in CH_2Cl_2 and washed with water. Drying over Na_2SO_4 and removal of the solvent gave 750 mg (54%) of **58**: mp 164–165 °C (EtOAc–petroleum ether); IR (KBr) 1745, 1725, 1250, 1170, 1075, 965 cm^{-1}. Anal. ($C_{21}H_{28}O_8$) C, H.

15-Acetoxy-4β-(methacryloyloxy)scirpene-3,8-dione (59). This compound was prepared in 78% yield from **58** using the procedure similar to that described for **54**: mp 165–166 °C (Et_2O–petroleum ether); IR (KBr) 1775, 1750, 1728, 1680, 1236, 1150, 1066 cm^{-1}. Anal. ($C_{21}H_{24}O_8$) C, H.

4β,15-Bis(methacryloyloxy)scirpene-3α,8β-diol (60). A solution of **56** (1.24 g, 2.6 mmol), methacryloyl chloride (1.36 g, 31 mmol), and triethylamine (1.01 g, 10 mmol) in 50 mL of CH_2Cl_2 was stirred at room temperature for 24 h. The usual workup gave 1.20 g (76%) of 4α,15-bis(methacryloyloxy)-3α,8β-bis-*O*-(2-tetrahydropyranyl)scirpene. Treatment of this material with pyridinium tosylate as described for **58** gave 595 mg (76%) of the title compound: mp 143–144 °C (Et_2O); IR (KBr) 3520, 3280, 1715, 1695, 1300, 1165, 965 cm^{-1}. Anal. ($C_{23}H_{30}O_8$) C; H: calcd, 6.96; found, 7.66.

4β,15-Bis(methacryloyloxy)scirpene-3,8-dione (61). This compound was prepared in 77% yield from **60** using the procedure similar to that described for **54**: mp 132–135 °C (Et_2O–petroleum ether): IR (KBr) 1772, 1725, 1685, 1150, 1060, 948 cm^{-1}. Anal. ($C_{23}H_{26}O_8 \cdot 0.125H_2O$) H; C: calcd, 64.17; found, 63.76.

Biological Testing. Antitumor Effects. The tumors and parameters used in evaluating the analogues are summarized in Table III. The analogues were dissolved in Me_2SO, and futher dilutions were made with saline. All drug injections were made intraperitoneally. Tests with P-388 and L1210 leukemias, B16 melanoma, and Lewis lung carcinoma were conducted as described before.[22] The percent T/C is defined as the median survival time (MST) of all mice in a drug-treated (T) group divided by MST of the tumor control (C) group × 100.

In the Colon 38 test, mice were treated daily for 5 days beginning on day 15 and again on day 26 post-implant. Mice bearing Colon 36 were treated daily for 5 days beginning on day 3 and again on day 14 post-implant. In these two tests, the antiumor activity was judged on (a) the relative median time for tumors to reach a predetermined size (e.g., 750 mg for Colon 36, and 1250 mg for Colon 38) in drug-treated (T) as compared to control (C) mice (i.e., T – C). These tests were conducted by Dr. T. H. Corbett of Southern Research Institute, Birmingham, AL.

Effects on Protein Synthesis in H-HeLa Cells. All procedures were carried out as described previously.[6] When used, H-HeLa cells were growing exponentially with a density of 4×10^5 cells/mL. Cultures of these cells were transferred to a medium lacking amino acids and serum, and were incubated at 37 °C for 15 min before administration of the test analogue. Then, L-[4,5-^{3}H]leucine (1 Ci/mmol) was added, together with a trichothecene analogue (time zero), and incubation was continued at 37 °C. The analogues were dissolved in Me_2SO so that the final concentration of Me_2SO in reaction mixtures never exceeded 1% (v/v). Samples of 1 mL each were taken at various times into 1 mL of 10% (w/v) trichloroacetic acid, held at 90 °C for 20 min, and then cooled in ice. Precipitated material was collected on Whatman GF/C glass-fiber disks, which were washed three times with 5% trichloroacetic acid, dried, and prepared for liquid scintillation counting according to standard procedures.[26]

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Notes

Synthesis and Some Pharmacological Properties of Z-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp(Phe-NH_2)-OH, a 32-β-Aspartyl Analogue of Cholecystokinin (Pancreozymin) 27–33

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The heptapeptide carbobenzoxy-L-tyrosyl(*O*-sulfate)-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-β-L-phenylalanine amine (Z-32-β-Asp-CCK-27–33) was synthesized and tested for its ability to stimulate amylase secretion from dispersed pancreatic acini in vitro, to increase protein secretion from cat pancreas in vivo, and to cause contraction of guinea pig gallbladder in situ. In increasing amylase secretion in vitro, the Z-32-β-Asp-CCK-27–33 was equal in efficacy with but approximatively one-third as potent as the Boc-CCK-27–33, and when tested in vivo its activity is approximately 10 Ivy dog units (Idu)/μg. In stimulation of the contraction of the gallbladder, it showed an activity lower than 1 Idu/μg. This analogue has more pancreozyminic activity than cholecystokin-like activity. This seems to indicate different affinities for the two receptors.

In an earlier study[1] on the synthesis and properties of the desamino derivative of the C-terminal heptapeptide segment of cholecystokinin (desamino-CCK-27–33), formation of a byproduct was observed.[2] It occurred during the preparation of the sulfate ester of the phenolic hydroxyl group of the N-terminal tyrosine residue. The properties and the conditions of the formation of this by

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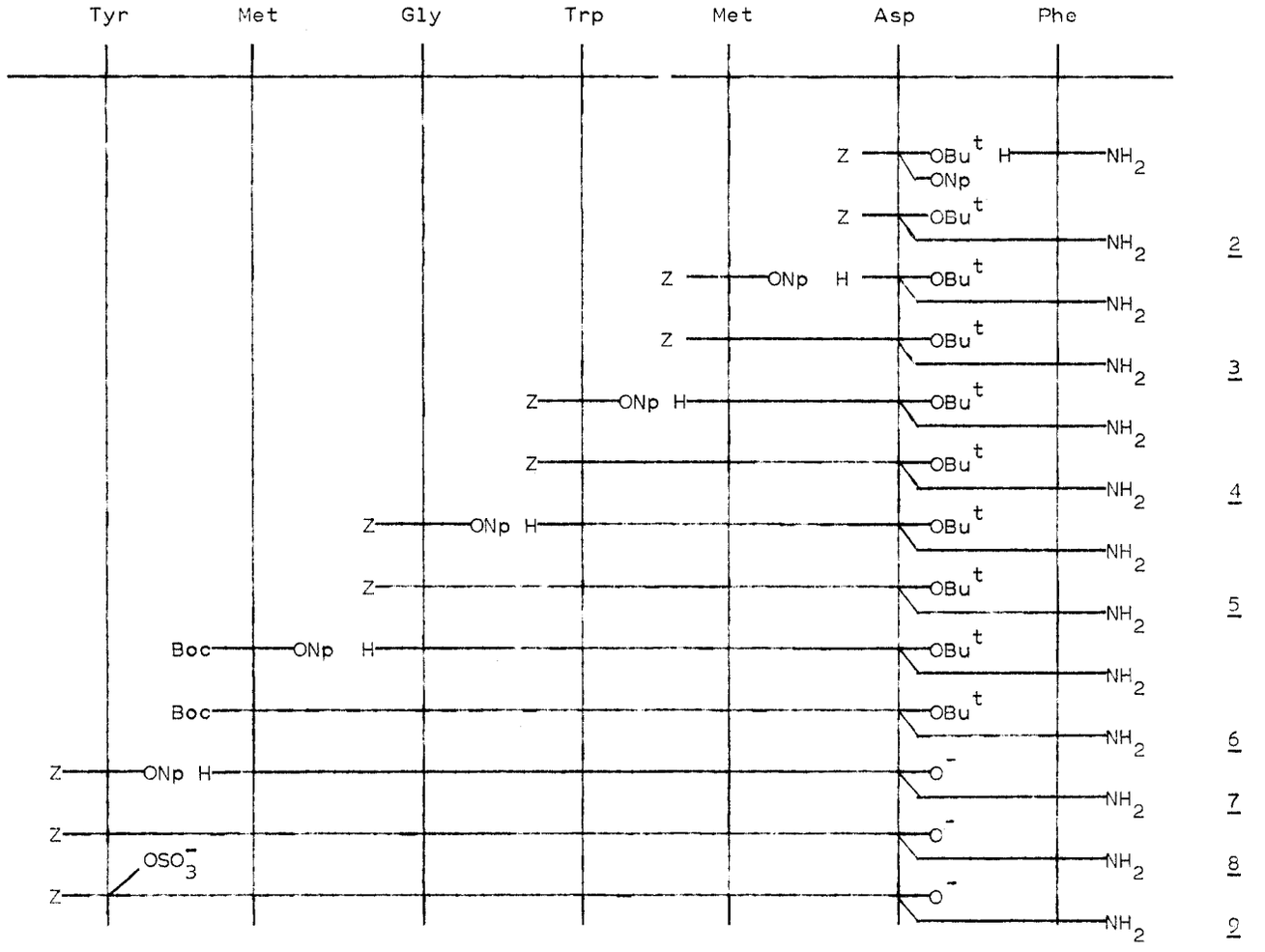
Chart I



product suggested that it was the asparagine-32 analogue of the desired peptide. The new compound had weak but definite pancreozyminic activity.[2] Therefore, if the so far tentative structural assignment for this analogue of CCK-27-33 is correct, an aspartyl residue with a free α-carboxyl group in position 32 is not necessary for hormonal activities.[3] To further explore the structural requirements for activity of residue 32, we undertook the synthesis of analogues with variations at this position. In this paper, we report the preparation and some pharmacological properties of the benzyloxycarbonyl derivative of the 32-β-Asp analogue of CCK–27–33 (Chart I).

Synthesis. The active ester, benzyloxycarbonyl-L-aspartic acid α-*tert*-butyl β-*p*-nitrophenyl ester, prepared for this synthesis was allowed to react with phenylalanine amide. The β-peptide derivative was partially deprotected by catalytic hydrogenation, and the resulting α-*tert*-butyl ester of β-L-aspartyl-L-phenylalanine amide was acylated with an active ester of benzyloxycarbonyl-L-methionine. The chain was lengthened by stepwise acylations[4] with active esters of benzyloxycarbonyl amino acids, exept in the preparation of the hexapeptide intermediate where *tert*-butyloxycarbonyl-L-methionine *p*-nitrophenyl ester was applied. Simultaneous removal of the N-terminal *tert*-butyloxycarbonyl group and *tert*-butyl ester group from the α-carboxyl of the penultimate aspartyl residue afforded the amphoteric hexapeptide amide, Met-Gly-Trp-Met-Asp(Phe-NH_2)-OH. Benzyloxycarbonyl-L-tyrosine was incorporated as an active ester. The partially protected heptapeptide derivative Z-Tyr-Met-Gly-Trp-Met-Asp(Phe-NH_2)-OH was treated with SO_3-pyridine complex to produce a sulfate ester group in the tyrosine side chain. The α-amino protecting group was not removed because earlier studies[5,6] showed that acyl derivatives of the C-terminal heptapeptide segment of CCK were more potent than the heptapeptide segment with the free α-amino group. In fact, the potency in the release of amylase from pancreatic cells or in the contraction of in situ gallbladder of the acetyl or the Boc heptapeptide was equal to that of the C-terminal octapeptide segment of the molecule, CCK–26–33.[7] The esterified material was

(3) In this respect, CCK is similar to the closely related peptides with gastrin sequences and gastrin-like activities or in which an aspartyl residue (or an electronic equivalent) has been shown to be important but not necessary for activity (Trout, H. H.; Grossman, M. I. *Nature (London), New Biol.* **1971**, *234*, 256. Morley, J. S. In "Hormonal Receptors in Digestive Tract Physiology"; Bonfils, S.; Fromageot, P.; Rosselin, G., Eds.; Elsevier: Amsterdam, 1972, p 4.

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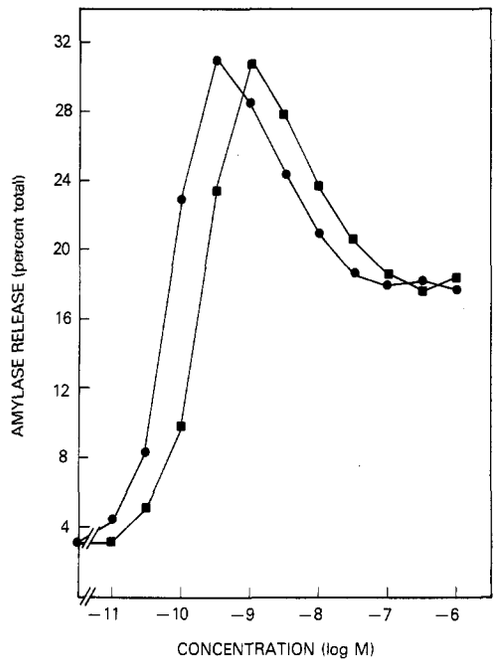

Figure 1. Amylase secretion by dispersed acini prepared from guinea pig pancreas. Closed circles represent results with Boc-CCK-27-33; closed squares represent results with the 32-β-Asp-CCK-27-33 analogue. Results are means from five separate experiments.

converted to the disodium salt and purified by chromatography on a column of silica gel. The purified compound was homogeneous by TLC and LC and gave the expected amino acid composition and elemental analysis. Its UV spectrum was essentially that of tryptophan. In the IR spectrum the characteristic band of a sulfate ester (1040 cm^{-1}) could be observed. The synthesis of the new analogue is summarized in Chart I.

Amylase Release from Pancreatic Acinar Cells. Amylase secretion by dispersed acini prepared from guinea pig pancreas was determined as previously described.[8] Acini from the pancreas of one animal were suspended in 150 mL of standard incubation solution composed of 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM KH_2PO_4, 1.2 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM sodium fumarate, 5 mM sodium glutamate, 5 mM sodium pyruvate, 2 mM glutamine, 5 mM theophylline, 11.5 mM glucose, 1% (w/v) bovine plasma albumin, 0.01% (w/v) soybean trypsin inhibitor, 1% (v/v) essential vitamin mixture, and 1% (v/v) Eagle's amino acid mixture. One milliliter of cell suspension was treated with 100% O_2 and incubated with the appropriate agent for 30 min at 37 °C. Amylase secretion was measured as the percentage of total cellular amylase that was released into the medium during the 30-min incubation. In each experiment, each value was determined in triplicate (Figure 1). With Boc-CCK-27-33 or the 32-β-Asp analogue, the shapes of dose–response curves for stimulation of amylase secretion were similar to those for native CCK as well as other C-terminal fragments and analogues of CCK.[9] In terms of the maximal stimulation of enzyme secretion, 32-β-Asp-CCK-27-33 was equal in efficacy to Boc-CCK-27-33. In terms of the lowest concentration that caused half-maximal stimulation of enzyme secretion, the β-Asp peptide was approximately one-third as potent as Boc-CCK-27-33.

Table I. Pancreozymin-like Activity in Vivo[a]

peptide	quantity	pancreatic response
none		0.066
standard CCK	3 Idu	0.40
32-β-Asp-CCK-27-33	0.5 μg	0.46
standard CCK	6 Idu	0.75
32-β-Asp-CCK-27-33	1.0 μg	0.97
standard CCK	12 Idu	1.08
32-β-Asp-CCK-27-33	2.0 μg	1.47
none		0.073

[a] Idu = Ivy dog units. Experiments were performed on an anesthetized cat with a cannulated pancreatic duct injected intravenously each 15 min with 2 clinical units of secretin, in addition to the peptide to be tested. The pancreatic response was measured as the optical density of the pancreatic juice secreted 30 min following injection of the peptide and diluted to 25 mL with physiological saline.

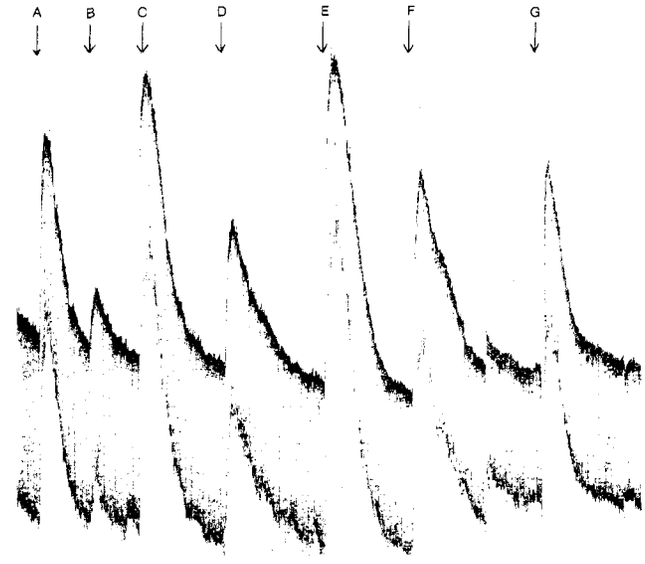

Figure 2. Contractile activities (guinea pig gallbladder). A, 0.2 Idu of standard CCK; B, 32-β-Asp-CCK-27-33 (0.07 μg); C, 0.4 Idu of standard CCK; D, 32-β-Asp-CCK-27-33 (0.14 μg); E, 0.8 Idu of standard CCK; F, 32-β-Asp-CCK-27-33 (0.28 μg); G, 0.2 Idu of standard CCK.

Protein Secretion from the Anesthetized Cat Pancreas. The benzyloxycarbonyl derivative of 32-β-Asp-CCK-27-33 was tested against standard CCK, according to the procedure of Jorpes and Mutt.[10] The peptide was dissolved to a concentration of 10 μg/3 mL of physiological saline containing, per milliliter, 1 mg of $NaHCO_3$ and 0.1 mg of L-cysteine. Aliquots of this solution were injected intravenously, alternatively with doses of standard CCK, into an anesthetized cat with a cannulated pancreatic duct,[10] the pancreas of which was being stimulated to secrete vigorously by the injection every 15 min of 2 clinical units of secretin. The pancreatic juice secreted in the 30-min period following the injection of CCK or of the 32-β-Asp analogue of CCK-27-33 was collected and diluted to 25 mL with physiological saline, and the optical density of the diluted juice was determined at 280 nm and 1-cm light path. The results are shown in Table I. It is evident that at these dose levels the pancreozyminic activity of the 32-β-Asp analogue of CCK-27-33 is approximately 10

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Idu/μg, which is in fairly close agreement with that of the in vitro amylase secretion assay.

Contractile Activity of the Guinea Pig Gallbladder. This assay was carried out according to Ljungberg.[11] A solution of 10 μg of the 32-β-Asp analogue of CCK-27-33 in 3 mL of physiological saline containing 1 mg of $NaHCO_3$ per mL and 0.1 mg of L-cysteine per mL was used. Aliquots of this solution were injected intravenously into an anesthetized guinea pig, alternatively with doses of standard CCK, and the contractile responses of the gallbladder were registered as described previously.[11] It is seen from Figure 2 that at the three doses levels tested the cholecystokinic activity of the 32-β-Asp analogue of CCK-27-33 was definitively weaker than 1 Idu/μg (0.7 Idu/μg calculated on E and G).

Conclusion

The 32-β-Asp analogue of CCK-27-33 showed more pancreozymin-like activity than cholecystokinin-like activity. A similar separation of the two kinds of activities has been observed with the desamino analogue of CCK-27-33. This compound showed relatively weak potency in the release of amylase from pancreatic acinar cells but it was at least as potent as CCK-26-33 in the stimulation of the contraction of the gallbladder of guinea pigs in situ.[1] These observations suggest that the exact nature of the residue in position 32 is more important for cholecystokinin-like activity than for pancreozymin-like activity. Thus, it seems possible to synthesize analogues with more specific activities than found in natural CCK.

Experimental Section

Capillary melting points are reported uncorrected. Thin-layer chromatographies (TLC) were performed on silica gel plates (Merck) developed with the following solvent systems: A, $CHCl_3$–MeOH, 9:1; B, $CHCl_3$–MeOH, 8:2; C, EtOAc–pyridine–AcOH–H_2O, 60:20:6:11; D, 1-butanol–H_2O–AcOH, 4:1:1; E, EtOAc–pyridine–AcOH–H_2O, 40:20:6:11. Spots were revealed with iodine vapors, ninhydrin, fluorescamin, charring, or by their absorption in the UV. For amino acids analysis, samples were hydrolyzed with constant-boiling HCl in evacuated, sealed ampules at 110 °C for 16 h. Analyses were performed by the Spackman Stein–Moore method on a Beckman–Spinco instrument. Optical rotations were measured with a 241 MC Perkin-Elmer polarimeter. The following abbreviations are used: DMF, dimethylformamide; DIEA, diisopropylethylamine; HOBt, hydroxybenzotriazole.

Benzyloxycarbonyl-L-aspartic Acid α-*tert*-Butyl β-*p*-Nitrophenyl Ester (1). To a cooled (0 °C) solution of *N*-(benzyloxycarbonyl)-L-aspartic acid α-*tert*-butyl ester[12] (15 g, 46.4 mmol) in ethyl acetate (250 mL) was added *p*-nitrophenol (7.8 g, 56 mmol), followed by dicyclohexylcarbodiimide (9.5 g, 46.4 mmol). After 0.5 h at 0 °C, stirring was continued at room temperature for 12 h. The precipitated *N,N'*-dicyclohexylurea was removed by filtration and rinsed with ethyl acetate. The filtrate was evaporated in vacuo, and the oily residue was dissolved in 95% ethanol (250 mL) containing 0.25 mL of AcOH and kept overnight in the refrigerator. The crystals that formed weighed 10.6 g. A second crop (4.2 g) was recovered similarly. The total yield was 14.4 g (71%): single spot on TLC, R_f 0.5 ($CHCl_3$); mp 77–79 °C; $[\alpha]^{23}_D$ −27.9° (*c* 1.08, DMF). Anal. ($C_{22}H_{24}N_2O_8$) C, H, N.

Benzyloxycarbonyl-α-*tert*-butyl-L-aspartyl-β-L-phenylalanine Amide (2). To a solution of L-phenylalanine amide trifluoroacetate (3 g, 10.8 mmol) in DMF (20 mL), cooled in an ice–water bath were added DIEA (3.84 g, 22 mmol) and HOBt[13] (1.64 g, 10.8 mmol), followed by a solution of α-*tert*-butyl β-*p*-nitrophenyl benzyloxycarbonyl-L-aspartate (4.9 g, 11 mmol) in DMF (20 mL). After 30 min, the solution was allowed to warm up to room temperature and was stirred overnight. At that time, the fluorescamine test was negative. The solvent was removed in vacuo, and the residue was triturated twice with a mixture of ethyl acetate/ether (1:1, 50 mL) and then with ether: a white powder was obtained, which was collected, washed with ether, and dried: yield 4.6 g (91%); single spot on TLC, R_f (A) 0.6; mp 171–173 °C; $[\alpha]^{23}_D$ −9.8° (*c* 1.02, DMF). Anal. ($C_{25}H_{31}N_3O_6$) C, H, N.

Benzyloxycarbonyl-L-methionyl-α-*tert*-butyl-L-aspartyl-β-L-phenylalanine Amide (3). A sample of compound **2** (4.5 g, 9.6 mmol) was hydrogenated in a mixture of ethanol (120 mL) and DMF (5 mL) in the presence of 10% Pd on charcoal. After the catalyst and solvents were removed, the oily residue was triturated with ether; the white product [R_f (B) 0.3] was dissolved in DMF (10 mL), cooled in an ice–water bath, and treated with benzyloxycarbonyl-L-methionine *p*-nitrophenyl ester[14] (4.04 g, 10 mmol), DIEA (1.7 mL), and HOBt (1.53 g, 10 mmol) in DMF (10 mL). After 0.5 h at 0 °C, the solution was kept at room temperature until the fluorescamine test became negative. The solution was evaporated in vacuo, and the residue was triturated with ethyl acetate (3 × 50 mL), filtered, washed with ether, and dried: yield 5.4 g; $[\alpha]^{23}_D$ −3.7° (*c* 1.17, DMF). Anal. ($C_{30}H_{40}N_4O_7S$) C, H, N.

Benzyloxycarbonyl-L-tryptophyl-L-methionyl-α-*tert*-butyl-L-aspartyl-β-L-phenylalanine Amide (4). Compound **3** (5.41 g, 9 mmol) was hydrogenated in a mixture (200 mL) of DMF/water/DIEA (10:2:1) in the presence of 10% Pd/$BaSO_4$ (600 mg). The solvent was concentrated in vacuo to about 20 mL after 12 h; on TLC, a single spot was observed, R_f (C) 0.45, R_f (D) 0.75. The black-colored solution was cooled in an ice–water bath and treated with benzyloxycarbonyl-L-tryptophan *p*-nitrophenyl ester[15] (4.33 g, 9.5 mmol), DIEA (1.6 mL), and HOBt (1.45 g, 9.5 mmol) in DMF (20 mL). After 0.5 h at 0 °C, the solution was kept at room temperature overnight. It was concentrated in vacuo to ca. 30 mL; the volume was adjusted to 150 mL with DMF, and the solution was passed through a column of neutral alumina (80 g); elution with DMF (200 mL) gave a clear colorless solution, which was evaporated in vacuo. Trituration of the residue with ethyl acetate and then with ether yielded a white solid. It was filtered and dried: yield 5.7 g (86%); single spot on TLC, R_f (A) 0.35; mp 224–225 °C; $[\alpha]^{23}_D$ −22.9° (*c* 1.18, DMF). Anal. ($C_{41}H_{50}N_6O_8S$) C, H, N.

Benzyloxycarbonylglycyl-L-tryptophyl-L-methionyl-α-*tert*-butyl-L-aspartyl-β-L-phenylalanine Amide (5). Compound **4** (4.9 g, 6.23 mmol) was hydrogenated overnight as described in the previous paragraph. The resulting solution (30 mL) was cooled in an ice–water bath and treated with benzyloxycarbonylglycine *p*-nitrophenyl ester[16] (2.31 g, 7 mmol), HOBt (1.07 g, 7 mmol), and DIEA (1.21 mL) in DMF (30 mL). The product, isolated as described for compound **4**, was recrystallized from a mixture of DMF/water: yield 4.68 g (89%); single spot on TLC, R_f (A) 0.35; $[\alpha]^{23}_D$ −14.5° (*c* 1.38, DMF). Anal. ($C_{43}H_{53}N_7O_9S$) C, H, N.

***tert*-Butyloxycarbonyl-L-methionylglycyl-L-tryptophyl-L-methionyl-α-*tert*-butyl-L-aspartyl-β-L-phenylalanine Amide (6).** After hydrogenation of **5** (4.06 g, 4.8 mmol) as described above, acylation was carried out in DMF with *tert*-butyloxycarbonyl-L-methionine *p*-nitrophenyl ester[17] (1.92 g, 5.2 mmol), HOBt (0.8 g, 5.2 mmol), and DIEA (0.8 mL). The protected hexapeptide (4.2 g, 93%) showed a single spot on TLC, R_f (A) 0.3, R_f (C) 0.65; mp 209–210 °C; $[\alpha]^{23}_D$ −19.1° (*c* 1.15, DMF). Anal. ($C_{45}H_{64}N_8O_{10}S_2$) C, H, N.

L-Methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-β-L-phenylalanine Amide (7). Trifluoroacetic acid (20 mL) containing anisole (1 mL) and thioanisole (1 mL) was cooled in an ice bath, while a stream of dry N_2 was passed over it. The protected hexapeptide **6** (1.5 g, 1.59 mmol) was added, and the solution was stirred at 0 °C under N_2 overnight. The well-stirred

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reaction mixture was diluted with ether (300 mL), the precipitate was filtered and washed several times with ethyl acetate and ether. This material was triturated and heated until it almost dissolved in a mixture (50 mL) of AcOEt/pyridine/AcOH/water (60:20:6:11). The solvent mixture was evaporated with a stream of N_2. The residue was treated with more of the same solvent mixture (50 mL), and the suspension was allowed to cool at room temperature, diluted with ethyl acetate (70 mL), and placed in the refrigerator. The next day the precipitate was collected, resuspended, and triturated with hot water. On standing at room temperature for 12 h and then overnight in the refrigerator, the white precipitate was filtered, washed with water, and dried: yield 1.1 g (85%); single spot on TLC, R_f (C) 0.7; mp 223–230; $[\alpha]^{23}_D$ −22.5° (c 1.2, DMF/1% DIEA). Anal. ($C_{36}H_{48}N_8O_8S_2 \cdot 2H_2O$) C, H, N.

Benzyloxycarbonyl-L-tyrosyl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-β-L-phenylalanine Amide (8). A solution of benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester (Bachem; 0.345 g, 0.79 mmol), DIEA (0.11 mL), and compound 7 (0.583 g, 0.71 mmol) in DMF (5 mL) was stirred at room temperature for 24 h. Evaporation of the solvent, followed by trituration with ethyl acetate and ether, yielded the heptapeptide 8 (0.68 g), which was purified by chromatography on a silica gel column (20 g) with EtOAc/pyridine/AcOH/water (60:20:6:11) as solvent: single spot on TLC, R_f (C) 0.4; mp 210–215 °C; $[\alpha]^{23}_D$ −15.4° (c 1.1, DMF). Anal. ($C_{53}H_{63}N_9O_{12}S_2 \cdot H_2O$) C, H, N.

Benzyloxycarbonyl(*O*-sulfate)-L-tyrosyl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-β-L-phenylalanine Amide (9). SO_3–pyridine complex (1.2 g; Aldrich) was added to a stirred solution of the peptide 8 (0.2 g) in dry pyridine (4 mL) and DMF (4 mL). Stirring was continued for 24 h and then the solvents were evaporated in vacuo. Cold water (5 mL) was added, followed by saturated sodium bicarbonate solution, dropwise, to maintain the pH at about 7 (pH paper). After about 1 h the solution remained neutral. A part of the product (0.125 g) separated and stayed in suspension. It was collected by centrifugation, washed with water, and dried in vacuo. A further crop (46 mg), less pure, was isolated by extraction of the aqueous solutions with 1-butanol. The first crop was purified by chromatography on a silica gel column (20 g) with EtOAc/pyridine/AcOH/water, 40:20:6:11, as eluent. Fractions containing the pure material (TLC) were concentrated in vacuo, pooled in 0.1 M NH_4OH, and lyophilized to yield 9 (82 mg). It was homogeneous on TLC, R_f (E) 0.25; R_f (D) 0.45; mp 185 °C dec; $[\alpha]^{23}_D$ −34.4° (c 0.96, DMF). In the IR spectrum, a strong sharp band at 1040 cm^{-1} indicates the presence of the sulfate ester group. The 32-β-Asp analogue of CCK–27–33 appeared as a sharp single peak on LC (MeOH/H_2O, 8:2, μ-Bondapak C_{18} column, flow rate 1.5 mL/min, detection at 254 nm, Waters instrument). After hydrolysis with 6 N HCl, amino acid analysis showed Asp, 1.03; Gly, 0.99; Tyr, 0.99; Phe, 1.06; Met, 2.00. Anal. ($C_{53}H_{61}N_9O_{15}S_3Na_2 \cdot 4H_2O$) C, H, N.

Effect of 4′-Halogen Substitution on the Mutagenicity of *trans*-4-Acetamidostilbene and *trans*-4-(*N*-Hydroxyacetamido)stilbene in the *Salmonella typhimurium* Test System

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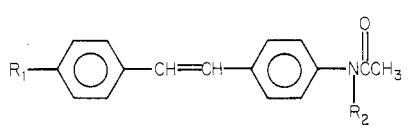
The effect of halogen substituents placed at the 4′ position of *trans*-4-acetamidostilbene (1, AAS) to alter the pattern of biotransformation and thus the mutagenicity of these derivatives was evaluated by comparing the mutagenic effects of 1 on *Salmonella typhimurium* TA-100 with the corresponding 4′-F (2), 4′-Cl (3), and 4′-Br (4) analogues. The mutagenic properties of *trans*-4-(*N*-hydroxyacetamido)stilbene (5) and its 4′-F (6), 4′-Cl (7), and 4′-Br (8) derivatives were also evaluated in this system. Both the amides (1–4) and hydroxamic acids (5–8) required the presence of a metabolic activating system prepared from hamster liver in order to produce a mutagenic effect. All of these compounds were mutagenic to TA-100. Their mutagenic potencies were markedly influenced by the 4′-halogen substituents, the relative mutagenic potencies of the amides being 2 (4′-F) > 1 (4′-H), 3 (4′-Cl) > 4 (4′-Br), while the hydroxamic acids followed the order of 1 (4′-H) > 2 (4′-F) > 3 (4′-Cl), 4 (4′-Br).

trans-4-Acetamidostilbene (1, AAS) is a carcinogenic



	R_1	R_2		R_1	R_2
1	H	H	5	H	OH
2	F	H	6	F	OH
3	Cl	H	7	Cl	OH
4	Br	H	8	Br	OH

N-aryl amide[1] which displys mutagenic activity when tested against several histidine-dependent tester strains of *Salmonella typhimurium* in the presence of a metabolic activation system.[2-4] Compound 1, like other carcinogenic and mutagenic *N*-aryl amides, must first be converted to its hydroxamic acid by N-oxidation of the amido nitrogen in order to display carcinogenic or mutagenic effects.[2,5,6] Formation of the ultimate carcinogen is believed to result by subsequent enzymatic and nonenzymatic reactions[7,8] involving the hydroxamic acid or its esters. Thus, the metabolic conversion of *N*-aryl amides to their hydroxamic acids is a critical step in the sequence of events that transform the parent compounds to their ultimate mutagenic and carcinogenic forms.

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