All compounds were administered (via an oral dosing needle placed in the esophagus) as a solution or suspension in 1% w/v methylcellulose solution.

With use of the above procedure, vehicle alone typically has little or no effect on blood pressure apart from a slight reduction (by 5–10%) at 6 h postdose.

Acknowledgment. We thank David Bragg, Helen Costema, Katharine Fish, and Graham Moore for skilled technical assistance.

Registry No. (±)-1, 75611-78-8; (±)-2, 94470-67-4; (±)-3, 103774-92-1; (±)-4, 94535-50-9; (−)-4 (carbamate), 94470-68-5; (±)-5, 94535-51-0; (+)-5 (carbamate), 94470-69-6; (±)-6, 103732-19-0; (±)-7, 103732-20-3; (±)-8 (isomer 1), 103751-07-1; (±)-8 (isomer 2), 103732-70-3; (±)-9, 103732-21-4; (±)-10, 103732-22-5; (±)-11, 103732-23-6; (±)-12, 103732-24-7; (±)-13, 103732-25-8; (±)-14, 103732-26-9; (±)-15, 103732-27-0; (±)-16, 103732-28-1; (±)-17, 103732-29-2; (±)-18, 103732-30-5; (±)-19, 103732-31-6; (±)-20, 103732-32-7; (±)-21, 103751-08-2; (±)-22, 103732-33-8; (±)-23, 103732-34-9; (±)-24, 103732-35-0; (±)-25, 103732-36-1; (±)-26, 103732-37-2; (±)-27, 103732-38-3; (±)-28, 103732-39-4; (±)-29, 103732-40-7; (±)-30, 103732-41-8; (±)-31, 103732-42-9; (±)-32, 103732-43-0; (±)-33, 103732-44-1; (±)-34, 103732-45-2; (±)-35, 103732-46-3; (±)-36, 103732-47-4; (±)-37, 103732-48-5; (±)-38, 103732-49-6; (±)-39, 103732-50-9; (±)-40, 103732-51-0; (±)-41, 103732-52-1; (±)-42, 103732-53-2; (±)-43, 103732-54-3; 44, 89080-74-0; 45, 89080-75-1; 46, 89080-73-9; 47, 89080-71-7; 48, 89080-72-8; 49, 103732-55-4; 50, 91236-94-1; 51, 91224-99-6; (±)-52a, 103732-57-6; (±)-52b (isomer 1), 103732-56-5; (±)-52b (isomer 2), 103732-72-5; (±)-52c, 103732-59-8; (±)-53a (isomer 1), 103774-93-2; (±)-53a (isomer 2), 103774-95-4; (±)-53b, 103732-60-1; (±)-53c, 103732-65-6; (±)-54b, 103732-62-3; 54c, 103732-61-2; (±)-55a, 103732-64-5; (±)-55a (epoxide), 103732-63-4; (±)-55b, 103732-66-7; (±)-56a, 103751-09-3; (±)-56b, 103751-10-6; (±)-57, 75611-72-2; (±)-58a, 103732-67-8; (±)-58b, 103774-94-3; (±)-59, 103732-68-9; (±)-59 (amine), 103732-70-3; (±)-60, 103732-69-0; $H_2N(CH_2)_3C-O_2CH_2CH_3$, 5959-36-4; $Cl(CH_2)_3COCl$, 4635-59-0; 6-nitro-2*H*-1-benzopyran, 16336-26-8; 2-methyl-6-nitro-2*H*-1-benzopyran, 103732-58-7; 6-formyl-2,2-dimethyl-2*H*-1-benzopyran, 69964-40-5.

Phenethyl Ester Derivative Analogues of the C-Terminal Tetrapeptide of Gastrin as Potent Gastrin Antagonists

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A series of phenethyl ester derivative analogues of the C-terminal tetrapeptide of gastrin, in which the phenylalanyl residue has been replaced by a phenethyl group and the peptide bond between aspartic acid and phenylalanine by an ester bond, were synthesized. None of these derivatives were able to stimulate gastric acid secretion in the anesthetized rat, whereas they inhibited gastrin-induced acid secretion with ED_{50} values between 0.02 and 1.5 mg/kg. Among these derivatives, Boc-βAla-Trp-Leu-Asp phenethyl ester (**9**) and Boc-βAla-Trp-Leu-Asp *p*-fluorophenethyl ester (**16**) were very potent in inhibiting gastrin-induced acid secretion. From these studies, the significant role of the C-terminal dipeptide of gastrin was pointed out. More particularly, the functional role of the phenylalanine through the C-terminal carboxamide and its binding role through its aromatic ring were demonstrated.

The search for active in vivo gastrin antagonists is still an interesting challenge that greatly depends on a good knowledge of the mechanism of action of gastrin. Structure–activity relationships were studied by Morley[1] on an unprecedented scale and showed that all the diverse biological activities of gastrin were found to be closely related to the C-terminal tetrapeptide amide portion of the molecule of sequence Trp-Met-Asp-Phe-NH_2.[2] Owing to the significance of tryptophan and phenylalanine residues, many analogues of the type Trp-Met-X-Phe-NH_2 were prepared, but they were devoid of antisecretory activity. Attempts to apply the "Robard's multi-subsite receptor model"[3] to the concept of gastrin antagonists was also unsuccessful.[4] Previous studies dealing with the significance of the phenylalanyl residue in C-terminal gastrin and cholecystokinin related peptides[5a] and our recent results[5b] allowed us to propose a functional rather than a binding role for this residue. C-Terminal gastrin related peptides lacking the phenylalanyl residue bind to the gastrin receptor while they are devoid of biological activity and are able to inhibit gastrin-induced acid secretion.[6] However, the loss of the phenylalanyl residue resulted in a significant decrease in the affinity of the peptide for the gastrin receptor. Recently we showed the importance of the peptide bonds of the C-terminal tetrapeptide of gastrin for eliciting biological activity, particularly of the bond between methionine and aspartic acid.[7] The pseudopeptide Boc-Trp-Leu-ψ(CH_2NH)-Asp-Phe-NH_2 analogue of the C-terminal tetrapeptide of gastrin, in which the peptide bond between leucine and aspartic acid had been replaced by a CH_2NH bond, was able to bind to the gastrin receptor with the same affinity as the tetrapeptide, but was devoid of biological activity. In fact, this pseudopeptide inhibited gastrin-induced acid secretion with ED_{50} value of 0.3 mg/kg,[7] whereas the pseudopeptide Boc-Trp-ψ-(CH_2NH)-Leu-Asp-Phe-NH_2, in which the peptide bond between tryptophan and leucine has been replaced by a CH_2NH bond, behaved as a complete agonist as potent as the tetrapeptide of natural sequence. From these results, we postulated that, for exhibiting agonist activity on acid secretion, the bond between methionine (or leucine) and

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(1) Morley, J. S. *Proc. R. Soc. London, B* **1968**, *170*, 97.
(2) Tracy, H. J.; Gregory, R. A. *Nature (London)* **1964**, *204*, 935.
(3) de Lean, A.; Munson, P. J.; Robard, D. *Mol. Pharmacol.* **1979**, *15*, 60.
(4) Morley, J. S. In *Gut Peptides and Ulcer*; Miyoshi, A., Ed.; Biomedical Research Foundation: Tokyo, **1982**; p 1.
(5) (a) Mazur, R. H. U.S. Patent 4 012 367, 1976. (b) Martinez, J.; Bali, J. P. *Regul. Pept.* **1984**, *9*, 259.
(6) Martinez, J.; Magous, R.; Lignon, M. F.; Laur, J.; Castro, B.; Bali, J. P. *J. Med. Chem.* **1984**, *27*, 1597.
(7) Martinez, J.; Bali, J. P.; Magous, R.; Laur, J.; Lignon, M. F.; Rodriguez, M.; Castro, B. *C. R. Seances Acad. Sci., Ser. I* **1985**, *300*, 437. Martinez, J.; Bali, J. P.; Rodriguez, M.; Castro, B.; Magous, R.; Laur, J.; Lignon, M. F. *J. Med. Chem.* **1985**, *28*, 1874.

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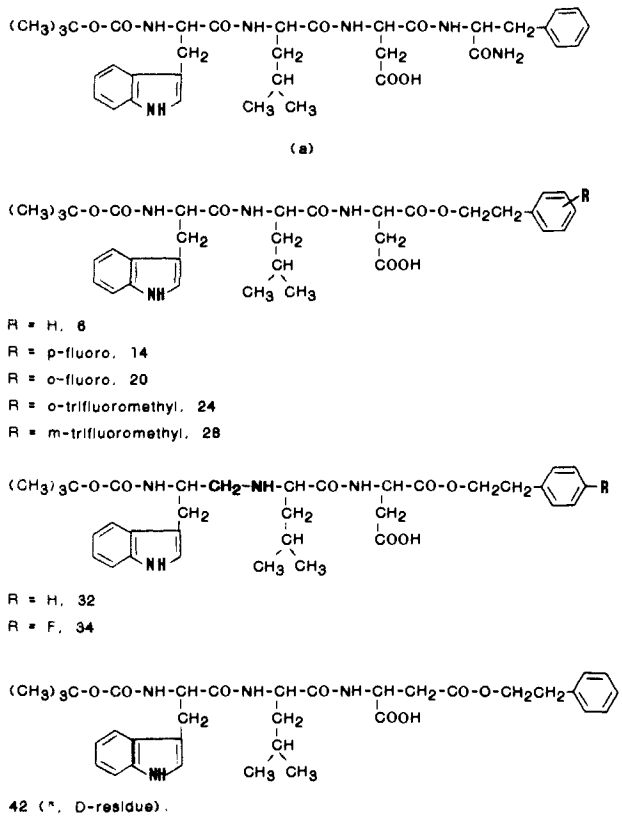


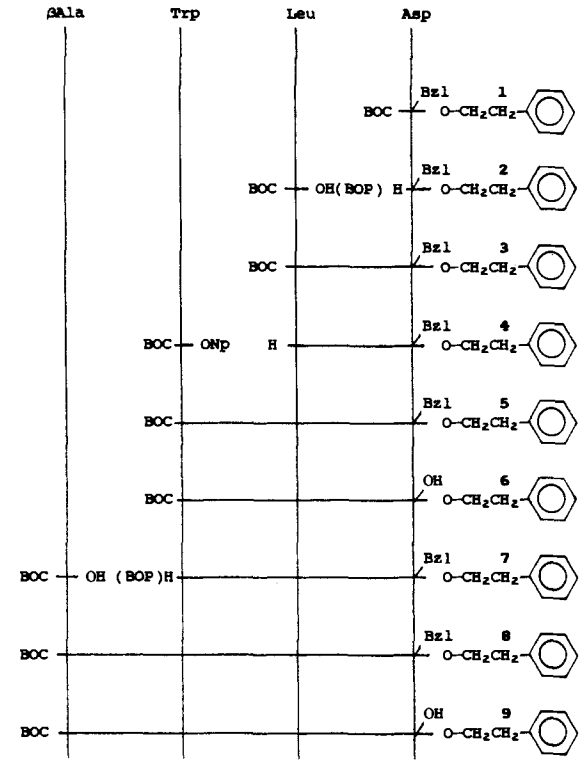
Figure 1. Molecular formulas of the synthetic peptide derivatives of the C-terminal tetrapeptide analogue of gastrin (a).

aspartic acid in the C-terminal tetrapeptide of gastrin should be an amide bond, a cleavable bond. The result of this cleavage at the site of action of the hormone, the dipeptide Asp-Phe-NH_2, might play a significant role in the biological activity. It has been demonstrated already that this dipeptide was able to exhibit agonist activity on acid secretion[8] at high concentration. Those reasons prompted us to investigate more in detail the structural requirements of the C-terminal dipeptide end of gastrin in relation to the biological responses. In this work, we examined the influence of the replacement of C-terminal phenylalanyl amide residue by a phenethyl alcohol and by phenethyl-substituted alcohols on in vivo gastrin-stimulated acid secretion in the anesthetized rat, according to Ghosh and Schild.[9] In the peptide derivatives synthesized in this work (Figure 1), leucine has been used instead of methionine. This change does not affect the biological activity,[10] but it makes easier the syntheses and enhances the stability of the compounds.

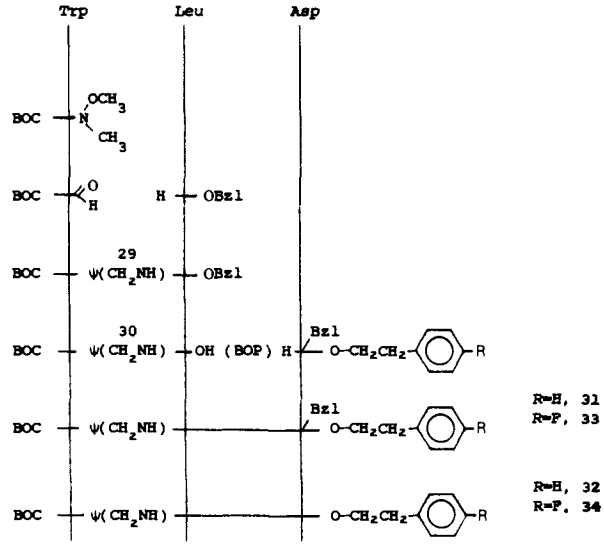
Chemistry

Compound Boc-Trp-Leu-Asp(Bzl) phenethyl ester (**5**) was synthesized according to Scheme I. The phenethyl ester of Boc-Asp(Bzl) (1) was prepared from the cesium salt of Boc-Asp(Bzl) and phenethyl bromide according to the procedure of Gisin[11] or from Boc-Asp(Bzl) and phenethyl bromide in the presence of DBU.[12] After partial deblocking with trifluoroacetic acid to yield compound **2**

Scheme I



Scheme II



and coupling with Boc-Leu in the presence of BOP as activating reagent,[13] compound **3** was obtained. It was partially deprotected by trifluoroacetic acid and allowed to react with the *p*-nitrophenyl ester of Boc-Trp[14] in the presence of HOBt as catalyst[15] to yield Boc-Trp-Leu-Asp(Bzl) phenethyl ester (**5**). It was partially deprotected by hydrogenation in the presence of Pd/C as catalyst to produce Boc-Trp-Leu-Asp phenethyl ester (**6**). Compound **9**, Boc-βAla-Trp-Leu-Asp phenethyl ester, was obtained by coupling Boc-βAla and the trifluoroacetate salt **7** with BOP as coupling reagent, followed by hydrogenation in the presence of Pd/C as catalyst in an ethanol/acetic acid mixture as described in Scheme I. Compounds **14**, **16**, **20**,

(8) Bertaccini, G. *Pharmacol. Future Man, Proc. Int. Congr. Pharmacol., 5th, 1972* **1972**, *5*, 336.
(9) Ghosh, M. N.; Schild, H. O. *J. Physiol. (London)* **1955**, *128*, 35.
(10) Martinez, J.; Bali, J. P.; Magous, R.; Laur, J.; Lignon, M. F.; Briet, C.; Nisato, D.; Castro, B. *J. Med. Chem.* **1985**, *28*, 273.
(11) Gisin, B. F. *Helv. Chim. Acta* **1973**, *56*, 1476.
(12) Ono, N.; Yamada, T.; Saito, T.; Tanaka, K.; Kaji, A. *Bull. Chem. Soc. Jpn* **1978**, *51*, 2401.
(13) Castro, B.; Dormoy, J. R.; Dourtoglou, B.; Evin, G.; Selve, C.; Ziegler, C. *Synthesis* **1976**, 751.
(14) Bodanszky, M.; Natarajan, S. *J. Org. Chem.* **1975**, *40*, 2495.
(15) König, W.; Geiger, R. *Chem. Ber.* **1973**, *106*, 3626.

Table I. Analytical and Physical Data of Synthetic Peptide Derivatives

peptide derivatives	mp, °C	$[\alpha]_D$,[a] deg	R_f[b]	anal. C, H, N
Boc-Asp(Bzl) phenethyl ester (1)	58–60	−20.7	(A) 0.96, (B) 0.88	$C_{24}H_{29}NO_6$
Boc-Leu-Asp(Bzl) phenethyl ester (3)	oil	−19.9	(A) 0.95, (B) 0.91	
Boc-Trp-Leu-Asp(Bzl) phenethyl ester (5)	135–138	−24	(A) 0.81, (B) 0.83	$C_{41}H_{50}N_4O_8$
Boc-Trp-Leu-Asp phenethyl ester (6)	95–100	−22.7	(B) 0.17, (F) 0.75	$C_{34}H_{44}N_4O_8$
Boc-βAla-Trp-Leu-Asp(Bzl) phenethyl ester (8)	163–166	−18.9	(A) 0.38, (B) 0.76	$C_{44}H_{55}N_5O_9$
Boc-βAla-Trp-Leu-Asp phenethyl ester (9)	100–103	−17.7	(B) 0.09, (F) 0.68	$C_{37}H_{49}N_5O_9$
Boc-Leu-Asp phenethyl ester (10)	65–68	−19.5	(A) 0.15, (B) 0.57	$C_{23}H_{34}N_2O_7$
Boc-Asp(Bzl) *p*-fluorophenethyl ester (11)	46–48	−20	(C) 0.87, (D) 0.92	$C_{24}H_{28}NO_6F$
Boc-Leu-Asp(Bzl) *p*-fluorophenethyl ester (12)	oil	−18.5	(C) 0.66, (D) 0.59	
Boc-Trp-Leu-Asp(Bzl) *p*-fluorophenethyl ester (13)	90–92	−23	(C) 0.17, (H) 0.74	$C_{41}H_{49}N_4O_8F$
Boc-Trp-Leu-Asp *p*-fluorophenethyl ester (14)	103–107	−19	(F) 0.74, (G) 0.10	$C_{34}H_{43}N_4O_8F$
Boc-βAla-Trp-Leu-Asp(Bzl) *p*-fluorophenethyl ester (15)	80–85	−22	(D) 0.61, (E) 0.84	$C_{44}H_{54}N_5O_9F$
Boc-βAla-Trp-Leu-Asp *p*-fluorophenethyl ester (16)	105–110	−14	(E) 0.42, (G) 0.79	$C_{37}H_{48}N_5O_9F$
Boc-Asp(Bzl) *o*-fluorophenethyl ester (17)	30–35	−10	(C) 0.89, (D) 0.96	$C_{24}H_{28}NO_6F$
Boc-Leu-Asp(Bzl) *o*-fluorophenethyl ester (18)	oil	−11	(C) 0.83, (D) 0.90	
Boc-Trp-Leu-Asp(Bzl) *o*-fluorophenethyl ester (19)	68–71	−25	(C) 0.44, (D) 0.87	$C_{41}H_{49}N_4O_8F$
Boc-Trp-Leu-Asp *o*-fluorophenethyl ester (20)	90–95 dec	−19	(E) 0.58, (G) 0.83	$C_{34}H_{43}N_4O_8F$
Boc-Asp(Bzl) *o*-(trifluoromethyl)phenethyl ester (21)	58–60	−14	(C) 0.89, (D) 0.96	$C_{25}H_{28}NO_6F_3$
Boc-Leu-Asp(Bzl) *o*-(trifluoromethyl)phenethyl ester (22)	oil	−15	(C) 0.83, (D) 0.93	
Boc-Trp-Leu-Asp(Bzl) *o*-(trifluoromethyl)phenethyl ester (23)	80–85	−18	(C) 0.42, (D) 0.92	$C_{42}H_{49}N_4O_8F_3$
Boc-Trp-Leu-Asp *o*-(trifluoromethyl)phenethyl ester (24)	95 dec	−11	(E) 0.60, (G) 0.82	$C_{35}H_{43}N_4O_8F_3$
Boc-Asp(Bzl) *m*-(trifluoromethyl)phenethyl ester (25)	oil	−12.9	(C) 0.67, (D) 0.69	
Boc-Leu-Asp(Bzl) *m*-(trifluoromethyl)phenethyl ester (26)	72–75	−18.2	(C) 0.68, (D) 0.61	$C_{31}H_{39}N_2O_7F_3$
Boc-Trp-Leu-Asp(Bzl) *m*-(trifluoromethyl)phenethyl ester (27)	55–60	−5.6	(C) 0.14, (E) 0.80	$C_{42}H_{49}N_4O_8F_3$
Boc-Trp-Leu-Asp *m*-(trifluoromethyl)phenethyl ester (28)	95–100	−19.6	(F) 0.74, (G) 0.12	$C_{35}H_{43}N_4O_8F_3$
Boc-Trp-ψ(CH_2NH)-Leu-OBzl (29)	100–102	−21.5	(D) 0.88, (E) 0.83	$C_{29}H_{37}N_3O_4$
Boc-Trp-ψ(CH_2NH)-Leu-OH (30)	220 dec	−20	(E) 0.20, (G) 0.34	$C_{22}H_{31}N_3O_4$
Boc-Trp-ψ(CH_2NH)-Leu-Asp(Bzl) phenethyl ester (31)	175 dec	−8.4	(D) 0.85, (E) 0.81	$C_{41}H_{52}N_4O_7$
Boc-Trp-ψ(CH_2NH)-Leu-Asp phenethyl ester (32)	120 dec	−12	(E) 0.29, (G) 0.82	$C_{34}H_{46}N_4O_7$
Boc-Trp-ψ(CH_2NH)-Leu-Asp(Bzl) *p*-fluorophenethyl ester (33)	175 dec	−10	(D) 0.86, (E) 0.84	$C_{41}H_{51}N_4O_7F$
Boc-Trp-ψ(CH_2NH)-Leu-Asp *p*-fluorophenethyl ester (34)	90 dec	−24	(E) 0.31, (G) 0.82	$C_{34}H_{45}N_4O_7F$
Boc-D-Trp-Leu-Asp(Bzl) phenethyl ester (35)	67–70	−14.1	(A) 0.71, (B) 0.88	$C_{41}H_{50}N_4O_8$
Boc-D-Trp-Leu-Asp phenethyl ester (36)	108–110 dec	−15.8	(B) 0.13, (F) 0.75	$C_{34}H_{44}N_4O_8$
Boc-βAla-D-Trp-Leu-Asp(Bzl) phenethyl ester (37)	153–156	−27.8	(A) 0.41, (B) 0.80	$C_{44}H_{55}N_5O_9$
Boc-βAla-D-Trp-Leu-Asp phenethyl ester (38)	105–110 dec	−20.4	(B) 0.09, (F) 0.71	$C_{37}H_{49}N_5O_9$
Boc-β(*p*-fluorophenethyl ester)-D-Asp-OBzl (39)	55–58	+17	(C) 0.87, (D) 0.92	$C_{24}H_{28}NO_6F$
Boc-Leu-β(*p*-fluorophenethyl ester)-D-Asp-OBzl (40)	71–73	+2.5	(C) 0.74, (D) 0.93	$C_{30}H_{39}N_2O_7F$
Boc-Trp-Leu-β(*p*-fluorophenethyl ester)-D-Asp-OBzl (41)	155–157	−3.8	(C) 0.32, (D) 0.91	$C_{41}H_{49}N_4O_8F$
Boc-Trp-Leu-β(*p*-fluorophenethyl ester)-D-Asp-OH (42)	100 dec	−13	(E) 0.49, (G) 0.77	$C_{34}H_{43}N_4O_8F$

[a] *c* 1, DMF. [b] Solvents: A, AcOEt/hexane, 8:2; B, acetone/hexane, 7:3; C, hexane/ethyl acetate, 1:1; D, ethyl acetate; E, $CHCl_3$/methanol/acetic acid, 85:10:5; F, AcOEt/pyr/AcOH/H_2O, 80:20:3:3; G, AcOEt/pyr/AcOH/H_2O, 80:20:5:10; H, $CHCl_3$/MeOH, 3%.

24, and 28 were synthesized according to the same scheme, starting by condensation of Boc-Asp(Bzl) with *p*-fluorophenethyl alcohol, *o*-fluorophenethyl alcohol, *m*-trifluorophenethyl alcohol, or *o*-fluorophenethyl alcohol in the presence of *N*,*N*-dicyclohexylcarbodiimide, with 4-(dimethylamino)pyridine as catalyst.[22] Compounds 32 and 34 were prepared according to Scheme II. The aldehyde of Boc-Trp, obtained by reduction with $LiAlH_4$ of the corresponding *N*,*O*-dimethyl hydroxamate,[16] was allowed to react with the *p*-toluenesulfonate salt of leucine benzyl ester, in a methanol/acetic acid mixture in the presence of $NaBH_4CN$ as reducing reagent,[17] to yield, after purification by silica gel chromatography, Boc-Trp-ψ(CH_2NH)-Leu-OBzl (29). Partial deprotection by hydrogenation in the presence of Pd/C as catalyst produced Boc-Trp-ψ(CH_2NH)-Leu-OH (30). Compound 30 was allowed to react with the TFA salt of Asp(Bzl) phenethyl ester or with the TFA salt of *p*-fluorophenethyl ester in the presence of BOP as coupling reagent to yield, respectively, Boc-Trp-ψ(CH_2NH)-Leu-Asp(Bzl) phenethyl ester (31) and Boc-Trp-ψ(CH_2NH)-Leu-Asp(Bzl) *p*-fluorophenethyl ester (33). No racemization occurred during this coupling by the oxazolone of Boc-Trp-ψ(CH_2NH)-Leu-OH during activation which cannot be formed. Partial deprotection of compounds 31 and 33 by hydrogenation in the presence of Pd/C as catalyst yielded Boc-Trp-ψ(CH_2NH)-Leu-Asp phenethyl ester (32) and Boc-Trp-ψ(CH_2NH)-Leu-Asp *p*-fluorophenethyl ester (34). Compound 42 was synthesized according to Scheme III. The α-benzyl ester of Boc-D-Asp was allowed to react with *p*-fluorophenethyl bromide in the presence of DBU to yield Boc-D-Asp(*p*-fluorophenethyl ester)-OBzl (39). Partial deprotection of 39 with trifluoroacetic acid and coupling with Boc-Leu in the presence of BOP as coupling reagent yielded Boc-Leu-D-Asp(*p*-fluorophenethyl ester)-OBzl (40). It was treated with trifluoroacetic acid and then allowed to react with Boc-Trp-ONp to produce Boc-Trp-Leu-Asp(*p*-fluorophenethyl ester)-OBzl (41), which was hydrogenated in the presence of Pd/C as catalyst to yield Boc-Trp-Leu-Asp(*p*-fluorophenethyl ester)-OH (42). Physical and analytical data of the derivatives synthesized in this work are reported in Table I.

Biological Results

Table II shows the results of the biological activities of compounds 6, 9, 10, 14, 16, 20, 24, 28, 32, 34, 36, 38, and 42 on acid secretion in the anesthetized rat according to Ghosh and Schild.[9] None of these compounds exhibited agonist activity at doses as high as 5 mg/kg. However,

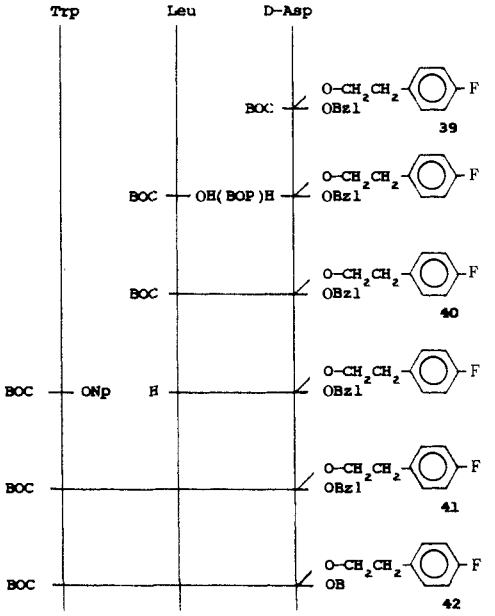
(16) Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, 676.
(17) Jacobson, K. A.; Marr-Leisy, D.; Rosenkranz, R. P.; Verlander, M.; Melmon, K. L.; Goodman, M. *J. Med. Chem.* **1983**, *26*, 492.
(18) Martinez, J.; Potier, P. *Trends Pharmacol. Sci.* **1986**, *7*, 139.
(19) Magous, R.; Bali, J. P. *Eur. J. Pharmacol.* **1982**, *82*, 47.
(20) Devilliers, R.; Castro, B.; Bali, J. P.; Martinez, J., in preparation.
(21) Magous, R.; Bali, J. P. *Regul. Pept.* **1983**, *7*, 233.
(22) Gilon, C.; Klausner, Y.; Hassner, A. *Tetrahedron Lett.* **1979**, 3811.

Table II. Antagonist Activity of Phenethyl Ester Peptide Derivatives, Analogues of the C-Terminal Tetrapeptide of Gastrin, on Gastrin-Induced Acid Secretion in the Anesthetized Rat, According to Ghosh and Schild[a]

peptide derivatives	ED_{50}, mg/kg
Boc-Trp-Leu-Asp phenethyl ester (**6**)	0.1
Boc-βAla-Trp-Leu-Asp phenethyl ester (**9**)	0.04
Boc-Leu-Asp phenethyl ester (**10**)	inactive
Boc-Trp-Leu-Asp *p*-fluorophenethyl ester (**14**)	0.08
Boc-βAla-Trp-Leu-Asp *p*-fluorophenethyl ester (**16**)	0.02
Boc-Trp-Leu-Asp *o*-fluorophenethyl ester (**20**)	0.1
Boc-Trp-Leu-Asp *o*-(trifluoromethyl)phenethyl ester (**24**)	1.5
Boc-Trp-Leu-Asp *m*-(trifluoromethyl)phenethyl ester (**28**)	>1
Boc-Trp-ψ(CH_2NH)Leu-Asp phenethyl ester (**32**)	1
Boc-Trp-ψ(CH_2NH)Leu-Asp *p*-fluorophenethyl ester (**34**)	0.1
Boc-D-Trp-Leu-Asp phenethyl ester (**36**)	0.8
Boc-βAla-D-Trp-Leu-Asp phenethyl ester (**38**)	0.08
Boc-Trp-Leu-β(*p*-fluorophenethyl ester)-D-Asp-OH (**42**)	inactive

[a] None of these compounds exhibited agonist activity at doses up to 3 mg/kg.

Scheme III



compounds **6**, **9**, **14**, **16**, **20**, **24**, **28**, **32**, **34**, **36**, and **38** were able to inhibit gastrin-induced acid secretion, whereas compounds **10** showed little antagonist activity and **42** was inactive. As it is indicated in Table II, the most potent compounds in inhibiting gastrin-induced acid secretion were the tripeptide derivatives **9** and **16**, with ED_{50} values of 0.04 and 0.02 mg/kg, respectively, and the dipeptide derivatives **6**, **14**, and **34**, with ED_{50} values of 0.1, 0.08, and 0.1 mg/kg, respectively. Compound **41** was less active, as well as other substituted phenethyl esters. Compounds **36** and **38** having a D-tryptophan in their sequence were quite active: ED_{50} values of 0.8 mg/kg for the peptide derivative **36** and 0.08 mg/kg for **38**.

Discussion

According to our hypothesis on one of the possible mechanisms of action of gastrin, the bond between methionine and aspartic acid might be cleaved, probably after the hormone is bound to its receptor. The result of this cleavage would be the liberation, at this precise point, of the dipeptide Asp-Phe-NH_2 (or a derivative), which might be of some importance for the biological response.[7] We already pointed out the significance of such fragments liberated from a larger molecule, leading to compounds at high concentrations at a precise and particular point.[18] According to previous studies, any change at the aspartyl residue resulted in less active or inactive compounds. We investigated the significance of chemical changes made at the phenylalanyl C-terminal residue on the biological activity on acid secretion, by the synthesis of the peptide derivatives Boc-Trp-Leu-Asp phenethyl ester (**6**) and Boc-βAla-Trp-Leu-Asp phenethyl ester (**9**). In these derivatives the phenylalanyl residue has been replaced by a phenethyl group and the amide bond by an ester bond. This modification led also to the suppression of the C-terminal amide, whereas the aromatic ring is still present and located at the same distance as in the natural tetrapeptide. Compounds **6** and **9** did not exhibit agonist activity on acid secretion but were potent inhibitors of gastrin-induced acid secretion (ED_{50} values of 0.1 and 0.04 mg/kg, respectively). Both peptide derivatives recognize the gastrin receptor. On the inhibition of [^{125}I]-(Nle^{11})-HG-13 binding to isolated gastric mucosal cells,[19] compound **6** showed an IC_{50} value of 7.5×10^{-7} M and compound **9** an IC_{50} value of 7.5×10^{-8} M, which are very close to that of the corresponding tetrapeptide Boc-Trp-Leu-Asp-Phe-NH_2 ($IC_{50} = 5 \times 10^{-7}$ M) and pentapeptide Boc-βAla-Trp-Leu-Asp-Phe-NH_2 ($IC_{50} = 5 \times 10^{-8}$ M). Extension on the N-terminal part of the molecule by a β-alanine proved again, either in vivo or in vitro, to increase the potency of the pseudopeptides. The major role played by the tryptophyl residue was again pointed out. The peptide derivative Boc-Leu-Asp phenethyl ester (**10**) did not show any activity when tested at doses up to 5 mg/kg. Owing to the high potency of compounds **6** and **9** in inhibiting gastrin-induced acid secretion, which confirms the particular role played by the C-terminal dipeptide, we synthesized peptide derivatives with fluoro- or trifluoromethyl-substituted phenethyl esters to make this C-terminal end more lipophilic. *p*-Fluorophenethyl ester derivatives **14** and **16**, *o*-fluorophenethyl ester derivative **20**, *o*-(trifluoromethyl)phenethyl ester derivative **24**, and *m*-(trifluoromethyl)phenethyl ester derivative **28** were prepared, and their activity on acid secretion was evaluated (Table II). None of these compounds exhibited agonist activity on acid secretion. Except for compounds **14** and **16**, which were very potent (ED_{50} values of 0.08 and 0.02 mg/kg, respectively), the other derivatives were less active in inhibiting gastrin-induced acid secretion. From these results we can conclude that substitution of the para position of the aromatic ring of the phenethyl group by a fluorine led to compounds with increased potency in inhibiting gastrin-induced acid secretion, whereas other substituted positions or substitution by a trifluoromethyl group led to less potent compounds. In order to increase the resistance against proteolytic enzymes of the more potent compounds **6** and **14**, at least at the tryptophan–leucine bond, we synthesized the pseudopeptides Boc-Trp-ψ(CH_2NH)-Leu-Asp phenethyl ester (**32**), and Boc-Trp-ψ(CH_2NH)-Leu-Asp *p*-fluorophenethyl ester (**34**)

(according to Scheme II) and the peptide derivatives Boc-D-Trp-Leu-Asp phenethyl ester (**36**) and Boc-βAla-D-Trp-Leu-Asp phenethyl ester (**38**). Compounds **32** and **34** were potent in inhibiting gastrin-induced acid secretion (ED_{50} values of 1 and 0.1 mg/kg, respectively) as well as **36** and **38** (ED_{50} values of 0.8 and 0.06 mg/kg, respectively). Finally we synthesized Boc-Trp-Leu-D-Asp(*p*-fluorophenethyl ester)-OH (**42**), according to Scheme III, in order to look at the significance of the distance of the aromatic ring to the carboxylate of the aspartyl residue and of this carboxylate to the peptide backbone. This compound was found inactive, either as an agonist on acid secretion or in inhibiting gastrin-induced acid secretion (tested at doses up to 5 mg/kg).

Conclusion

The results obtained in this work strongly support an important role for the C-terminal dipeptide of gastrin. Particularly, the C-terminal phenylalanine amide seems to play a significant role, more specifically, a binding role through the aromatic moiety and a functional role through the C-terminal amide group. Phenethyl ester derivative analogues of the C-terminal tetrapeptide of gastrin did not exhibit activity on acid secretion, whereas they were very potent in inhibiting gastrin-induced acid secretion. Compounds **6** and **9** were able to recognize the gastrin receptor with high affinity, showing that the C-terminal amide group is not essential for the binding to the gastrin receptor, but is crucial for the biological activity. The search of gastrin antagonists by modifying the chemical structure of the C-terminal dipeptide of gastrin Asp-Phe-NH_2, without disturbing the special location of the carboxylate and of the aromatic moiety, is a promising field. As another known example, proglumide, which mimics the C-terminal end of gastrin, particularly the C-terminal tripeptide,[20] is a gastrin antagonist.[21] It seems that restricted chemical changes that can be made at the C-terminal dipeptide of gastrin that will produce antagonists of this C-terminal dipeptide might be a way of interfering in the mechanism of action of gastrin and of producing gastrin antagonists.

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 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, 8:2; B, acetone/hexane, 7:3; C, hexane/ethyl acetate, 1:1; D, ethyl acetate; E, $CHCl_3$/methanol/acetic acid, 85:10:5; F, AcOEt/pyr/AcOH/H_2O, 80:20:3:3; G, AcOEt/pyr/AcOH/H_2O, 80:20:5:10; H, $CHCl_3$/MeOH, 3%. Peptide derivatives were located with charring reagent or ninhydrin. Column chromatographies were performed with silica gel 60, 60–229 mesh, ASTM (Merck). L-Amino acids and derivatives were from Bachem (Switzerland). All reagents and solvents were of analytical grade. BOP was recrystallized from acetone and ether. The following abbreviations were used: DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DIEA, *N,N*-diisopropylethylamine; BOP, benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene(1,5,5); DCC, *N,N'*-dicyclohexylcarbodiimide. Other abbreviations used were those recommended by the IUPAC-IUB Commission (*Eur. J. Biochem.* 1984, *138*, 9–37).

Synthesis. The peptide derivatives described in this work were synthesized according to the general procedures detailed in this section for the preparation of compounds **6** and **9**, unless syntheses are described. All compounds used for biological tests were dissolved in 0.1 N NH_4OH, filtered on Millipore (0.45 μm), and lyophilized. The purity of the lyophilized compounds was checked again by TLC.

(*tert*-Butyloxycarbonyl)-β-benzyl-L-aspartic Acid Phenethyl Ester (1). To a solution of the cesium salt of Boc-(β-benzyl)-L-aspartic acid (4.55 g, 10 mmol) in DMF (100 mL) was added phenethyl bromide (2.03 g, 11 mmol) with vigorous stirring. After the mixture was allowed to stand overnight at room temperature, the solvent was removed in vacuo ($t < 40$ °C) and the residue dissolved in ethyl acetate (250 mL) and washed with a saturated sodium bicarbonate solution (2 × 50 mL), water (2 × 50 mL), a 10% citric acid solution (2 × 50 mL), and water (2 × 50 mL). The organic layer was dried over sodium sulfate and then concentrated in vacuo. The residue, triturated with a mixture of ether/hexane, gave a white powder, yield 3.6 g (85%): R_f (A) 0.96, (B) 0.88; mp 58–60 °C; $[\alpha]_D$ −20.7° (*c* 1, DMF). Anal. ($C_{24}H_{29}NO_6$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-leucyl-β-benzyl-L-aspartic Acid Phenethyl Ester (3). Compound **1** (4.39 g, 10 mmol) was treated with trifluoroacetic acid (25 mL). After standing at room temperature for 30 min, ether (300 mL) was added and the solvents were concentrated in vacuo ($t < 40$ °C). The oily residue was triturated several times with ether and dried in vacuo over KOH. It was dissolved in DMF (30 mL) containing Boc-L-Leu (1.21 g, 5.5 mmol) and BOP (2.21 g, 5 mmol). The solution was cooled in an ice–water bath, and DIEA (1.8 mL) was added. After being allowed to stand overnight at room temperature, the reaction mixture was treated as described for compound **1**. The crude oil was purified by silica gel chromatography with a mixture of ethyl acetate/hexane 1:1 as eluent to yield pure compound **3** as an oil (2.20 g, 82%): R_f (A) 0.95, (B) 0.91; $[\alpha]_D$ −20° (*c* 1.1, DMF).

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucyl-β-benzyl-L-aspartic Acid Phenethyl Ester (5). Compound **3** (2 g, 3.7 mmol) was treated with trifluoroacetic acid (15 mL). After standing at room temperature for 30 min, ether (300 mL) was added and the solvents were concentrated in vacuo ($t < 40$ °C). The oily residue was triturated several times with ether and dried in vacuo over KOH. It was dissolved in DMF (20 mL) in the presence of Boc-L-Trp-ONp[14] (1.45 g, 3.5 mmol) and HOBt (0.54 g, 3.5 mmol). The solution was cooled in an ice–water bath, and DIEA (1.24 mL, 7 mmol) was added. After being allowed to stand overnight at room temperature, the reaction mixture was treated as described for compound **1**. The crude oil was purified by silica gel chromatography with a mixture of ethyl acetate/hexane 1:1 as eluent to yield pure compound **5**. It gave a white powder upon trituration with ether, yield 2.36 g (88%): R_f (A) 0.81, (B) 0.83; mp 135–138 °C; $[\alpha]_D$ −24° (*c* 1.3, DMF). Anal. ($C_{41}H_{50}N_4O_8$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartic Acid Phenethyl Ester (6). Compound **5** (0.73 g, 1 mmol) was dissolved in 95% ethanol (50 mL) and hydrogenated in the presence of a 10% Pd/C catalyst. After 4 h, no more starting material could be detected by TLC. The catalyst was removed by filtration and the solvent concentrated in vacuo ($t < 40$ °C). The residue gave a white powder upon trituration with ether. It was filtered, rinsed several times with a mixture of ether/hexane, and dried in vacuo over P_2O_5, yield 0.48 g (76%): R_f (F) 0.75, (B) 0.17; mp 95–100 °C; $[\alpha]_D$ −22.7° (*c* 1.3, DMF). Anal. ($C_{34}H_{44}N_4O_8$) 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.

(*tert*-Butyloxycarbonyl)-β-alanyl-L-tryptophyl-L-leucyl-β-benzyl-L-aspartic Acid Phenethyl Ester (8). Compound **5** (0.73 g, 1 mmol) was treated with trifluoroacetic acid (5 mL). After the mixture was allowed to stand at room temperature for 30 min, ether (300 mL) was added and the solvents were concentrated in vacuo ($t < 40$ °C). The oily residue was triturated several times with ether and dried in vacuo over KOH. It was dissolved in DMF (10 mL) containing Boc-β-alanine (0.19 g, 1 mmol) and BOP (0.4 g, 0.9 mmol). The solution was cooled in an ice–water bath, and DIEA (0.4 mL) was added. After being allowed to stand overnight at room temperature, the reaction mixture was treated as described for compound **1**. The crude oil was purified by silica gel chromatography with a mixture of ethyl acetate/hexane 7:3 as eluent to yield pure compound **8**. It gave a white powder upon trituration with ether, yield 0.59 g (83%): R_f (A) 0.38, (B) 0.76; mp 163–166 °C; $[\alpha]_D$ −19° (*c* 1.2, DMF). Anal. ($C_{44}H_{55}N_5O_9$) C, H, N.

(*tert*-Butyloxycarbonyl)-β-alanyl-L-tryptophyl-L-leucyl-L-aspartic Acid Phenethyl Ester (9). Compound 8 (0.4 g, 0.5 mmol) was dissolved in 95% ethanol (20 mL) and hydrogenated in the presence of a 10% Pd/C catalyst. After 5 h, no more starting material could be detected by TLC. The catalyst was removed by filtration and the solvent concentrated in vacuo ($t < 40$ °C). The residue gave a white powder upon trituration with ether. It was collected by filtration, rinsed several times with a mixture of ether/hexane, and dried in vacuo over P_2O_5, yield 0.28 g (79%): R_f (F) 0.68, R (B) 0.09; mp 100–103 °C; $[\alpha]_D$ −17.7 (*c* 1.7, DMF). Anal. ($C_{37}H_{49}N_5O_9$) 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.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2NH)-L-leucine Benzyl Ester (29). (*tert*-Butyloxycarbonyl)-L-tryptophanal[7] (1.44 g, 5 mmol) was dissolved in a mixture of methanol/acetic acid 99:1 (30 mL) containing the *p*-toluenesulfonate salt of leucine benzyl ester (1.76 g, 4.5 mmol). Sodium cyanoborohydride (10 mmol) was added portionwise during 45 min. After 1 h, no more leucine benzyl ester could be detected by TLC. The reaction mixture was cooled in an ice–water bath, and a saturated sodium bicarbonate solution (100 mL) was added with stirring, followed by ethyl acetate (150 mL). The organic layer was collected, washed with water (1 × 20 mL), dried over sodium sulfate, and concentrated in vacuo ($t < 40$ °C). The residue was purified by chromatography on silica gel, with ethyl acetate as eluent, to yield a pure compound, which gave a white powder by trituration with ether, yield 1.5 g (68%): R_f (D) 0.88, (E) 0.83; mp 100–102 °C; $[\alpha]_D$ −21.5° (*c* 1, DMF). Anal. ($C_{29}H_{37}N_3O_4$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2NH)-L-leucine (30). Compound **29** (1.47 g, 3 mmol) was hydrogenated in 95% ethanol in the presence of Pd/C as catalyst. After removal of the catalyst by filtration, the solvent was concentrated in vacuo and the resulting residue gave a white powder upon tituration with ether, yield 1.5 g (87%): R_f (E) 0.20, (G) 0.34; mp 220 °C dec; $[\alpha]_D$ −20° (*c* 1, DMF). Anal. ($C_{22}H_{31}N_3O_4$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2NH)-L-leucyl-(β-benzyl)-L-aspartic Acid Phenethyl Ester (31). Compound **30** (0.40 g, 1 mmol) was allowed to react with the TFA salt **2** (1.1 mmol) in the presence of BOP as coupling reagent (0.44 g, 1 mmol). After cooling in an ice–water bath, DIEA (0.35 mL, 2 mmol) was added and the reaction mixture was allowed to stand overnight at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate, washed with a sodium bicarbonate solution, washed with water, dried over sodium sulfate, and concentrated in vacuo. It was purified by silica gel chromatography using ethyl acetate/hexane as eluent, then ethyl acetate, yield 0.5 g (71%): R_f (D) 0.85, (E) 0.81; mp 175 °C dec; $[\alpha]_D$ −8.4° (*c* 1, DMF). Anal. ($C_{41}H_{52}N_4O_7$) C, H, N.

Compound **33** was similarly prepared from **30** and the TFA salt of Asp(Bzl) *p*-fluorophenethyl ester.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-ψ(CH_2NH)-L-leucyl-L-aspartic Acid Phenethyl Ester (32). Compound **31** (0.72 g, 1 mmol) was dissolved in 95% ethanol (30 mL) and hydrogenated in the presence of a 10% Pd/C catalyst. After 4 h, no more starting material could be detected by TLC. The catalyst was removed by filtration and the solvent concentrated in vacuo ($t < 40$ °C). The residue gave a white powder upon trituration with ether. It was filtered, rinsed several times with a mixture of ether/hexane, and dried in vacuo over P_2O_5, yield 0.45 g (72%): R_f (E) 0.29, (G) 0.82; mp 120 °C dec; $[\alpha]_D$ −12.2° (*c* 0.9, DMF). Anal. ($C_{34}H_{46}N_4O_7$) C, H, N. For biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore biological tests, this compound was dissolved in 0.1 N NH_4OH, filtered on Millipore (0.2 μm), and lyophilized. The purity of the lyophilized material was checked by TLC.

Compound **34** was obtained according the same procedure, by hydrogenation of **33**.

Biological Tests. Gastric acid secretion was determined in vivo in the reperfused rat stomach according to the method of Ghosh and Schild.[9] 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 Prof. E. Wunch, Max Planck Institute, München) and compounds **6**, **9**, **10**, 14, **16**, **20**, **24**, **28**, **32**, **34**, **36**, **38**, or **42** were dissolved in 0.9% NaCl and bolus injected intravenously. 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 and of gastrin (80 pmol were usually employed). The amount of H^+ secreted in the presence of various doses of the peptides was related to the amount of H^+ secreted after gastrin alone and expressed as percent of inhibition. The mean H^+ secretion after gastrin injection was 203 ± 28 μmol of H^+/nmol of peptide ($n = 17$).

Binding Studies. Isolation of rabbit gastric cells was carried out by the collagenase/EDTA procedure previously described.[19] 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 a 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 200*g* and then washed twice with medium A. This procedure gave about 5×10^7 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.[19] After purification by DE-52 ion-exchange chromatography, the mono-iodinated 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 the presence of 1×10^{-6} M cold (Nle^{11})-HG-13.