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Synthesis of Five Cholecystokinin-like Peptides and Determination of Their Analgesic Effects[1)]

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Five CCK (cholecystokinin)-like peptides [CCK(27—32)amide, CCK-6, CCK-7, Boc–CCK-7 and nonsulfated CCK-7] were prepared by stepwise condensation in combination with fragment condensation by the active ester procedure. The analgesic effects of these CCK-like peptides were measured in the writhing test. The ED_{50} value of CCK-7 was 1500 nmol/kg (1.8 mg/kg). Boc–CCK-7 appeared to reduce writhing between doses of 1 and 8 mg/kg but did not show a dose-dependent response. Other synthetic peptides produced no response even at a dose of 8 mg/kg. Our results suggested that CCK-7 is one of the shortest CCK-fragments that can retain the analgesic effect.

Keywords——cholecystokinin; peptide synthesis; CCK-7; writhing test; analgesic effect

There have been many studies on the structure–activity relationships of CCK (cholecystokinin)-like peptides for gallbladder contraction or amylase release,[2)] but few on the analgesic effect. Zetler[3)] reported that CCK octapeptide (CCK-8) and caerulein produced analgesic effects after subcutaneous injection in mice and that pentagastrin (CCK-5) was inactive, but other CCK fragments have not been tested.

It seemed interesting to investigate the analgesic effects of other CCK fragments. Thus, we undertook the synthesis of five CCK-like peptides, CCK(27—32)amide, CCK-6, CCK-7, Boc–CCK-7 and nonsulfated CCK-7(CCK-7-NS), and measured their analgesic effects using the writhing method according to the procedure of Zetler.[3)] The synthesis of CCK(27—32)amide has not been reported, but syntheses of CCK-6, CCK-7, Boc–CCK-7 and CCK-7-NS have been carried out by stepwise condensation.[2b,c)] In our procedure, stepwise condensation and fragment condensation were employed, using the active ester procedure. The present report describes the synthesis of five CCK-like peptides, as well as the determination of their analgesic activity.

CCK(27—32)amide was synthesized as shown in Fig. 1. The α-amino functions of amino acids and peptides were protected with the Boc group. The C-terminal peptide fragment, Boc–Met–Asp–NH_2 (I) was synthesized by the coupling of H–Asp–NH_2[4)] with Boc–Met–OCp.[5)] After deprotection of I by TFA, Boc–Trp–OCp[5)] was coupled to produce Boc–Trp–Met–Asp–NH_2 (II). Boc–Met–Gly–OH (III) was produced from Boc–Met–OCp[5)] and Gly, led to Boc–Met–Gly–OCp, and coupled with H–Trp–Met–Asp–NH_2 (obtained by deprotection of II with TFA). The resulting Boc–pentapeptide amide (IV) was deprotected with TFA and a tyrosine residue was incorporated at the N-terminal using Boc–Tyr–OSu[6)] to produce Boc–Tyr–Met–Gly–Trp–Met–Asp–NH_2 (V). Compound V was sulfated with pyridine–sulfur trioxide complex in anhydrous pyridine solution to form protected CCK(27—32)amide, which was deprotected with TFA after treatment with sodium carbonate. Finally, the resulting CCK(27—32)amide (VI) was purified by chromatography on diethyl aminoethyl (DEAE) Sephadex A-25 using ammonium carbonate aqueous solution as the eluting solvent,

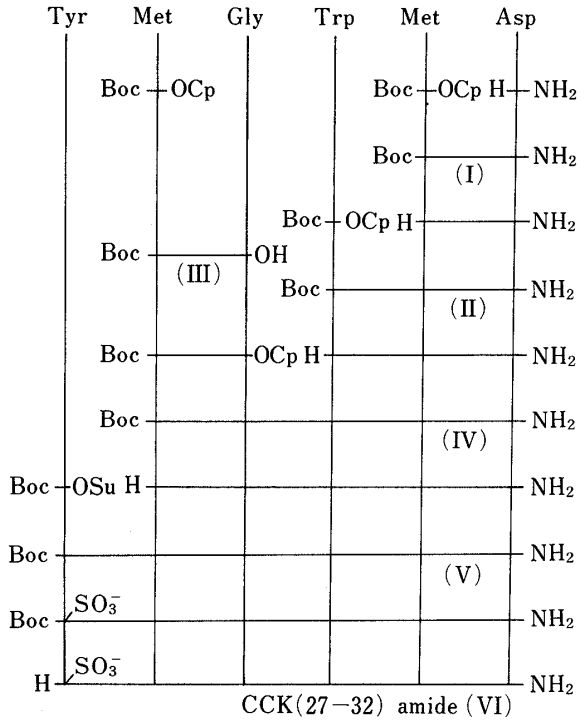

Fig. 1. Synthetic Scheme for CCK(27—32)-amide

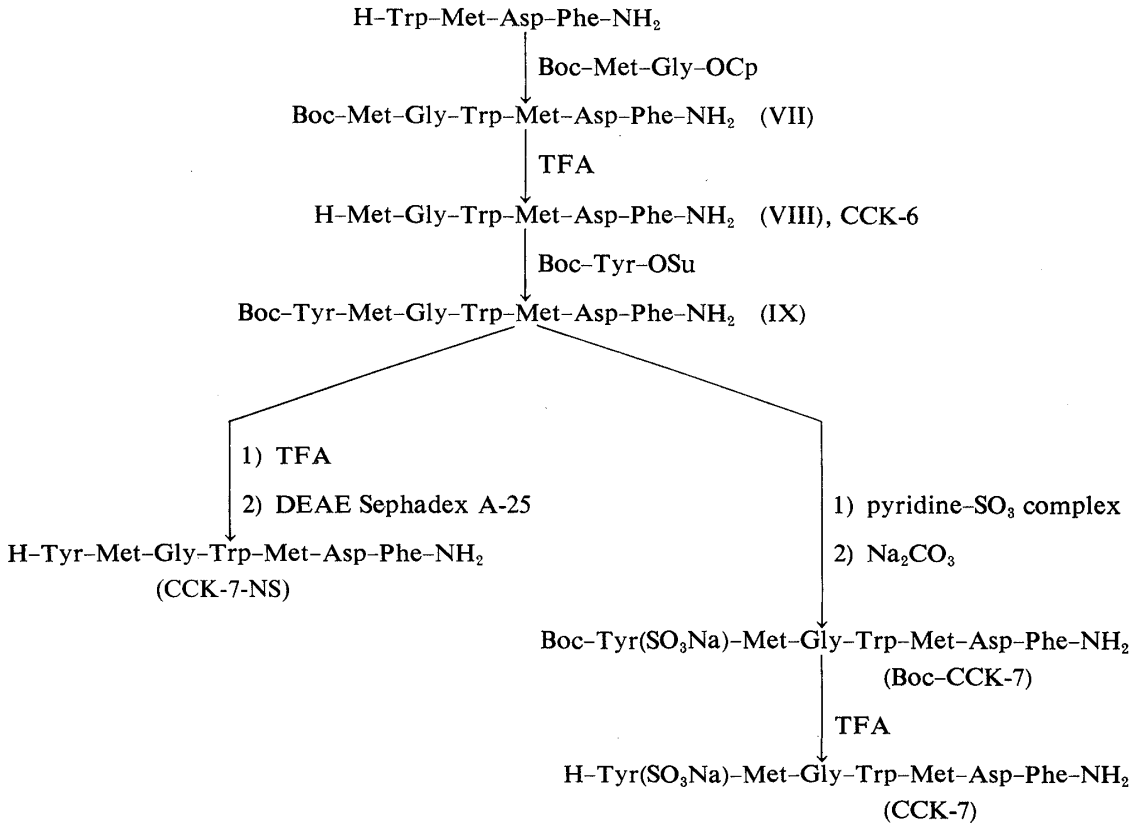

Fig. 2. Synthetic Methods for CCK-6, CCK-7, Boc–CCK-7 and CCK-7-NS

and lyophilized.

The synthetic methods for CCK-6, Boc–CCK-7, CCK-7 and CCK-7-NS are summarized in Fig. 2. H–Trp–Met–Asp–Phe–NH_2[7] was linked with Boc–Met–Gly–OCp to give Boc–Met–Gly–Trp–Met–Asp–Phe–NH_2 (VII), which was deprotected with TFA to afford

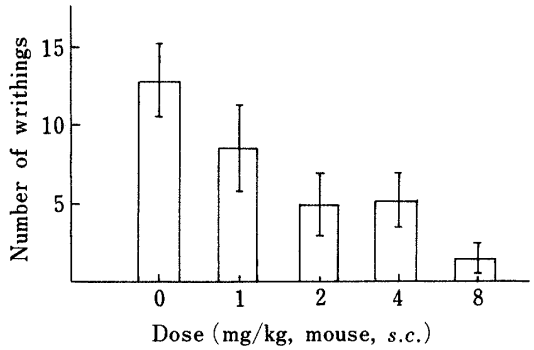

Fig. 3. Effects of CCK-7 on AcOH-Induced Writhing

CCK-6 (VIII). Reaction of VIII with Boc–Tyr–OSu[6] provided Boc–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2 (IX). Compound IX was deprotected with TFA and purified by column chromatography on DEAE Sephadex A-25 using ammonium carbonate aqueous ethanol solution as the eluting solvent, to give H–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2 (CCK-7-NS). On the other hand, IX was sulfated with pyridine–sulfur trioxide complex in anhydrous pyridine, and then treated with sodium carbonate to produce crude Boc–CCK-7. This material was purified by silica gel column chromatography to afford Boc–CCK-7. In order to obtain CCK-7, crude Boc–CCK-7 was deprotected with TFA, purified by silica gel column chromatography and Sephadex G-25 column chromatography and then lyophilized.

The synthesized CCK-like peptides were shown to be homogeneous by thin-layer chromatography (TLC) on silica gel and gave the expected elemental analyses. Amino acid analyses of acid hydrolysates of these peptides were in good agreement with the theoretically expected values. In the infrared (IR) spectra of CCK(27—32)amide, Boc–CCK-7 and CCK-7, the characteristic band (1050 cm^{-1})[8] due to a sulfate ester was observed.

The analgesic effects of these CCK-like peptides were examined in the writhing test according to Zetler.[3] Writhing was elicited by intraperitoneal injection of 0.6% (v/v) acetic acid (10 ml/kg) into ddY male mice weighing 18—22 g. The solutions of the peptides in distilled water or 0.05 M sodium bicarbonate were administered subcutaneously 10 min before the acetic acid injection. The number of writhings occurring between 10 and 20 min after injection of acetic acid was counted. CCK-7 reduced the number of writhings dose-dependently (Fig. 3). From these data the ED_{50} value was calculated to be 1500 nmol/kg (1.8 mg/kg). Boc–CCK-7 appeared to reduce writhing at doses between 1 mg/kg and 8 mg/kg, but did not produce a dose-dependent responce. CCK-6, CCK(27—32)amide and CCK-7-NS gave no response even at a dose of 8 mg/kg.

Zetler reported that the ED_{50} value of CCK-8 in the writhing test was 790 nmol/kg (0.88 mg/kg).[3] Our results suggest that CCK-7 is one of the shortest CCK-fragment that can retain the analgesic effect, and that the tyrosine sulfate residue is essential for the analgesic effect.

Experimental

The melting points are uncorrected. Optical rotations were measured with a model DIP-181 polarimeter (Japan Spectroscopic Co.). Amino acid analyses of acid hydrolysates were performed with a JEOL JLC-6AH amino acid analyzer. IR spectra were measured with a Shimadzu IR-400 infrared spectrophotometer. Secondary ion mass spectra (SIMS) were recorded on a Hitachi M-80B mass spectrometer. Elementary analyses were carried out with a Yanagimoto MT-3 CHN Corder. Ascending TLC was performed on silica gel TLC plates (Kieselgel 60 F_{254}, Merck) using the following solvent systems: Rf^1 AcOEt–pyridine–AcOH–H_2O (60:20:6:11); Rf^2 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2); Rf^3 *n*-BuOH–AcOH–pyridine–H_2O (60:20:6:24); Rf^4 benzene–AcOH (3:1).

Boc–Met–Asp–NH_2 (I)——Et_3N (11.2 ml) was added to H–Asp–NH_2[4] (5.3 g) in H_2O (60 ml), followed by addition of a solution of Boc–Met–OCp[5] (17.2 g) in THF (60 ml) at 0 °C. The mixture was stirred at 7—8 °C

overnight. The solvent was evaporated off *in vacuo* and the residue was dissolved in a saturated sodium bicarbonate solution (100 ml), which was washed with ether (100 ml). The aqueous layer was acidified with 6 M HCl under cooling with ice and extracted with AcOEt (100 ml). The extract was dried over $MgSO_4$, and the solvent was evaporated off *in vacuo*. The residue was triturated with ether and crystallized from AcOEt. Yield 8.6 g (59.2%). mp 126.5—128.5 °C (lit.[9] 126—129 °C). $[\alpha]_D^{20}$ −29.0° (c=1.0, MeOH) [lit.[9] $[\alpha]_D$ −27° (c=1, DMF)]. Rf^1 0.68, Rf^2 0.70. DCHA salt, mp 177—178 °C. $[\alpha]_D^{20}$ +5° (c=1.0, MeOH). *Anal.* Calcd for $C_{45}H_{25}N_3O_6S \cdot C_{12}H_{23}N$: C, 57.33; H, 8.88; N, 10.28. Found: C, 57.34; H, 9.11; N, 10.32.

Boc–Trp–Met–Asp–NH_2 (II)——I (2.5 g) was added to a solution of TFA (15 ml) containing thioanisole (0.8 ml) and the mixture was stirred at 0 °C for 2 h. Ether (170 ml) was added to cause precipitation. The precipitate was washed with ether (30 ml) and dried over KOH *in vacuo* to yield H–Met–Asp–NH_2·TFA. This material was dissolved in DMF (20 ml) and Et_3N (1.9 ml), Boc–Trp–OCp (3.3 g) and HOBt (0.9 g) were added successively at 0 °C. The mixture was stirred at 7—8 °C for 2 d, and the solvent was evaporated off *in vacuo*. The residue was dissolved in H_2O (50 ml) containing Et_3N (0.97 ml) and washed with AcOEt (30 ml). The aqueous solution was acidified with citric acid to yield a precipitate, which was recrystallized from MeOH. Yield 1.6 g (42.2%). mp 210—212 °C [lit.[9] 210 °C (dec.)]. $[\alpha]_D^{20}$ −17.0° (c=1.2, DMF) [lit.[9] $[\alpha]_D$ −27.1° (c=1, DMF)]. Rf^1 0.68, Rf^2 0.73, Rf^4 0.12. *Anal.* Calcd for $C_{25}H_{35}N_5O_7S$: C, 54.63; H, 6.42; N, 12.74. Found: C, 54.69; H, 6.44; N, 12.92.

Boc–Met–Gly–OH (III)——A solution of Boc–Met–OCp[5] (26.7 g) in THF (100 ml) was added to a mixture of Gly (5.25 g) and Et_3N (9.8 ml) in H_2O (50 ml) under cooling with ice-water, and stirred overnight at room temperature. The reaction mixture was concentrated to about 50 ml. After addition of H_2O (50 ml), the water layer was washed with AcOEt (2 × 50 ml), acidified with citric acid and extracted with AcOEt (3 × 100 ml). The organic layer was washed with brine (3 × 100 ml) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave the residue, which was recrystallized from AcOEt–petroleum ether. Yield 16.2 g (75.5%). mp 125—126 °C (lit.[10] 122—123 °C). $[\alpha]_D^{20}$ −18.4° (c=1, MeOH) [lit.[10] $[\alpha]_D^{21}$ −12.2° (c=1.2, DMF)]. *Anal.* Calcd for $C_{12}H_{22}N_2O_5S$: C, 47.04; H, 7.24; N, 9.14. Found: C, 47.28; H, 7.50; N, 9.19.

Boc–Met–Gly–Trp–Met–Asp–NH_2 (IV)——2,4,5-Trichlorophenol (7.03 g) was added to a solution of III (10.9 g) in DMF (50 ml), followed by addition of a solution of DCC (7.71 g) in DMF (15 ml) at 0 °C. The mixture was stirred for 2 h at 0 °C then overnight at 7—8 °C. Dicyclohexylurea was removed by filtration and the solvent was evaporated off *in vacuo*. The residue was dissolved in THF (30 ml) and insoluble material was removed by filtration. Petroleum ether was added to the filtrate, and the resulting solid was collected by filtration to yield Boc–Met–Gly–OCp (15.2 g). This compound was contaminated with a small amount of III, but was used without further purification in the next coupling reaction. Compound II (1.0 g) was treated with TFA (4 ml) in the presence of thioanisole (0.2 ml) at 0 °C for 1 h. Ether (50 ml) was added to form a precipitate, which was collected by filtration, washed with ether (20 ml) and dried over KOH *in vacuo* to yield H–Trp–Met–Asp–NH_2·TFA. This material was dissolved in DMF–H_2O (10 ml, 2 ml) and neutralized with NEt_3. After addition of the above-mentioned Boc–Met–Gly–OCp (1.0 g) and HOBt (0.2 g), the mixture was stirred at 7—8 °C overnight. The solvent was evaporated off *in vacuo* and the residue was dissolved in H_2O (30 ml) containing NEt_3 (0.5 ml). The aqueous solution was washed with AcOEt (30 ml), then acidified with citric acid at 0 °C. The resulting precipitates were collected by filtration, washed with H_2O (30 ml) and recrystallized from iso-PrOH–H_2O (2:1, 150 ml). Yield 600 mg (45.2%). mp 250 °C (dec.). $[\alpha]_D^{20}$ −18.0° (c=1.2, DMF). Rf^1 0.56, Rf^2 0.70. Amino acid ratio in an acid hydrolysate: Asp, 1.00; Gly, 1.01; Met, 1.98 (average recovery, 86%). *Anal.* Calcd for $C_{32}H_{47}N_7O_9S_2$: C, 52.09; H, 6.42; N, 13.29. Found: C, 52.24; H, 6.51; N, 13.16.

Boc–Tyr–Met–Gly–Trp–Met–Asp–NH_2 (V)——Compound IV (300 mg) was treated with TFA (2 ml) containing thioanisole (0.1 ml) at 0 °C for 1.5 h. Ether was added to yield a precipitate, which was collected by filtration, washed with ether and dried over KOH *in vacuo*. This material was added to a mixture of DMF (5 ml) and Et_3N (1.5 ml) at 0 °C. The reaction mixture was stirred for 30 min, then Boc–Tyr–OSu[6] (0.16 g) was added and the whole was stirred overnight at 7—8 °C. The solvent was evaporated off *in vacuo* and the residue was dissolved in a mixture of H_2O (15 ml) and Et_3N (0.1 ml), and acidified with citric acid at 0 °C. The resulting precipitate was collected by filtration and washed with ether. This material was dissolved in ammonia water and purified by chromatography on a Sephadex G-25 column (3 × 37 cm) with 0.05 M ammonium carbonate as the eluent. The eluate was monitored by measuring the ultraviolet (UV) absorption at 254 nm. Fractions No. 11—16 (16 ml each) were combined and lyophilized to yield a white powder, which was added to 3% AcOH (10 ml) and stirred for 10 min under cooling with ice-water. Insoluble material was collected by filtration and washed with H_2O (20 ml). Yield 160 mg (43.3%). mp 240 °C (dec.). $[\alpha]_{365}^{20}$ −29.0° (c=0.48, pyridine). Rf^1 0.58, Rf^2 0.73. Amino acid ratio in an acid hydrolysate: Asp, 1.02; Gly, 0.98; Met, 1.97; Tyr, 0.99 (average recovery, 87.2%). *Anal.* Calcd for $C_{41}H_{56}N_8O_{11}S_2 \cdot 0.5H_2O$; C, 54.11; H, 6.31; N, 12.31. Found: C, 54.05; H, 6.26; N, 12.17.

CCK(27—32)amide [VI, H–Tyr(SO_3Na)–Met–Gly–Trp–Met–Asp–NH_2]——Compound V (100 mg) and pyridine–sulfur trioxide complex (190 mg) were added to anhydrous pyridine (5 ml). The mixture was stirred at 7—8 °C for 5 d. The solvent was evaporated off *in vacuo* and sodium carbonate (300 mg) in cold water (15 ml) was added to the residue, followed by stirring at 0 °C for 1 h. The aqueous solution was washed with AcOEt (30 ml) and applied to a Sephadex G-25 column (30 × 37 cm). The column was developed with 0.05 M ammonium carbonate. Each fraction (15 ml) was monitored by measuring the UV absorption at 254 nm and fractions No. 13—15 were combined

and lyophilized several times to yield Boc–Tyr(SO_3Na)–Met–Gly–Trp–Met–Asp–NH_2 (120 mg) as a white hygroscopic powder. mp 245 °C (dec.). $[\alpha]_D^{20}$ −8.0° (c=2.2, 1 M NH_4OH). Rf^1 0.22, Rf^2 0.70. This compound (80 mg) was treated with TFA (2.0 ml) containing thioanisole (0.1 ml) at 0 °C for 1 h. After evaporation of the TFA *in vacuo*, the residue was triturated with ether, and then purified by DEAE Sephadex A-25 column chromatography. The column (1.2 × 18.5 cm) was eluted with a linear gradient of ammonium carbonate (from 0.1 to 0.7 M) and the eluate was monitored by measuring the UV absorption at 254 nm. Fractions No. 40—48 (13 ml each) were collected and lyophilized four times to give a hygroscopic powder. Yield 29 mg (40.2%). mp 185—195 °C (dec.). $[\alpha]_D^{20}$ −12.0° (c= 0.97, 0.3 M NH_4OH). Rf^1 0.13, Rf^2 0.31. IR ν_{max}^{KBr} cm^{-1}: 1050 (SO_3). Amino acid ratio in an acid hydrolysate: Asp, 1.02; Gly, 1.00; Met, 1.97; Tyr, 1.01 (average recovery, 87.2%). *Anal.* Calcd for $C_{36}H_{47}N_8NaO_{12}S_3 \cdot 2.5H_2O$: C, 46.05; H, 5.56; N, 11.93. Found: C, 45.59; H, 5.47; N, 12.39.

Boc–Met–Gly–Trp–Met–Asp–Phe–NH_2 (VII)——NEt_3 (0.25 mg) was added to a solution of H–Trp–Met–Asp–Phe–$NH_2 \cdot TFA$[7] (3.55 g) in DMF (30 ml) under cooling with ice. The mixture was stirred for 10 min, then Boc–Met–Gly–Ocp (2.43 g, prepared from III, 2,4,5-trichlorophenol and DCC) and HOBt (0.68 g) were added and the whole was stirred overnight at 7—8 °C. The reaction mixture was concentrated to about 5 ml and poured into ice-water (360 ml) containing AcOH (3 ml) with stirring. The resulting precipitate was collected by filtration, washed successively with H_2O (50 ml), AcOEt (2 × 20 ml) and EtOH (2 × 20 ml), and then recrystallized from EtOH. Yield 2.91 g (65.8%). mp 200—201 °C (dec.). $[\alpha]_D^{20}$ −26.5° (c=2.0, DMF). This compound contained a small amount of impurity with a high Rf value, but was used as such in the next reaction. For analysis, a pure sample was obtained as follows. Crude VII (200 mg) was dissolved in pyridine (20 ml), celite (5 g) was added, and the pyridine was evaporated off *in vacuo*. The residue was applied to a silica gel column (4 × 80 cm), equilibrated and eluted with AcOEt–pyridine–AcOH–H_2O (60 : 20 : 6 : 11), and the desired fractions were collected. After removal of the solvent, the residue was recrystallized from EtOH to yield purified VII (175 mg). mp 199—200 °C (dec.) [lit.[2b] 200—201 °C (dec.), lit.[8] 184—185 °C (dec.)]. $[\alpha]_D^{20}$ −27.5° (c=2.0, DMF) [lit.[2b] $[\alpha]_D^{25}$ −30.3° (c=1.4, DMF), lit.[8] $[\alpha]_D^{23}$ −19.7° (c=2.0, DMF)]. Rf^1 0.52, Rf^2 0.74, Rf^3 0.76. Amino acid ratio in an acid hydrolysate: Asp, 1.01; Gly, 0.99; Met, 1.99; Phe, 1.00; NH_3, 1.05 (average recovery, 82.6%). *Anal.* Calcd for $C_{41}H_{56}N_8O_{10}S_2 \cdot 3H_2O$: C, 52.44; H, 6.66; N, 11.93. Found: C, 52.14; H, 6.48; N, 11.79.

CCK-6 (VIII, H–Met–Gly–Trp–Met–Asp–Phe–NH_2)——Compound VII (1.85 g) was added to a mixture of TFA (8 ml) and thioanisole (0.4 ml) at 0 °C, followed by stirring for 1 h. Ether was added and the resulting precipitate was collected by filtration, washed with ether and dried over KOH *in vacuo* to yield the crude TFA salt of VIII (VIIIa, 1.9 g). This material was used for the next coupling reaction, to produce IX, without further purification. A sample for analysis and biological testing was purified by column chromatography in the same manner as described for VII. The solvent of the eluant was evaporated off *in vacuo* and the residue was triturated in water to give VIII. mp 212—213 °C (dec.) [lit.[2c] 214—216 °C (dec.)]. $[\alpha]_D^{26}$ −22° (c=1.1, DMF) [lit.[2c] $[\alpha]_D^{25}$ −20.2° (c=1.1, DMF)]. Rf^1 0.19, Rf^2 0.68, Rf^3 0.71. Amino acid ratio in an acid hydrolysate: Asp, 1.01; Gly, 0.95; Met, 2.02; Phe, 1.01; NH_3, 1.05 (average recovery, 85.3%). *Anal.* Calcd for $C_{36}H_{48}N_8O_8S_2 \cdot H_2O$: C, 53.85; H, 6.28; N, 13.96. Found: 54.06; H, 6.49; N, 13.79.

Boc–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2 (IX)——Et_3N (0.14 ml) was added to a solution of VIIIa (899 mg) in DMF (10 ml) at 0 °C and the mixture was stirred for 10 min. After addition of Boc–Tyr–OSu[6] (378 mg), the mixture was stirred at 7—8 °C for 2 d, then concentrated to about 4 ml *in vacuo*, and poured into ice-water (50 ml) containing AcOH (0.2 ml) with stirring. The resulting precipitate was collected by filtration, suspended in AcOEt (8 ml) at 60 °C, stirred for 10 min, then cooled at 5 °C. The precipitate was collected by filtration. Yield 835 mg (79.7%). mp 184—185 °C (dec.) (lit.[2b] 181—182.5 °C). $[\alpha]_D^{20}$ −27.0° (c=2, DMF) [lit.[2b] $[\alpha]_D^{25}$ −26.6° (c=2, DMF)]. Rf^1 0.6. Amino acid ratio in an acid hydrolysate: Asp, 1:02; Gly, 0.97; Met, 2.00; Tyr, 1.01; Phe, 1.01; NH_3, 1.17 (average recovery, 83.5%). *Anal.* Calcd for $C_{50}H_{65}N_9O_{12}S_2 \cdot 1.5H_2O$: C, 55.85; H, 6.37; N, 11.72. Found: C, 56.12; H, 6.50; N, 11.68.

H–Tyr–Met–Gly–Trp–Met–Asp–Phe–NH_2 (CCK-7-NS)——Compound IX (150 mg) was treated with TFA (1 ml) containing thioanisole (0.05 ml) at 0 °C for 1.5 h. Ether was added to yield a precipitate, which was collected by filtration, washed with ether (2 × 20 ml) and dried over KOH *in vacuo* to give CCK-7-NS TFA salt (150 mg). This material (80 mg) was purified by chromatography on a DEAE Sephadex A-25 column (1.1 × 4 cm) with 0.1 M ammonium carbonate as an eluent. The elutions was monitored by measuring the UV absorption at 254 nm. Fractions (No. 30—50, 6 ml each) were combined and lyophilized repeatedly to yield CCK-7-NS (41 mg). mp 203—205 °C (dec.). $[\alpha]_D^{20}$ −38° (c=1, DMF). Rf^1 0.28, Rf^2 0.71, Rf^3 0.69. SIMS m/z: 949 (MH^+). Amino acid ratio in an acid hydrolysate: Asp, 1.04; Gly, 1.00; Met, 1.98; Tyr, 1.01; Phe, 0.98; NH_3, 1.21 (average recovery, 84.3%). *Anal.* Calcd for $C_{45}H_{57}N_9O_{10}S_2 \cdot 3H_2O$: C, 53.93; H, 6.33; N, 12.58. Found: C, 54.05; H, 6.37; N, 12.40.

Boc–CCK-7 [Boc–Tyr(SO_3Na)–Met–Gly–Trp–Met–Asp–Phe–NH_2]——Pyridine–sulfur trioxide complex (668 mg) was added to a solution of IX (629 mg) in anhydrous pyridine (20 ml), and the mixture was stirred at room temperature for 2 d. The solvent was evaporated off *in vacuo* and a cold solution of sodium carbonate (657 mg) in H_2O (40 ml) was added to the residue. After being stirred in an ice-bath for 30 min, the mixture was extracted with *n*-BuOH (4 × 50 ml). The solvent was evaporated off *in vacuo* to yield crude Boc–CCK-7 (790 mg). This compound (395 mg) was purified by column chromatography on a silica gel column (4 × 80 cm) with AcOEt–pyridine–AcOH–

H_2O (60:20:6:11) as the eluent and the fractions (13 ml each, Rf^1 0.22) were monitored by TLC. The desired fractions (No. 155—188) were collected and the solvent was evaporated off *in vacuo*. The residue was crystallized from 95% EtOH to give Boc–CCK-7. Yield, 180 mg (47.9%). mp 219—220 °C (dec.). $[\alpha]_D^{20}$ −23° (c=1, DMF). Rf^1 0.22, Rf^2 0.70, Rf^3 0.68. IR ν_{max}^{KBr} cm^{-1}: 1050 (SO_3). Amino acid ratio in an acid hydrolysate: Asp, 1.01; Gly, 0.98; Met, 1.97; Tyr, 1.04; Phe, 1.01; NH_3 1.10 (average recovery, 81.5%). *Anal.* Calcd for $C_{50}H_{63}N_9Na_2O_{15}S_3 \cdot 4.5H_2O$: C, 47.92; H, 5.79; N, 10.06. Found: C, 47.97; H, 5.68; N, 9.70.

CCK-7 [H–Tyr(SO_3Na)–Met–Gly–Trp–Met–Asp–Phe–NH_2]——Crude Boc–CCK-7 (360 mg) mentioned above was added to a mixture of TFA (5 ml) and thioanisole (0.5 ml). The reaction mixture was stirred at 0 °C for 30 min, and then added to ether (100 ml) with stirring to form a precipitate. The precipitate was collected by filtration, washed with ether (30 ml) and dried over KOH to give crude CCK-7 (296 mg). This material was dissolved in MeOH (5 ml) and applied to a silica gel column (2.8 × 50 cm), which was developed with AcOEt–pyridine–AcOH–H_2O (60:20:6:11). Fractions (tube No. 155—200, 13 ml each, Rf^1 0.11) were collected and the solvent was evaporated off to give a solid residue (249 mg). This compound (155 mg) was further purified by Sephadex G-25 column chromatography. The column (2.2 × 100 cm) was developed with H_2O–EtOH (80:20). Fractions (tube No. 24—33, 10 ml each) were combined and lyophilized to give a hygroscopic powder. Yield 119 mg. mp 197—210 °C (dec.). $[\alpha]_D^{20}$ −10° (c=0.8, 80% DMF). Rf^1 0.11, Rf^2 0.49, Rf^3 0.56. IR ν_{max}^{KBr} cm^{-1}: 1050 (SO_3). SIMS m/z: 1164 (MH^+). Amino acid ratio in an acid hydrolysate: Asp, 1.01; Gly, 0.98; Met, 1.96; Tyr, 1.01; Phe, 1.02; NH_3, 1.01 (average recovery, 86%). *Anal.* Calcd for $C_{45}H_{56}N_9NaO_{13}S_3 \cdot TFA \cdot 3.5H_2O$: C, 46.00; H, 5.26; N, 10.27. Found: C, 45.91; H, 5.35; N, 10.51.

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References and Notes

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The customary L indication for amino acid residue is omitted. Standard abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochemistry* **5**, 2485 (1966); **6**, 362 (1967); **11**, 1726 (1972)]. Other abbreviations used are: Boc, *tert*-butyloxycarbonyl; OCp, 2,4,5-trichlorophenyl ester; OSu, *N*-hydroxysuccinimido ester; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; DCHA, dicyclohexylamine; AcOEt, ethyl acetate; AcOH, acetic acid; PrOH, propanol; *n*-BuOH, *n*-butanol; HOBt, 1-hydroxybenztriazole.
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