g, 9.1 mmol). After 15 min the reaction was poured into H_2O, affording 1.3 g (94%) of **9**.

4,5-Dichloro-6-methoxyphthalic Anhydride (10). A mixture of Ac_2O (150 mL) and compound **6** (23.85 g, 0.09 mol) was stirred and slowly distilled at 125–133 °C at atmospheric pressure over a 3.5- period. After the solution was cooled, 18.75 g (84%) of **10**, which melted at 220–222 °C, was obtained. Anal. ($C_9H_4Cl_2O_4$) C, H.

4,5-Dichloro-6-methoxy-2-phenyl-1*H*-indene-1,3(2*H*)-dione (11). A mixture of **10** (4.94 g, 0.02 mol), $C_6H_5CH_2CO_2H$ (2.72 g, 0.02 mol), Ac_2O (7.55 mL, 0.08 mol), and Et_3N (8.35 mL, 0.06 mol) was heated at reflux for 0.75 h. The volatile constituents were removed at reduced pressure. The residue was treated with 95% EtOH (20 mL), and the solution was heated to reflux, poured into 2% NaOH (450 mL), stirred for 6 h, and filtered. The filtrate was acidified with 6 N HCl to precipitate 4.85 g (76%) of **11**, which melted at 175–178 °C after recrystallization from EtOH. Anal. ($C_{16}H_{10}Cl_2O_3$) C, H.

4,5-Dichloro-6-methoxy-2-methyl-2-phenyl-1*H*-indene-1,3(2*H*)-dione (12). A mixture of **11** (0.5 g, 0.156 mmol), K_2CO_3 (0.432 g, 3.12 mmol), and CH_3I (0.8 mL, 12.5 mmol) in DMF (20 mL) was stirred at 35 °C for 3 h and then poured into H_2O (100 mL) to give 485 mg (93%) of **12**, which melted at 174–175.5 °C after recrystallization from C_6H_{12}–C_6H_6. Anal. ($C_{17}H_{12}Cl_2O_3$) C, H.

[(7′-Chloro-1′,3′-dihydro-6′-methyl-1′,3′-dioxospiro[cyclopentane-1,2′-[2*H*]inden]-5′-yl)oxy]acetonitrile (13). A stirred mixture of **2c** (9.3 g, 0.035 mol), $ClCH_2CN$ (2.83 g, 0.0375 mol), K_2CO_3 (5.2 g, 0.0375 mol), and KI (6.2 g, 0.0375 mol) in DMF (100 mL) was heated at 65 °C for 2.5 h and then poured into H_2O (1.5 L), affording 10.7 g (100%) of **13**, which melted at 137–139 °C after recrystallization from EtOH–H_2O. Anal. ($C_{16}H_{14}ClNO_3$) C, H, N.

4′-Chloro-5′-methyl-6′-(1*H*-tetrazol-5-ylmethoxy)spiro[cyclopentane-1,2′-[2*H*]indene]-1′,3′-dione (14). A stirred mixture of **13** (9.0 g, 0.03 mol), NaN_3 (3.9 g, 0.06 mol), and NH_4Cl (3.2 g, 0.06 mol) in DMF (150 mL) was heated at 95 °C for 2 h and then poured into dilute HCl to give 9.9 g (95%) of **14**, which melted at 211–212 °C after recrystallization from AcOH–H_2O. Anal. ($C_{16}H_{15}ClN_4O_3$) C, H, N.

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Synthesis of Potent Heptapeptide Analogues of Cholecystokinin

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Nine new analogues of acetyl-CCK-heptapeptide (Ac-Tyr(SO_3H)^2-Met^3-Gly^4-Trp^5-Met^6-Asp^7-Phe^8-NH_2) were synthesized by solid-phase methodology. In a first series, the Asp^7 residue was replaced by hydroxy amino acid sulfate esters. In another series, Gly^4 was substituted by D-Ala, while Trp^5 and Met^6 were replaced by their D enantiomer. The introduction of the sulfate ester was performed with a new, mild, crystalline, and stable reagent, pyridinium acetyl sulfate. Each analogue that contained Tyr(SO_3H)^2 and a hydroxy amino acid sulfate ester [Ser(SO_3H), Thr(SO_3H), or Hyp(SO_3H)] in position 7 proved to be more potent (1.9, 1.7, and 3.0 times, respectively) than CCK-8 in vitro (isolated gallbladder strips). While devoid of gastrin-like activity in vivo, these analogues had potent anticonvulsive activity. The analogues containing a D-amino acid residue were less potent than the parent compound in vitro. The D-Ala^4 replacement, however, yielded a compound that was 40% as potent as CCK-8 in the in vitro test but showed prolonged duration of action on sphincter Oddi. While the 7-substituted Ac-CCK heptapeptides are among the most potent CCK analogues reported so far, the D-Ala^4 replacement resulted, for the first time, in prolonged activity in vivo.

The gastrointestinal peptide hormone cholecystokinin-pancreozymin exists in different molecular forms, including CCK-39, CCK-33, CCK-12, and CCK-8.[1] All biologically active CCK fragments contain a tyrosine *O*-sulfate in position 27. The active center of the molecule is the C-terminal heptapeptide[2] (CCK-27-33 or CCK-7), but the shortest naturally occurring biologically active fragment is the C-terminal octapeptide[3] (CCK-26-33 or CCK-8).[4] The N-terminal amino group of CCK-7, however, is not necessary for cholecystokinetic activity,[5] and similarly, the *N*-acetyl derivative of CCK-7 shows the same potency as CCK-8 in the pancreas amylase release test.[6] On the basis of these observations and an earlier study[7] that demonstrated that the aspartyl residue of the tetragastrin (H-Trp-Met-Asp-Phe-NH_2) could be replaced by the electronically equivalent serine *O*-sulfate, we replaced the Asp^7 residue of Ac-CCK-7 by the sulfate ester of serine, threonine, or 3-hydroxyproline. Since it was also reported that

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Table I. Biological Potencies of CCK Analogues

compd	peptide sequence	in vivo gastrinic act., %	in vitro cholecystokinetic act., %	anticonvulsive act., relative %	
				tonic seizure	time of death
gastrin-5	H-Gly-Trp-Met-Asp-Phe-NH_2	100	0	62[a]	38[a]
CCK-8	H-Asp-Tyr(SO_3Na)-Met-Gly-Trp-Met-Asp-Phe-NH_2	0	100	100[a]	100[a]
1	Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2	0	190	66[a]	121[a]
2	Ac-Tyr-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2	40	0.3		
3	Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2	0	166	146[a]	115[a]
4	Ac-Tyr-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2	25	0.2		
5	Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2	0	300	79[a]	121[a]
6	Ac-Tyr-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2	0	0.3		
7	Ac-Tyr(SO_3Na)-Met-DAla-Trp-Met-Asp-Phe-NH_2	0	40	100[a]	115[a]
8	Ac-Tyr(SO_3Na)-Met-Gly-DTrp-Met-Asp-Phe-NH_2	0	0.7	65[a]	45[a]
9	Ac-Tyr(SO_3Na)-Met-Gly-Trp-DMet-Asp-Phe-NH_2	0	0.6	21	0

[a] Significant vs. saline-treated control groups.

the main metabolic cleavage sites of the CCK-8 were between Gly-Trp or Trp-Met,[8] we substituted these amino acids with their D enantiomer and Gly with D-alanine, in order to increase enzymic resistance. Although tetragastrin analogues with D-Trp or D-Met replacement have low biological potency,[9] it was reasoned that similar replacements could result in potent CCK-7 analogues, since the conformation of tetragastrin and CCK-7 were reported to be very different.[10]

Synthesis. The following analogues were synthesized: Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2 (**1**), Ac-Tyr-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2 (**2**), Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2 (**3**), Ac-Tyr-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2 (**4**), Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2 (**5**), Ac-Tyr-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2 (**6**), Ac-Tyr(SO_3Na)-Met-DAla-Trp-Met-Asp-Phe-NH_2 (**7**), Ac-Tyr(SO_3Na)-Met-Gly-DTrp-Met-Asp-Phe-NH_2 (**8**), and Ac-Tyr(SO_3Na)-Met-Gly-Trp-DMet-Asp-Phe-NH_2 (**9**).

The nonsulfated heptapeptides were synthesized on methylbenzhydrylamino resin with Boc-protected amino acids by a standard procedure published earlier.[11] The β-COOH of Asp was protected with OBzl; the alcoholic hydroxyl group of Ser, Thr, and Hyp was protected with the benzyl group. Tyr was used as Boc-Tyr(2,6-dichlorobenzyl); other amino acid side chains were unprotected. Each coupling was made with dicyclohexylcarbodiimide (DCC). N-terminal acetylation was performed on the resin. The peptides were cleaved from the resin with HF in the presence of scavangers. After removal of HF under high vacuum, the peptides were dissolved in DMF, the resin was separated by filtration, and the compounds were precipitated with ether. Peptides were sulfated with a new reagent (pyridinium acetyl sulfate, $CH_3COOSO_3^-C_5H_6NH^+$, PAS).[12] This mild sulfating agent did not cause side reactions similar to those known to occur with other sulfation methods (oxidation of Met, sulfonation of Tyr and Trp, etc.) and reacted (~100-fold) faster with alcoholic than with phenolic hydroxyls (unpublished results). This allowed us to consistently synthesize two analogues from a nonsulfated peptide: one of them containing only one sulfate ester on Ser, Thr, or Hyp (**2**, **4** and **6**, respectively) and the other two *O*-sulfates included on the Tyr residue (**1**, **3**, and **5**). Structures were supported by amino acid analysis after $Ba(OH)_2$ hydrolysis according to Doolittle.[13] Under those conditions tyrosine *O*-sulfate was stable and eluted with the standard sodium citrate buffer between cysteic acid and Asp. Peptides were purified by preparative RP-HPLC as follows: after dilution of the reaction mixture with distilled water, the colloidal solution was filtered on Celite and directly loaded onto the HPLC C_{18} column. This mild procedure allowed minimal deterioration of Trp, Met, and Asp by avoiding rotary evaporation of pyridine and DMF. In view of the known acid lability of the tyrosine *O*-sulfate, separations were performed with 0.1 M, pH 6.5, ammonium–acetate buffer, and all analogues were isolated as sodium salts. If a reaction mixture contained a mono- and a disulfate ester, the more hydrophylic disulfate ester was eluted prior to the monosulfate under the conditions reported here. Pure fractions were pooled and lyophilized.

Measurement of Biological Activities. The peptides were tested for their ability to stimulate gallbladder contraction, for gastric acid secretion, and for their anticonvulsive effect. Gastrin-like activities were measured on perfused rat stomach by conductometric titration of HCl liberated as a result of intravenous (iv) administration of the test solution.[14] Cholecystokinetic activity of different CCK analogues was measured by a modification of the bioassay for CCK[15] originally described by Berry and Flower.[16] In vivo assay for cholecystokinin-like activity was carried out (analogues **7**–**9**) in dogs, according to the method of Wyatt.[17] The anticonvulsive potency of the peptides against picrotoxin-induced (6 mg/kg sc) convulsions was measured in mice by the method of Zetler.[18]

Results and Discussion

Biological results, a preliminary account of which was reported in ref 12, are summarized in Table I. For the first time, highly potent Ac-CCK-7 analogues were synthesized. Replacement of Asp[7] by Thr(SE), Ser(SE), or Hyp(SE) increased CCK activity to 166, 190, and 300%,

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respectively. This was unexpected, since the carboxyl of the Asp[7] residue had been reported to be necessary for cholecystokinetic activity. In contrast, our results would indicate that the presence of an anion is necessary in that position. The high CCK activity of **5** suggests that a preferred conformation is stabilized by introducing a rigid Hyp ring into the molecule. As expected, the analogues that did not contain Tyr(SE) in position 2 (**2** and **4**) possessed only minimal CCK activity, while the same analogues proved to behave as gastrin agonists. The only exception was **6**, which had *no* gastrin-like and practically *no* CCK-like activity. While this latter result was expected (in view of the absence of tyrosine *O*-sulfate), the lack of gastrin-like activity might be explained by the inability of the peptide to assume the conformation (involving "stacking" between Trp and Phe) necessary for hormone–receptor interaction, as reported for tetra- and pentagastrin.[19,20] The presence of hydroxyproline *O*-sulfate in position 7 may stabilize a preferred CCK conformation and at the same time may prevent the formation of a preferred gastrin conformation. The finding that tetragastrin and CCK-7 have different steric structures (Durieux et al.[10]) supports this hypothesis.

Among the peptides containing D-amino acids, only the D-Ala analogue (**7**) was active. Its cholecystokinetic potency, measured on isolated gallbladder strip, was 40% that of CCK-8, while its high integrated in vivo potency (250%) suggests that it may be more resistant to enzymic degradation. The low in vivo potencies of analogues **8** (3%) and **9** (2%) suggest that the position of the side chain of Trp and Met plays an important role in hormone–receptor interactions. Furthermore, the presence of a small amount of L isomer, which would contribute toward the activities of **8** and **9**, cannot be excluded.

None of the analogues tested for anticonvulsive effect at the same single dose (on a molar basis) were found to be more potent than CCK-8. Analogues **1**, **3**, and **5**, as well as **7**, appeared equipotent in delaying the time of death of picrotoxin-treated mice. A detailed analysis of the anticonvulsive activity of CCK peptides revealed that unsulfated CCK-8 was equipotent with CCK-8 in antagonizing picrotoxin effects in mice after intraperitoneal administration; in fact, pentagastrin also exhibited anticonvulsive activity, and tetragastrin (CCK tetrapeptide) was the shortest structure that could exert such an effect.[21,22] Parallel antagonistic effects of CCK-8 and analogues on picrotoxin- and electroshock-induced seizures and on other behavioral effects were found in rats after intracerebroventricular administration.[23,24] This suggests that CNS effects on CCK-8 could mainly be mediated through receptors different from those in the periphery, which are responsible for the specific cholecystokinetic effect of CCK-8. This is also supported by previous receptor-binding studies.[25,26] The conformational change caused by desulfation of CCK-8[10] might be irrelevant for the brain CCK receptors.

In summary, while the 7-substituted Ac-CCK-heptapeptides are among the most potent CCK analogues reported so far, the D-Ala[4] replacement resulted, for the first time, in prolonged activity in vivo.

Table II

step	reagents and operations	mixing times
1	CH_2Cl_2 wash, 30 mL (2 times)	3 min
2	CH_3OH wash, 15 mL (2 times)	3 min
3	CH_2Cl_2 wash, 30 mL (3 times)	3 min
4	50% TFA + 50% ethanedithiol in CH_2Cl_2, 20 mL (2 times)	10 + 17 min
5	2-propanol wash containing 2% ethanedithiol	3 min
6	CH_2Cl_2 wash, 30 mL (2 times)	3 min
7	triethylamine (10%) in CH_2Cl_2, 30 mL (2 times)	3 + 3 min
8	CH_3OH wash, 15 mL (2 times)	3 min
9	CH_2Cl_2 wash, 30 mL (3 times)	3 min
10	Boc-protected amino acid (3 mmol) in CH_2Cl_2 (20 mL) or contained DMF (10%) in CH_2Cl_2 for Boc-Trp-OH; plus DCC (3 mmol) in CH_2Cl_2	60 min
11	CH_3OH wash, 15 mL (2 times)	3 min
12	CH_2Cl_2 wash, 30 mL (2 times)	3 min
13	Ac_2O (3 M solution) in CH_2Cl_2, 20 mL (2 times)	10 + 7 min

Experimental Section

An automatic peptide synthesizer (Beckman, Model 990B) was used for solid-phase syntheses. Protected amino acids were bought from Bachem (Torrance). All solvents were reagent or HPLC grade. TFA and Et_3N were redistilled. The standard cycle of coupling, acetylation of unreacted amino groups, and deblocking for 3 g of resin is shown in Table II.

Peptide retention times (t_R) were measured by RP-HPLC under isocratic conditions [Vydac C_{18} column, 24 × 0.46 cm, 5 μm, pore size 300 Å; flow rate 2 mL/min, ammonium acetate buffer (0.1 M), pH 6.5, contained 24% CH_3CN, UV detection at 210 nm]. Preparative HPLC purification was carried out on a Waters Prep LC500 liquid chromatograph using custom-made cartridges. (5.7 × 30 cm, Vydac C_{18} 15-20 μm, pore size 300 Å). Optical rotations were measured on a Perkin-Elmer 141 spectropolarimeter. Peptide hydrolyses were performed in 4 M methanesulfonic acid containing 0.2% tryptamine (24 h, 110 °C, in a sealed ampule under high vacuum). Hydrolysis in 0.2 M $Ba(OH)_2$ (110 °C, 24 h) was used to determine sulfate esters in peptides. Tyr(SO_3H) values are compared to Phe (= 1.00). Analyses were carried out on a Beckman automatic amino acid analyzer (Model 121M). TLC was performed on precoated silica gel plates (60F-254, 0.2-mm thick, Merck) with the following solvent systems: A, ethyl acetate–1-butanol–acetic acid–water (50:35:10:5, v/v); B, 70% ethyl acetate–30% 1-butanol–acetic acid–water (7:2:1, v/v).

Pyridinium Acetyl Sulfate (PAS).[12,20] To a mixture of acetic anhydride (28.4 mL, 0.3 mol) and pyridine (8.05 mL, 0.1 mol) was added dropwise at −15 °C under stirring concentrated H_2SO_4 (5.4 mL, 0.1 mol). After 5 min, pyridinium acetyl sulfate was precipitated with ether (300 mL), filtered off, washed 3 times with ether, and dried in a desiccator over P_2O_5 and KOH in vacuo: yield 18.4 g (83%); mp 75 °C; IR 4050 (sharp) cm^{-1}. Anal. Calcd for $C_7H_9O_5NS$ (219.2): C, 38.3; H, 4.1. Found: C, 38.1; H, 3.9.

Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2 (1) and Ac-Tyr-Met-Gly-Trp-Met-Ser(SO_3Na)-Phe-NH_2 (2). The protected acetyl-heptapeptide Ac-Tyr(2,6-Cl_2Bzl)-Met-Gly-

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Trp-Met-Ser(Bzl)-Phe was synthesized on MBHA resin (3.0 g, 1.2 mmol) by the standard procedure. Acetylation on the resin after standard deblocking (3 M acetic anhydride in CH_2Cl_2, 30 mL) yielded acetyl-heptapeptidyl-resin (4.3 g), which was treated with HF (60 mL, 30 min, 0 °C) containing anisole (6 mL) and ethyl methyl sulfide (3 mL). After HF evaporation, the crude peptide was extracted in DMF (10 mL) and precipitated with ether (300 mL), yielding the nonsulfated crude acetyl-heptapeptide (700 mg, 61%; R_f (A) 0.75). This peptide (650 mg, 0.66 mmol) was dissolved in DMF–pyridine (1:1) (10 mL) and sulfated with PAS (880 mg, 4 mmol). After 24 h, the reaction mixture was diluted with water (800 mL), the pH was adjusted to 7.0 with 4 N NaOH, the mixture was filtered on Celite, and the filtrate was purified on a RP-HPLC column (flow rate 75 mL/min; pH 6.5, ammonium acetate buffer, 0.1 M, acetonitrile linear gradient: from 12 to 24% in 60 min). The pure **1** and **2** containing fractions were lyophilized twice, yielding 1 (150 mg, 19.5%) and **2** (190 mg, 27%).

Characterization of 1: single spot on TLC, R_f (A) 0.18; $[\alpha]^{20}_D$ −14.06° (*c* 1, DMF); t_R = 4.2 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.00; Phe, 0.98; Met, 1.8; Trp, 0.86; Tyr, 0.90; Ser, 0.94. Amino acid analysis after $Ba(OH)_2$ hydrolysis: Phe, 1.00; Tyr(SE), 0.96.

Characterization of **2**: single spot on TLC, R_f (A) 0.51; $[\alpha]^{20}_D$ −15.32° (*c* 1, DMF); t_R = 7.9 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.0; Phe, 1.05; Met, 1.86; Trp, 0.89; Tyr, 0.94; Ser, 0.92. Amino acid analysis after $Ba(OH)_2$ hydrolysis: no traces of Tyr(SE).

Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2 (3) and Ac-Tyr-Met-Gly-Trp-Met-Thr(SO_3Na)-Phe-NH_2 (4). Acetyl-heptapeptide Ac-Tyr-Met-Gly-Trp-Met-Thr-Phe-NH_2 [600 mg, 51%; R_f (A) 0.85] was synthesized on MBHA resin (3.0 g, 1.2 mmol) by the standard method. Sulfation of the crude acetyl-heptapeptide (585 mg, 0.6 mmol) in DMF–pyridine (1:1) (8 mL) with PAS (660 mg, 3 mmol; 24 h reaction) and subsequent purification with RP-HPLC (as described for **1** and **2**) yielded **3** (135 mg, 19%) and **4** (210 mg, 32.7%).

Characterization of **3**: single spot on TLC, R_f (A) 0.22; $[\alpha]^{20}_D$ −15.32° (*c* 1, DMF); t_R = 4.4 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.0; Phe, 1.05; Met, 1.95; Trp, 0.90; Tyr, 0.92; Thr, 0.98. Amino acid analysis after $Ba(OH)_2$ hydrolysis: Phe, 1.00; Tyr(SE), 0.92.

Characterization of **4**: single spot on TLC, R_f (A) 0.64; $[\alpha]^{20}_D$ −17.85° (*c* 1, DMF); t_R = 8.25 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.0; Phe, 1.02; Met, 1.88; Trp, 0.95; Tyr, 1.02; Thr, 0.96. Amino acid analysis after $Ba(OH)_2$ hydrolysis: no traces of Tyr(SE).

Ac-Tyr(SO_3Na)-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2 (5) and Ac-Tyr-Met-Gly-Trp-Met-Hyp(SO_3Na)-Phe-NH_2 (6). Ac-Tyr-Met-Gly-Trp-Met-Hyp-Phe-NH_2 [420 mg, 36%; R_f (A) 0.81] was synthesized on MBHA resin (3.0 g, 1.2 mmol) by the standard method. Sulfation of the acetyl-heptapeptide (400 mg, 0.40 mmol) in DMF–pyridine (1:1) (4 mL) with PAS (440 mg, 2 mmol; 24 h) and purification by RP-HPLC, yielded **5** (147 mg, 31%) and **6** (161 mg, 37%).

Characterization of **5**: single spot on TLC, R_f (A) 0.1; $[\alpha]^{20}_D$ −19.39° (*c* 1, DMF); t_R = 4.5 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.0; Phe, 1.05; Met, 1.82; Trp, 0.95; Tyr, 0.98; Hyp, 0.92; Amino acid analysis after $Ba(OH)_2$ hydrolysis: Phe, 1.00; Tyr(SE), 0.95.

Characterization of **6**: single spot on TLC, R_f (A) 0.51; $[\alpha]^{20}_D$ −26.6° (*c* 1, DMF); t_R = 8.7 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Gly, 1.0; Phe, 0.96; Met, 1.90; Trp, 0.92; Tyr, 1.04; Hyp, 1.02; Amino acid analysis after $Ba(OH)_2$ hydrolysis: no traces of Tyr(SE).

Ac-Tyr(SO_3Na)-Met-D-Ala-Trp-Met-Asp-Phe-NH_2 (7). Ac-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH_2 [143 mg, 36.4%; R_f (B) 0.45] was synthesized on MBHA resin (3.0 g, 1.2 mmol). Sulfation of this peptide (401 mg, 0.4 mmol) in DMF–pyridine (1:1) (4 mL) with PAS (440 mg, 2 mmol; 24 h) and purification with RP-HPLC yielded **7** (210 mg, 52.3%): single spot on TLC, R_f (B) 0.12; $[\alpha]^{20}_D$ −21.2° (*c* 1, DMF); t_R = 3.4 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Phe, 1.00; Asp, 1.05; Ala, 0.98; Met, 1.94; Tyr, 0.92; Trp, 0.90.

Ac-Tyr(SO_3Na)-Met-Gly-D-Trp-Met-Asp-Phe-NH_2 (8). Ac-Tyr-Met-Gly-D-Trp-Met-Asp-Phe-NH_2 [650 mg, 54.8%; R_f (A) 0.43] was synthesized on MBHA resin (3.0 g, 1.2 mmol). Sulfation of this peptide (600 mg, 0.6 mmol) in DMF–pyridine (1:1) (10 mL) with PAS (660 mg, 3 mmol; 24 h) and purification with RP-HPLC yielded **8** (201 mg, 46%): single spot on TLC, R_f (B) 0.11; $[\alpha]^{20}_D$ 14.2° (*c* 1, DMF); t_R = 3.8 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis, Phe, 1.00; Asp, 1.02; Gly, 0.98; Met, 1.85; Tyr, 0.95; Trp, 0.92.

Ac-Tyr(SO_3Na)-Met-Gly-Trp-D-Met-Asp-Phe-NH_2 (9). Ac-Tyr-Met-Gly-Trp-D-Met-Asp-Phe-NH_2 [838 mg, 71%; R_f (B) 0.42] was synthesized on MBHA resin (3.0 g, 1.2 mmol). Sulfation of the acetylheptapeptide (780 mg, 0.79 mmol) in DMF–pyridine (1:1) (8 mL) with PAS (880 mg, 4 mmol; 24 h) and purification with RP-HPLC yielded **9** (382 mg, 44.3%): single spot on TLC, R_f (B) 0.11; $[\alpha]^{20}_D$ −20.6° (*c* 1, DMF); t_R = 3.75 min (single peak); IR 1050 (sharp) cm^{-1}. Amino acid analysis: Phe, 1.00; Asp, 1.05; Gly, 1.06; Met, 1.90; Tyr, 0.96; Trp, 0.92.

Biological Tests. Gastrin-like activities were measured by the method of Halter et al.[14] on male Wistar rats (~250-g body weight) with conductometric titration of HCl secreted after iv administration of the peptides to be tested (10, 20, 40, 80, 160 and 320 ng) in physiological saline. Pentagastrin was used as standard in doses of 10, 20, 40, and 80 ng. Lowest limit of detection of the agonist effect of an analogue compared to pentagastrin was 1–2%. The effect of each compound was measured on 10 animals. Biological activity of pentagastrin was taken as 100%, and relative potency of different CCK heptapeptide analogue was expressed as a percentage.

Cholecystokinetic activity was measured on isolated rabbit gallbladder strips[15,16] placed in an organ bath containing Krebs bicarbonate solution (20 mL), gassed with a mixture of 95% O_2 and 5% CO_2. The temperature was maintained at 37 °C. Peptides were dissolved in physicological saline and added directly into the bath. Tension changes induced by peptides were measured with a force-displacement transducer and recorded on Grass Model 7 polygraph. In each experiment, dose–response curves were generated with CCK-8 and with CCK heptapeptide analogues. Five determinations were made for each compound. The maximal response to each peptide and the dose required to produce half the maximal response (ED_{50}) were both obtained by direct observation and by Dowd–Riggs linear transformation[27] of the Michaelis–Menten constant. The tension response is plotted on the ordinate, and the response-to-dose ratio is plotted on the abscissa. The *y*-intercept gives the calculated maximal response, and the slope of the linear portion of the curve gives the ED_{50}. Biological activity of heptapeptide analogues of CCK and CCK-8 were compared on the basis of ED_{50} values. The activity of CCK-8 was considered to be 100%. Results are expressed as a percentage as the mean plus or minus the standard error of the mean. Variance was between 5 and 10%.

The Oddi-sphincter relaxing effect of the CCK analogues (**7–9**) was measured according to the method of Wyatt[17] on Nembutal-anesthesized dogs with perfused ductus choledochus (physiological saline, 37 °C), and the volume (drop number) of the perfused solution was registered. Each compound was administered intravenously to five animals. CCK-8 was used as a standard. Statistical analysis of the results was carried out as described above.

For the measurement of *anticonvulsive activity*, male CFLP mice weighing 25–35 g were used. All peptides were dissolved in physiological saline and were administered intraperitoneally at a dose of 0.8 μmol/kg (1 mg/kg for CCK-8) 10 min prior to the injection of picrotoxin at a dose of 6 mg/kg subcutaneously. The dose selected was approximately the ED_{50} of CCK-8 against picrotoxin-induced seizures. Following the administration of the convulsant drug, the latencies until the first tonic seizure and the time of death were recorded. Animals were observed for 60 min, and if an animal did not die or did not show tonic seizures within 60 min, a score of 60 was recorded. In the saline-treated control group, the mean time of death was 30–35 min. For each analogue, the percentage prolongations of the mean control latencies were calculated and are expressed as relative values compared to that of CCK-8. The number of animals was 10 in each group. Statistical comparisons were made by Student's *t* test between peptide-treated and saline-treated control groups.

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Registry No. 1, 89596-94-1; 2, 89618-30-4; 3, 89596-95-2; 4, 89618-31-5; 5, 89596-96-3; 6, 89596-97-4; 7, 89596-98-5; 8, 89596-99-6; 9, 89673-88-1; Ac-Tyr-Met-Gly-Trp-Met-Ser-Phe-NH_2, 89597-00-2; Ac-Tyr-Met-Gly-Trp-Met-Thr-Phe-NH_2, 89597-01-3; Ac-Tyr-Met-Gly-Trp-Met-Hyp-Phe-NH_2, 89597-02-4; Ac-Tyr-Met-D-Ala-Trp-Met-Asp-Phe-NH_2, 89597-03-5; Ac-Tyr-Met-Gly-D-Trp-Met-Asp-Phe-NH_2, 88457-85-6; Ac-Tyr-Met-Gly-Trp-D-Met-Asp-Phe-NH_2, 88495-33-4.

Histamine H_2 Receptor Antagonists. 1. Synthesis of *N*-Cyano and *N*-Carbamoyl Amidine Derivatives and Their Biological Activities

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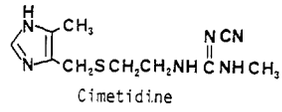
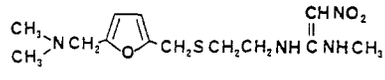
A large number of *N*-cyano amidine derivatives were prepared as potential histamine H_2 receptor antagonists and evaluated for their inhibitory action on histamine-stimulated chronotropic response of isolated right atria from guinea pigs. Several selected compounds were assessed as inhibitors of gastric acid secretion induced by histamine in anesthetized dogs. Of these compounds, furan (**8c**) and [(diaminomethylene)amino]thiazole derivatives (**16c**) were found to be more potent than cimetidine in both assays. In contrast to the guanidine series, methyl substitution at the terminal nitrogen of the cyano amidines was detrimental to the activities. Furthermore, acid hydrolysis of the cyano amidines gave carbamoyl amidines, which proved to be more active than the cyano amidines, the converse of the case for guanidines. 3-[[[2-[(Diaminomethylene)amino]-4-thiazolyl]methyl]thio]-*N'*-carbamoylpropionamidine (**16d**) was the most potent of all the compounds tested and was approximately 30 times more active in vitro and 50 times more active in vivo than cimetidine.

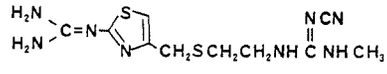
Burimamide was first demonstrated as a histamine H_2 receptor antagonist by Black et al. in 1972.[1] Structural modification of this prototype led to the more potent antagonists, metiamide[2] and cimetidine.[3] Cimetidine is a



Cimetidine



Ranitidine

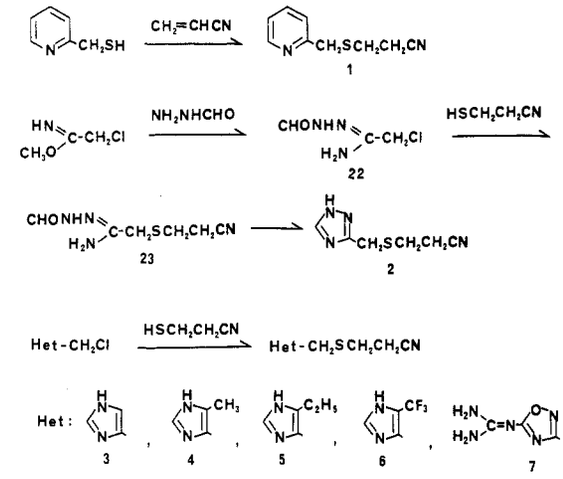


Tiotidine

well-known histamine H_2 receptor antagonist and used widely as an effective inhibitor of gastric acid secretion in the treatment of peptic ulcers and associated gastrointestinal disorders. Recent studies have shown that structurally unique nonimidazole derivatives, such as ranitidine[4] and tiotidine,[5] are more active antagonists than cimetidine.

In general, the structures of these antagonists are composed of three fundamental substructures: substituted heterocyclic components, i.e., methylimidazole, [(dimethylamino)methyl]furan, or [(diaminomethylene)amino]thiazole, connected by a (methylthio)ethyl chain to an essentially neutral end group, such as cyanoguanidine or 1,1-diamino-2-nitroethylene.[6] More recently, a new class of potent histamine H_2 receptor antagonist, in which the end group was replaced by a 3,4-diamino-1,2,5-thiadiazole 1-oxide or 1,1-dioxide, was reported.[6,7]

Scheme I



Our efforts to find a new type of H_2 antagonist focused on replacing the usual cyanoguanidine and 1,1-diamino-2-nitroethylene moieties with the cyano amidine and carbamoyl amidine moieties. We now report in this paper the preparation and the pharmacological profile of the cyano and carbamoyl amidine derivatives as H_2 antihistaminic agents and discuss the structure–activity relationships.

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