ANALOGUES OF THE CHOLECYSTOKININ C-TERMINAL TETRAPEPTIDE*

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Substituted phenylethyl amides and phenylethyl esters *Va – Vj* derived from the carboxyterminal tetrapeptide part of the cholecystokinin were synthesized and their biological properties assayed. In the original CCK tetrapeptide structure Trp-Met-Asp-Phe-NH_2 the Met was replaced by Lys(Pamc) and the terminal Phe-NH_2 was replaced by Phe-$NHCH_2C_6H_5$ or Phe-$OCH_2C_6H_5$ moiety with a various degree of alkylation in the Ph ring. A bioassay revealed that these simple CCK analogues were selectively bound to A receptors from pancreas, whereas no binding to B receptors from brain was found. Some of the compounds behaved as weak inhibitors of CCK activity on gall bladder and guinea pig ileum contractions without any effect in anorectic assay.

Cholecystokinin (CCK) is a linear polypeptide** consisting of 33 amino acid residues which stimulates the gastrointestinal motility and gall bladder contractions as well as pancreatic amylase secretion[2,3]. In addition, CCK is involved in the food intake control[4]. Some of its fragments have been identified in gastrointestinal tract as well as in brain of several animal species[5–9]. Structure–activity relationship studies[10,11] have shown that the C-terminal octa- and heptapeptides (CCK-8 and CCK-7) reproduce the entire range of biological activities of CCK. Beside CCK-8 itself, particularly *N*-protected CCK-7 derivatives exhibit high CCK potency[12–18]. In the molecule of the cholecystokinin and its shorten fragments the *O*-sulfotyrosine residue was found to be necessary for full agonistic activity in periphery through interaction with a receptor of the type A.

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**The nomenclature and symbols of amino acids and peptides obey the published recommendations[1]. In addition to common symbols we use the following symbols: BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate; HOBt, 1-hydroxybenzotriazol; DMF, dimethylformamide; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; DIEA, *N*-ethyldiisopropylamine; TFA, trifluoroacetic acid; Pamc, phenylaminocarbonyl.

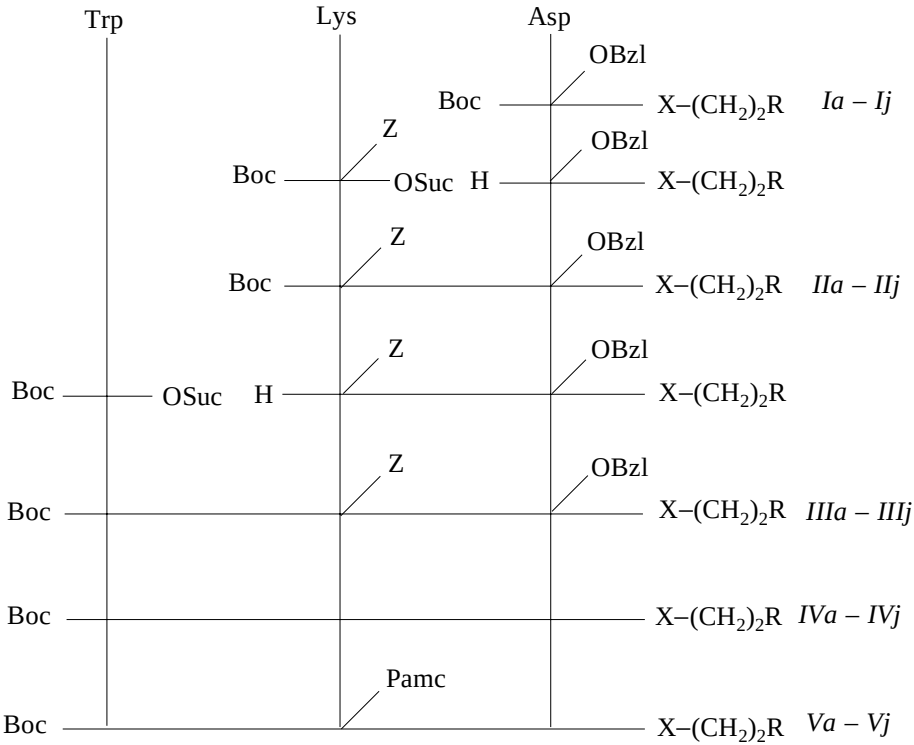
Recently, also non-sulfated tetrapeptides derived from the CCK C-terminus were described as potent and selective CCK-A receptor agonists[19,20] in which the Met residue was replaced by N^{ε}-substituted Lys derivatives. This replacement extensively changed selectivity of the corresponding analogues to receptors. While Boc-CCK-4 is selectively recognized only by the CCK-B receptor, Met substitution for the N^{ε}-substituted Lys in this tetrapeptide elicits affinity to CCK-A receptor, too.

In our work we combined this structural pattern with a modification of the aspartylphenylalanine moiety in this C-terminal tetrapeptide for corresponding phenylethyl amides or phenylethyl esters of the aspartyl residue[21–23] with an idea to obtain extremely shortened analogues of CCK with affinity to A receptor behaving like CCK antagonists. Phenyl nucleus in these compounds was also alkylated to different degree since we previously observed the relationships between the phenylalanine ring alkylation and a potency of the corresponding analogues to bind to CCK receptors and to prolong biological effect in several organs[16,24].

These analogues were prepared step-wise in solution from the corresponding phenylethyl amides *Ia – Ie* and phenylethyl esters *If – Ij* of Boc-Asp(OBzl)-OH (Scheme 1). The amides *Ia – Ie* were obtained by an acylation of the corresponding phenylethylamines by means of Boc-Asp(OBzl)-OH, mediated by BOP in DMF in the presence of DIEA. The esters *If – Ij* were prepared by a reaction of the corresponding phenylethyl bromides with cesium salt[25] of Boc-Asp(Bzl)-OH. Boc-protecting group of the amides and esters *Ia – Ij* was split off by TFA and the corresponding trifluoroacetates were coupled with Boc-Lys(Z)-OSuc in the presence of DIEA (refs[26,27]) to obtain amides and esters *IIa – IIj*. The next step was essentially similar to previous one with the exception that the trifluoroacetates of the compounds *IIa – IIj* were coupled with Boc-Trp-OSuc in the presence of HOBt and DIEA to obtain the compounds *IIIa – IIIj*. Protected peptides *IIIa – IIIj* were hydrogenated on palladium black to split off the benzyl and benzyloxycarbonyl protection in the side-chains of the Asp and Lys residues, respectively, and to get the compounds *IVa – IVj*. In the last step the N^{ε}-amino group of Lys was acylated by phenyl isocyanate to yield analogues *Va – Vj*. After purification by the preparative TLC and HPLC these compounds were compared with the tetrapeptide *VI* in a binding study using receptors isolated from pancreas and brain[16]. Their activity to stimulate an anorexia and gall bladder[14] and guinea pig ileum contractions[28] was assayed, too.

Boc-Trp-Lys(CONHPh)-Asp-Phe-NH_2

VI



I – V	R	X	*I – V*	R	X
a	C_6H_5	NH	*f*	C_6H_5	O
b	3-MeC_6H_4	NH	*g*	3-MeC_6H_4	O
c	2,4,6-$Me_3C_6H_2$	NH	*h*	2,4,6-$Me_3C_6H_2$	O
d	2,3,4,5,6-Me_5C_6	NH	*i*	2,3,4,5,6-Me_5C_6	O
e	3,4-$(OCH_2O)C_6H_3$	NH	*j*	3,4-$(OCH_2O)C_6H_3$	O

SCHEME 1

As it can be seen from the Tables I and II both amides and esters tested were, contrary to the tetrapeptide *VI*, selectively bound only to receptor of A type from the pancreatic acini and no binding to the B receptor from the brain was detected. It seems that substitution of the carboxyterminal phenylalanine amide moiety in *VI* for phenylethyl amide or phenylethyl ester does not, in general, interfere with binding of these tripeptides to corresponding A receptor, however, decreases the binding in comparison with the parent tetrapeptide due to change in a conformation of the C-terminus. Interestingly, the assay on gall bladder and guinea pig contraction showed only a weak inhibitory activity in compounds *Vc*, *Vd*, *Vf* and *Vh* which were studied in details. Thus, binding of these tripeptide analogues to A receptors, enabled by the presence of the substituted Lys residue, is relatively weak due to the replacement of the carboxyterminal Phe-NH_2 for the corresponding phenylethyl ester and amide structures. On the other hand, this binding creates a complex peptide–receptor which can not accept a biologically active conformation necessary for triggering the agonistic effect of CCK and thus the receptor occupacy of the receptor is followed by antagonistic activity. Most of the analogues were tested in anorectic assay (intrinsic activity of the compounds applied i.p. was tested in a range of 0.4 – 400 μg/kg; their inhibitory activity was determined after i.p. injection of the compound in a range 4 – 400 μg/kg and i.p. injection of the CCK-8 in a dose corresponding to ED_{50}, 9 μg/kg, added after 10 min), too, however neither agonistic nor inhibitory potency has been found in these series of the short CCK derivatives.

EXPERIMENTAL

Melting points were measured on Kofler block and were not corrected. Samples for elemental analysis were dried over P_2O_5 or KOH at room temperature at 150 Pa. The peptide samples for amino acid analysis were hydrolyzed by 6 M HCl at 110 °C for 20 h, Trp containing samples were hydrolyzed under the same conditions with 4% thioglycolic acid added. The amino acid analyses were performed on a Durrum D-500 (Durrum Instrument, Palo Alto, U.S.A.) or a Mikrotechna (Prague, The Czech Republic) amino acid analyzers. Mass spectrometry with FAB technique was used for determination of M^+ of the corresponding peptides (ZAB-EQ spectrometer of VG Analytical, Manchester, U.K.). Optical rotations were obtained on polarimeter Perkin–Elmer 141 MCA at 22 °C. Analytical TLC were performed on Silufol plates (Kavalier, The Czech Republic) in the following systems: 10% ethyl acetate in light petroleum (A); 20% ethyl acetate in light petroleum (B); 30% ethyl acetate in light petroleum (C); 30% heptane in acetone (D). The detection was carried out with UV light at 254 nm. For preparative TLC the glass plate of size 20 × 50 cm or 13 × 27 cm were used with 4 mm thick silica gel (30 – 60 μ) layer containing a luminofor. For HPLC a Spectra Physics instrument with an SP 8800 pump, an SP 8450 UV detector and an SP 4290 integrator was used. The analytical HPLC was carried out on a 15 × 0.4 cm Vydac column (The Separations Group, Hesperia, U.S.A.), flow rate 60 ml/h, detection at 222 nm, mobile phase 70% methanol in 0.05% aqueous TFA. The preparative HPLC was done on 25 × 1.0 cm column packed with the same stationary phase, flow rate 180 ml/h, mobile phase 60 – 80% of methanol in 0.05% aqueous TFA, detection at 280 nm. Analytical electrophoresis was carried out in a moist chamber on a Whatman 3MM paper (20 V/cm) in 6% acetic acid (pH 2.4) and in a pyridine–acetate buffer (pH 6.7) for 60 min. Just before use, all amino

TABLE I
In vitro bioactivities of phenylethyl amides and phenylethyl esters *V*

Compound	Gall Bladder Contraction		GP Ileum Contraction	
	EC_{50} [a]	IC_{50} [b]	EC_{50} [a]	IC_{50} [b]
Va	0[c]	NT[d]	0	0
Vb	0	NT	NT	NT
Vc	0	0	NT	1 200
Vd	0	0	0	1 400
Vf	0	750	0	500
Vh	0	1 400	0	0
Vi	0	NT	0	0
Vj	0	NT	0	0
VI	34.3	NT	37.3	NT

[a] Agonist activity, nmol/l; tested in the range $10^{-9} - 10^{-6}$ mol/l. [b] Antagonist activity, nmol/l; tested in the range $10^{-8} - 10^{-6}$ mol/l. [c] 0 Means without effect in a range tested. [d] NT Means not tested.

TABLE II
Affinity of phenylethyl amides and phenylethyl esters *V* to CCK A and B receptors

Compound	A receptor IC_{50} [a]	B receptor IC_{50} [a]
Va	2 000	0[b]
Vb	9 000	0
Vc	300	0
Vd	300	0
Vf	3 000	0
Vh	400	0
Vi	2 000	0
Vj	3 000	0
VI	4.3	330

[a] Compounds were tested in a range of $10^{-11} - 10^{-5}$ mol/l; IC_{50} (nmol) was determined as the concentration of the peptide that inhibited 50% of the specific binding of [^{125}I]BH-CCK-8 in each tissue. [b] 0 Means without effect in range tested.

acid derivatives were tested for homogeneity by thin-layer chromatography (Silufol plates, Kavalier, The Czech Republic) in the systems: 2-butanol–98% formic acid–water (75 : 13.5 : 11.5) (S1); 2-butanol–25% aqueous ammonia–water (85 : 7.5 : 7.5) (S2); 1-butanol–acetic acid–water (4 : 1 : 1) (S3); 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4). Detection was with iodine, ninhydrin or by the chlorination method. Solvents were evaporated in vacuo on a rotary evaporator (bath temperature 30 °C); DMF was evaporated at 30 °C and 150 Pa. The standard compound Boc-Trp-Lys(Pamc)-Asp-Phe-NH_2 (*VI*) was prepared following the literature[20].

N^{α}-Boc-O^{δ}-Bzl-Aspartic Acid Phenylethyl Amides *Ia – Ie*

To a solution of the corresponding β-phenylethylamine hydrochloride (11 mmol) in DMF (30 ml) the Boc-Asp(OBzl)-OH (3.3 g, 10 mmol), BOP (4.4 g, 10 mmol) and DIEA (1.9 ml, 11 mmol) were added and the mixture was stirred at room temperature for 20 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate and washed with 1 M $NaHCO_3$ (3 × 50 ml), water (3 × 50 ml), 10% citric acid (3 × 50 ml) and water (3 × 50 ml). After drying over Na_2SO_4, the solvent was evaporated to yield corresponding phenylethyl amide as a white solid. The amides were purified by a preparative TLC on silica in a solvent system 30% of ethyl acetate in light petroleum. The analytical data are given in Table III.

TABLE III
Analytical data on N^{α}-Boc-O^{δ}-Bzl-aspartic acid phenylethyl amides *Ia – Ie*

Compound	M.p., °C Yield, %	Formula M.w./$M^{+,a}$	Calculated/Found			$[\alpha]_D$, ° *c*, DMF	TLC[b]	Electrophoresis[c]		
			% C	% H	% N			$E^{Gly}_{2.4}$	$E^{His}_{2.4}$	$E^{His}_{5.7}$
Ia	105 – 106	$C_{24}H_{30}N_2O_5$	67.59	7.09	6.57	−15.9	0.85	1.10	0.71	0.75
	52	426.5/326.3	67.74	7.30	6.65	0.25				
Ib	104 – 106	$C_{25}H_{32}N_2O_5$	68.19	7.32	6.36	−14.4	0.87	1.08	0.69	0.73
	31	440.5/340.3	68.34	7.53	6.42	0.31				
Ic	105 – 108	$C_{27}H_{36}N_2O_5$	69.21	7.74	5.98	−7.7	0.63	1.02	0.59	0.68
	44	468.5/368.3	69.43	7.94	5.73	0.28				
Id	110 – 113	$C_{29}H_{40}N_2O_5$	70.13	8.12	5.64	−12.9	0.74	0.98	0.58	0.63
	77	496.7/396.5	69.88	8.02	5.75	0.34				
Ie	oil	$C_{25}H_{30}N_2O_7$	63.82	6.43	5.95	−10.6	0.29	1.03	0.68	1.02
	43	470.5/370.4	63.64	6.56	5.87	0.29				

[a] Peak corresponding to calculated mass without Boc was detected as M^+. [b] Solvent system 30% ethyl acetate in light petroleum. [c] After deprotection by TFA.

N^{α}-Boc-O^{δ}-Bzl-Aspartic Acid Phenylethyl Esters *Ie – Ij*

To a solution of corresponding β-phenylethyl bromide (2 mmol) in DMF (5 ml) the cesium salt of Boc-Asp(OBzl)-$O^{-}Cs^{+}$ (1.4 g, 3 mmol) and dibenzo-18-crown 6 (1.4 g, 4 mmol) were added and the mixture stirred at 50 °C for 24 h. The solvent was evaporated in vacuo and the residue was dissolved in ethyl acetate (100 ml) and washed with 1 M $NaHCO_3$ (3 × 50 ml), water (3 × 50 ml), 10% citric acid (3 × 50 ml) and water (3 × 50 ml). After drying over Na_2SO_4, the solvent was evaporated in vacuo to yield corresponding phenylethyl ester. The compounds *Ie – Ij* were purified by a preparative TLC on silica in a solvent system 10% of ethyl acetate in light petroleum. The analytical data are given in Table IV.

N^{α}-Boc-N^{ε}-Z-Lysyl-O^{δ}-Bzl-Aspartic Acid Phenylethyl Amides and Phenylethyl Esters *IIa – IIj*

N^{α}-Boc protecting group of the amides and esters *Ia – Ij* (3 mmol) was removed by trifluoroacetic acid (12 ml) over 30 min. After evaporation of TFA corresponding trifluoroacetate was dried in desiccator over KOH, dissolved in DMF (3 ml) and added dropwise to the solution of Boc-Lys(Z)-OSuc (2.6 g, 5 mmol) in the presence of DIEA (0.9 ml, 5 mmol) at room temperature. After stirring for 24 h, the solvent was evaporated in vacuo and the residue was dissolved in ethyl acetate and washed with 1 M $NaHCO_3$ (3 × 50 ml), water (3 × 50 ml), 10% citric acid (3 × 50 ml) and water (3 × 50 ml).

TABLE IV
Analytical data on N^{α}-Boc-O^{δ}-Bzl-aspartic acid phenylethyl esters *If – Ij*

Compound	Yield, %	Formula M.w./M^{+},[a]	Calculated/Found			$[\alpha]_D$, °, *c*, DMF	TLC	Electrophoresis[b]		
			% C	% H	% N			$E_{2.4}^{Gly}$	$E_{2.4}^{His}$	$E_{5.7}^{His}$
If	36	$C_{24}H_{29}NO_6$	67.43	6.84	3.28	–24.8	0.33[c]	1.06	0.75	0.88
		427.5/327.3	67.65	6.66	3.14	0.31				
Ig	77	$C_{25}H_{31}NO_6$	68.01	7.08	3.17	–20.4	0.64[d]	1.05	0.71	0.76
		441.5/341.3	68.34	7.25	3.32	0.33				
Ih	38	$C_{27}H_{35}NO_6$	69.06	7.51	2.98	–20.3	0.42[c]	0.92	0.63	0.70
		469.6/369.4	69.20	7.43	3.46	0.27				
Ii	67	$C_{29}H_{39}NO_6$	70.25	7.90	2.81	–19.1	0.40[c]	0.90	0.61	0.68
		497.6/397.5	70.34	7.79	2.76	0.25				
Ij	93	$C_{25}H_{29}NO_8$	63.68	6.20	2.97	–18.7	0.46[d]	1.02	0.70	0.71
		471.5/371.2	63.72	6.33	2.86	0.26				

[a] Peak corresponding to calculated mass without Boc was detected as M^{+}. [b] After deprotection by TFA. [c] Solvent system: 10% ethyl acetate in light petroleum. [d] Solvent system: 20% ethyl acetate in light petroleum.

After drying over Na_2SO_4, the solvent was evaporated in vacuo and the pure amides *IIa – IIe* were obtained on crystallization from ether–light petroleum and pure esters *IIf – IIj* on crystallization from the solvent mixture ethyl acetate, ether, heptane and light petroleum. The analytical data are given in Table V.

TABLE V
Analytical data on N^{α}-Boc-N^{ϵ}-Z-lysyl-O^{δ}-Bzl-aspartic acid phenylethyl amides and phenylethyl esters *IIa – IIj*

Compound	M.p., °C Yield, %	Formula M.w./$M^{+,a}$	Calculated/Found			$[\alpha]_D$, ° *c*	TLC[b]	Electrophoresis[c]		
			% C	% H	% N			$E^{Gly}_{2.4}$	$E^{His}_{2.4}$	$E^{His}_{5.7}$
IIa	105 – 110	$C_{38}H_{48}N_4O_8$	66.26	7.02	8.13	–20.2[d]	0.75	0.84	0.53	0.46
	40	688.8/588.6	66.32	7.22	8.05	0.35				
IIb	100 – 103	$C_{39}H_{50}N_4O_8$	66.65	7.17	7.97	–16.6[d]	0.82	0.74	0.50	0.32
	63	702.9/602.7	66.48	7.32	7.85	0.29				
IIc	150 – 154	$C_{41}H_{54}N_4O_8$	67.38	7.45	7.67	–19.7[d]	0.82	0.79	0.57	0.43
	51	730.9/630.7	67.45	7.19	7.58	0.32				
IId	112 – 113	$C_{43}H_{58}N_4O_8$	68.05	7.70	7.38	–17.9[d]	0.79	0.75	0.54	0.43
	66	758.9/658.7	68.14	7.78	7.28	0.34				
IIe	111 – 112	$C_{39}H_{48}N_4O_{10}$	63.92	6.60	7.65	–20.7[d]	0.75	0.75	0.51	0.76
	31	732.8/632.6	63.86	6.43	7.44	0.41				
IIf	oil	$C_{38}H_{47}N_3O_9$	66.17	6.87	6.09	–9.1[d]	0.81	0.73	0.54	–
	76	689.8/589.6	66.24	6.97	6.23	0.24				
IIg	oil	$C_{39}H_{49}N_3O_9$	66.55	7.02	5.97	–10.9[d]	0.81	0.68	0.53	–
	85	703.8/603.6	66.39	7.24	5.79	0.27				
IIh	103 – 105	$C_{41}H_{53}N_3O_9$	67.29	7.30	5.74	–15.3[d]	0.80	0.63	0.41	–
	78	731.9/631.7	67.32	7.36	5.63	0.32				
IIi	oil	$C_{43}H_{57}N_3O_9$	67.96	7.56	5.53	–12.8[e]	0.74	0.74	0.55	–
	92	759.9/659.7	67.84	7.29	5.47	0.30				
IIj	85 – 86	$C_{39}H_{47}N_3O_{11}$	63.83	6.46	5.73	–11.2[e]	0.81	0.63	0.46	–
	36	733.8/633.6	63.76	6.53	5.88	0.27				

[a] Peak corresponding to calculated mass without Boc was detected as M^+. [b] Solvent system: 30% heptane in acetone. [c] After deprotection by TFA. [d] In DMF. [e] In MeOH.

N^{α}-Boc-Tryptophyl-*N^{ε}*-Z-Lysyl-*O^{δ}*-Bzl-Aspartic Acid Phenylethyl Amides and Phenylethyl Esters *IIIa – IIIj*

N^{α}-Boc protecting group of the amides and esters *IIa – IIj* (3 mmol) was removed by trifluoroacetic acid (12 ml) over 30 min. After evaporation of TFA corresponding trifluoroacetates were dried in desiccator over KOH for 5 h, then dissolved in DMF (2 ml) and added to the solution of Boc-Trp-OSuc (1.1 mmol, 0.46 g), HOBt (1 mmol, 0,13 g) and DIEA (2 mmol, 0.35 ml) in DMF (3 ml). The reaction mixtures were stirred at room temperature for 24 h and solvent was evaporated in vacuo.

TABLE VI
Analytical data on *N^{α}*-Boc-tryptophyl-*N^{ε}*-Z-lysyl-*O^{δ}*-Bzl-aspartic acid phenylethyl amides and phenylethyl esters *IIIa – IIIj*

Compound	M.p., °C Yield, %	Formula M.w./$M^{+,b}$	$[\alpha]_D$, ° *c*, DMF	TLC[a]
IIIa	160 – 168	$C_{49}H_{58}N_6O_9$	−17.3	0.78
	53	875.0/774.41	0.31	
IIIb	155 – 156	$C_{50}H_{60}N_6O_9$	−17.9	0.76
	80	889.0/788.5	0.28	
IIIc	178 – 179	$C_{52}H_{64}N_6O_9$	−17.0	0.81
	80	917.1/816.4	0.29	
IIId	166 – 167	$C_{54}H_{68}N_6O_9$	−15.3	0.77
	76	945.2/844.5	0.35	
IIIe	130 – 133	$C_{50}H_{58}N_6O_{11}$	−23.7	0.77
	80	919.1/818.4	0.38	
IIIf	135 – 138	$C_{49}H_{57}N_5O_{10}$	−27.3	0.77
	68	876.0/775.4	0.34	
IIIg	60 – 61	$C_{50}H_{59}N_5O_{10}$	−24.1	0.67
	81	890.1/789.5	0.27	
IIIh	120 – 121	$C_{52}H_{63}N_5O_{10}$	−15.6	0.80
	90	918.1/817.4	0.29	
IIIi	133 – 138	$C_{54}H_{67}N_5O_{10}$	−22.8	0.79
	32	946.2/845.5	0.32	
IIIj	80 – 82	$C_{50}H_{57}N_5O_{12}$	−17.9	0.77
	83	920.0/819.5	0.30	

[a] Solvent system 30% heptane in acetone. [b] Peak corresponding to calculated mass without Boc was detected as M^+.

The residues were dissolved in ethyl acetate and washed with 1 M $NaHCO_3$ (3 × 50 ml), water (3 × 50 ml), 10% citric acid (3 × 50 ml) and water (3 × 50 ml). After drying over Na_2SO_4, the solvent was evaporated in vacuo. The oily amides were then triturated with ether and the esters with light petroleum to obtain purified protected peptides *IIIa – IIIj* as white solids. The analytical data are given in Table VI.

TABLE VII
Analytical data on N^{α}-Boc-tryptophyl-N^{ε}-Pamc-lysyl-aspartic acid phenylethyl amides and phenylethyl esters *Va – Vj*

Compound	M.p., °C	Formula M.w./$M^{+,c}$	TLC[a]	R_T[b]
Va	104 – 107	$C_{41}H_{51}N_7O_8$ 769.9/669.7	0.62	8.20
Vb	118	$C_{42}H_{53}N_7O_8$ 783.9/683.3	0.65	10.52
Vc	128 – 132	$C_{44}H_{57}N_7O_8$ 812.0/711.8	0.67	21.93
Vd	100 – 105	$C_{46}H_{61}N_7O_8$ 840.0/739.8	0.78	42.12
Ve	97 – 99	$C_{42}H_{51}N_7O_{10}$ 813.9/713.7	0.63	8.46
Vf	115	$C_{41}H_{50}N_6O_9$ 770.9/670.7	0.68	8.36
Vg	112	$C_{42}H_{52}N_6O_9$ 784.9/684.7	0.67	10.85
Vh	92 – 98	$C_{44}H_{56}N_6O_9$ 812.9/712.6	0.72	22.41
Vi	110 – 114	$C_{46}H_{60}N_6O_9$ 841.0/740.8	0.78	35.81
Vj	90 – 92	$C_{42}H_{50}N_6O_{11}$ 814.9/714.7	0.65	7.72

[a] Solvent system 30% heptane in acetone. [b] HPLC retention time, min; 70% MeOH in 0.05% aqueous TFA. [c] Peak corresponding to calculated mass without Boc was detected as M^+.

N$^{\alpha}$-Boc-Tryptophyl-Lysyl-Aspartic Acid Phenylethyl Amides and Phenylethyl Esters *IVa – IVj*

The protected amides or esters *IIIa – IIIj* (1 mmol) in methanol (20 ml) were stirred in the presence of 5% Pd–C (0.6 g) at ambient pressure of hydrogen for 5 – 8 h at room temperature. A progress of the hydrogenation was monitored by TLC, by disappereance of starting material. The reaction mixtures were filtered, the catalyst was repeatedly washed with DMF and the solvent evaporated in vacuo. The partially protected peptides *IVa – IVj* with M^+ corresponding to their molecular weights were used in the next synthetic step without purification.

N$^{\alpha}$-Boc-Tryptophyl-*N*$^{\varepsilon}$-Pamc-Lysyl-Aspartic Acid Phenylethyl Amides and Phenylethyl Esters *Va – Vj*

The solutions of phenylethyl amides or phenylethyl esters of *N*$^{\alpha}$-Boc-tripeptides *IVa – IVj* (0.7 mmol), phenyl isocyanate (0.09 ml, 0.9 mmol) and DIEA (0.19 ml, 1.1 mmol) in DMF (5 ml) were stirred at room temperature for 20 h. The solvent was evaporated in vacuo and crude products were purified by preparative TLC on silica in the solvent system 30% heptane in acetone. The pure compounds *IVa – IVj* were obtained by semipreparative HPLC. The analytical data are given in Table VII.

Biological Activity Assays

Guinea pig gall bladder and guinea pig ileum contraction abilities were estimated according to Lonovics[29] and Sugg[28]. For the anorectic effect determination a test was used as previously described[30]. Binding studies were performed using dispersed acini from rat pancreas according to the methods of Jensen[11] and Rodrigues[31] and using guinea-pig brain membranes following the procedures of Pelaprat[32] and Rodrigues[31], respectively.

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