the Ames bacterial assay, and is not metabolized to procainamide (dog). The compound is orally bioavailable (41–98%, dog, 3 mg/kg, po; elimination $t_{1/2} \approx 2.7$ h) and shows efficacy with a duration >2 h in canine models of reentrant ventricular tachycardia. Further details of medicinal chemistry and preclinical pharmacology are given in the supplementary material or will be reported in forthcoming publications.

Supplementary Material Available: Synthetic procedure for **3a**, pharmacology procedures (intracellular electrophysiology, extracellular electrophysiology, intraduodenal bioavailability, and PES protocol) and additional V_{max} and CT data (Table III) (14 pages). Ordering information is given on any current masthead page.

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Received September 22, 1986

Articles

Synthesis and Biological Activity of Partially Modified Retro-Inverso Pseudopeptide Derivatives of the C-Terminal Tetrapeptide of Gastrin

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The effects of partial retro-inverso modifications of selected peptide bonds of the N-terminal tetrapeptide of gastrin have been studied. In some of the synthesized compounds, the phenylalanyl residue has been replaced by the (*R,S*)-2-benzylmalonyl, 3-phenylpropionyl, benzylcarbamoyl, or benzyloxycarbonyl moieties. All pseudopeptides showed affinity for the gastrin receptor, in vitro, with potencies varying from $IC_{50} = 10^{-7}$ to $IC_{50} = 10^{-4}$ M. These compounds exhibited little or no activity on acid secretion in the anesthetized rat but were able to antagonize the action of gastrin. Among the most potent were Boc-Trp-Leu-gAsp-CO-$CH_2CH_2C_6H_5$ (**20**) ($ED_{50} = 0.15$ μM/kg), Boc-Trp-Leu-gAsp-m(*R,S*)Phe-NH_2 (**3**) ($ED_{50} = 0.15$ μM/kg), and Boc-Trp-gLeu-D-Asp-m(*R,S*)Phe-NH_2 (**7**) ($ED_{50} = 0.3$ μM/kg).

Gastrin, a polypeptide hormone, has been isolated from hog antral mucosa by Gregory and Tracy[1] and exists as gastrin I (sulfated form) and gastrin II (unsulfated form). Gastrin exhibits a wide range of biological actions, the most potent of which is stimulation of gastric acid secretion and antral smooth muscle activity.[2] Extensive structure–function relation studies in several laboratories have provided convincing evidence on the role of the C-terminal tetrapeptide of gastrin and of each of its amino acid residues. The biological potency of the gastrins is related to the C-terminal tetrapeptide amide Trp-Met-Asp-Phe-NH_2.[3] Replacement of the tryptophan lowers but does not destroy the activity. Many changes can be made at the methionine position without a resulting loss of activity (particularly the replacement by leucine or norleucine), whereas all changes affecting the aspartic acid lead to very weak or inactive compounds.[4] Although some modifications are permitted at the phenylalanyl residue, its suppression led to compounds that are able to antagonize the action of gastrin on acid secretion.[5] Removal of the terminal amide function resulted in completely inactive derivative on acid secretion,[4] whereas these compounds were able to recognize the gastrin receptor and to antagonize the action of gastrin on acid secretion.[6,7] We recently showed the significance of the peptide bonds of the C-terminal tetrapeptide of gastrin for the biological activity, particularly of the bond between methionine and aspartic acid.[8] For exhibiting biological activity on acid secretion, the bond between methionine and aspartic acid should be a peptide bond. Pseudopeptide derivatives in which this peptide bond has been replaced by a noncleavable bond, e.g., a CH_2NH bond, were shown to bind to the gastrin receptor and to inhibit gastrin-induced acid secretion[8,9] ($IC_{50} = 0.3$ μM, $ED_{50} = 0.45$ μM/kg). These results prompted us to hypothesize about the role of the C-terminal dipeptide of gastrin that might be the result of the

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0022-2623/87/1830-0758$01.50/0

Scheme I

Z-D-Asp(OBzl)-OH + H-D-Leu-NH_2

↓

Z-D-Asp(OBzl)-D-Leu-NH_2 (1)

↓ $PhI(CF_3CO_2)_2$

[Z-D-Asp(OBzl)-g(D-Leu)-H·TFA]

↓ Boc-Trp-OSu

Boc-Trp-gLeu-(OBzl)Asp-D-Z (2)

↓ 1. H_2, Pd/C; 2. H_2N-CO-CH($CH_2C_6H_5$)-COOSu

Boc-Trp-gLeu-D-Asp-m(*R,S*)Phe-NH_2 (3)

Scheme II

Boc-Leu-Asp(OBzl)-NH_2 (4)

↓ $PhI(CF_3CO_2)_2$

[Boc-Leu-gAsp(OBzl)-H·TFA]

↓ H_2N-CO-CH($CH_2C_6H_5$)-COOH

Boc-Leu-gAsp(OBzl)m(*R,S*)Phe-NH_2 (5)

↓ 1. TFA; 2. Boc-Trp-OSu

Boc-Trp-Leu-gAsp(OBzl)-m(*R,S*)Phe-NH_2 (6)

↓ H_2, Pd/C

Boc-Trp-Leu-gAsp-m(*R,S*)Phe-NH_2 (7)

in vivo cleavage of the gastrin on its receptor and to investigate in more detail the significance of the bond between methionine and aspartic acid. Partial retro-inverso modification involving the reversal of the direction of the peptide bond in the backbone, associated with inversion of each chiral center,[10] resulted, in some cases, in isomers retaining biological activity.[11] These modifications have been applied to enhance enzymatic resistance of a number of peptide hormones with success. In this work, we synthesized retro-inverso modified peptide derivatives analogues of the C-terminal tetrapeptide of gastrin. The activity of these pseudopeptides was evaluated in vivo on acid secretion in the anesthetized rat[12] and in vitro for their ability to inhibit the binding of [^{125}I]-(Nle^{11})-HG-13 to its receptor[13] on isolated rabbit gastric mucosal cells.

Chemistry

Schemes I–IV outline the syntheses of Boc-Trp-gLeu-D-Asp-m(*R,S*)Phe-NH_2 (3), Boc-Trp-Leu-gAsp-m(*R,S*)-Phe-NH_2 (7), Boc-Trp-gNle-D-Asp-CO-$CH_2CH_2C_6H_5$ (14), Boc-Trp-gNle-D-Asp-CO-NH-$CH_2C_6H_5$ (15), Boc-Trp-Leu-gAsp-CO-$CH_2CH_2C_6H_5$ (20), and Boc-Trp-Leu-gAsp-CO-NH-$CH_2C_6H_5$ (21). Treatment of the dipeptide Z-D-Asp(OBzl)-DLeu-NH_2 (1) with [bis(trifluoroacetoxy)-iodo]benzene[14] produced the *gem*-diamino derivative as its trifluoroacetate salt, which after reaction with the succinimido ester of Boc-Trp[15] yielded the partially retro-inverso peptide Boc-Trp-gLeu-D-Asp(OBzl)-Z (2). Hydrogenolysis of 2 over Pd/C as catalyst and coupling

Scheme III

Boc-D-Asp(OBzl)-OH + H-D-Nle-NH_2

↓

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

↓ TFA

[H-D-Asp(OBzl)-D-Nle-NH_2·TFA

↓

R-CO-D-Asp(OBzl)-D-Nle-NH_2 (R = -$CH_2CH_2C_6H_5$ (10), R = -NH-$CH_2C_6H_5$ (11))

↓ $PhI(CF_3CO_2)_2$

[R-CO-D-Asp(OBzl)-g(D-Nle)-H·TFA]

↓ Boc-Trp-OSu

Boc-Trp-gNle-D-Asp(OBzl)-CO-R (R = -$CH_2CH_2C_6H_5$ (12), R = -NH-$CH_2C_6H_5$ (13))

↓ H_2·Pd/C

Boc-Trp-gNle-D-Asp-CO-R (R = -$CH_2CH_2C_6H_5$ (14), R = -NH-$CH_2C_6H_5$ (15))

Scheme IV

Boc-Leu-Asp(OBzl)-NH_2 (4)

↓ $PhI(CF_3CO_2)_2$

[Boc-Leu-gAsp(OBzl)-H·TFA]

↓

Boc-Leu-gAsp(OBzl)-CO-R (R = -$CH_2CH_2C_6H_5$ (16), R = -NH-$CH_2C_6H_5$ (17))

↓ 1. TFA; 2. Boc-Trp-OSu

Boc-Trp-Leu-gAsp(OBzl)-CO-R R = -$CH_2CH_2C_6H_5$ (18), R = -NH-$CH_2C_6H_5$ (19))

↓ H_2, Pd/C

Boc-Trp-Leu-gAsp-CO-R (R = -$CH_2CH_2C_6H_5$ (20), R = -NH-$CH_2C_6H_5$ (21))

with the succinimido ester of (*R,S*)-2-benzylmalonic acid monoamide, prepared according to Anderson et al.[15] from (*R,S*)-2-benzylmalonic acid monoamide,[19] yielded Boc-Trp-gLeu-D-Asp-m(*R,S*)Phe-NH_2 (3) (Scheme I). We were unable to separate the two diastereoisomers and to get each of them in pure isomeric form. Compound 7, Boc-Trp-Leu-gAsp-m(*R,S*)Phe-NH_2, was synthesized according to Scheme II. The *gem*-diamino derivative was obtained as its TFA salt by treating the dipeptide Boc-Leu-Asp-(Bzl)-NH_2 (4) with [bis(trifluoroacetoxy)iodo]benzene and was allowed to react with (*R,S*)-2-benzylmalonic acid monoamide in the presence of *N*-[3-(dimethylamino)-propyl]-*N'*-ethylcarbodiimide hydrochloride[16] and HOBt[20] to produce Boc-Leu-gAsp(OBzl)-m(*R,S*)Phe-NH_2 (5). It was treated with trifluoroacetic acid and allowed to react with Boc-Trp-OSu to produce Boc-Trp-Leu-gAsp-(OBzl)-m(*R,S*)Phe-NH_2 (6), which after hydrogenolysis in

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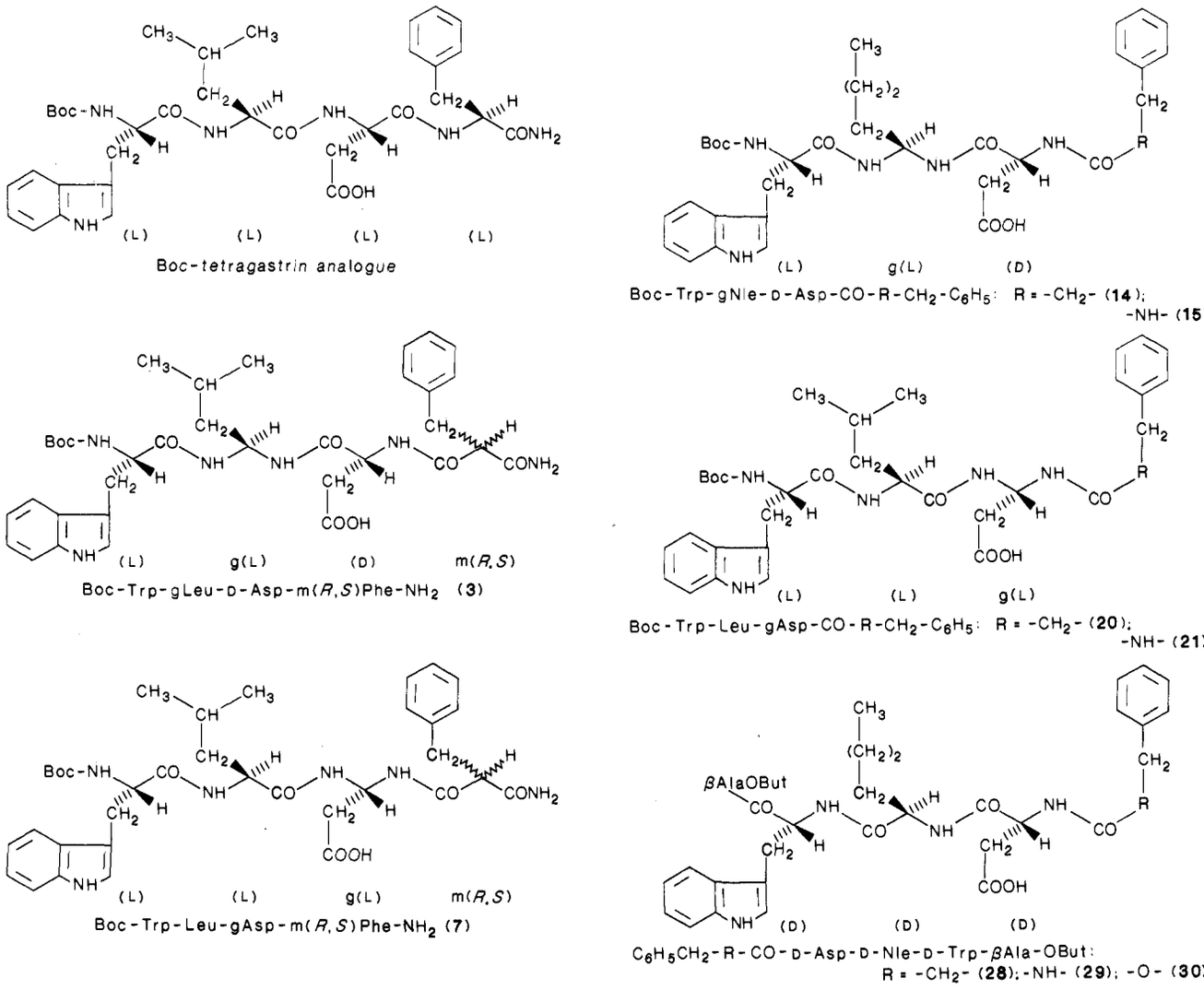

Figure 1. Structures of partially retro-inverso peptide derivatives of the C-terminal tetrapeptide of gastrin.

the presence of Pd/C as catalyst yielded Boc-Trp-Leu-gAsp-m(*R*,*S*)Phe–NH_2 (**7**). As in the case of compound **3**, attempts to separate the diastereoisomers failed. Compound **8** was obtained by condensation of Boc-D-Asp(OBzl) with D-Nle-NH_2 by the mixed anhydride method, using isobutyl chloroformate.[17] After partial deprotection with TFA, it was allowed to react with the succinimido ester of 3-phenylpropionic acid or with benzyl isocyanate to yield $C_6H_5CH_2CH_2CO$-D-Asp(OBzl)-D-Nle-NH_2 (**10**) and $C_6H_5CH_2NH$-CO-D-Asp(OBzl)-D-Nle-NH_2 (**11**), respectively. Treatment of **10** and **11** with [bis(trifluoroacetoxy)iodo]benzene produced the TFA salts of the *gem*-diamino norleucine analogues. They were allowed to react with Boc-Trp-OSu to produce compounds **12** and **13**, which after hydrogenolysis in the presence of Pd/C yielded Boc-Trp-gNle-D-Asp-CO-$CH_2CH_2C_6H_5$ (**14**) and Boc-Trp-gNle-D-Asp-CO-NH-$CH_2C_6H_5$ (**15**). The syntheses of compounds **20** and **21** are described in Scheme IV. Compounds **28–30** were prepared by standard methods in solution, and their syntheses are described in the Experimental Section.

Biological Activity and Discussion

Compounds **3**, **7**, **14**, **15**, **20**, **21**, and **28–30** were tested for their ability to stimulate acid secretion, to inhibit gastrin-induced acid secretion in the in situ perfused rat stomach,[12] and to inhibit the binding of [^{125}I]-(Nle^{11})-HG-13 to its receptors. Results are summarized in the Table I. The data presented in this work indicate that most of the peptide analogues of the C-terminus of gastrin retain conformational requirements for binding to the gastrin receptor. Particularly, analogues **7** and **20**, in which Asp has been converted to *gem*-diamino and Phe to malonyl derivative or replaced by a 3-phenylpropionyl group, respectively, are very potent in inhibiting the binding of [^{125}I]-(Nle^{11})-HG-13 to its receptors (IC_{50} = 10^{-7} and 5 × 10^{-6} M, respectively). Compound **7** showed little agonist activity, whereas **20** was completely inactive in stimulating acid secretion. Both derivatives were highly potent in inhibiting gastrin-induced acid secretion (ED_{50} = 0.3 and 0.15 μM/kg, respectively). Compound **3**, Boc-Trp-gLeu-D-Asp-m(*R*,*S*)Phe-NH_2, is also very potent in inhibiting the binding of [^{125}I]-(Leu^{11})-HG-13 to its receptors (IC_{50} = 10^{-6} M) and in antagonizing the action of gastrin (ED_{50} = 0.15 μM/kg), confirming the importance of the peptide bond between Leu and Asp for gastrin activity.[8] The other peptide derivatives **14**, **15**, **21**, and **28–30** exhibited less affinity for the gastrin receptor and were less active in inhibiting gastrin-induced acid secretion. As an interesting point, it should be noted that the duration of the antagonistic activity of the derivatives synthesized in this work (particularly compounds **3**, **7**, and **20**) is enhanced (in fact, the length of time the antagonist effect was observed was increased up to 2–3 h). This can probably be explained by their metabolic stability: the presence of D residues, of nonpeptidic moieties and of *gem*-diamino analogues might, in fact, protect them from enzymatic cleavage.

Table I. Biological Activities of Partially Retro-Inverso Peptide and Peptide Derivatives of the C-Terminal Tetrapeptide of Gastrin on the Binding to the Gastrin Receptor and on the Activity on Acid Secretion[a]

compounds	binding: IC_{50}, μM	biological activity: agonist	biological activity: antagonist: ED_{50}, $\mu M/kg$
Boc-Trp-Leu-Asp-Phe-NH_2	0.3	****	
proglumide	2500		350
Boc-Trp-gLeu-D-Asp-m(*R,S*)-Phe-NH_2 (**3**)	1.2 ± 0.5	*	0.15 ± 0.04
Boc-Trp-Leu-gAsp-m(*R,S*)-Phe-NH_2 (**7**)	0.09 ± 0.03	*	0.3 ± 0.03
Boc-Trp-gNle-D-Asp-CO-$CH_2CH_2C_6H_5$ (**14**)	110 ± 20	*	2.5 ± 0.2
Boc-Trp-gNle-D-Asp-CO-N-H-$CH_2C_6H_5$ (**15**)	15 ± 2	*	1.1 ± 0.1
Boc-Trp-Leu-gAsp-CO-$CH_2CH_2C_6H_5$ (**20**)	6 ± 2		0.15 ± 0.04
Boc-Trp-Leu-gAsp-CO-NH-$CH_2C_6H_5$ (**21**)	25 ± 5		0.9 ± 0.08
$C_6H_5CH_2CH_2$-D-Asp-D-Nle-D-Trp-βAla-OBut (**28**)	81 ± 6		10.4 ± 1.3
$C_6H_5CH_2NH$-CO-D-Asp-D-Nle-D-Trp-βAla-OBut (**29**)	78 ± 7		5.6 ± 0.9
$C_6H_5CH_2OCO$-D-Asp-D-Nle-D-Trp-βAla-OBut (**30**)	75 ± 6		4.3 ± 0.8

[a] At a dose of 3 mg/kg, these compounds exhibited $\simeq 1/4$ of the agonist activity (*) of Boc-Trp-Leu-Asp-Phe-NH_2 (which has the full activity of tetragastrin) (****). No agonist activity means that we do not observe any activity at doses as high as 3 mg/kg. IC_{50} values were determined from the curves representing the inhibitory effect of the various peptide derivatives on gastrin binding to isolated rabbit mucosal cells. The values are the mean ± SD of at least five separate experiments. Isolated cells (5×10^6/mL) were incubated in Earle's medium with [^{125}I]-(Leu^{11})-HG-13 (20 pM) for 30 min at 37 °C in the presence or not of various concentrations of the peptide derivatives. ED_{50} values were determined from the curves representing the inhibitory effect of the peptide derivatives on gastrin-induced acid secretion in the anesthetized rat (according to Ghosh and Schild). Peptide derivatives together with 80 pmol of (Nle^{15})-HG (submaximal dose) were injected iv in small volumes (0.05–0.1 mL) at different doses. The pH was recorded for 40 min and the percent of inhibition was calculated for each dose of peptide as the ratio of acid output induced by peptide derivatives plus gastrin to acid output induced by gastrin alone. The values are the mean ± SD of at least three separate experiments, with no more than four injections made on the same animal.

Conclusion

From the results obtained in this work, it appears that the structures of partially retro-inverso peptide analogues of the C-terminal tetrapeptide of gastrin share topochemical and, at least in part, spatial similarities. The significance of the peptide bond between leucine and aspartic acid for the biological activity is confirmed, whereas it appears less crucial for the binding to the gastrin receptor. Along with incorporation of nonpeptidic moieties, the partial retro-inverso modification in the C-terminal tetrapeptide of gastrin led to derivatives having high affinity for the gastrin receptor but no or little activity on acid secretion. These compounds, however, are very potent in inhibiting gastrin-induced acid secretion.

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'E.N.S.C.M. (Montpellier, France). Ascending TLC were performed on precoated plates of silica gel 60 F_{254} (Merck) with the following solvent systems (by volume): A, ethyl acetate; B, ethyl acetate/hexane, 7:3; C, AcOEt/pyr/AcOH/H_2O, 80:20:5:10; D, chloroform/methanol/acetic acid, 85:10:5; E, AcOEt/pyr/AcOH/H_2O, 80:20:3:3; F, ethyl acetate/hexane, 1:1; G, chloroform/methanol/acetic acid, 55:30:15. Peptide derivatives were located with charring reagent or ninhydrin. Column chromatographies were performed with silica gel 60, 60–229 mesh, ASTM (Merck). L- and D-amino acids and derivatives were from Bachem. All reagents and solvents were of analytical grade. The following abbreviations were used: DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DIEA, *N,N*-diisopropylethylamine; NMM, *N*-methylmorpholine; DCC, *N,N'*-dicyclohexylcarbodiimide. 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*-diamino alkyl residue derived from the specified amino acid. The prefix m represents the malonic acid residue derived from the amino acid specified by the three-letters notation (Chorev et al. *J. Med. Chem.* **1983**, *26*, 129). Configuration designation of the retro-inverso residues follows those of the amino acid.

Z-D-Asp(OBzl)-D-Leu-NH_2 (1). To a cold (−15 °C) solution of (benzyloxycarbonyl)-β-benzyl-D-aspartic acid (2.0 g, 5.60 mmol) in DMF (10 mL) were added *N*-methylmorpholine (0.63 mL, 5.60 mmol) and isobutyl chloroformate[17] (0.76 mL, 5.60 mmol). After 5 min of stirring at −15 °C, D-leucinamide (0.791 g, 6.0 mmol) was added. The mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. Upon addition of a 10% aqueous citric acid solution (150 mL) with vigorous stirring, a white precipitate formed, which was collected by filtration, thoroughly washed with water (3 × 80 mL), a saturated sodium bicarbonate solution (3 × 80 mL), and water to neutrality, and dried in vacuo over phosphorus pentoxide. Yield 2.47 g (94%); R_f (A) 0.66; mp 158–160 °C; $[\alpha]^{20}_D$ +13.5° (*c* 1.04, DMF). Anal. ($C_{25}H_{31}N_3O_6$) C, H, N.

Boc-Trp-gLeu-D-Asp(OBzl)-Z (2). To a suspension of dipeptide 1 (2.2 g, 4.7 mmol) in a mixture of acetonitrile/water (1:1, 20 mL), was added [bis(trifluoroacetoxy)iodo]benzene[14] (2.12 g, 4.92 mmol). After 3 h of stirring at room temperature, a clear solution resulted and no more starting material could be detected by TLC (solvent A). The solvents were removed under reduced pressure to leave a white solid residue that was dried in vacuo over P_2O_5. It was dissolved in DMF (5 mL) containing DIEA (0.80 mL, 4.7 mmol) and Boc-Trp-OSu[15] (1.6 g, 4 mmol). The mixture was stirred 2 h at room temperature. The expected compound (**2**) was isolated according to the procedure described for 1. Yield 2, 79 g (96%); R_f (B) 0.77; mp 185 °C dec. Anal. ($C_{40}H_{49}N_5O_8$) C, H, N.

Boc-Trp-gLeu-D-Asp-m(*R,S*)Phe-NH_2 (3). Compound **2** (2.0 g, 2.75 mmol) was partially deprotected by hydrogenation for 4 h at room temperature under atmospheric pressure, in DMF (30 mL) and in the presence of 10% Pd/C as catalyst. Filtration of the catalyst, evaporation of the solvent under reduced pressure ($t < 40$ °C), and trituration of the resulting residue in diethyl ether afforded a white solid (1.08 g, 2.14 mmol). To a solution of (*R,S*)-2-benzylmalonic acid monoamide succinimido ester (0.667 g, 2.3 mmol), prepared according to Anderson et al.[19] from the corresponding (*R,S*)-2-benzylmalonic acid monoamide,[19] DCC, and *N*-hydroxysuccinimide, in DMF (5 mL) were added the deprotected material (1.08 g, 2.14 mmol) and DIEA (0.37 mL, 2.14 mmol). After 4 h of stirring at room temperature, addition of a 10% aqueous citric acid solution (100 mL) yielded a white precipitate that was collected on a sinter glass funnel, washed with water (3 × 50 mL), ethyl acetate (3 × 50 mL), diethyl ether (3 × 50 mL), and dried in vacuo over P_2O_5. Yield 1.05 g (52%); R_f (C) 0.68 and 0.75 (corresponding to the two diastereoisomers): mp 210 °C dec; $[\alpha]^{20}_D$ +7.0 (*c* 1.4, DMF). Anal. ($C_{35}H_{46}N_6O_8$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Boc-Leu-gAsp(OBzl)-m(*R,S*)Phe-NH_2 (5). Boc-Leu-Asp(OBzl)-NH_2[5] (4) (2.5 g, 5.74 mmol) was converted into its corresponding *gem*-diamino derivative in the presence of [bis(trifluoroacetoxy)iodo]benzene (2.6 g, 6.03 mmol) as described for compound **2**. To a solution of the TFA salt of the *gem*-diamino compound (5.74 mmol) in DMF (15 mL) were added DIEA (0.99 mL, 5.74 mmol), (*R,S*)-2-benzylmalonic acid monoamide (1.11 g, 5.74 mmol), and HOBt[21] (0.78 g, 5.74 mmol). The mixture was cooled down to 0 °C and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride[16] (1.1 g, 5.74 mmol) was added. After overnight stirring at room temperature, addition of a 10%

aqueous citric acid solution (100 mL) afforded a white precipitate that was collected by filtration, washed with water (3 × 80 mL), acetone (10 mL), and diethyl ether (3 × 80 mL), and dried in vacuo over P_2O_5. Yield 2.15 g (65%); R_f (A) 0.62; mp 184–187 °C; $[\alpha]^{20}_D$ −3.0° (*c* 1.3, DMF). Anal. ($C_{31}H_{42}N_4O_7$) C, H, N.

Boc-Trp-Leu-gAsp(OBzl)-m(*R*,*S*)Phe-NH$_2$ (6). Compound **5** (1 g, 1.72 mmol) was partially deprotected with trifluoroacetic acid (3 mL) for 30 min. Addition of diethyl ether (100 mL) with vigorous stirring yielded a white precipitate that was collected by filtration, washed several times with diethyl ether, and dried in vacuo over KOH pellets. This TFA salt (1.72 mmol) was dissolved in DMF (3 mL) in the presence of DIEA (0.30 g, 1.72 mmol) and Boc-Trp-OSu[15] (0.642 g, 1.60 mmol). After 2 h of stirring at room temperature, the expected compound **6** was isolated as described for **2**. Yield 1.20 g (98%); R_f (A) 0.48; R_f (D) 0.80; mp 200–205 °C; $[\alpha]^{20}_D$ −9.9° (*c* 1.03 DMF). Anal. ($C_{42}H_{52}N_6O_8$) C, H, N.

Boc-Trp-Leu-gAsp-m(*R*,*S*)Phe-NH$_2$ (7). Compound **6** (1 g, 1.3 mmol) was partially deprotected by hydrogenation for 4 h at room temperature under atmospheric pressure, in a mixture of ethanol/DMF (1:1, 80 mL) and in the presence of 10% Pd/C as catalyst. Filtration of the catalyst, evaporation of the solvent under reduced pressure ($t < 40$ °C), and trituration of the resulting residue in diethyl ether afforded a white solid that was collected by filtration, washed several times with diethyl ether, and dried in vacuo (1.08 g, 2.14 mmol). Yield 0.84 g (95%); R_f (E) 0.40 and 0.45 corresponding to the two diastereoisomers; mp 225 °C dec; $[\alpha]^{20}_D$ −14.4° (*c* 1.05 DMF). Anal. ($C_{35}H_{46}N_6O_8$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Boc-D-Asp(OBzl)-D-Nle-NH$_2$ (8). To a cold (−15 °C) solution of (benzyloxycarbonyl)-β-benzyl-D-aspartic acid (2.7 g, 8.35 mmol) in DMF (10 mL) were added *N*-methylmorpholine (0.94 mL, 8.35 mmol) and isobutyl chloroformate[17] (1.13 mL, 8.35 mmol). After 5 min of stirring at −15 °C, D-Nle-NH$_2$ (1.17 g, 9.0 mmol) was added. The mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. The product was isolated as described for **1**. Yield 3.46 g (95%); R_f (A) 0.62; mp 140–145 °C; $[\alpha]^{20}_D$ +18.4° (*c* 0.98, DMF). Anal. ($C_{22}H_{33}N_3O_6$) C, H, N.

3-Phenylpropionic Acid Succinimido Ester (9). To a cold (0 °C) solution of 3-phenylpropionic acid (5 g, 33.3 mmol) and *N*-hydroxysuccinimide (5.75 g, 50 mmol) in ethylene glycol dimethyl ether (40 mL) was added DCC (6.87 g, 33.3 mmol). After overnight stirring at room temperature, DCU was removed by filtration and the filtrate was diluted with ethyl acetate (200 mL). This solution was washed with a cold 2% aqueous bicarbonate solution (3 × 60 mL), water (50 mL), 1 N aqueous $KHSO_4$ solution (100 mL), and brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to leave a white solid that was recrystallized in a mixture of ethyl acetate/hexane. Yield 5.69 g (69%); R_f (F) 0.45; mp 112–115 °C. Anal. ($C_{13}H_{13}NO_4$) C, H, N.

C$_6$H$_5$CH$_2$CH$_2$-CO-D-Asp(OBzl)-D-Nle-NH$_2$ (10). Compound **8** (1.45 g, 3.34 mmol) was partially deprotected in TFA according to the usual procedure. The TFA salt was dissolved in DMF (5 mL), and DIEA (0.574 mL, 3.34 mmol), and 3-phenylpropionic acid succinimido ester (**9**) (0.816 g, 3.3 mmol) were added. After 2 h of stirring at room temperature, the product was isolated as described for compound **1**. Yield 1.36 g (87%); R_f (A) 0.39; mp 174–177 °C; $[\alpha]^{20}_D$ +18.9° (*c* 1.2, DMF). Anal. ($C_{26}H_{33}N_3O_5$) C, H, N.

C$_6$H$_5$CH$_2$-NH-CO-D-Asp(OBzl)-D-Nle-NH$_2$ (11). Compound **8** (1.65 g, 3.78 mmol) was partially deprotected in TFA according to the usual procedure. The TFA salt was dissolved in ethyl acetate (25 mL), and DIEA (0.651 g, 3.78 mmol) and benzyl isocyanate (0.47 mL, 3.80 mmol) were added. A precipitate formed instantly and was collected on a sinter glass funnel, washed with ethyl acetate (3 × 40 mL) and diethyl ether (3 × 40 mL), and dried in vacuo. Yield 1.6 g (92%); R_f (A) 0.48; mp 208–212 °C; $[\alpha]^{20}_D$ +1.9° (*c* 1.2, DMF). Anal. ($C_{25}H_{32}N_4O_5$) C, H, N.

Boc-Trp-gNle-D-Asp(OBzl)-CO-CH$_2$CH$_2$C$_6$H$_5$ (12). Compound **10** (1.3 g, 2.78 mmol) was converted into its corresponding *gem*-diamino derivative in the presence of [bis(trifluoroacetoxy)iodo]benzene (1.26 g, 2.92 mmol) as described for compound **2**. To a solution of the TFA salt of the *gem*-diamino compound (2.78 mmol) in DMF (5 mL) were added DIEA (0.48 mL, 2.78 mmol) and Boc-Trp-OSu (0.883 g, 2.2 mmol). After overnight stirring at room temperature, the desired product was isolated as described for compound **1**. Yield 1.52 g (95%); R_f (A) 0.68; mp 173–178 °C; $[\alpha]^{20}_D$ +5.6° (*c* 1, DMF). Anal. ($C_{41}H_{51}N_5O_7$) C, H, N.

Boc-Trp-gNle-D-Asp(OBzl)-CO-NH-CH$_2$C$_6$H$_5$ (13). This compound was obtained from derivative **10** (1.5 g, 3.2 mmol) as described for **12**. Yield 1.43 g (69%); R_f (A) 0.65; R_f (D) 0.85; mp 210 °C dec; $[\alpha]^{20}_D$ −2.3° (*c* 1.09, DMF). Anal. ($C_{40}H_{50}N_6O_7$) C, H, N.

Boc-Trp-gNle-D-Asp-CO-CH$_2$CH$_2$C$_6$H$_5$ (14). Compound **12** (1.43 g, 1.96 mmol) was partially deprotected by hydrogenation as described for compound **7** to afford **14** as a white powder. Yield 0.95 g (80%); R_f (D) 0.76; mp 190 °C dec; $[\alpha]^{20}_D$ +9.8° (*c* 1.1, DMF). Anal. ($C_{34}H_{45}N_5O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Boc-Trp-gNle-D-Asp-CO-NH-CH$_2$C$_6$H$_5$ (15). Compound **13** (1.35 g, 1.86 mmol) was partially deprotected by hydrogenation as described for compound **7** to afford **15** as a white powder. Yield 1.1 g (93%); R_f (D) 0.45; mp 200 °C dec; $[\alpha]^{20}_D$ −5.3° (*c* 1, DMF). Anal. ($C_{33}H_{44}N_6O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Boc-Leu-gAsp(OBzl)-CO-CH$_2$CH$_2$C$_6$H$_5$ (16). Boc-Leu-Asp(OBzl)-NH$_2$ (**4**) (1 g, 2.30 mmol) was converted into its corresponding *gem*-diamino derivative in the presence of [bis(trifluoroacetoxy)iodo]benzene (1.04 g, 2.41 mmol) as described for compound **2**. To a solution of the TFA salt of the *gem*-diamino compound (2.30 mmol) in DMF (8 mL) were added DIEA (0.4 mL, 2.3 mmol) and 3-phenylpropionic acid succinimido ester **9** (0.495 g, 2.0 mmol). After 3 h of stirring at room temperature, the solvent was removed under reduced pressure at $t < 40$ °C and the residue applied on a silica gel chromatography column (eluent ethyl acetate/hexane). Pure fractions were pooled to yield, after removal of the solvent, compound **16** as a white solid. It was recrystallized in a mixture of ethyl acetate/hexane. Yield 0.70 g (65%); R_f (F) 0.52; mp 155–158 °C; $[\alpha]^{20}_D$ −8.7° (*c* 1.1, DMF). Anal. ($C_{30}H_{41}N_3O_6$) C, H, N.

Boc-Leu-gAsp(OBzl)-CO-NH-CH$_2$C$_6$H$_5$ (17). Boc-Leu-Asp(OBzl)-NH$_2$ (**4**) (1 g, 2.30 mmol) was converted into its corresponding *gem*-diamino derivative in the presence of [bis(trifluoroacetoxy)iodo]benzene (1.04 g, 2.41 mmol) as described for compound **2**. To a solution of the TFA salt of the *gem*-diamino compound (2.30 mmol) in ethyl acetate (10 mL) were added DIEA (0.4 mL, 2.3 mmol) and benzyl isocyanate (0.285 mL, 2.30 mmol). After 2 h of stirring at room temperature, the precipitate that formed was collected by filtration, washed the diethyl ether (3 × 10 mL), and dried in vacuo. Yield 0.64 g (51%); R_f (B) 0.59; mp 175–178 °C; $[\alpha]^{20}_D$ −10.1° (*c* 1, DMF). Anal. ($C_{20}H_{40}N_4O_6$) C, H, N.

Boc-Trp-Leu-gAsp(OBzl)-CO-CH$_2$CH$_2$C$_6$H$_5$ (18). Compound **16** (0.60 g, 1.11 mmol) was partially deprotected in TFA according to the usual procedure. The TFA salt was dissolved in DMF (5 mL), DIEA (0.19 mL, 1.11 mmol) and Boc-Trp-OSu (0.42 g, 1.05 mmol) were added, and the mixture was stirred for 2 h at room temperature. Compound **18** was isolated as described for **1**. Yield 0.7 g (92%); R_f (B) 0.68; mp 175–185 °C; $[\alpha]^{20}_D$ −14.8° (*c* 1, DMF). Anal ($C_{41}H_{51}N_5O_7$) C, H, N.

Boc-Trp-Leu-gAsp(OBzl)-CO-NH-CH$_2$C$_6$H$_5$ (19). This compound was synthesized as described above from **17** (0.55 g, 1.01 mmol). Yield 0.53 g (77%); R_f (A) 0.67; mp 200 °C dec; $[\alpha]^{20}_D$ −15.7° (*c* 1.1, DMF). Anal. ($C_{40}H_{50}N_6O_7$) C, H, N.

Boc-Trp-Leu-gAsp-CO-CH$_2$CH$_2$C$_6$H$_5$ (20). Compound **18** was hydrogenated as described for **7** to afford **20** as a white solid. Yield 0.26 g (87%); R_f (D) 0.68; mp 205 °C dec; $[\alpha]^{20}_D$ −13.8° (*c* 1.3, DMF). Anal. ($C_{34}H_{45}N_5O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Boc-Trp-Leu-gAsp-CO-NH-CH$_2$C$_6$H$_5$ (21). Compound **19** was hydrogenated as described for **7** to yield **21** as a white solid. Yield 0.32 g (80%); R_f (D) 0.53; mp 150 °C dec; $[\alpha]^{20}_D$ −17.4 (*c* 1.2, DMF). Anal. ($C_{33}H_{44}N_6O_7$) C, H, N. For biological tests,

this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Z-D-Trp-βAla-OBut (22). To a cold (−15 °C) solution of Z-D-Trp (4.23 g, 12.5 mmol) in DMF (15 mL) were added NMM (1.4 mL, 12.5 mmol) and isobutyl chloroformate (1.7 mL, 12.5 mmol). After 5 min, βAla-OBut, HCl (2.5 g, 13.8 mmol) was added. The mixture was stirred at 0 °C for 1 h and at room temperature for an additional hour. The solvent was removed in vacuo to leave a residue that was dissolved in ethyl acetate (200 mL). It was washed with a 10% aqueous citric acid solution (3 × 50 mL), water (2 × 50 mL), a saturated sodium bicarbonate solution (3 × 50 mL), water (50 mL), and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure at $t < 40$ °C to leave a white powder. Yield 5.20 g (90%); R_f (B) 0.75; R_f (F) 0.33; mp 55–60 °C; $[\alpha]^{20}_D$ +22.0° (c 1.1, DMF). Anal. ($C_{26}H_{31}N_3O_5$) C, H, N.

Z-D-Nle-D-Trp-βAla-OBut (24). Compound **22** (4.75 g, 10.2 mmol) was hydrogenated in ethanol according to the usual procedure, in the presence of acetic acid (1 mL). The partially deprotected peptide **23** was obtained as an acetate salt. To a cold (−15 °C) solution of Z-D-Nle (2.79 g, 10.5 mmol) in DMF (10 mL) were added NMM (1.18 mL, 10.5 mmol) and isobutyl chloroformate (1.43 mL, 10.5 mmol). After 5 min, compound **23** was added followed by DIEA (1.75 mL, 10.2 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature for an additional hour. The reaction mixture was treated as described for **22**. Recrystallization in a mixture of acetone/water yielded a white solid. Yield 4.50 g (76%); R_f (B) 0.58; mp 162–163 °C; $[\alpha]^{20}_D$ +10.2° (c 1.1 DMF). Anal. ($C_{32}H_{42}N_4O_6$) C, H, N.

Z-D-Asp(OBzl)-D-Nle-D-Trp-βAla-OBut (26). Compound **24** (3.95 g, 6.83 mmol) was hydrogenated in ethanol according to the usual procedure, in the presence of acetic acid (1 mL). The partially deprotected peptide **25** was obtained as an acetate. To a cold (−15 °C) solution of Z-D-Asp(OBzl) (2.47 g, 6.9 mmol) in DMF (10 mL) were added NMM (0.78 mL, 6.9 mmol) and isobutyl chloroformate (0.94 mL, 6.9 mmol). After 5 min. compound **25** was added followed by DIEA (1.18 mL, 6.8 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature for an additional hour. The reaction mixture was treated as described for **22**. Yield 3.54 g (66%); R_f (B) 0.67; mp 148–150 °C; $[\alpha]^{20}_D$ +12.5° (c 1.2, DMF). Anal. ($C_{43}H_{53}N_5O_9$) C, H, N.

H-D-Asp-D-Nle-D-Trp-βAla-OBut (27). Compound **26** (3.35 g, 4.27 mmol) was hydrogenated at room temperature and atmospheric pressure in DMF (50 mL) for 6 h in the presence of 10% Pd/C catalyst. Removal of the catalyst by filtration followed by evaporation of the solvent under reduced pressure and trituration in diethyl ether afforded **27** as a white solid. Yield 1.67 g (70%); R_f (G) 0.63; mp 230 °C dec; $[\alpha]^{20}_D$ +22.3° (c 1.03, DMF). Anal. ($C_{28}H_{41}N_5O_7$) C, H, N.

$C_6H_5CH_2CH_2CO$-D-Asp-D-Nle-D-Trp-βAla-OBut (28). To a solution of **27** (0.26 g, 0.465 mmol) in DMF (3 mL) was added 3-phenylpropionic acid succinimido ester (**9**) (0.148 g, 0.6 mmol). After 3 h of stirring at room temperature, the solvent was removed under reduced pressure at $t < 40$ °C and the residue applied on a silica gel chromatography column (eluent chloroform/methanol/acetic acid, 100:10:5). Pure fractions were pooled to yield, after removal of the solvent and trituration of the residue with diethyl ester, compound **28** as a white solid. Yield 0.19 g (59%); R_f (D) 0.69; mp 190 °C dec; $[\alpha]^{20}_D$ +34.2° (c 1.07, DMF). Anal. ($C_{37}H_{49}N_5O_8$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

$C_6H_5CH_2$-NH-CO-D-Asp-D-Nle-D-Trp-βAla-OBut (29). To a solution of **27** (0.40 g, 0.715 mmol) in DMF (3 mL) was added benzyl isocyanate (0.095 mL, 0.77 mmol). After 3 h of stirring at room temperature, the product **29** was isolated as described for **28**. Yield 0.28 g (56%); R_f (D) 0.47; mp 165–169 °C; $[\alpha]^{20}_D$ +22.7° (c 1.21, DMF). Anal. ($C_{36}H_{48}N_6O_8$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

$C_6H_5CH_2$-O-CO-D-Asp-D-Nle-D-Trp-βAla-OBut (30). To a cold solution of **27** (0.40 g, 0.715 mmol) in DMF (3 mL) was added DIEA (0.123 mL, 0.715 mmol) and benzyloxycarbonyl chloride (0.107 mL, 0.715 mmol). After 30 min of stirring at room temperature, the desired compound was precipitated by addition of a 10% aqueous citric acid solution (20 mL). The precipitate was collected by filtration and washed several times with water. It was dissolved in ethyl acetate (20 mL) and dried over $MgSO_4$. Addition of hexane (50 mL) afforded a white solid. It was collected by filtration, washed several times with hexane, and dried in vacuo over KOH pellets. Yield 0.27 g (53%); R_f (D) 0.75; mp 150–155 °C; $[\alpha]^{20}_D$ +22.2° (c 1.02, DMF). Anal. ($C_{36}H_{47}N_5O_9$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.4 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Biological Tests. Gastric acid secretion was determined in vivo in the reperfused rat stomach according to the method of Ghosh and Schild.[12] The gastric pouch of an anesthetized rat (urethane ip) was continuously washed at 30 °C with a propionate–succinate solution. The cumulative pH was recorded with time and used as an index of acid secretion. Synthetic (Leu^{15})-human gastrin I (gift from Professor E. Wunsch, Max Plank Institute, Munchen) and compounds **3**, **7**, **14**, **15**, **20**, **21** and **28–30** were dissolved in 0.9% NaCl and bolus injected intravenously. Before a new bolus injection of compound, the rat stomach was washed until stabilization of basal acid secretion occurred. No more than four injections were made on the same animal. The amount of H^+ secreted was determined by the pH difference between stimulated and basal recorded traces. The inhibitory effect of synthetic peptides was measured after simultaneous bolus injection of the compounds in water alkaline solution (0.01 N NaOH) and of gastrin (80 pmol was usually employed). The amount of H^+ secreted in the presence of various doses of the peptides was reported to the amount of H^+ secreted after gastrin alone and expressed as percentage of inhibition. The mean H^+ secretion after gastrin injection was 203 ± 28 μmol H^+/nmol. of peptide (n = 17).

Binding Studies. Isolation of gastric cells from rabbit stomachs was carried out by the collagenase/EDTA procedure previously described.[13] Fundic mucosa was scraped and tissues were chopped into small cubes and then dispersed in medium A (132 mM NaCl, 5.4 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 25 mM Hepes, 0.2% glucose, 0.2% bovine serum albumin, 0.02% phenol red, pH 7.4) (gassed O_2/CO_2) containing 0.30 mg/mL collagenase. After 15-min incubation at 37 °C, tissue fragments were allowed to settle, and the medium was discarded. The fragments were washed in Ca^{2+}-free medium A containing 2 mM EDTA and then incubated in the same medium for 10 min. The fragments were transferred to medium A containing fresh 0.30 mg/mL collagenase and incubated for 15 min at 37 °C with continuous gassing (O_2/CO_2). The cell suspension was centrifuged for 15 min at 200g and then washed twice with medium A. This procedure gave about 2 × 10^7 cells/g of wet mucosa with 95% viability (trypan blue exclusion). The mixed population contained 45% parietal cells. (Nle^{11})-HG-13 was iodinated according to a modification of the already described chloramine T procedure.[13] After purification by DE-52 ion exchange chromatography, the monoiodinated peptide was obtained with full biological activity. Specific gastrin binding was determined by incubation in medium B (Earle's balanced salt medium without bicarbonate and containing 10 mM Hepes and 0.2% BSA, pH 7.4) of 20 pM labeled (Nle^{11})-HG-13 (≈40 000 cpm/mL) for 30 min at 37 °C with 5 × 10^6 cells/mL ± various concentrations of peptides or unlabeled (Nle^{11})-HG-13. Nonsaturable binding was determined as the amount of radioactivity associated with cells in presence of 1 × 10^{-6} M of cold (Nle^{11})-HG-13.