Synthesis and Biological Activity of Some Partially Modified Retro-Inverso Analogues of Cholecystokinin

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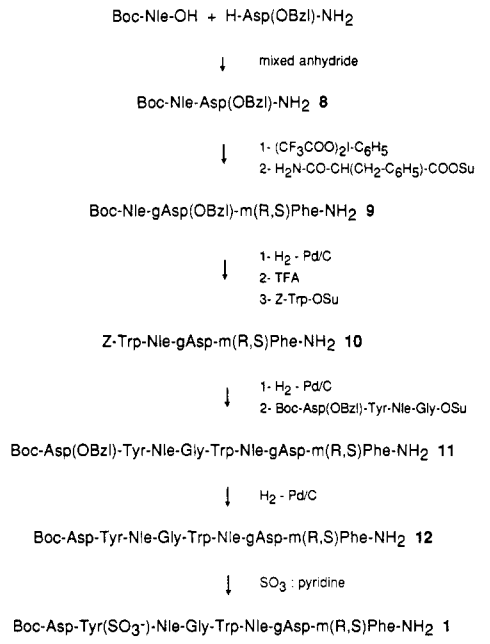
Syntheses of some partially modified retro-inverso analogues of the C-terminal octa- or heptapeptide of cholecystokinin are described. These analogues (in which the C-terminal carboxamide was deleted or not) were obtained by reverting one or several peptide bonds in the parent molecule. All these compounds were able to inhibit binding of labeled CCK-8 to rat pancreatic acini and guinea pig brain membranes and to stimulate amylase release from rat pancreatic acini with various potencies. Some of these derivatives reproduce only part of the biological response of CCK on amylase release.

We recently described a series of analogues of cholecystokinin (CCK) in which the C-terminal phenylalanine residue was replaced by 2-phenylethylamine or 2-phenylethanol.[1,2] These compounds were able to exhibit part of the biological response of CCK on isolated rat pancreatic acini, giving maximal stimulation of enzyme secretion, but with no decrease in the response at supramaximal concentrations.[1] However, these analogues were unable to promote phosphoinositide breakdown in rat pancreatic acini.[3,4] CCK analogues combining the modifications described above at the C-terminal and substituted at the tryptophan residue in position 30 by its D enantiomer behaved as potent CCK-receptor antagonists in rat and guinea pig pancreatic acini and in isolated smooth-muscle cells from rabbit gastric mucosa.[2,5,6] We thus pointed out the functional role of the C-terminal amide moiety and the crucial importance of the tryptophane residue in the biological activity of CCK. In the gastrin series, suppression of the C-terminal amide function produced in all cases competitive gastrin antagonists.[7,8] In order to increase the chemical and enzymatic stability of CCK analogues and because we have shown that replacement of peptide bonds can produce CCK analogues exhibiting interesting biological activities,[9,10] we decided to investigate some retro-inverso modifications in this series. The partial retro-inverso modification[11] involves the reversal of the direction of one or several peptide bonds, by introduction of a *gem*-diaminoalkyl residue and of a malonyl residue, associated with inversion of configuration of the residues in between, in order to retain general topochemistry of the parent molecule. These modifications, which have been applied with success to enhance enzymatic resistance to a number of peptide hormones, resulted in some cases in isomers retaining biological activity[12] or, on the contrary, to antagonists of the parent peptide.[13] In this work, in attempting to design enzyme-resistant CCK analogues, we synthesized CCK derivatives containing partial retro-inverso modifications of selected peptide bonds, in combination or not with the deletion of the C-terminal amide function of the CCK sequence. The following compounds were synthesized: Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**1**), Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (**2**), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (**3**), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**4**), Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (**5**), Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**6**), and Boc-Asp-Tyr-

Scheme I



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Scheme II

Boc-Nle-Asp(OBzl)-NH_2 **8**

↓ 1- $(CF_3COO)_2I$-C_6H_5 2- C_6H_5-CH_2-CH_2-COOH / BOP

Boc-Nle-gAsp(OBzl)-CO-CH_2-CH_2-C_6H_5 **13**

↓ 1- TFA 2- Z-Trp-OSu

Z-Trp-Nle-gAsp(OBzl)-CO-CH_2-CH_2-C_6H_5 **14**

↓ 1- H_2 - Pd/C 2- Boc-Asp(OBzl)-Tyr-Nle-Gly-OSu

Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-gAsp-CO-CH_2-CH_2-C_6H_5 **15**

↓ H_2 - Pd/C

Boc-Asp-Tyr-Nle-Gly-Trp-Nle-gAsp-CO-CH_2-CH_2-C_6H_5 **16**

↓ SO_3 : pyridine

Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-CO-CH_2-CH_2-C_6H_5 **2**

Scheme III

Boc-Nle-OSu + H-Gly-NH_2

↓

Boc-Nle-Gly-NH_2 **17**

↓ 1- $(CF_3COO)_2I$-C_6H_5 2- Z-D-Trp / BOP

Boc-Nle-gGly-D-Trp-Z **18**

↓ 1- H_2 - Pd/C 2- Z-D-Nle / mixed anhydride

Boc-Nle-gGly-D-Trp-D-Nle-Z **19**

↓ 1- H_2 - Pd/C 2- Z-D-Asp(OBzl) / mixed anhydride

Boc-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z **20**

↓ 1- TFA 2- Boc-Tyr-OSu

Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z **21**

↓ 1- H_2 - Pd/C 2- R-CO-OSu

Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp-R
R = m(R,S)Phe-NH_2 **22**
R = C_6H_5-CH_2-CH_2-CO **23**

↓ SO_3 pyridine

Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-R
R = m(R,S)Phe-NH_2 **3**
R = C_6H_5-CH_2-CH_2-CO **4**

Scheme IV

Boc-Asp(OBzl)-Phe-NH_2

↓ 1- $(CF_3COO)_2I$-C_6H_5 2- For-OtCp

Boc-Asp(OBzl)-gPhe-For **24**

↓ 1- TFA 2- Boc-Nle-OSu

Boc-Nle-Asp(OBzl)-gPhe-For **25**

↓ 1- TFA 2- Z-Xxx / BOP

Z-Xxx-Nle-Asp(OBzl)-gPhe-For
Xxx = Trp **26**
Xxx = D-Trp **27**

↓ 1- H_2 - Pd/C 2- Boc-Tyr-Nle-Gly-OSu

Boc-Tyr-Nle-Gly-Xxx-Nle-Asp-gPhe-For
Xxx = Trp **28**
Xxx = D-Trp **29**

↓ SO_3 : pyridine

Boc-Tyr(SO_3^-)-Nle-Gly-Xxx-Nle-Asp-gPhe-For
Xxx = Trp **5**
Xxx = D-Trp **6**

(SO_3^-)-gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (**7**). Some of these modifications (e.g. compounds **1** and **2**) were shown to lead to gastrin antagonists in the gastrin series.[13] Compound **3** and **4** were obtained by introducing a gGly and the following D-amino acid residues, combined with either a mPhe-NH_2 (compound **3**), or by suppressing the primary amide function (compound **4**). Compound **5** was designed to further investigate the role of the C-terminal amide of CCK, by inversion of the CO–NH_2 bond (NHCOH in place of $CONH_2$),[14] whereas compound **6** combines the same modification and the reversal of configuration of the tryptophane residue. Retro-inverso peptide **7** was designed to prevent enzymatic cleavage between residues 28 and 29 of CCK, which is the major degradation process in rat hypothalamic synaptosomes.[15]

Chemistry

Syntheses of compounds **1–6** (outlined in Schemes I–IV) were carried out in solution. Synthesis of compound **7** has been reported elsewhere.[16] Treatment of the dipeptide Boc-Nle-Asp(OBzl)-NH_2 (**8**) (obtained from Boc-Nle and Asp(OBzl)-NH_2 through a mixed anhydride coupling) with [bis(trifluoroacetoxy)iodo]benzene[17] led to the corresponding trifluoroacetate salt of the *gem*-diamino alkyl derivative, which was coupled to benzylmalonic acid monoamide succinimido ester,[13] to lead to compound **9**. After removal of protecting groups and coupling to Z-Trp-OSu (synthesized according to Anderson et al.[18]), Z-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**10**) was obtained. Deprotection of compound **10**, coupling to Boc-Asp(OBzl)-Tyr-Nle-Gly-OSu,[2] hydrogenolysis in the presence of Pd/C as catalyst of the benzyl ester, and subsequent sulfation of the tyrosine residue (with pyridine–sulfur trioxide complex, as already described[2]) produced the retro-inverso peptide Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**1**). Attempts to separate the two enantiomers were unsuccessful because of racemization of the malonyl residue, as it has already been observed in substance P,[19] enkephalin,[20] and somatostatin[21] analogues.

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Table I. Physical and Analytical Data of the Partially Modified Retro-Inverso Peptide Analogues of the C-Terminal Octa- or Heptapeptide of Cholecystokinin Used in the Biological Tests

compounds	mp, °C	$[\alpha]_D$, deg (*c*, DMF)	*t*r, min (A/B)[e]	R_f[c]
Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**1**)[d]	180 dec	−14.4 (0.75)	(**1A**) 10.54 and (**1B**) 14.18 (45:55)[a]	0.12 and 0.10
Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (**2**)	160 dec	−20.3 (1.13)	18.26 (45:55)[a]	0.18
Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (**3**)[d]	110 dec	+8.0 (0.89)	(**3A**) 8.91 and (**3B**) 9.80 (30:70)[a]	0.28
Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**4**)	145 dec	+16.2 (0.94)	8.60 (32:68)[a]	0.30
Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (**5**)	130 dec	−12.7 (0.70)	22.18 (37:63)[b]	0.25
Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**6**)	160 dec	−8.6 (0.94)	18.40 (37:63)[b]	0.25
Boc-Asp-Tyr(SO_3^-)-gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (**7**)	190 dec	−11.2 (0.16)	7.98 (40:60)[a]	0.27

[a] HPLC purifications were run on a Merck–Hitachi instrument with a Beckman Ultrasphere ODS (5 μm) 10 × 250 mm column at a flow rate of 3 mL/min. [b] Beckman Ultrasphere ODS (10 μm) 21.2 × 150 mm column at a flow rate of 7 mL/min. [c] Solvent: ethyl acetate/pyridine/acetic acid/water, 80:20:5:10. [d] Mixture of diastereomers. [e] A mixture of 0.05 M ammonium acetate, pH 6.5 (A), and methanol (B), with UV detection at 279 nm.

Table II. Physical and Analytical Data of the Synthetic Peptide Derivatives

compounds	mp, °C	$[\alpha]_D$, deg (*c*, DMF)	R_f[a]	Anal. C, H, N
Boc-Nle-Asp(OBzl)-NH_2 (**8**)	115–117	−40.6 (0.84)	A, 0.54; B, 0.78	$C_{22}H_{33}N_3O_6$
Boc-Nle-gAsp(OBzl)-(*RS*)-mPhe-NH_2 (**9**)	162–164	−0.2 (1.39)	B, 0.71; C, 0.98	$C_{31}H_{42}N_4O_7$
Z-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**10**)	240 dec	−14.1 (1.17)	D, 0.76 and 0.59	$C_{38}H_{44}N_6O_8$
Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**11**)	235 dec	−12.2 (1.20)	D, 0.83 and 0.60	$C_{63}H_{80}N_{10}O_{15}$
Boc-Asp-Tyr-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**12**)	160 dec	−20.5 (1.36)	D, 0.42 and 0.34	$C_{56}H_{74}N_{10}O_{15}$
Boc-Nle-gAsp(OBzl)-$COCH_2CH_2C_6H_5$ (**13**)	141–145	−4.7 (1.57)	A, 0.91; B, 0.96	$C_{30}H_{41}N_3O_6$
Z-Trp-Nle-gAsp(OBzl)-$COCH_2CH_2C_6H_5$ (**14**)	208–210	−16.0 (1.10)	A, 0.61; B, 0.84	$C_{44}H_{49}N_5O_7$
Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (**15**)	210 dec	−16.1 (1.21)	C, 0.47; D, 0.91	$C_{62}H_{79}N_9O_{14}$
Boc-Asp-Tyr-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (**16**)	175 dec	−22.8 (1.48)	C, 0.18; D, 0.52	$C_{55}H_{73}N_9O_{14}$
Boc-Nle-Gly-NH_2 (**17**)	89–91	−7.0 (0.84)	A, 0.12; B, 0.51	$C_{13}H_{25}N_3O_4$
Boc-Nle-gGly-D-Trp-Z (**18**)	130–132	+13.7 (0.94)	A, 0.62; B, 0.84	$C_{31}H_{41}N_5O_6$
Boc-Nle-gGly-D-Trp-D-Nle-Z (**19**)	170–172	+6.0 (1.47)	A, 0.60; B, 0.84	$C_{37}H_{52}N_6O_7$
Boc-Nle-gGly-D-Trp-Nle-D-Asp(OBzl)-Z (**20**)	180 dec	+8.1 (0.83)	A, 0.52; B, 0.77	$C_{48}H_{63}N_7O_{10}$
Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z (**21**)	195 dec	+9.4 (0.80)	B, 0.64	$C_{57}H_{72}N_8O_{12}$
Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (**22**)	200 dec	+16.6 (1.36)	C, 0.42 and 0.38	$C_{52}H_{69}N_9O_{12}$
Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**23**)	185 dec	+19.9 (1.42)	C, 0.80; D, 0.94	$C_{51}H_{68}N_8O_{11}$
Boc-Asp(OBzl)-gPhe-For (**24**)	130–131	−12.3 (0.81)	A, 0.52; B, 0.74	$C_{25}H_{31}N_3O_6$
Boc-Nle-Asp(OBzl)-gPhe-For (**25**)	159–161	−21.8 (1.11)	A, 0.50; B, 0.69	$C_{31}H_{42}N_4O_7$
Z-Trp-Nle-Asp(OBzl)-gPhe-For (**26**)	230–232	−24.1 (0.94)	B, 0.50; C, 0.95	$C_{45}H_{50}N_6O_8$
Z-D-Trp-Nle-Asp(OBzl)-gPhe-For (**27**)	205–208	−7.2 (1.06)	B, 0.51; C, 0.95	$C_{45}H_{50}N_6O_8$
Boc-Tyr-Nle-Gly-Trp-Nle-Asp-gPhe-For (**28**)	190 dec	−13.6 (0.95)	C, 0.22; D, 0.62	$C_{52}H_{69}N_9O_{12}$
Boc-Tyr-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**29**)	190 dec	−23.0 (0.69)	C, 0.38; D, 0.74	$C_{52}H_{69}N_9O_{12}$

[a] Solvents: A, ethyl acetate/hexane, 7:3; B, ethyl acetate; C, chloroform/methanol/acetic acid, 85:10:5; D, ethyl acetate/pyridine/acetic acid/water, 80:20:5:10.

The trifluoroacetate salt of the *gem*-diamino alkyl derivative described above, (prepared from compound **8**), was coupled with BOP[22] to 3-phenylpropionic acid to afford compound **13**. After deprotection with trifluoroacetic acid and coupling to Z-Trp-OSu, compound Z-Trp-Nle-gAsp-(OBzl)-$COCH_2CH_2C_6H_5$ (**14**) was obtained. Deprotection of compound **14**, followed by coupling to Boc-Asp-(OBzl)-Tyr-Nle-Gly-OSu,[2] hydrogenolysis of the benzyl ester, and sulfation of the tyrosine residue, afforded Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2$-$CH_2C_6H_5$ (**2**).

Conversion of Boc-Nle-Gly-NH_2 (**17**) to the corresponding gem-diamino derivative and coupling to Z-Trp with BOP[22] afforded compound **18**, which led to Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z (**21**) after successive partial deprotections and couplings to Z-D-Nle, Z-D-Asp(OBzl), and Boc-Tyr, as described in Scheme III. Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (**3**) and Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**4**) were obtained from compound **21**, after partial deprotection, coupling to (*RS*)-mPhe-NH_2 and 3-phenylpropionic acid respectively through their succinimido esters, and subsequent sulfation (Scheme III). The two diastereomers of compound **3** were identified by HPLC, but they were not separated.

The dipeptide Boc-Asp(OBzl)-Phe-NH_2[23] was converted to the corresponding *gem*-diamino derivative, which was formylated with 2,4,5-trichlorophenyl formate[24] to lead to compound **24** containing the retro-inverso analogue of the C-terminal $CONH_2$ function, i.e. NH-For. From Boc-Asp(OBzl)-gPhe-For (**24**), a sequence of partial deprotections and couplings afforded Z-Trp-Asp(OBzl)-gPhe-For (**26**) and Z-D-Trp-Nle-Asp(OBzl)-gPhe-For (**27**). They were hydrogenated, coupled to Boc-Tyr-Nle-Gly-OSu,[2] and sulfated to lead respectively to Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (**5**) and Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**6**) (Scheme IV).

Analytical and physical data of the final compounds are listed in Table I, those of the intermediates are in Table II. Compounds **1–7** were identified by ^1H NMR spectroscopy. ^1H NMR spectra of compounds **5** and **6** showed the presence of two sets of conformers, because of the *Z*/*E* isomerism of the formamide bond (ratio 1/3).

Biological Results and Discussion

All retro-inverso peptides (compounds **1–7**) were tested for their ability to stimulate in vitro amylase release from rat pancreatic acini[25] (Figures 1 and 2) and to inhibit the

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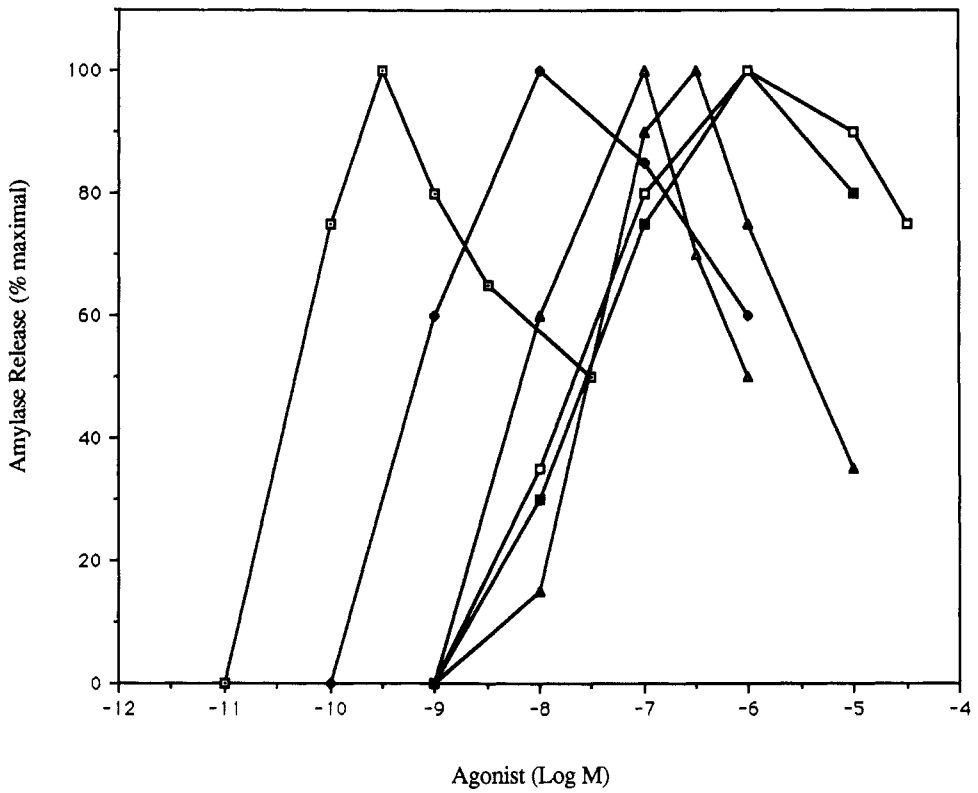

Figure 1. The effects of Boc-[Nle^{28},Nle^{31}]-CCK-7 (⊡) and of partially modified retro-inverso peptides Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (1) (◆), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (3) (■), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (4) (□), Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (5) (▲), and Boc-Asp-Tyr(SO_3^-)-gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (7) (△) on amylase release from rat pancreatic acini. Dispersed rat pancreatic acini were prepared and amylase release was measured according to previously described procedures.[26] Amylase activity was determined by the method of Ceska et al.[33] with Phadebas reagent. Amylase release was measured as the difference of amylase activity at the end of incubation that was released in the extracellular medium, with and without secretagogue, and expressed as percent of maximal stimulation. In each experiment, each value was determined in duplicate, and the results given are the means from at least three separate experiments.

Table III. Biological Activities of the Partially Modified Retro-Inverso Peptide Analogues of the C-Terminal Octa- or Heptapeptide of Cholecystokinin on the Binding to the Pancreatic and Brain CCK Receptors and on the Activity on Amylase Release from Rat Pancreatic Acini

	rat pancreatic acini		guinea pig brain membranes
	amylase stimulation: EC_{50}, nM	binding: IC_{50}, μM	binding: IC_{50}, μM
Boc[Nle^{28},Nle^{31}]-CCK 7	0.05	0.0015	0.0003
Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (1)[a]	0.7	0.1	0.01
Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (2)	130 (p)[b]	0.1	0.04
Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (3)	30	0.02	1.0
Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (4)	17	0.04	0.1
Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (5)	30	0.5	0.03
Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (6)	1700 (p)[b]	4.0	0.1
Boc-Asp-Tyr(SO_3^-)gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (7)	6	0.5	0.002

[a] Mixture of diastereomers. [b] (p): plateau.

binding of [^{125}I]BH-CCK-8 to isolated rat pancreatic acini[26] (Figure 3) and to guinea pig brain membranes[27] (Figure 4). All compounds were demonstrated by HPLC to remain unaffected in the incubation conditions. They were compared in the same experiments with the potent CCK analogue Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2 (Boc-[Nle^{28},Nle^{31}]-CCK-7).[28] The results are summarized in Table III. All compounds are less potent in inhibiting binding of [^{125}I]BH-CCK-8 to guinea pig brain membranes (half-maximal inhibition ranging from 2 to 2000 nM) than Boc-[Nle^{28},Nle^{31}]-CCK-7 (half-maximal inhibition at 0.3 nM). Compound **7**, the most potent, is able to recognize CCK central receptors with a good apparent affinity (half-maximal inhibition at 2 nM), in accordance with Charpentier et al.[29] These results are in good agreement with our previous observation[30] that chemical modifications affecting the C-terminal tetrapeptide of CCK lead to compounds exhibiting a decreased apparent affinity for central CCK receptors as compared to peripheral CCK receptors. Furthermore, the half-maximal inhibition value (2 nM) observed for compound **7** is very close to the one reported for CCK-4 (Trp-Met-Asp-Phe-NH_2, $IC_{50} \approx 1.7$ nM).[31] In compounds **1–6**, the structure of the parent

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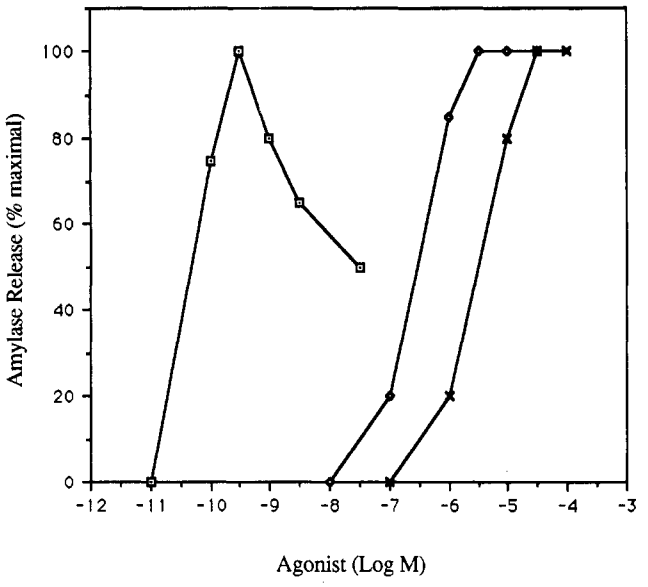

Figure 2. The effects of Boc-[Nle^{28},Nle^{31}]-CCK-7 (□) and of partially modified retro-inverso peptides Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (**2**) (◇) and Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**6**) (×) on amylase release from rat pancreatic acini. Dispersed rat pancreatic acini were prepared and amylase release was measured according to previously described procedures.[26] Amylase activity was determined by the method of Ceska et al.[33] with Phadebas reagent. Amylase release was measured as the difference of amylase activity at the end of incubation that was released in the extracellular medium, with and without secretagogue, and expressed as percent of maximal stimulation. In each experiment, each value was determined in duplicate, and the results given are the means from at least three separate experiments.

molecule was mainly modified in the C-terminal part, whereas in compound **7** the modification only affects the bond between the Nle^{28} and Gly^{29} residues. In contrast with the results obtained in the gastrin series,[13] none of the retro-inverso peptides synthesized in this work behave as CCK antagonists. On stimulation of amylase release from rat pancreatic acini, compounds **1**, **3–5**, and **7** have a biphasic dose–response curve. As the peptide derivative concentration increases, amylase stimulation increases, becomes maximal, and decreases at supramaximal concentrations (Figure 1). On the other hand, compounds **2** and **6** show a plateau of maximal stimulation at high concentrations (Figure 2). This type of dose–response curve was first observed by our group with CCK analogues in which the C-terminal amide function had been deleted[1,2] (i.e. Boc-Tyr(SO_3)-Nle-Gly-Trp-Nle-Asp-$OCH_2CH_2C_6H_5$ or Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-NH-$CH_2CH_2C_6H_5$). As expected, compound **2**, which does not contain the C-terminal amide group shows a plateaued dose–response curve. Surprisingly, Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**4**) does not exhibit a plateau of stimulation but a biphasic dose–response curve, although it does not contain the C-terminal amide function. This result is in apparent contradiction with our previous conclusions pointing out the crucial role of the C-terminal amide function in the inhibition of amylase secretion at supramaximal concentrations of the agonist[1] and indicates that the C-terminal primary amide function is not the only structural requirement responsible for the downstroke of the dose–response curve at supramaximal concentrations. These findings suggest that spatial orientation of side chains of the C-terminal amino acid residues of the CCK molecule might be of some importance for the full biological response on amylase secretion. Compounds **5** and **6** were designed to investigate the functional role of the C-terminal amide of CCK, by simply inverting the direction of the $CONH_2$ bond. According to our previous results,[1,2] we believed that the integrity of the C-terminal amide moiety was an essential requirement for full CCK-like biological activity. Unexpectedly, again, compound **5** behaves as a full agonist of CCK, with, however, a reduced potency as compared to Boc-[Nle^{28},Nle^{31}]-CCK-7. Interestingly, compound **6** which is identical with compound **5**, but contains a D-tryptophan, is able to induce maximal response of amylase release from isolated rat pancreatic acini, with no decrease at supramaximal concentrations. However, the weak potency of compound **5** makes it difficult to ascertain the maximal response at high concentrations such as 1 mM. Nevertheless, it is interesting to notice that replacement of L-tryptophan by D-tryptophan leads to an analogue exhibiting the full response of amylase release from rat pancreatic acini to an analogue exhibiting the maximal response, with no decrease at supramaximal concentrations.

All partial retro-inverso analogues of cholecystokinin described in this work have a decreased apparent affinity for the CCK pancreatic receptors as compared to Boc-[Nle^{28},Nle^{31}]-CCK-7, in contrast with most of the retro-inverso analogues of tetragastrin, which were able to bind to the gastrin receptor with the same apparent affinity as tetragastrin.

Conclusions

From this study on partially modified retro-inverso analogues of cholecystokinin, it appears once again that the integrity of the C-terminal tetrapeptide of CCK is an essential requirement for efficient binding to central CCK receptors. Although retro-inverso modifications are not supposed to alter the general topochemistry of the extended conformation of the parent molecule, we have obtained CCK analogues less potent in binding to CCK pancreatic receptors than Boc-[Nle^{28},Nle^{31}]-CCK-7. This can be due to the fact that "bioactive conformation" in partially modified retro-inverso analogues can be different from that of the parent peptide. However, some of the structure–activity relationships observed in this study remain poorly understood and actually unexplained. Further works in order to ascertain both the role of the C-terminal amide moiety, of its spatial orientation, and of the chirality of tryptophan residue of CCK, in relation with CCK receptors, are in progress in our laboratory.

Experimental Section

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, 7:3; B, AcOEt; C, chloroform/methanol/acetic acid, 85:10:5; D, AcOEt/pyridine/acetic acid/water, 80:20:5:10. 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 (5 μm) 10 × 250 mm column at a flow rate of 3 mL/min or on a Beckman Ultrasphere ODS (10 μm) 21.2 × 150 mm column at a flow rate of 7 mL/min, with a mixture of ammonium acetate 0.05 M, pH 6.5, and methanol, with UV detection at 279 nm. ^{1}H NMR spectra were run on a Brücker 360 instru-

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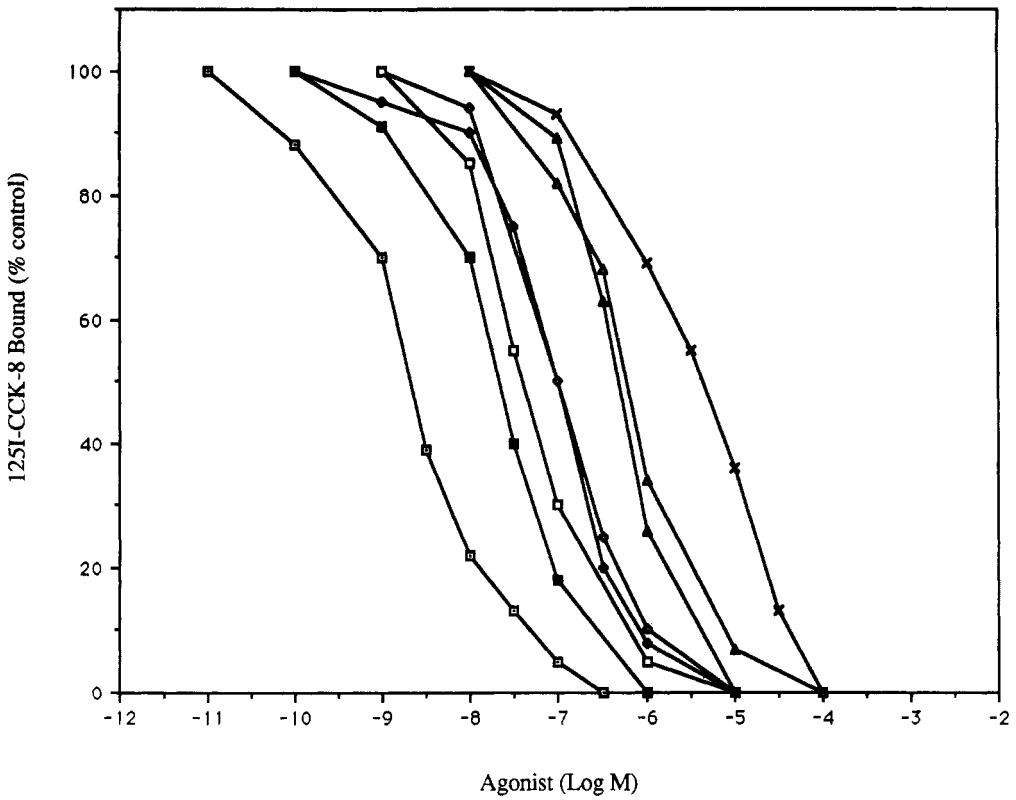

Figure 3. Abilities of Boc-[Nle^{28},Nle^{31}]-CCK-7 (⊡) and of partially modified retro-inverso peptides Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (1) (◆), Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (2) (◇), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (3) (■), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (4) (□), Boc-Tyr-(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (5) (▲), Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (6) (×), and Boc-Asp-Tyr(SO_3^-)-gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (7) (△) to inhibit binding of labeled [^{125}]BH-CCK-8 (Amersham) to rat pancreatic acini. Binding of [^{125}I]BH-CCK-8 was performed as described by Jensen et al.[26] Acini were incubated for 30 min at 37 °C with various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 or of partially modified retro-inverso peptides 1–7, plus 10 pM labeled CCK-8. Values are expressed as the percentage of the value obtained with labeled CCK-8 alone. In each experiment, each value was determined in duplicate, and the results given are the means from at least three separate experiments.

ment at 293 K. Amino acids and derivatives were purchased from Bachem (Switzerland). All reagents and solvents were of analytical grade. The following abbreviations were used: DMF, dimethylformamide; HOBT, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; DIEA, *N*,*N*-diisopropylethylamine; BOP, (benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate; NMM, *N*-methylmorpholine; IBCF, isobutyl chloroformate; TFA, trifluoroacetic acid. [^{125}I]BH-CCK-8 is ^{125}I-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionyl-CCK-8. Other abbreviations used were those recommended by the IUPAC–IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9–37). The standard three-letter notation for amino acid residues preceded by the prefix g represents the *gem*-diaminoalkyl residue derived from the specified amino acid. The prefix m represents the malonic acid residue derived from the amino acid specified by the three-letter notation (Chorev et al. *J. Med. Chem.* **1983**, *26*, 129). Configuration designations of the modified residues follows those of the amino acids.

Boc-Nle-Asp-(OBzl)-NH_2 (8). To a cold (0 °C) solution of Boc-Nle (2.80 g, 12.1 mmol) in DMF (20 mL) were successively added NMM (1.36 mL, 12.1 mmol) and IBCF (1.64 mL, 12.1 mmol). After 5 min of stirring, the trifluoroacetate salt of Asp-(OBzl)-NH_2 (3.73 g, 11.1 mmol) was added, followed by DIEA (1.91 mL, 11.1 mmol), and the solution was stirred for 30 min at 0 °C and 30 min more at room temperature. The expected compound precipitated upon addition of saturated aqueous sodium bicarbonate (200 mL). It was collected by filtration, washed with saturated aqueous sodium bicarbonate, water, 1 M potassium hydrogen sulfate, and water, and dried in vacuo, yield 4.26 g (88%). Physical and analytical data are reported in Table II.

Boc-Nle-gAsp(OBzl)-(*RS*)-mPhe-NH_2 (9). To a suspension of dipeptide 8 (2.0 g, 4.59 mmol), in a mixture of acetonitrile and water (1:1 20 mL), was added [bis(trifluoroacetoxy)iodo]benzene[17] (2.15 g, 5.0 mmol). After 3 h of stirring at room temperature, a clear solution resulted and no more starting material could be detected by TLC. The solvents were removed under reduced pressure to leave a residue that was dried in vacuo over phosphorus pentoxide. It was added to a solution of (*R*,*S*)-2-benzylmalonic acid monoamide succinimido ester[13] (1.16 g, 4.0 mmol) in DMF (20 mL), followed by DIEA (0.80 mL, 4.59 mmol). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (200 mL). It was collected by filtration, washed with 1 M potassium hydrogen sulfate, water, saturated aqueous sodium bicarbonate, water, ethyl acetate, and ether, and dried in vacuo, yield 1.20 g (51%). Physical and analytical data are reported in Table II.

Z-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (10). Compound 9 (1.1 g, 1.89 mmol) was deprotected by hydrogenation for 4 h at room temperature and atmospheric pressure, in a mixture of DMF and 95% ethanol (1:1, 100 mL). The catalyst was filtered off and the filtrate was concentrated under reduced pressure to leave a residue which was dissolved in TFA. After 30 min at room temperature, addition of ether afforded the trifluoroacetate salt of Nle-gAsp-(*RS*)-mPhe-NH_2 as a white precipitate (0.750 g, 1.48 mmol, 79%), which was dried in vacuo over KOH pellets. It was added to a solution of Z-Trp-OSu[18] (0.609 g, 1.4 mmol) in DMF (5 mL), followed by DIEA (0.25 mL, 1.48 mmol). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (200 mL). It was collected by filtration, washed with 1 M potassium hydrogen sulfate, water, and ether, and dried in vacuo, yield 0.870 g (87%). Physical and analytical data are reported in Table II.

Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (11). Compound 10 (0.420 g, 0.589 mmol) was hydrogenated for 7 h in a mixture of DMF, acetic acid, and water (15:1:1, 50 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a residue,

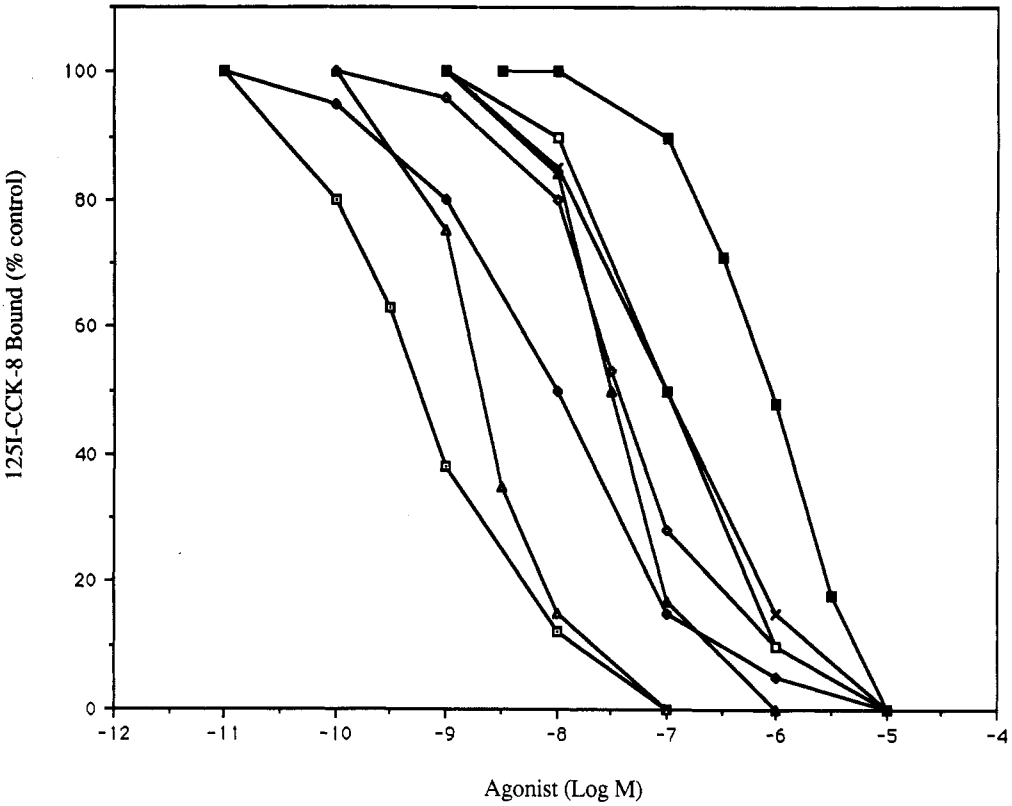

Figure 4. Abilities of Boc-[Nle^{28},Nle^{31}]-CCK-7 (⊡) and of partially modified retro-inverso peptides Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (**1**) (◆), Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-CO-CH_2-CH_2-C_6H_5 (**2**) (◇), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (**3**) (■), Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (**4**) (□), Boc-Tyr-(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (**5**) (▲), Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (**6**) (×), and Boc-Asp-Tyr(SO_3^-)-gNle-mGly-Trp-Nle-Asp-Phe-NH_2 (**7**) (△) to inhibit binding of labeled [^{125}I]BH-CCK-8 (Amersham) to guinea pig brain membranes. Binding of [^{125}I]BH-CCK-8 was performed as described by Pelaprat et al.[27] Brain membranes were incubated for 40 min at 25 °C with various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 or of partially modified retro-inverso peptides **1–7**, plus 15 pM labeled CCK-8. Values are expressed as the percentage of the value obtained with labeled CCK-8 alone. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7. In each experiment, each value was determined in duplicate, and the results given are the means from four separate experiments.

which crystallized upon trituration with ether. It was collected, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of Boc-Asp(OBzl)-Nle-Gly-OSu[2] (0.391 g, 0.52 mmol) in DMF (5 mL), followed by DIEA (0.10 mL, 0.589 mmol). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 0.1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 0.1 M potassium hydrogen sulfate, water, and ether, and dried in vacuo, yield 0.582 g (92%). Physical and analytical data are reported in Table II.

Boc-Asp-Tyr-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (12). Compound **11** (0.515 g, 0.423 mmol) was hydrogenated for 4 h in a mixture of DMF, acetic acid, and water (30:5:5, 40 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a residue, which crystallized upon trituration with ether. It was collected, washed with ether, and dried in vacuo over KOH pellets, yield 0.470 g (98%). Physical and analytical data are reported in Table II.

Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-(*RS*)-mPhe-NH_2 (1). To a solution of compound **12** (0.250 g, 0.222 mmol) in a mixture of DMF (2 mL) and pyridine (2 mL) was added SO_3–pyridine complex (1.5 g). After overnight stirring at room temperature, the solvents were concentrated under reduced pressure, and the excess complex was hydrolyzed at 0 °C 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 1 M potassium hydrogen sulfate and extracted with *n*-BuOH (3 × 20 mL). The organic layers were washed with water and concentrated under reduced pressure to leave a solid residue, which was triturated with ether, collected, and dried in vacuo. It was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 60:20:5:10) to afford compound **1** as a white solid, yield 0.154 g (57%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Boc-Nle-gAsp(OBzl)-$COCH_2CH_2C_6H_5$ (13). To a suspension of dipeptide **8** (2 g, 4.59 mmol) in a mixture of acetonitrile and water (1:1, 20 mL), was added [bis(trifluoroacetoxy)iodo]benzene[17] (2.15 g, 5.0 mmol). After 3 h of stirring at room temperature, a clear solution resulted and no more starting material could be detected by TLC. The solvents were removed under reduced pressure to leave a residue that was dried in vacuo over phosphorus pentoxide. The residue was added to a solution of 3-phenylpropionic acid (1.35 g, 9.0 mmol) and BOP[22] in DMF (20 mL), followed by DIEA (2.33 mL, 13.59 mmol). After 1 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (150 mL). The organic layer was washed with aqueous 1 M potassium hydrogen sulfate (3 × 100 mL), water (100 mL), aqueous saturated sodium bicarbonate (3 × 100 mL), and brine, dried over magnesium sulfate, and concentrated in vacuo to leave a residue that crystallized upon trituration in a mixture of ether and hexane (1:3). It was collected by filtration, washed with hexane, and dried in vacuo, yield 1.97 g (80%). Physical and analytical data are reported in Table II.

Z-Trp-Nle-gAsp(OBzl)-$COCH_2CH_2C_6H_5$ (14). Compound **13** (1.40 g, 2.59 mmol) was treated with TFA for 30 min at room temperature. Upon addition of ether, the partially deprotected material precipitated. It was collected by filtration, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of Z-Trp-OSu[18] (1.04 g, 2.4 mmol) in DMF (15 mL), followed by DIEA (0.45 mL, 2.59 mmol). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (200 mL). It was collected by filtration, washed with 1 M potassium hydrogen

sulfate, water, saturated aqueous sodium bicarbonate, water, and ether, and dried in vacuo, yield 1.63 g (89%). Physical and analytical data are reported in Table II.

Boc-Asp(OBzl)-Tyr-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (15) was synthesized from compound **14** (0.511 g, 0.673 mmol), after hydrogenation and coupling to Boc-Asp(OBzl)-Tyr-Nle-Gly-OSu[2] (0.497 g, 0.660 mmol) as described for compound **11**, yield 0.766 g (98%). Physical and analytical data are reported in Table II.

Boc-Asp-Tyr-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (16). Synthesized from compound **15** (0.650 g, 0.553 mmol), after hydrogenation as described for compound **12**, yield 0.590 g (98%). Physical and analytical data are reported in Table II.

Boc-Asp-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-gAsp-$COCH_2CH_2C_6H_5$ (2) was synthesized from compound **16** (0.250 g, 0.230 mmol), as described for compound **1**. Compound **2** was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 80:20:5:10), yield 0.175 g (65%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Boc-Nle-Gly-NH_2 (17). To a solution of Boc-Nle-OSu[18] (4.1 g, 12.48 mmol) in DMF (20 mL) were successively added glycinamide hydrochloride (1.75 g, 14.0 mmol) and DIEA (2.41 mL, 14.0 mmol). After 2 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (250 mL). This solution was washed with a aqueous 1 M potassium hydrogen sulfate (3 × 100 mL), water (100 mL), aqueous saturated sodium bicarbonate (3 × 100 mL), and brine, dried over magnesium sulfate, and concentrated in vacuo to leave a residue that crystallized upon standing in the cold, yield 3.07 g (86%). Physical and analytical data are reported in Table II.

Boc-Nle-gGly-D-Trp-Z (18). To a suspension of dipeptide **17** (2.98 g, 10.37 mmol), in a mixture of acetonitrile and water (1:1, 60mL), was added [bis(trifluoroacetoxy)iodo]benzene[17] (4.51 g, 10.50 mmol). After 3 h of stirring at room temperature, a clear solution resulted and no more starting material could be detected by TLC. The solvents were removed under reduced pressure to leave a residue that was dried in vacuo over phosphorus pentoxide. The residue was added to a solution of Z-D-Trp (3.38 g, 10.0 mmol) and BOP[22] (4.42 g, 10.0 mmol) in DMF (50mL), followed by NMM (2.3 mL, 20.37 mmol). After 3 h of stirring at room temperature, the reaction mixture was treated as described for compound **17**. Compound **18** crystallized upon trituration in ether, yield 2.48 g (43%). Physical and analytical data are reported in Table II.

Boc-Nle-gGly-D-Trp-D-Nle-Z (19). Compound **18** (2.3 g, 3.97 mmol) was hydrogenated in a mixture of DMF, acetic acid, and water (50:3:3, 56 mL) as already described. To a cold (0 °C) solution of Z-D-Nle (1.06 g, 4.0 mmol) in DMF (10mL) were successively added NMM (0.45 mL, 4.0 mmol) and IBCF (0.54 mL, 4.0 mmol). After 5 min of stirring, the partially deprotected material obtained above was added, followed by DIEA (0.68 mL, 3.97 mmol), and the solution was stirred for 30 min at 0 °C and 30 min at room temperature. The expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 1 M potassium hydrogen sulfate, water, saturated aqueous sodium bicarbonate, and water, and dried in vacuo, yield 2.23 g (81%). Physical and analytical data are reported in Table II.

Boc-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z (20) was synthesized from compound **19** (2.1 g, 3.03 mmol), after hydrogenation and coupling to Z-D-Asp(OBzl) through a mixed anhydride, as described for compound **19**, yield 1.91 g (70%). Physical and analytical data are reported in Table II.

Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-Z (21). Compound **20** (1.8 g, 2.0 mmol) was treated with TFA in the usual way. The partially deprotected material was added to a solution of Boc-Tyr-OSu[18] (0.719 g, 1.90 mmol) in DMF (10mL), followed by DIEA (0.35 mL, 2.0 mmol). After 2 h of stirring at room temperature, the expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 1 M potassium hydrogen sulfate, water, saturated aqueous sodium bicarbonate, water, and ether, and dried in vacuo, yield 1.76 g (87%). Physical and analytical data are reported in Table II.

Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (22). Compound **21** (0.850 g, 0.80 mmol) was hydrogenated for 5 h in a mixture of DMF, acetic acid, and water (40:5:5, 100 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a residue, which crystallized upon trituration with ether. It was collected, washed with ether and dried in vacuo over KOH pellets. It was added to a solution of (*R,S*)-2-benzylmalonic acid monoamide succinimido ester[13] (0.225 g, 0.777 mmol) in DMF (5 mL). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 0.1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 0.1 M potassium hydrogen sulfate, water, and ether, and dried in vacuo, yield 0.623 g (79%). Physical and analytical data are reported in Table II.

Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-(*RS*)-mPhe-NH_2 (3) was synthesized from compound **22** (0.300 g, 0.296 mmol), as described for compound **1**. Compound **3** was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 80:20:5:10), yield 0.175 g (65%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Boc-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (23). Compound **21** (0.690 g, 0.65 mmol) was hydrogenated for 5 h in a mixture of DMF, acetic acid, and water (40:5:5, 80 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a residue, which crystallized upon trituration with ether. It was collected, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of 3-phenylpropionic acid succinimido ester[13] (0.153 g, 0.62 mmol) in DMF (5 mL). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 0.1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 0.1 M potassium hydrogen sulfate, water, and ether, and dried in vacuo, yield 0.495 g (82%). Physical and analytical data are reported in Table II.

Boc-Tyr(SO_3^-)-Nle-gGly-D-Trp-D-Nle-D-Asp-$COCH_2CH_2C_6H_5$ (4) was synthesized from compound **23** (0.250 g, 0.259 mmol), as described for compound **1**. Compound **4** was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 80:20:5:10), yield 0.100 g (37%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Boc-Asp(OBzl)-gPhe-For (24). To a suspension of Boc-Asp(OBzl)-Phe-NH_2[23] (3.50 g, 7.45 mmol) in a mixture of acetonitrile and water (1:1, 60 mL), was added [bis(trifluoroacetoxy)iodo]benzene[17] (3.44 g, 8.0 mmol). After 4 h of stirring at room temperature, a clear solution resulted and no more starting material could be detected by TLC. The solvents were removed under reduced pressure to leave a residue that was dried in vacuo over phosphorus pentoxide. It was added to a solution of 2,4,5-trichlorophenyl formate[24] (1.70 g, 7.50 mmol) in DMF (20 mL), followed by DIEA (1.30 mL, 7.45 mmol). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 1 M potassium hydrogen sulfate (200 mL). The precipitate was collected by filtration, washed with 1 M potassium hydrogen sulfate, water, saturated aqueous sodium bicarbonate, water, and ether, and dried in vacuo, yield 2.48 g (71%). Physical and analytical data are reported in Table II.

Boc-Nle-Asp(OBzl)-gPhe-For (25) was synthesized from compound **24** (2.2 g, 4.68 mmol), after partial deprotection by TFA and coupling to Boc-Nle-OSu.[18] It was isolated according to the procedure described for compound **24**, yield 2.14 g (78%). Physical and analytical data are reported in Table II.

Z-Trp-Nle-Asp(OBzl)-gPhe-For (26) was synthesized from compound **25** (0.70 g, 1.2 mmol) after partial deprotection by TFA and a BOP[22]-mediated coupling to Z-Trp. It was isolated according to the procedure described for compound **24**, yield 0.905 g (94%). Physical and analytical data are reported in Table II.

Z-D-Trp-Nle-Asp(OBzl)-gPhe-For (27) was synthesized from compound **25** (0.827 g, 1.42 mmol) after partial deprotection by TFA and a BOP[22]-mediated coupling to Z-D-Trp. It was isolated according to the procedure described for compound **24**, yield 1.11 g (97%). Physical and analytical data are reported in Table II.

Boc-Tyr-Nle-Gly-Trp-Nle-Asp-gPhe-For (28). Compound **26** (0.803 g, 1.0 mmol) was hydrogenated for 5 h in a mixture of DMF, acetic acid, and water (30:5:5, 40 mL) in the presence of a 10% Pd/C catalyst at room temperature and atmospheric pressure. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to leave a residue, which crystallized upon trituration with ether. It was collected, washed with ether, and dried in vacuo over KOH pellets. It was added to a solution of Boc-Tyr-Nle-Gly-OSu[2] (0.493 g, 0.90 mmol) in DMF (5 mL). After 3 h of stirring at room temperature, the expected compound precipitated upon addition of 0.1 M potassium hydrogen sulfate (100 mL). It was collected by filtration, washed with 0.1 M potassium hydrogen sulfate, water, and ether, and dried in vacuo, yield 0.850 g (93%). Physical and analytical data are reported in Table II.

Boc-Tyr-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (29) was synthesized from compound **27** (0.803 g, 1.0 mmol), after hydrogenation and coupling to Boc-Tyr-Nle-Gly-OSu[2] as described for compound **28**, yield 0.900 g (99%). Physical and analytical data are reported in Table II.

Boc-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-Asp-gPhe-For (5). Synthesized from compound **28** (0.250 g, 0.246 mmol), as described for compound **1**. Compound **5** was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 80:20:5:10), yield 0.169 g (62%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Boc-Tyr(SO_3^-)-Nle-Gly-D-Trp-Nle-Asp-gPhe-For (6) was synthesized from compound **29** (0.260 g, 0.257 mmol), as described for compound **1**. Compound **6** was purified by silica gel chromatography (eluent: ethyl acetate/pyridine/acetic acid/water 80:20:5:10), yield 0.128 g (46%). It was finally purified by HPLC and lyophilized. Physical and analytical data are reported in Table I.

Biological Evaluations. Male guinea pigs (280–300 g) were obtained from le Centre d'Elevage d'Animaux de Laboratoire (Ardenay, France); male Wistar rats (180-200 g) were from Effa-Credo (Saint Germain l'Arbresle, France). Hepes was from Boehringer-Mannheim; purified collagenase was from Serva (Garden City Park, NY); soybean trypsin inhibitor was from Sigma (St. Louis, MO); Eagle's basal amino acid medium (100 times concentrated) was from GIBCO (Grand Island, NY); essential vitamin mixture (100 times concentrated) was from Microbiological Associates (Bethesda, MD); Bovine Plasma Albumin (fraction V) was from Miles Laboratories Inc. (Elkhart, IN); Phadebas amylase test was from Pharmacia Diagnostics (Piscataway, NJ), and ^{125}I-labeled *N*-succinimidyl-3-(4-hydroxyphenyl)propionyl-CCK-8 ([^{125}I]BH-CCK-8) was from Amersham Corp. (Buckinghamshire, UK). Unless otherwise stated, the standard incubation solutions contained 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH_2PO_4, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 mg/mL bacitracin, 0.2% (w/v) albumin, 0.03% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) essential vitamin mixture. The incubation solution was equilibrated with 95% O_2, 5% CO_2 as the gas phase. Dispersed acini from rat pancreas were prepared according to the previously described modifications[25] of the method of Peikin et al.[32] Guinea pig brain membranes were prepared following the procedures described by Pelaprat et al.[27] Amylase release was measured using the procedure already described[26] for preparation of guinea pig pancreatic acini. Briefly, acini were resuspended in the standard incubation solution complemented with 1% bovine serum albumin, 1 mM calcium, and 5 mM theophylline containing about 1 mg of protein/mL, and samples (1 mL) were incubated at 37 °C for 30 min. Amylase activity was determined by the method of Ceska et al.[33] using the Phadebas reagent. Amylase release was measured as the difference of amylase activity at the end of incubation that was released into the extracellular medium, with and without secretagogue and expressed as the percentage of maximal stimulation obtained with Boc-[Nle^{28},Nle^{31}]-CCK-7 (40 ± 5% of the total amylase contained in the acini) minus the basal amylase secretion (10 ± 2% of the total amylase contained in the acini) obtained without secretagogue.

Binding Studies. Binding of [^{125}I]CCK-8 to rat pancreatic acini was performed as previously described.[26] Briefly, samples (0.5 mL containing ≈ 1 mg/mL protein) were incubated with the appropriate peptide concentrations for 30 min at 37 °C in the presence of 10 pM [^{125}I]CCK-8 plus various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7. After centrifugation at 10000g for 10 min and washings, the radioactivity associated with the acinar pellet was measured. Values are expressed as the percentage of the value obtained with labeled CCK-8 alone. The specific activities of the various preparations used in our experiments was 2000 Ci/mmol. Acini from three rat pancreata were suspended in 100 mL of standard incubation solution. Specific binding in the absence of any unlabeled CCK-peptide was 13 ± 3% of the total radioactivity present in the sample. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7 and was always less than 15% of the total binding. Binding of [^{125}I]CCK-8 to guinea pig brain membranes was performed according to Pelaprat et al.[27] The buffer used was 50 mM Tris·HCl, 5 mM $MgCl_2$, 0.1 mg/mL bacitracin, pH 7.4 (Tris/$MgCl_2$/bacitracin buffer). Briefly, displacement experiments were performed by incubation of 1 mL of brain membranes (approximately 0.5 mg of protein) in the presence of 15 pM [^{125}I]CCK-8 for 60 min at 25 °C in the presence of various concentrations of Boc-[Nle^{28},Nle^{31}]-CCK-7 or compounds to be tested in a total volume of 1 mL. Nonspecific binding was determined in the presence of 1 μM Boc-[Nle^{28},Nle^{31}]-CCK-7 and was always less than 25% of the total binding. Total binding was about 10% of the total radioactivity contained in the sample.

Registry No. (*R*)-**1**, 121918-60-3; (*S*)-**1**, 121961-24-8; **2**, 121961-23-7; (*R*)-**3**, 121888-28-6; (*S*)-**3**, 121959-94-2; **4**, 121888-29-7; **5**, 121888-30-0; **6**, 121959-91-9; **7**, 122044-11-5; **8**, 94236-49-4; (*R*)-**9**, 121888-31-1; (*S*)-**9**, 121959-95-3; (*R*)-**10**, 121888-32-2; (*S*)-**10**, 121959-96-4; (*R*)-**11**, 121888-33-3; (*S*)-**11**, 121959-97-5; (*R*)-**12**, 121888-34-4; (*S*)-**12**, 121959-98-6; **13**, 121888-35-5; **14**, 121888-36-6; **15**, 121888-37-7; **16**, 121888-38-8; **17**, 121888-39-9; **18**, 121888-40-2; **19**, 121888-41-3; **20**, 121888-42-4; **21**, 121888-43-5; (*R*)-**22**, 121888-44-6; (*S*)-**22**, 121959-99-7; **23**, 121888-45-7; **24**, 121888-46-8; **25**, 121888-47-9; **26**, 121888-48-0; **27**, 121959-92-0; **28**, 121888-49-1; **29**, 121959-93-1; BOC-Nle-OH, 6404-28-0; H-Asp(OBzl)-NH_2·TFA, 92762-94-2; (*R,S*)-$H_2NCOCH(CH_2Ph)COOSu$, 109318-34-5; H-Nle-gAsp-(*R*)-mPhe-NH_2·TFA, 121888-51-5; H-Nle-gAsp-(*S*)-mPhe-NH_2·TFA, 121960-01-8; H-Trp-Nle-gAsp-(*R*)-mPhe-NH_2, 121888-52-6; H-Trp-Nle-gAsp-(*S*)-mPhe-NH_2, 121960-02-9; BOC-Asp(OBzl)-Nle-Gly-OSu, 121888-53-7; $PhCH_2CH_2COOH$, 501-52-0; BOC-Nle-OSu, 36360-61-9; H-Gly-NH_2·HBr, 55264-42-1; Z-D-Trp-OH, 2279-15-4; Z-D-Nle-OH, 15027-14-2; Z-D-Asp-(OBzl)-OH, 5241-62-3; BOC-Tyr-OSu, 20866-56-2; $PhCH_2CH_2COOSu$, 109318-10-7; BOC-Asp(OBzl)-Phe-NH_2, 60058-69-7; Z-Trp-OH, 7432-21-5; BOC-Tyr-Nle-gGly-D-Trp-D-Nle-D-Asp(OBzl)-H, 121888-55-9; Z-Trp-Nle-Asp(OBzl)-gPhe-H, 121888-54-8; Z-Trp-OSu, 50305-28-7; BOC-Tyr-Nle-Gly-OSu, 119005-56-0; amylase, 9000-92-4.

Supplementary Material Available: Tables IV–IX listing ^{1}H NMR data for **1–6** and a table listing analysis data for **8–29** (7 pages). Ordering information is given on any current masthead page.

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