**. Yield 96% (2.05 g); TLC R_f(G) 0.68, (E) 0.25; mp 180–183 °C; $[\alpha]_D$ +7.5 (*c* 0.8, DMF).

Z-His(Z)-Leu-β-homo-D-Phe-NH_2 ***4d***. It was prepared from compound **4c** (1.99 g, 5.08 mmol) as described for compound **1d**. Yield 91% (2.65 g); TLC R_f(G) 0.72, (F) 0.39; mp 168–170 °C; $[\alpha]_D$ +4 (*c* 1.5, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-D-Phe-NH_2 ***4e***. Compound **4d** (2.56 g, 3.67 mmol) was hydrogenated as described for compound **1b'**. Yield 98%. The expected HCl salt (0.2 g, 0.41 mmol) was dissolved in DMF containing compound **1g'** (0.33 g, 0.41 mmol), BOP (0.18 g, 0.41 mmol) and NMM (0.136 mL, 1.23 mmol). The reaction mixture was stirred for 2 h and treated as described for compound **1h**. Yield 58% (0.29 g); TLC R_f(M) 0.49, (L) 0.4; mp 230 °C dec; $[\alpha]_D$ –2 (*c* 0.8, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-β-homo-D-Phe-NH_2 ***4***. Prepared from compound **4e** (0.2 g, 0.164 mmol) as described for compound **1**. Yield 98% (0.198 g). It was purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu$\Psi(CH_2CH_2)$Leu-NH_2 ***5***

Boc-β-homo-Leu-OH ***5a***. Boc-Leu-OH, H_2O (15 g, 60 mmol) was homologated as previously described [17] to lead to Boc-β-homo-Leu-OH. Yield 81% (12 g); TLC R_f(E) 0.55, (D) 0.42; oil; $[\alpha]_D$ –12 (*c* 1.2, DMF).

Boc-β-homo-Leu-N(OCH_3)CH_3 ***5b***. Boc-β-homo-Leu-OH **5a** (11 g, 44.84 mmol), BOP (21.81 g, 49.32 mmol) and NMM (10.4 mL, 94.16 mmol) were added to a mixture of O,N-dimethylhydroxylamine hydrochloride (4.81 g, 49.32 mmol) in DMF. After 3 h, saturated sodium bicarbonate solution (50 mL) was added under stirring, followed by ethyl acetate (50 mL). The organic layer was washed with saturated sodium bicarbonate (2 x 50 mL), water, 1 M potassium hydrogen sulfate (2 x 50 mL), water, dried over sodium sulfate and then concentrated in vacuo. The expected product was purified by silica gel column chromatography with ethyl acetate/hexane (3:7) as eluent, to yield a clear oil. Yield 63% (8.14 g); TLC R_f(C) 0.74, (B) 0.64; $[\alpha]_D$ –35 (*c* 1.2, DMF).

Boc-β-homo-LeuΨ(CH=CH)-(CH_2-$CH(CH_3)_2$)-COOEt ***5c***. To a cold (0 °C) solution of compound **5b** (7 g, 24.27 mmol) in ether (200 mL), $AlLiH_4$ (1.38 g, 36.41 mmol) was added portionwise over 15 min. After 15 min, ethyl acetate was added, followed by 1 M potassium hydrogen sulfate. The organic layer was washed with 1 M potassium hydrogen sulfate, water, dried over sodium sulfate. The solvent was removed in vacuo. The product was precipitated with hexane, collected by filtration, washed with hexane and dried in vacuo over KOH. Yield 70% (3.89 g) **5c'**. To a cold (0 °C) solution of $(EtO)_2$–(P=O)–CH–[CH_2–$CH(CH_3)_2$] (0.5 g, 13.08 mmol) in DME (50 mL), 60% NaH (0.96 g, 24.02 mmol) was added. After 1 h, compound **5c'** was added under stirring. The reaction mixture was stirred over 2 h, then ethyl acetate (200 mL) followed by 1 M potassium hydrogen sulfate were added. The organic layer was washed with 1 M potassium hydrogen sulfate, water, dried over sodium sulfate and then concentrated in vacuo. The expected product was purified by silica gel column chromatography, with ethyl acetate/hexane (1:5) as eluent, to give a clear oil. Yield 90% (4.18 g); TLC R_f(B) 0.72/0.78, (A) 0.63/0.73; $[\alpha]_D$ –8 (*c* 1, DMF).

Boc-Leu$\Psi(CH_2$-$CH_2)$Leu-OEt ***5d***. Compound **5c** (4 g, 11.25 mmol) was dissolved in 95% ethanol and hydrogenated in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the solvent was concentrated in vacuo to give a clear oil. Yield 97% (3.9 g); TLC R_f(E) 0.7, (C) 0.37; $[\alpha]_D$ +53.5 (*c* 0.8, DMF).

(3R,6R)-3,6-(isobutyl)-piperidin-2-one ***5e'*** *and (3S,6R)-3,6-(isobutyl)-piperidin-2-one* ***5e''***. Compound **5d** was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane. The expected TFA salt was dissolved in pyridine (40 mL) and the reaction mixture was stirred in refluxing pyridine over 2 h. The solvent was concentrated in vacuo up to 2/3 and 1 M potassium hydrogen sulfate was added. The expected product was precipitated, collected by filtration, washed with water. The two diastereoisomers were separated by silica gel column chromatography with ethyl acetate/hexane (5:5) as eluent, and were identified by ^{1}H-NMR. Yield **5e'** 43% (0.76 g) and **5e''** 42% (0.74 g); TLC **5e'** R_f(E) 0.62, (C) 0.21 and TLC **5e''** R_f(G) 0.62, (E) 0.55; mp **5e'** 103–104 °C and mp **5e''** 89–90 °C; **5e'** $[\alpha]_D$ –36 (*c* 0.6, DMF) and **5e''** $[\alpha]_D$ –5 (*c* 1, DMF); Anal $C_{13}H_{25}N_1O_1$ (C, H, N) for both compounds. ^{1}H-NMR values (δ ppm): **5e'**: 7.2 (m, 1H, NH); 3.3 (m, 1H, H6); 2.11 (m, 1H, H3); 1.73 (m, 1H, H5); 1.72 (m, 1H, H4); 1.67 (m, 1H, Hγ); 1.67 (m, 1H, Hγ); 1.54 (m, 1H, Hβ); 1.47 (m, 1H, H4'); 1.38 (m, 1H, H6); 1.32 (m, 1H, Hβ); 1.19 (m, 1H, Hβ); 1.19 (m, 1H, Hβ); 0.89–0.83 (d, 6H, Hδ, *J* = 6.5 Hz); 0.86–0.84 (d, 6H, Hd, *J* = 6.5 Hz); observed Noe between H3 and H6. **5e''**: 7.09 (m, 1H, NH); 3.27 (m, 1H, H6); 2.04 (m, 1H, H3); 1.88 (m, 1H, H5); 1.88 (m, 1H, H4); 1.70 (m, 1H, Hβ); 1.69 (m, 1H, Hγ); 1.69 (m, 1H, Hγ); 1.32 (m, 1H, Hβ); 1.27 (m, 1H, H4'); 1.22 (m, 1H, H6); 1.17 (m, 1H, Hβ); 1.13 (m, 1H, Hβ); 0.88–0.82 (d, 6H, Hδ, *J* = 6.5 Hz); 0.86–0.84 (d, 6H, Hd, *J* = 6.5 Hz); no observed Noe.

Boc-Leu$\Psi(CH_2$-$CH_2)$Leu-OH ***5f***. Compound **5e'** (0.7 g, 3.31 mmol) was hydrolyzed in 1 N HCl/H_2O (2:1) (25 mL). After 5 h under stirring, the reaction mixture was cooled (0 °C)

and 1 N NaOH was added up to neutral pH, followed by dioxane (40 mL) and Boc_2O (0.86 g, 3.97 mmol). The pH reaction was controlled to obtain a basic solution (pH 10) by addition of 1 N NaOH. After 2 h, the aqueous solution was washed with ether (2 x 100 mL), followed by hexane (2 x 100 mL). The aqueous layer was acidified by 1 M potassium hydrogen sulfate. The aqueous layer was extracted with ethyl acetate (2 x 100 mL), the organic solution washed with water, dried over sodium sulfate and concentrated in vacuo to give a white powder. Yield 95% (1.03 g); TLC R_f(E) 0.65, (C) 0.14; mp 72–74 °C ; $[\alpha]_D$ –12 (*c* 1, DMF).

Boc-LeuΨ*(CH_2-CH_2)Leu-NH_2* ***5g***. To a solution of compound **5f** (0.9 g, 2.73 mmol) in DMF cooled at –20 °C, NMM (0.3 mL, 2.73 mmol) and IBCF (0.37 mL, 2.73 mmol) were added. After 5 min under stirring, 0.97 mL of NH_4OH (13.65 mmol) was added. The reaction mixture was stirred for 10 min. The expected product was precipitated with water, collected by filtration, washed with water and hexane. It was dried in vacuo over KOH pellets. Yield 60% (0.57 g); TLC R_f(G) 0.66, (E) 0.34; mp 148–150 °C; $[\alpha]_D$ +10 (*c* 1.4, DMF).

Z-His(Z)-LeuΨ*(CH_2-CH_2)Leu-NH_2* ***5h***. Compound **5g** (0.5 g, 1.52 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane. The expected TFA salt was dissolved in DMF containing Z-His(Z)-OH, EtOH (0.75 g, 1.59 mmol), BOP (0.7 g, 1.59 mmol) and NMM (0.34 mL, 3.11 mmol). After 2 h a saturated sodium bicarbonate solution (300 mL) was added under stirring, followed by ethyl acetate (200 mL). The organic layer was washed with a saturated bicarbonate solution (3 x 100 mL), water, 1 M potassium hydrogen sulfate (3 x 100 mL), water, dried over sodium sulfate and then concentrated in vacuo. The residue was precipitated with hexane, collected by filtration, and then dried in vacuo over KOH. Yield 60% (0.57 g); TLC R_f(J) 0.57, (I) 0.27; mp 110–120 °C; $[\alpha]_D$ –12 (*c* 0.9, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2CH_2)Leu-NH_2* ***5i***. Compound **5h** (0.5 g, 0.79 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.14 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH to yield compound **5i'**. Yield 98%. This compound **5i'** (0.34 g, 0.77 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.62 g, 0.77 mmol), BOP (0.34 g, 0.77 mmol) and NMM (0.25 mL, 2.31 mmol). After 2 h at room temperature, the expected compound was treated as described for compound **1h**. Yield 60% (0.53 g); TLC R_f(C) 0.6, (B) 0.2; mp 193–196 °C; $[\alpha]_D$ –9 (*c* 0.9, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2CH_2)Leu-NH_2* ***5***. Prepared from compound **5i** (0.2 mg, 0.173 mmol) as described for compound **1**. Yield 94% (0.19 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2CH_2)D-Leu-NH_2* ***6***

Boc-LeuΨ*(CH_2-CH_2)D-Leu-OH* ***6a***. Prepared from compound **5e"** (0.7 g, 3.31 mmol) as described for compound **5f**. Yield 82% (0.89 g) of a clear oil. TLC R_f(E) 0.67, (D) 0.66; $[\alpha]_D$ –10 (*c* 1.6, DMF).

Boc-LeuΨ*(CH_2-CH_2)D-Leu-NH_2* ***6b***. Prepared from compound **6a** (0.8 g, 2.41 mmol) as described for compound **5g** (yield 62%, 0.49 g). TLC R_f(G) 0.62, (F) 0.58; mp 109–111 °C; $[\alpha]_D$ +3.5 (*c* 0.9, DMF).

Z-His(Z)-LeuΨ*(CH_2-CH_2)D-Leu-NH_2* ***6c***. Prepared from compound **6b** (0.4 g, 1.21 mmol) as described for compound **5h** (yield 78%, 0.59 g). TLC R_f(M) 0.55, (L) 0.29; mp 130–132 °C; $[\alpha]_D$ –11 (*c* 0.7, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2CH_2)D-Leu-NH_2* ***6d***. Prepared from compound **6c** (0.5 g, 0.79 mmol) as described for compound **5i**. Yield 48% (0.43 g); TLC R_f(G) 0.52, (E) 0.42; mp 210–212 °C; $[\alpha]_D$ –12.5 (*c* 1.2, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2CH_2)D-Leu-NH_2* ***6***. Prepared from compound **6d** (0.2 g, 0.173 mmol) as described for compound **5j**. Yield 90% (0.18 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2)Leu-NH_2 7 and 7'*

Boc-LeuΨ*(CH=CH)–(CH_2–$CH(CH_3)_2$)–(COOEt)* ***7a***. Prepared from Boc-Leu-$N(OCH_3)CH_3$ (10 g, 36.44 mmol) as described for compound **5c**. Yield 11% (1.36 g) of a clear oily compound after purification by chromatography on silica gel ; TLC R_f(A) 0.62.

Boc-LeuΨ*(CH_2)L,D-Leu-OEt* ***7b***. Prepared from compound **7a** (1.3 g, 3.78 mmol) as described for compound **5d**. Yield 95% (0.7 g) of a clear oil; TLC R_f(D) 0.7, (C) 0.42.

(3S or 3R, 5S)-3,5-isobutyl-pyrrolidin-2-one ***7c***. Prepared from compound **7b** (0.6 g, 1.74 mmol) as decribed for compounds **5e'** and **5e"**. Yield 72% (0.25 g) of a mixture of two diastereoisomers which could not be separated by silica gel column chromatography. TLC R_f(G) 0.7, (E) 0.55.

Boc-LeuΨ*(CH_2)L,D-Leu-OH* ***7d***. Prepared from compound **7c** (0.24 g, 1.21 mmol) as described for compound **5f**. Obtained as an oil. Yield 58% (0.22 g); TLC R_f(D) 0.58, (C) 0.45; anal $C_{17}H_{33}N_1O_4$ (C, H, N).

Boc-LeuΨ*(CH_2)L,D-Leu-NH_2* ***7e***. Prepared from compound **7d** (0.2 g, 0.63 mmol) as described for compound **5g**. Obtained as an oil. Yield 89% (0.18 g); TLC R_f(G) 0.45, (F) 0.21; anal $C_{19}H_{34}N_2O_3$ (C, H, N).

Z-His(Z)-LeuΨ*(CH_2)L,D-Leu-NH_2* ***7f***. Prepared from compound **7e** (0.18 g, 0.57 mmol) as described for compound **5h**. Obtained as an oil. Yield 67% (0.24 g); TLC R_f(G) 0.52, (D) 0.19; anal $C_{34}H_{45}N_5O_6$ (C, H, N).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2)L,D-Leu-NH_2* ***7g***. This compound was synthesized from compound **7f** (0.24 g, 0.387 mmol) as described for compound **5i**. Yield 40% (0.15 g) after purification by chromatography on silica gel; TLC R_f(M) 0.62, (L) 0.38; mp 170–178 °C dec; $[\alpha]_D$ –10.3 (*c* 1.3, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ*(CH_2)L,D-Leu-NH_2 7 and 7'*. Synthesized from compound **7g** (0.15 g, 0.13 mmol) as described for compound **5j**. Yield 80% (0.12 g) of a white powder. The two diastereoisomers **7** and **7'** were separated and purified by HPLC then lyophilized. Their absolute configuration was not assigned.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2NH)Leu-NH_2 ***8***

Z-LeuΨ(CH_2NH)Leu-NH_2 ***8a***. Z-Leu-N(OCH_3)CH_3 (5 g, 16.21 mmol) was dissolved in anhydrous DME (50 mL) and the reaction mixture was cooled to 0 °C. $AlLiH_4$ (0.92 g, 24.32 mmol) was added portionwise over 15 min. After an additionnal 30 min stirring, ethyl acetate (100 mL) was added, followed by a 1 M potassium hydrogen sulfate solution (200 mL). The reaction mixture was vigorously stirred for 30 min. The organic layer was washed with 1 M potassium hydrogen sulfate and water, dried over sodium sulfate and concentrated in vacuo. The resulting oily aldehyde was dissolved in a mixture of methanol–acetic acid (99:1, 100 mL) containing TFA, H-Leu-NH_2 (4.27 g, 17.5 mmol). Sodium cyanoborohydride (3 g, 48.63 mmol) dissolved in methanol (50 mL) was added portionwise over 45 min. After 2 h, methanol was removed in vacuo. After addition of saturated bicarbonate solution (100 mL) the expected product was precipitated. It was collected by filtration, washed with hexane and dried over KOH pellets: yield 66% (3.89 g); TLC R_f(D) 0.5, (C) 0.18; mp 123–126 °C; $[\alpha]_D$ –32.3 (*c* 1, DMF); anal $C_{20}H_{31}N_3O_4$ (C, H, N).

Z-His(Z)-LeuΨ(CH_2NH)Leu-NH_2 ***8b***. Prepared from compound **8a** (1 g, 2.46 mmol) as described for compound **1b**. Yield 77% (1.2 g); TLC R_f(H) 0.83, (G) 0.37; $[\alpha]_D$ +6 (*c* 0.6, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2NH)Leu-NH_2 ***8c***. Compound **8b** (0.93 g, 1.46 mmol) was hydrogenated as described for compound **1b'**. Yield 97% of a clear oil. The expected HCl salt (0.67 g, 1.4 mmol) was dissolved in DMF containing compound **1g'** (1.13 g, 1.4 mmol), BOP (0.62 g, 1.4 mmol) and NMM (0.62 mL, 5.6 mmol). The reaction mixture was stirred for 2 h and treated as described for compound **1h**. Yield 94% (1.52 g); TLC R_f(J) 0.66, (G) 0.36; mp 183–186 °C; $[\alpha]_D$ +4 (*c* 0.9, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2NH)Leu-NH_2 ***8***. Prepared from compound **8c** (0.44 g, 0.337 mmol) as described for compound **1**. Yield 88% (0.38 g). It was purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2O)Nle-NH_2 ***9***

Boc-Leucinol ***9a***. To a cold (0 °C) solution of Boc-Leu-OH, H_2O (10 g, 40.11 mmol) in DME, NMM (4.45 mL, 40.11 mmol) and IBCF (5.46 mL, 40.11 mmol) were added. The NMM salt was collected by filtration and washed with DME. $NaBH_4$ (2.28 g, 60 mmol) was added to the reaction mixture. After 10 min, water (100 mL) was added, followed by ethyl acetate (100 mL). The organic layer was washed with a saturated sodium bicarbonate solution (2 x 50 mL), water, dried over sodium sulfate and concentrated in vacuo to give a clear oil: yield 98% (8.54 g); TLC R_f(C) 0.48, (B) 0.19; $[\alpha]_D$ –26 (*c* 1, MeOH).

2(R)-Bromo-hexanoic acid ***9b***. H-D-Nle-OH (10 g, 76.23 mmol) was dissolved in a mixture of 2.5 N sulfuric acid–water (1:1) (160 mL) containing KBr (32.13 g, 0.27 mmol). The reaction mixture was cooled to 0 °C before the slow addition of $NaNO_2$ (8.41 g, 122 mmol). After 2 h at 0 °C and 2 h at room temperature in the dark, water (100 mL) was added followed by ether (100 mL). The organic layer was washed with ether (2 x 100 mL), dried over sodium sulfate and concentrated in vacuo to give a clear oil: yield 86% (12.8 g); TLC R_f(G) 0.58; $[\alpha]_D$ +4 (*c* 4, DMF).

N-[2(R)-Bromo-4-hexanoyl]-L-Leu-ol ***9c***. Compound **9a** (8 g, 36.8 mmol) was partially deprotected with TFA. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane, dried over sodium sulfate to yield the TFA salt **9a'** (98%). Compound **9b** (5.45 g, 30.49 mmol) was dissolved in DME (20 mL). The reaction mixture was cooled to 0 °C before the addition of HOSu (5.26 g, 45.73 mmol) and DCC (6.29 g, 30.49 mmol). After 2 h, the salts were removed by filtration and washed with DME (2 x 5 mL). Compound **9a'** (3.75 g, 32 mmol) was added to the solution containing the bromo-hexanoic acid *N*-hydroxusuccinimide ester, followed by DIEA (5.5 mL, 32 mmol). After 3 h, the reaction mixture was treated as described for compound **1c**. Yield 41% (3.67 g); TLC R_f(E) 0.68, (D) 0.37; $[\alpha]_D$ –2.3 (*c* 1, DMF).

(2S,5S)-2-butyl-5-isobutyl-1,4-oxazin-3-one ***9d***. Compound **9c** (3 g, 10.2 mmol) was dissolved in anhydrous DME (25 mL) cooled to –15 °C. 60% NaH (0.15 g, 10.7 mmol) was added to the reaction mixture. After 1 h at –15 °C and 2 h at room temperature, ethyl acetate (100 mL) was added followed by 1 M potassium hydrogen sulfate (100 mL). The organic layer was washed with water, dried over sodium sulfate and concentrated in vacuo. The expected product was purified by silica gel column chromatography, with ethyl acetate/hexane (5:5) as eluent. It was precipitated with hexane, collected by filtration and dried over KOH pellets: yield 74% (1.6 g); TLC R_f(C) 0.42, (B) 0.26; mp 68–70 °C; $[\alpha]_D$ –72 (*c* 0.7, DMF).

Boc-LeuΨ(CH_2O)Nle-OH ***9e***. Compound **9d** (1.55 g, 7.3 mmol) was dissolved in acetonitrile (6 mL) containing DMAP (0.09 g, 0.73 mmol) and Boc_2O (1.9 g, 8.72 mmol). The reaction mixture was stirred for 12 h at room temperature. Ethyl acetate (100 mL) was added followed by 1 M potassium hydrogen sulfate (100 mL). The organic layer was washed with 1 M potassium hydrogen sulfate, dried over sodium sulfate and concentrated in vacuo. The residue was dissolved in THF (40 mL) and 2 N NaOH (5.45 mL, 10.9 mmol) was added. The reaction mixture was stirred for 4 h at room temperature. The solvent was removed in vacuo and 1 M potassium hydrogen sulfate (100 mL) was added followed by ethyl acetate (100 mL). The organic layer was washed with water, dried over sodium sulfate and concentrated in vacuo to give a white powder: yield 98% (2.36 g); TLC R_f(G) 0.58, (E) 0.46; mp 58–60 °C; $[\alpha]_D$ –63 (*c* 1.2, DMF).

Boc-LeuΨ(CH_2O)Nle-NH_2 ***9f***. Prepared from compound **9e** (2.23 g, 6.73 mmol) as described for compound **1a**: yield 83% (1.82 g); TLC R_f(E) 0.46, (C) 0.42; mp 148–150 °C; $[\alpha]_D$ –56 (*c* 0.5, DMF).

Z-His(Z)-LeuΨ(CH_2O)Nle-NH_2 ***9g***. Prepared from compound **9f** (1.73 g, 5.23 mmol) as described for compound **1b** to give the compound as a powder: yield 62% (2.05 g); TLC R_f(G) 0.7, (F) 0.58; mp 125–127 °C; $[\alpha]_D$ –7 (*c* 0.7, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2O)Nle-NH_2 ***9h***. Compound **9g** (1.92 g, 3.02 mmol) was hydrogenated as described for compound **1b'**: yield 99%. The expected HCl salt (0.31 g, 0.71 mmol) was dissolved in DMF containing compound **1g'** (0.57 g, 0.71 mmol), BOP (0.31 g, 0.708 mmol) and NMM (0.23 mL, 2.12 mmol). The reaction mixture was stirred for 2 h and the expected product was precipitated by ethyl acetate: yield 71% (0.58 g); TLC R_f(N) 0.82, (M) 0.43; mp 230 °C dec; $[\alpha]_D$ –14 (*c* 0.4, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-LeuΨ(CH_2O)Nle-NH_2 ***9***. Prepared from compound **9h** (0.15 g, 0.129 mmol) as described for compound **1**: yield 80% (0.12 g). It was purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2 ***10***

Boc-Aic-NH_2 ***10a***. To a solution of Boc-Aic-OH (2 g, 7.2 mmol) in DMF cooled at −20 °C, NMM (0.79 mL, 7.2 mmol) and IBCF (0.97 mL, 7.2 mmol) were added. After 5 min under stirring, 2.6 mL of NH_4OH (36 mmol) were added. The reaction mixture was stirred for 10 min. The expected compound was precipitated with water, collected by filtration, washed with water and hexane. It was dried over KOH pellets: yield 55% (1.09 g); TLC R_f(E) 0.64, (D) 0.35, (C) 0.12; mp 78–80 °C.

Boc-Leu-Aic-NH_2 ***10b***. Compound **10a** (0.98 g, 3.54 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 60%. The expected TFA salt (0.6 g, 2 mmol) was dissolved in DMF containing Boc-Leu-OH, H_2O (0.52 g, 2.1 mmol), BOP (0.93 g, 2.1 mmol) and NMM (0.45 mL, 4.1 mmol). The reaction mixture was stirred for 1 h at room temperature and treated as described for compound **1b**: yield 55% (0.43 g); TLC R_f(E) 0.68, (D) 0.37, (C) 0.12; mp 87–92 °C; $[\alpha]_D$ −52 (*c* 0.8, DMF).

Z-His(Z)-Leu-Aic-NH_2 ***10c***. Compound **10b** (0.4 g, 1 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 89%. The expected TFA salt (0.36 g, 0.89 mmol) was dissolved in DMF containing Z-His(Z)-OH, EtOH (0.44 g, 0.93 mmol), BOP (0.41 g, 0.93 mmol) and NMM (0.2 mL, 1.82 mmol). The reaction mixture was stirred for 2 h at room temperature and treated as described for compound **1b**: yield 98% (0.6 g); TLC R_f(G) 0.58, (F) 0.28; mp 103–105 °C; $[\alpha]_D$ −18 (*c* 0.9, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2 ***10d***. Compound **10c** (0.5 g, 0.72 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.13 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue which solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH: yield 98%. This compound (0.3 g, 0.6 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.48 g, 0.6 mmol), BOP (0.26 g, 0.6 mmol) and NMM (0.19 mL, 1.8 mmol). After 2 h at room temperature, the reaction mixture was treated as described for compound **1h**: yield 60% (0.43 g); TLC R_f(M) 0.43, (L) 0.37; mp 160–164 °C (dec); $[\alpha]_D$ −15 (*c* 1, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Aic-NH_2 ***10***. Prepared from compound **10d** (0.15 mg, 0.123 mmol) as described for compound **1**: yield 98% (0.148 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2 ***11***

Boc-Tic-NH_2 ***11a***. To a solution of Boc-Tic-OH (2 g, 7.2 mmol) in DMF cooled at −20 °C, NMM (0.79 mL, 7.2 mmol) and IBCF (0.97 mL, 7.2 mmol) were added. After 5 min under stirring, 2.57 mL of NH_4OH (36 mmol) were added. After 10 min, water (200 mL) was added under stirring, followed by ethyl acetate (100 mL). The organic layer was washed with water, dried over sodium sulfate and then concentrated in vacuo to give a clear oil: yield 92% (1.83g); TLC R_f(D) 0.39, (C) 0.18.

Boc-Leu-Tic-NH_2 ***11b***. Compound **11a** (1.75 g, 6.33 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 91%. The expected TFA salt (1.68 g, 5.78 mmol) was dissolved in DMF containing Boc-Leu-OH, H_2O (1.44 g, 5.78 mmol), BOP (1.54 g, 3.49 mmol) and NMM (1.28 mL, 11.56 mmol). The reaction mixture was stirred for 1 h at room temperature. The expected compound was treated as described for compound **1a**: yield 66% (1.49 g); TLC R_f(E) 0.59, (D) 0.3; mp 66–68 °C; $[\alpha]_D$ −15 (*c* 1.5, DMF).

Z-His(Z)-Leu-Tic-NH_2 ***11c***. Compound **11b** (1.39 g, 3.57 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 98%. The expected TFA salt (1.41 g, 3.5 mmol) was dissolved in DMF containing Z-His(Z)-OH, EtOH (1.64 g, 3.49 mmol), BOP (1.54 g, 3.49 mmol) and NMM (0.77 mL, 6.98 mmol). The reaction mixture was stirred for 2 h at room temperature. The expected compound was treated as described for compound **1a**: yield 81% (1.96 g); TLC R_f(G) 0.7, (F) 0.52; mp 78–81 °C; $[\alpha]_D$ −7 (*c* 1, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2 ***11d***. Compound **11c** (1 g, 1.44 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.26 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue which solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH: yield 98%. This compound (0.12 g, 0.246 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.198 g, 0.246 mmol), BOP (0.1 g, 0.246 mmol) and NMM (0.082 mL, 0.738 mmol). After 2 h at room temperature, the expected compound was treated as described for compound **1a**: yield 84% (0.25 g); TLC R_f(M) 0.44, (L) 0.36; mp 150–153 °C dec; $[\alpha]_D$ −5 (*c* 1, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-Tic-NH_2 ***11***. Prepared from compound **11d** (0.15 mg, 0.123 mmol) as described for compound **1**: yield 98% (0.148 g). It was finally purified by HPLC and lyophilized.

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH_2 ***12***

Boc-D-Tic-NH_2 ***12a***. Boc-D-Tic-OH (2 g, 7.2 mmol) was treated as described for compound **4b** to produce a white product: yield 70% (1.39 g); TLC R_f(E) 0.64, (D) 0.48, (C) 0.16; mp 60–70 °C dec; $[\alpha]_D$ +3 (*c* 1, DMF).

Boc-Leu-D-Tic-NH_2 ***12b***. Compound **12a** (1.16 g, 6.58 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 91%. The expected TFA salt (1.74 g, 5.99 mmol) was dissolved in DMF

containing Boc-Leu-OH, H_2O (1.56 g, 6.28 mmol), BOP (2.77 g, 6.28 mmol) and NMM (1.36 mL, 12.27 mmol). The reaction mixture was stirred for 1 h at room temperature. The expected compound was treated as described for compound **1a**: yield 61% (1.43 g); TLC R_f(E) 0.68, (D) 0.46, (D) 0.15; mp 73–75 °C; $[\alpha]_D$ –14 (*c* 1.3, DMF).

Z-His(Z)-Leu-D-Tic-NH₂ ***12c***. Compound **12b** (1.33 g, 3.41 mmol) was partially deprotected with trifluoroacetic acid. After 30 min at room temperature, the trifluoroacetic acid was removed in vacuo, coevaporated with hexane and the product was precipitated upon addition of ether: yield 98%. The expected TFA salt (1.3 g, 3.22 mmol) was dissolved in DMF containing Z-His(Z)-OH, EtOH (1.51 g, 3.22 mmol), BOP (1.42 g, 3.22 mmol) and NMM (0.71 mL, 6.44 mmol). The reaction mixture was stirred for 2 h at room temperature. The expected compound was treated as described for compound **1b**: yield 98% (2.19 g); TLC R_f(G) 0.59, (F) 0.26; mp 75–77 °C; $[\alpha]_D$ –4 (*c* 1.6, DMF).

Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH₂ ***12d***. Compound **12c** (1.5 g, 2.15 mmol) was hydrogenated at room temperature in DMF/EtOH: 10:1 (100 mL) containing 11 N hydrochloric acid (0.39 mL) in the presence of a 10% Pd/C catalyst. After 2 h, the catalyst was removed by filtration and the filtrate concentrated in vacuo to leave a residue that solidified upon trituration in ether. It was collected, washed with ether, dried in vacuo over KOH: yield 98%. This compound (0.24 g, 0.49 mmol) was dissolved in DMF containing Boc-D-Phe-Gln-Trp-Ala-Val-Gly-OH **1g'** (0.4 g, 0.49 mmol), BOP (0.21 g, 0.49 mmol) and NMM (0.163 mL, 1.47 mmol). After 2 h stirring at room temperature, the expected compound was treated as described for compound **1a**: yield 50% (0.3 g); TLC R_f(M) 0.47, (L) 0.29; mp 150–153 °C (dec); $[\alpha]_D$ –2 (*c* 1, DMF).

H-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-D-Tic-NH₂ ***12***. This was prepared from compound **12d** (0.15 mg, 0.123 mmol) as described for compound **1**: yield 98% (0.148 g). It was finally purified by HPLC and lyophilized.

Pharmacology

In vitro studies: All compounds were dissolved in dimethylsulfoxide (Merck Art 2951) and then appropriately diluted. Final solutions used for testing did not contain more than 1% DMSO.

Materials

HEPES, D-glucose, calcium chloride, soybean trypsin inhibitor, bacitracin, sodium chloride, potassium chloride, magnesium chloride, sodium pyruvate, sodium fumarate were from Sigma Chemical Co, St Louis, MO; purified collagenase (0.88 PZ U/mg) was from Serva Feinbiochemica GmbH & Co, Heidelberg; glutamine, MEM non essential aminoacids (100X), essential vitamin mixture were from Gibco Life Technologies Ltd, Scotland; Phadebas amylase test reagent was from Pharmacia France SA; bovine plasma albumin (fraction V, pH 7) was from Euromedex, Schiltigheim; (3-[^{125}I]Iodotyrosyl^{15})Gastrin Releasing Peptide was from Amersham France SA.

The buffer used for rat pancreatic acini preparation contained 25.5 mL HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 5 mM sodium pyruvate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01% (w/v) trypsin inhibitor, 1% (v/v) aminoacid mixture and 1% (v/v) essential vitamine mixture.

The binding solution (pH 7.4) was Krebs–Henseleit buffer from Sigma Chemical Co, St Louis, MO, supplemented with 1% (w/v) bovine plasma albumin. For washing, Krebs–Henseleit buffer was supplemented with 4% (w/v) bovine plasma albumin.

Tissue preparation

Male Wistar rats (200–300 g) were obtained from the Pharmacological Breeding Center of Montpellier University. Dispersed acini from rat pancreas were prepared using the modification [24] of the method described previously [25].

Binding of (3-[^{125}I]iodotyrosyl^{15})Gastrin releasing peptide to acini

Briefly, samples (0.5 mL containing about 1 mg/mL protein) were incubated for 60 min at 37 °C [26] in the presence of 20 pM (3-[^{125}I]Tyr^{15})Gastrin releasing peptide and various concentrations of bombesin analogs. After centrifugation at 10 000 *g* and two washings, the radioactivity associated with the acinar pellet was measured [27]. Binding in the absence of any unlabeled bombesin-peptide was 7.5% of the total radioactivity present in the sample. Nonspecific binding was determined in the presence of 10 μM of unlabeled bombesin and was always less than 25% of the total binding. These results were expressed as a percentage of the specific binding.

Amylase release test

Dispersed acini were suspended in 0.5 mL of standard incubation media containing about 1 mg of protein/mL; samples were incubated for 30 min at 37 °C and amylase release was measured as described previously [28, 29]. Amylase activity was determined by the method of Ceska [30], using the Phadebas reagent. Amylase release was calculated as the percentage of maximal amylase activity obtained with optimal concentration of the reference BN.

Effect of peptides on bombesin-stimulated amylase release

Antagonist activity was determined as described previously [31]. Various concentrations of analogues to be tested were incubated in the presence of 1 nM BN that causes maximal stimulation of amylase secretion.

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