The ^{13}C T_1 experiment used an inversion-recovery pulse sequence with a relaxation delay D_1 = 4 s and averaging 2800 scans into an 8K data block. The experiment was repeated for 15 values of VD ranging from 0.01 to 3 s. The T_1 values were calculated by using the Bruker DISNMR program. The 180° pulse had been calibrated and the value was 19 μs in $CDCl_3$ and in CD_3OD solution.

FAB-PI mass spectra were obtained on a Nermag 1030 instrument fitted with an Ion Tech fast atom gun. Xenon and argon were used as the bombarding species. Glycerol was used as the matrix to dissolve the samples.

Acknowledgment. We would like to thank Dr. B. Bachet and J. P. Mornon for the crystal structure coordinates of roxithromycin.[12] We thank Laboratoire Roussel (Paris) for their scientific and financial support during this research.

Registry No. 1, 80214-83-1; 2, 114-07-8.

Supplementary Material Available: Table S1 listing ^1H and ^{13}C NMR relaxation times in $CDCl_3$ and CD_3OD solution for roxithromycin (1); concentration effect on the chemical shifts of hydroxyl groups for roxithromycin (1) and erythromycin (2) in $CDCl_3$ solution (Graph S1); chemical shifts variation with pH of the *N,N*-dimethylamino group for roxithromycin (1) and erythromycin (2) in D_2O solution (10^{-3} M) (Graph S2); Figure S1 is an FAB mass spectrum of a roxithromycin (1) and erythromycin (2) mixture (5 pages). Ordering information is given on any current masthead page.

Carboxylic Acids and Tetrazoles as Isosteric Replacements for Sulfate in Cholecystokinin Analogues

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A series of analogues of the satiety-inducing peptide cholecystokinin (CCK-8) was prepared in which the sulfated tyrosine required for activation of peripheral receptors was replaced with a carboxy(alkyl)- or tetrazolyl(alkyl)-phenylalanine to investigate whether an organic acid could serve the role of the sulfate group at the receptor. The necessary intermediates were prepared by previously reported procedures or by alkylation of carboxy(alkyl)- or tetrazolyl(alkyl)phenylmethyl bromides with a glycine-derived anion followed by protecting-group manipulations, and these were incorporated into derivatives of acetyl-CCK-7 using solid-phase synthesis. Peptide analogues were evaluated in a CCK-binding assay for affinity for either peripheral (CCK-A) receptors using homogenated rat pancreatic membranes as the receptor source or for central (CCK-B) receptors using bovine striatum as the receptor source. They were further evaluated for effects on food intake in rats after intraperitoneal (ip) injection. A number of the compounds reported are active in the CCK-A receptor binding assay although less potent than acetyl-CCK-7 and decrease food intake with comparable potency to acetyl-CCK-7. In a meal feeding model designed to assess appetite suppressant activity, acetyl-CCK-7 has an ED_{50} of 7 nmol/kg ip, while the ED_{50}s of Ac-Phe(4-CH_2CO_2H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (**28**) and Ac-Phe[4-(tetrazol-5-yl)]-Met-Gly-Trp-Met-Asp-Phe-NH_2 (**34**) were 9 and 11 nmol/kg ip, respectively. An analogue of **28** lacking the N-terminal acetamido group, 3-[4-(carboxymethyl)-phenyl]propanoyl-Met-Gly-Trp-Met-Asp-Phe-NH_2 (**50**), was also active in the meal feeding assay with an ED_{50} of 3 nmol/kg ip. Its anorexic effect was blocked by simultaneous administration of the CCK-A receptor antagonist MK 329, indicating that the observed anorexic activity is mediated by CCK-A receptors. We conclude from this work that the requirement for a negative charge at the CCK-A receptor provided in the natural substrate by a sulfate group can be satisfied by organic acids.

A number of neurohormones and secretory peptides such as cholecystokinin,[1] fibronectin,[2] hirudin,[3] and sulfakinin[4] contain a sulfated tyrosine, which is necessary for full expression of their biological activity. Because of the lability of tyrosine sulfates,[5] other investigators have developed methodology for their replacement in peptide analogues by phosphono-,[6] (phosphonomethyl)-,[7] and (sulfomethyl)phenylalanines.[7,8] We have been concerned for some time with the design of analogues of the putative satiety-signalling peptide cholecystokinin (CCK) as appetite suppressants, and in particular with the discovery of suitable stable and less polar replacements for the tyrosine sulfate which might ultimately be compatible with an orally active CCK mimetic. In this paper we report that analogues of CCK in which the tyrosine sulfate is substituted by either an appropriate (carboxymethyl)-phenylalanine or a tetrazolylphenylalanine retain binding affinity to the peripheral (CCK-A) receptor subtype[9] and, moreover, possess equivalent potency to CCK-7 as appetite suppressants in rats.

CCK represents a family of gut and neurohormones of which the 33 amino acid peptide CCK-33 and the equipotent eight amino acid peptide CCK-8 (1) are the most abundant circulating bioactive forms. Peripheral activities of CCK include stimulation of enzyme secretion from pancreatic acinar cells, inhibition of gastric emptying,

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§ Department of Pharmacology.

(1) Mutt, V.; Jorpes, J. E. *Eur. J. Biochem.* **1968**, *6*, 156.
(2) Liu, M.-C.; Lipmann, F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 34.
(3) Braun, P. J.; Hofsteenge, J.; Stone, S. R. *Biochemistry* **1988**, *27*, 6517. Stone, S. R.; Hofsteenge, J. *Biochemistry* **1986**, *25*, 4622.
(4) Nachamn, R. J.; Holman, G. M.; Haddon, W. F.; Ling, N. *Science* **1986**, *234*, 71. Nachamn, R. J.; Holman, G. M.; Cook, B. J.; Haddon, W. F.; Ling, N. *Biochem. Biophys. Res. Commun.* **1986**, *140*, 357. Nachamn, R. J.; Holman, G. M.; Haddon, W. F.; Hayes, T. K. *Pept. Res.* **1989**, *2*, 171.
(5) Bettelheim, F. R. *J. Am. Chem. Soc.* **1954**, *76*, 2838.
(6) Petrakis, K. S.; Nagabhushan, T. L. *J. Am. Chem. Soc.* **1987**, *109*, 2831.
(7) Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621.
(8) Marseigne, I.; Dor, R. C.; Durieux, C.; Pelaprat, D.; Reibaud, M.; Blanchard, J. C.; Roques, B. P. *J. Med. Chem.* **1989**, *32*, 445.
(9) Moran, T. H.; Robinson, M. S.; Goldrich, M. S.; McHugh, P. R. *Brain Res.* **1986**, *362*, 175. Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. *J. Med. Chem.* **1989**, *32*, 13. Freidinger, R. F. *Med. Res. Rev.* **1989**, *9*, 271.

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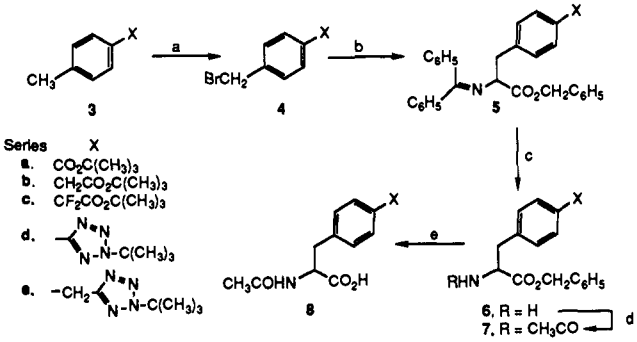
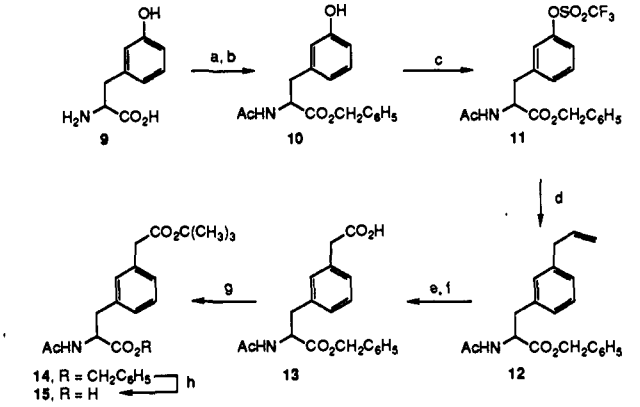
Scheme I[a]



[a] (a) NBS, benzoyl peroxide, CCl_4; (b) $[(C_4H_9)_4N]_2SO_4$, NaOH, benzyl diphenylmethyleneglycinate, CH_2Cl_2; (c) pTsOH, CH_3CN, H_2O; (d) acetic anhydride, NEt_3, CH_2Cl_2; (e) H_2, Pd(C), EtOH.

Scheme II[a]



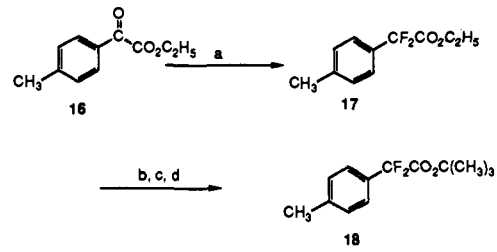
[a] (a) Benzyl alcohol, p-TsOH, toluene, reflux, 4 h; (b) acetic anhydride, Na_2CO_3, H_2O, CH_2Cl_2; (c) Phenyltriflimide, NEt_3, CH_2Cl_2 (85%); (d) Allyltributyltin, $[(C_6H_5)_3P]_2PdCl_2$, DMF, 90 °C, 0.5 h (85%); (e) catalytic $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O, 25 °C, 1 h; (f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, $(CH_3)_3COH$, 0 °C, 2 h (63%, (2) steps); (g) DMF di-*tert*-butyl acetal, toluene 55 °C (84%); (h) 10% Pd(C), EtOH, 3 h, room temperature.

stimulation of gall bladder contraction, and stimulation of vagal afferents terminating in the nucleus of the solitary tract and the area postrema. Exogenous administration of CCK to animals[10] or to man[11] decreases meal size and elicits in rats a sequence of behavior mimicking normal satiety. These effects have prompted the proposal that CCK released endogenously from the small intestine is a peripherally acting signalling hormone mediating postprandial satiety.[12] Since acylated CCK-7 has equivalent potency to its longer homologues[13] and is a full agonist at CCK-A receptors, we have initiated our structure–activity studies with acetyl-CCK-7 (**2**).

H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
1

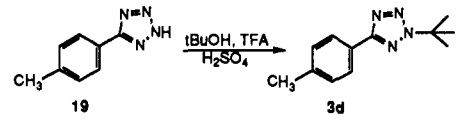
Ac-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2
2

Scheme III[a]

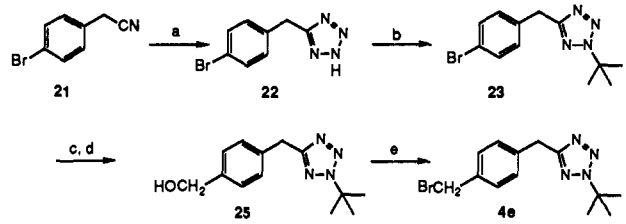


[a] (a) DAST; (b) KOH, CH_3CN, H_2O; (c) oxalyl chloride, toluene; (d) *tert*-butyl alcohol.

Scheme IV



Scheme V[a]



[a] (a) NaN_3, NH_4Cl, DMF, 90 °C, 48 h; (b) *tert*-butyl alcohol, TFA, 1 equiv H_2SO_4, room temperature 24 h; (c) $(C_6H_5)_3P$, $(C_4H_9)_3N$, *tert*-butyl alcohol, H_2O, $[(C_6H_5)_3P]_2PdCl_2$, (200 psi) CO, 100 °C, 48 h; (d) DIBAL, toluene, 0 °C; (e) $(C_6H_5)_3P$, CBr_4, Et_2O, 3 h.

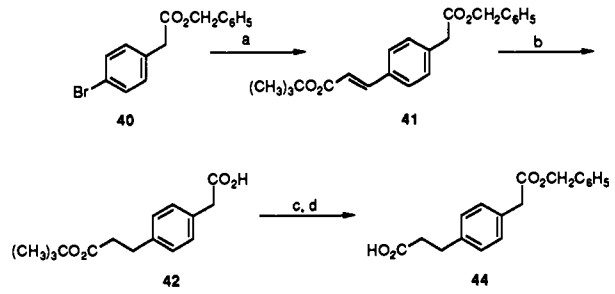
Chemistry

The new phenylalanine derivatives required for this work were prepared according to Schemes I and II. Scheme I details the reaction between an activated nucleophilic glycine and the appropriate benzyl bromides **4** by using the general methodology developed by O'Donnell.[14] Subsequent routine protecting-group transformations then lead to the racemic *N*-acetylphenylalanine derivatives **8a-e**. The transformations described in Scheme II featuring a palladium-catalyzed allylation of triflate **11** derived from *m*-tyrosine leading to *m*-(carboxymethyl)-phenylalanine **15** are similar to those previously described for the synthesis of *p*-(carboxymethyl)phenylalanines.[15]

The benzyl derivatives required for the glycine alkylation chemistry were synthesized as outlined in Schemes III–V. We found it expedient to convert ethyl α,α-difluorophenylacetate derivative **17**, obtained by (diethylamido)-sulfur trifluoride (DAST) treatment of ethyl phenylglyoxylate **16**, to the corresponding *tert*-butyl ester **18** as shown in Scheme III due to the lability of the ethyl ester during peptide synthesis. In Schemes IV and V, the readily available 5-phenyltetrazoles **19** and **22** were protected as their 2-*tert*-butyl derivatives **3d** and **23**, respectively, by treatment with *tert*-butyl alcohol in the presence of trifluoroacetic acid and sulfuric acid. These *tert*-butyl groups proved exceptionally stable, but were readily removed by treatment with HF during cleavage of the resulting peptides from the resin. Bromide **23** was further

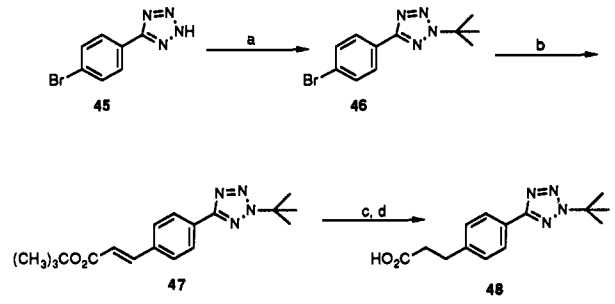
(10) Antin, J. J.; Gibbs, J.; Holt, J.; Young, R. C.; Smith, G. P. *J. Comp. Physiol. Psychol.* **1975**, *89*, 784–790.
(11) Smith, G. P.; Gibbs, J. In *Human Obesity*; Wurtman, R. J., Wurtman, J. J., Eds.; Academy of Science: New York, 1987; pp 132–136.
(12) Smith, G. P. In *Eating and Its Disorders*: Stunkard, A. J., Stellar, E., Ed.; Raven: New York, 1984, pp 67–75. C. A. Baile, A.; McLaughlin, C. L.; Della-Fera M. A. *Physiol. Rev.* **1986**, *66*, 172–234.
(13) Bodanszky, M.; Tolle, J. C.; Gardner, J. D.; Walker, M. D.; Mutt, V. *Int. J. Pept. Protein Res.* **1980**, *16*, 402.
(14) O'Donnell, M. J.; Boniece, J. M.; Earp, S. E. *Tetrahedron Lett.* **1978**, 2641.
(15) Tilley, J. W.; Sarabu, R.; Wagner, R.; Mulkerins, K. *J. Org. Chem.* **1990**, *55*, 906.

Scheme VI[a]



[a] (a) *tert*-butylacrylate, $(C_2H_5)_3N$, DMF, $[(C_6H_5)_3P]_2PdCl_2$; (b) H_2, Pd(C); (c) benzyl bromide, K_2CO_3, DMF; (d) HCO_2H.

Scheme VII[a]



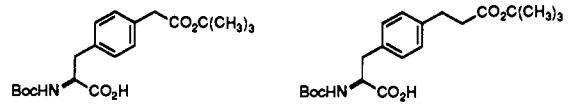
[a] (a) *tert*-butyl alcohol, TFA, 1 equiv H_2SO_4, room temperature 24 h; (b) *tert*-butyl acrylate, $(C_2H_5)_3N$, $[(C_6H_5)_3P]_2PdCl_2$, DMF; (c) H_2, Pd(C), EtOH; (d) TFA.

elaborated into benzyl bromide derivative **4e** by palladium-catalyzed carbonylation to the corresponding acid, DIBAL reduction to alcohol **25**, and carbon tetrabromide/triphenylphosphine-mediated bromination as outlined in Scheme V.

Since activity at CCK-A receptors tolerates acyl groups on tyrosine ranging in size from acetyl to a 26 amino acid peptide (i.e. CCK-33) and is not critically dependent on the presence of an N-terminal acyl moiety,[16] we were interested to determine whether analogues such as **28** and **34** might be further simplified by deletion of their N-terminal amino groups. To this end the substituted phenyl bromides **40** and **46** were coupled with *tert*-butyl acrylate in the presence of Pd^0 to give the corresponding cinnamic acid derivatives **41** and **47**, respectively. Hydrogenation of the double bond and transformation of the ester groups as outlined in Schemes VI and VII then led to the desired starting materials for peptide synthesis, **44** and **48**.

Peptides **2**, **26–37**, and **49–51** listed in Tables I and II were prepared by standard solid-phase methods utilizing either the Fmoc/*tert*-butyl strategy, employing [[4-(methyloxy)phenyl]acetamido]methyl (PAM) resin and ammonolysis for cleavage (method A) or the Boc/benzyl strategy, employing benzhydrylamine (BHA) resin and HF cleavage[17] (method B). Coupling reactions were mediated by DCC/HOBT and monitored by the ninhydrin test.[18] Attempted HF-mediated cleavage of the resin-bound peptides leading to **28–30** was complicated by Friedel–Crafts chemistry involving the (carboxyalkyl)phenylalanines. Thus synthesis of the carboxyl derivatives was accomplished on a PAM resin by using method A in which the resin-bound peptides were first deprotected by trifluoroacetic acid treatment and then cleaved by ammonolysis. The crude peptides were purified to homogeneity by preparative HPLC on a Micro Bondapack C-18 column and their purity was verified by analytical HPLC. They were characterized by FAB MS and amino acid analysis after acid hydrolysis.

Where the racemic acetylated analogues **8a–e** were employed in the synthesis, the resulting diastereomeric peptides were separated by HPLC. The chirality of the phenylalanine derivatives in the separated diastereomers was determined by hydrolysis with 6 N hydrochloric acid, esterification of the individual amino acids with 2-propanol in hydrochloric acid, and acylation with perfluoropropanoic anhydride prior to gas chromatographic analysis on a Chirasil-Val chiral column.[19] Of the substituted phenylalanine derivatives, the isomer with the longer retention time was taken to be the *S* enantiomer based on our past experience with amino acids.[20] In the case of **28**, this stereochemical assignment was confirmed by direct comparison of racemic (carboxymethyl)phenylalanine with the corresponding previously described homochiral derivative **38**.[15]



Results and Discussion

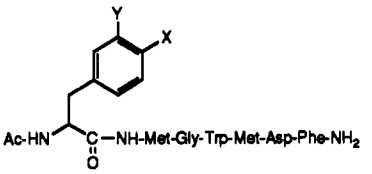
Receptor binding activity for the CCK-A and CCK-B receptor subtypes was determined with solubilized membranes prepared from fresh pancreatic tissue, obtained from fasted rats, or bovine striatum, respectively, as previously described by Van Dijk[21] and detailed in the Experimental Section. Nonspecific binding was determined in the presence of 1 μM native Ac-CCK-8 and subtracted from all samples to determine specific binding. The concentration of the peptides listed in Tables I and II necessary to inhibit 50% of total specific ([^{3}H]propanoyl)-CCK-8 binding (IC_{50} value) was determined by log–probit analysis. In general, data for active compounds were confirmed by duplicate experiments. The IC_{50} for Ac-CCK-8 under these conditions was ca. 1 nM for both tissue preparations.

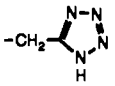
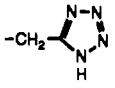
Test peptides were evaluated for their ability to suppress food intake in a meal-fed rat model. Male Sprague–Dawley rats (200–250 g) were trained to take their daily meals during two 1-h periods separated by a 2-h interval for 4–5 days prior to test peptide administration. On the test day peptides were given by ip injection 15 min before the first meal to groups of five or six rats and the amount of food eaten during each meal was determined. The treated groups were compared to the control groups by using the *t* test. Data are expressed as percent of saline-treated control food intake or as the dose which caused 50% inhibition of the control intake (ED_{50}) as determined from log–probit analysis of the data.

The results summarized in Table I indicate that most of the carboxy- and tetrazolylphenylalanine-containing peptide derivatives prepared bind to CCK receptors and suppress food intake in rats. As is typical for CCK in this

(16) Bodanszky, M.; Martinez, J.; Walker, M.; Gardner, J. D.; Mutt, V. *J. Med. Chem.* **1980**, *23*, 82.
(17) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6442.
(18) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 594.
(19) Frank, H.; Nicholson, J. T.; Bayer, E. *J. Chromatogr.* **1978**, *167*, 187. Frank, H.; Woiwode, W.; Nicholson, G.; Bayer, E. *Liebigs. Ann. Chem.* **1981**, 354.
(20) Meienhofer, J. *Biopolymers* **1981**, *20*, 1761.
(21) Van Dijk, A.; Richards, J. C.; Trzeciak, A.; Gillesen, D.; Mohler, H. *J. Neurosci.* **1984**, *4*, 1021.

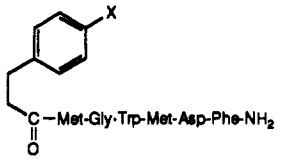
Table I. Tyrosine Sulfate Substituted Analogues



no.	Phe Deriv stereo.	method	X	Y	CCK binding assay; IC_{50}, nM pancreas	CCK binding assay; IC_{50}, nM striatum	feeding assay % control food intake[a] dose, μg/kg ip	1st meal	2nd meal	ED_{50} (first meal), nmol/kg ip
2	*S*		OSO_3H(Ac-CCK-7)	H	0.60	4.4	32	6 ± 1**	141 ± 6**	
							10	36 ± 2**	137 ± 13	7
							3	81 ± 8	118 ± 11	
26	*S*	A	CO_2H	H	30	62	320	12 ± 6**	165 ± 8**	
							32	79 ± 12	140 ± 6*	
27	*R*	A	CO_2H	H	210	68	320	85 ± 4	146 ± 6*	
28	*S*	A	CH_2CO_2H	H	2.6	54	320	1 ± 0**	116 ± 14	
							16	38 ± 5**	179 ± 20*	9
							3	76 ± 6*	139 ± 16	
29	*R*	A	CH_2CO_2H	H	21	145	320	31 ± 19**	141 ± 8	
							32	75 ± 10**	114 ± 8	
30	*S*	A	$CH_2CH_2CO_2H$	H	390	620	320	103 ± 5	114 ± 7	
31	*S*	A	CF_2CO_2H	H	140	92	100	16 ± 5**	196 ± 15**	~23
							32	43 ± 10**	200 ± 6**	
32	*R*	A	CF_2CO_2H	H	170	200	100	41 ± 6**	211 ± 13**	
33	*R,S*	A	H	CH_2CO_2H	23	53	320	0 ± 0**	163 ± 13**	
							100	7 ± 1**	168 ± 21**	19
							32	40 ± 9**	169 ± 11**	
							10	68 ± 9*	123 ± 8	
34	*S*	B		H	3.0	0.50	16	32 ± 10**	191 ± 10**	~11
							3	79 ± 10	156 ± 8*	
35	*R*	B		H	120	76	16	89 ± 6	145 ± 12	
36	*S*	B		H	67	18	100	16 ± 3**	179 ± 14**	
							32	25 ± 4**	170 ± 12**	14
							10	65 ± 4**	179 ± 9**	
							3	85 ± 6*	118 ± 9	
37	*R*	B		H	380	62	320	47 ± 13**	170 ± 21*	
							100	58 ± 6**	159 ± 9*	218
							32	80 ± 6*	119 ± 28	

[a] Data represent mean ± SEM for each treatment group; *$p < 0.5$, **$p < 0.01$ relative to control food intake.

Table II. Desaminotyrosine Sulfate Substituted CCK Analogues



no.	X	method	CCK binding assay; IC_{50}, nM pancreas	CCK binding assay; IC_{50}, nM striatum	feeding assay % control food intake[a] dose, μg/kg ip	1st meal	2nd meal	ED_{50} (first meal), nmol/kg ip
49	OSO_3H	B	0.25	3.1	32	34 ± 22**	113 ± 12	
50	CH_2CO_2H	A	26	22	10	16 ± 10**	184 ± 21**	
					3	50 ± 8**	172 ± 24**	3
					1	78 ± 12	155 ± 8*	
51		B	35	5	32	41 ± 5**	159 ± 13*	
					10	70 ± 8**	133 ± 4	19
					3	82 ± 8	142 ± 11*	

[a] Data represent mean ± SEM for each treatment group; *$p < 0.5$, **$p < 0.01$ relative to control food intake.

model, the active compounds induce a dose-dependent hyperphagia in the second feeding period. While in the present work no attempt has been made to make the peptide derivatives resistant to degradation, our experience indicates that longer acting analogues suppress food intake during the second meal as well.[22]

In the pancreatic (CCK-A) receptor binding assay, the most potent compounds are the carboxymethyl analogue **28** and tetrazole **34**, each of which are only about 4-fold less potent than Ac-CCK-7. Both of these compounds are also nearly as potent as Ac-CCK-7 at suppressing food intake. Carboxyphenylalanine derivative **26** is several fold less potent that its homologue **28** in both the CCK-A receptor binding assay and the feeding assay while the corresponding carboxyethyl analogue **30** is 100-fold weaker in the binding assay and does not inhibit food intake at 320 μg/kg. Since these compounds were intended to mimic the activity of a sulfuric acid derivative, we were interested to determine whether a strong acid would be more effective. To this end, α,α-difluoro analogue **31** was tested and, surprisingly, was found to be relatively weakly active in both assays. Previous results which indicated that Ac-[*m*-Tyr(OSO_3H)]-CCK-7 retains most of the potency of the para isomer[23] prompted us to synthesize *m*-carboxymethyl analogue **33**, which was found to be active, but less somewhat less potent than **28**. Of the two tetrazoles tested, the tetrazolylphenylalanine derivative **34** is nearly identical with **28** in overall profile while the homologous (tetrazolylmethyl)phenylalanine derivative **36** is considerably less potent in the CCK-A receptor binding assay, but only slightly less effective in the feeding assay. This finding may be a result of altered metabolism or distribution of **36** relative to the other CCK analogues reported herein or alternatively, since activation of the pancreatic receptor is not directly responsible for the effects of CCK on food intake[24], may reflect subtle differences between the putative satiety receptor and the pancreatic receptor. In each case analogues derived from the *R* enantiomer of the phenylalanine derivatives proved less active than the corresponding *S* enantiomer in both the binding and the meal feeding assays.

In order to provide for simpler analogues, we also prepared the "desamino" analogues of CCK-7 and the two most interesting compounds **28** and **34** as shown in Table II. We were pleased to note that both the carboxymethyl derivative **50** and tetrazole **51** were comparable in the food intake assays to their parent compounds although each was about 1 order of magnitude less potent in the CCK-A receptor binding assay.

In order to confirm that the anorexic effect of these compounds is in fact mediated through a CCK receptor and not due to nonspecific effects, **50** was evaluated to determine if its anorexic effect could be blocked by the CCK-A antagonist MK-329. In addition, **50** was tested in combination with the CCK-B antagonist L-365,260, which has been shown to be ineffective in blocking the anorexic effect of exogenously administered CCK.[25] Initially, groups of five to seven rats were fasted overnight, administered vehicle or drug by ip injection, and were presented with food cups for 1 h. The average food consumed during the test period was compared with vehicle-treated controls and ED_{50} values were determined from log–probit analysis of effective anorexic doses. The dose–response curve for **50** generated in this overnight food deprivation paradigm is given in Figure 1; the ED_{50} for this compound is 3 nmol/kg, the same as that determined in the two-meal feeding assay discussed above. In the second phase, the rats were administered 3 nmol/kg of **50** alone or combined with 100 μg/kg of either MK 329 or L-365,260. The results, summarized in Figure 2 show that the CCK-A receptor antagonist MK 329, but not the CCK-B antagonist L-365,260, blocks the anorexic effect of **50**, indicating that this compound and, by implication, the other active compounds reported herein suppress food intake via stimulation of peripheral CCK receptors.

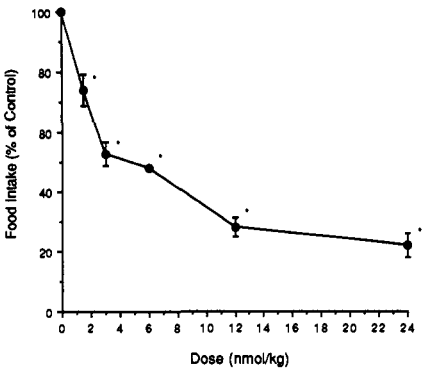


Figure 1. Mean ± SEM of food intake expressed as a percent of control intake after injection (ip) of 0.5% DMSO and saline (control) or one of five doses of **50**. $*p < 0.05$, **50** vs control. $n = 7$ for all doses except 24 nmol/kg where $n = 5$.

In conclusion, we have demonstrated that for peripherally activating CCK analogues, either a carboxymethyl or tetrazole can substitute for the tyrosine sulfate group without significant loss of activity. We postulate that the function of the sulfate group is to provide a suitably disposed negative charge to accommodate an ion dipole interaction necessary for receptor activation and that this negative charge can equally well be provided by an organic acid. Since analogues in which the carboxyl group is in the meta position or attached directly to the aromatic ring retain activity, it appears that the N-terminal chain in CCK has sufficient flexibility to achieve the necessary positioning of the carboxyl group when bound to the receptor without a major free-energy cost. These findings represent a significant step toward the design of orally active CCK analogues, particularly since ample precedent exists for the masking of carboxyl groups in the form of prodrugs. It will be interesting to determine whether the (carboxyalkyl)- and tetrazolylphenylalanines represent isosteric replacements for tyrosine sulfates and phosphates in general.

Experimental Section

For the preparation of the compounds described in Schemes I–VI melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian XL-200 or XL-400 spectrometer with tetramethylsilane used as an internal reference. Electron impact (EI, 70 eV) and fast ion bombardment (FAB) mass spectra were taken on VG ZAB-1F or VG 70E mass spectrometers. NMR and MS data were in accord with each of the assigned structures. Elemental analyses of the new intermediates reported were obtained for the elements indicated and were within 0.4% of the calculated values except as noted. Preparative high-pressure liquid chromatography (HPLC) was performed on silica gel Prep-Pak 500 cartridges using a Water Associates Prep LC 500A. Flash silica gel chromatography employed Kiesel gel 60 (230–400 mesh) as supplied by E. Merck, Darmstadt, Germany, under a nitrogen pressure of 2–5 psi. DMF was dried over Linde 3A sieves and triethylamine was distilled from calcium hydride. Extracts were dried over $MgSO_4$ except as indicated. Concentration refers to removal of solvent under aspirator pressure with a Büchi rotary evaporator.

For the synthesis of the peptides listed in Tables I and II,

(22) Danho, W.; Makofske, R. C.; Swistok, J.; Michalewsky, J.; Gabriel, T.; Nelson, D.; Mackie, G.; Triscari, J., manuscript in preparation.
(23) Stanfield, C. F.; Felix, A. M.; Danho, W. *Pept. Res.* **1990**, *2*, 69.
(24) Moran, T. H.; Shnayder, L.; Hostetler, A. M.; McHugh, P. R. *Am. J. Physiol.* **1988**, *255*, R1059.
(25) Dourish, C. T.; Ruckert, A. C.; Tattersall, F. D.; Iversen, S. D. *Eur. J. Pharmacol.* **1989**, *173*, 233.

solvents for all washings and couplings were measured to volumes of 10–20 mL/g of resin. Couplings were performed with the DCC/HOBt procedure. Coupling reactions were monitored by the Kaiser ninhydrin test[18] to determine whether coupling was complete at step seven of method A, the Fmoc synthetic protocol, or at step 16 of method B, the Boc synthetic protocol (vide infra). EDT refers to ethanedithiol and DIPEA refers to diisopropylethylamine. High-pressure liquid chromatography (HPLC) was conducted on an LDC apparatus consisting of a Constametric I pump, a Constametric III pump, a Gradient Master solvent programmer and mixer, and a Spectromonitor III variable-wavelength UV detector. Analytical HPLC chromatography was performed on reversed phase with Waters Micro Bondapack C-18 columns (0.4 × 25 cm). Preparative HPLC separations were run on a (2.3 × 30 cm) Micro Bondapack C-18 column (E. S. Industries, Marlton, NJ); in both cases, a precolumn of Whatman Co:Pell ODS pellicular packing was used. Preparative purification was carried out directly on the crude peptide by HPLC. The peptides were applied in a minimum volume of 50% AcOH and eluted with a slow gradient (4 h) of 5–65%, 0.022% TFA/CH_3CN, at a flow rate of 8.0 mL/min, with monitoring of the 280-nm absorption of the effluent. Fractions were collected at 3-min intervals and cuts were made after inspection by analytical HPLC. Fractions, judged to be greater than 97% pure, were pooled and lyophilized. Amino acid analyses and FAB MS of the individual peptides were performed and the expected values were obtained in each case.

For the separation of the diastereomeric peptides, samples of the purified mixtures were dissolved in 0.5 mL of 0.2 N NH_4OH and applied to a (1.25 × 30 cm) Micro Bondapak C-18 column from E. S. Industries which was eluted with a linear gradient of 0.01 M NH_4Ac and acetonitrile as noted in the individual examples. The 280-nm absorption of the column effluent was monitored and the material corresponding to the two peaks was collected separately.

To determine the enantiomeric identities of the substituted phenylalanine moieties of **26–29**, **31**, **32**, and **34–37**, a 1-mg sample of each of the purified peptides was dissolved in 0.5 mL of 6 N HCl and sealed under vacuum. The vial was heated at 110 °C for 18 h and allowed to cool. The hydrolysate was evaporated to dryness and then heated in a sealed reaction vessel in 3 N HCl in 2-propanol for 1 h at 110 °C. The reaction mixture was evaporated to dryness and dissolved in 0.3 mL of ethyl acetate and 0.2 mL of pentafluoropropionic anhydride. After heating in a sealed reaction vessel for 10 min at 150 °C, the solvent and excess reagent were evaporated under a stream of nitrogen. The residue was dissolved in 1.0 mL of CH_2Cl_2 and was analyzed by gas chromatography on a Hewlett-Packard 5710A instrument equipped with a 50 m × 0.28 mm Chirasil-Val III capillary column. The column temperature was programmed from 90 to 200 °C at a rate of 4 °C/min using hydrogen as the carrier gas and FID detection. The retention times of the substituted phenylalanines derived from each of the separated diastereomeric peptides were compared and that with the longer retention time was taken to be the *S* enantiomer based on our experience.[20] In the case of **28**, this assignment was confirmed by direct comparison with compound **38**,[15] which was treated in the above fashion and employed as a reference standard.

4-(Bromomethyl)benzoic Acid 1,1-Dimethylethyl Ester (4a). *N*-Bromosuccinimide (NBS; 35.8 g, 0.20 mol) was added in several portions to a solution of 1,1-dimethylethyl 4-methylbenzoate (38.0 g, 0.20 mol) and benzoyl peroxide (0.36 g, 1.5 mmol) in carbon tetrachloride (1200 mL) at vigorous reflux. Another portion of benzoyl peroxide (0.36 g, 1.5 mmol) was added toward the final addition of NBS. After the foaming subsided, the reaction mixture was cooled to room temperature, washed with water (2 × 60 mL), and dried. Filtration and concentration provided an oil that was purified by HPLC eluting with a mixture of hexane and ethyl acetate to give 21.4 g (40%) of **4a** as a colorless oil contaminated with a small amount of dibrominated material as indicated by NMR. This material was used in the next step without further purification.

4-(Bromomethyl)benzeneacetic acid 1,1-dimethylethyl ester (4b) was prepared from 25.0 g (0.12 mol) of 4-methylbenzeneacetic acid 1,1-dimethylethyl ester by using the above procedure for **4a** to give 10.9 g (32%) of **4b**: ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 3.51 (s, 2 H), 4.47 (s, 2 H), 7.24 (d, 2 H, J = 8 Hz), 7.34 (d, 2 H, J = 8 Hz). Anal. $C_{13}H_{17}BrO_2$ (C, H, Br).

4-(Bromomethyl)-α,α-difluorobenzeneacetic acid 1,1-dimethylethyl ester (4c) was prepared from 8.0 g (0.03 mol) of **18** by using the above procedure for **4a** to give 7.7 g (73%) of **4c** as a colorless oil which was used directly in the next step.

5-[4-(Bromomethyl)phenyl]-2-(1,1-dimethylethyl)-2*H*-tetrazole (4d) was prepared from 18.0 g (0.08 mol) of **3d** by using the above procedure for **4a**, eluting with ethyl acetate–hexane to give 17.4 g (71%) of **4d** as a colorless oil contaminated with a small amount of dibrominated material. This material was used in the next step without further purification: ^1H NMR ($CDCl_3$) δ 1.79 (s, 9 H), 4.53 (s, 2 H), 7.50 (d, 2 H, J = 8 Hz), 8.14 (d, 2 H, J = 8 Hz).

(±)-4-[(1,1-Dimethylethoxy)carbonyl]-α-[(diphenylmethylene)amino]benzenepropanoic Acid Phenylmethyl Ester (5a). Following the general procedure of O'Donnell,[14] tetrabutyl ammonium sulfate (3.0 g, 9.0 mmol) was added to a biphasic mixture consisting of 10% aqueous sodium hydroxide (75 mL), benzyl diphenylmethyleneglycinate (3.05 g, 9.3 mmol), and **4a** (3.0 g, 9.0 mmol) in CH_2Cl_2 (58 mL). After 5 h of vigorous stirring at room temperature, the reaction mixture was diluted with ether (300 mL), and the layers were separated. The organic layer was washed with water (3 × 30 mL), dried (K_2CO_3), and filtered. Concentration provided an oil that was purified by preparative HPLC eluting with a mixture of hexane and ethyl acetate to give 1.7 g (36%) of **5a** as an oil. Anal. $C_{34}H_{33}NO_4$ (C, H, N).

(±)-4-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-α-[(diphenylmethylene)amino]benzenepropanoic acid phenylmethyl ester (5b) was prepared by using the above procedure for **5a**. Thus from 3.1 g (11 mmol) of **4b** there was obtained 2.9 g (50%) of **5b** as a thick oil: ^1H NMR (DMSO) δ 1.34 (s, 9 H), 3.04 (m, 1 H, β-C*H*H), 3.15 (m, 1 H, β-CH*H*), 4.10 (m, 1 H, α-CH), 5.15 (s, 2 H), 6.93 (d, 2 H, J = 8 Hz), 7.05 (d, 2 H, J = 8 Hz), 7.30 (m, 15 H). Anal. $C_{35}H_{35}NO_4$ (C, H, N).

4-[1,1-Difluoro-2-(1,1-dimethylethoxy)-2-oxoethyl]-α-[(diphenylmethylene)amino]benzenepropanoic Acid Phenylmethyl Ester (5c). Lithium diisopropylamide (8.8 mmol) was generated in THF (25 mL) at 0 °C and chilled to −78 °C. After a solution of benzyl (diphenylmethylene)glycinate (2.9 g, 8.8 mmol) in THF (5 mL) was added (15 min), HMPA (1.6 g, 8.8 mmol) was added dropwise, the mixture was stirred for 15 min and **4c** (2.7 g, 8.0 mmol) in THF (5 mL) was added slowly. The reaction mixture was warmed to room temperature over 2 h and was partitioned between saturated aqueous NH_4Cl (50 mL) and ether (30 mL). The aqueous layer was extracted with ether (2 × 30 mL), and the combined organic layers were washed with brine (20 mL) and dried. Filtration and concentration yielded an oil that was purified by flash chromatography on silica gel (pretreated with 10% triethylamine in hexane) eluting with hexane–ethyl acetate (9:1) to give 3.2 g (71%) of **5c** as a colorless oil. Anal. $C_{35}H_{33}F_2NO_4$ (H, N); C: calcd, 73.70; found, 73.25.

(±)-4-[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]-α-[(diphenylmethylene)amino]benzenepropanoic acid phenylmethyl ester (5d) was prepared by using the above procedure for **5a**. Thus from 16.0 g (54 mmol) of **4d** there was obtained 10.9 g (59%) of **5d** as a thick oil: ^1H NMR ($CDCl_3$) δ 1.79 (s, 9 H), 3.30 (m, 2 H), 4.32 (m, 1 H), 5.14 (d, 1 H, J = 12 Hz), 5.22 (d, 1 H, J = 12 Hz), 6.56 (s, 2 H), 7.11 (d, 2 H, J = 8 Hz), 7.30 (m, 11 H), 7.56 (d, 2 H, J = 8 Hz), 7.95 (d, 2 H, J = 8 Hz). Anal. $C_{34}H_{33}N_5O_2$ (C, H, N).

(±)-4-[[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]methyl]-α-[(diphenylmethylene)amino]benzenepropanoic Acid Phenylmethyl Ester (5e) was prepared by using the above procedure for **5a**. Thus from 2.0 g (6.5 mmol) of **4e** there was obtained 1.9 g (50%) of **5e** as a thick oil. Anal. $C_{35}H_{35}N_5O_2$ (C, H, N).

(±)-4-[(1,1-Dimethylethoxy)carbonyl]-α-aminobenzenepropanoic Acid Phenylmethyl Ester *p*-Toluenesulfonate (6a). A mixture of 11.5 g (22 mmol) of **5a** and *p*-toluenesulfonic acid monohydrate (4.18 g, 22 mmol) was stirred in a 10/1 mixture of acetonitrile and water (640 mL) for 2 h at room temperature. Concentration provided a solid which was recrystallized from ethanol–ether to give 8.1 g (67%) of **6a**, mp 154–157 °C. Anal. $C_{28}H_{33}NO_7S$ (C, H, N, S).

(±)-4-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-α-aminobenzenepropanoic acid phenylmethyl ester *p*-toluenesulfonate (**6b**) was prepared by using the above procedure for **6a**. Thus from 6.9 g (13 mmol) of **5b** there was obtained 6.1 g (87%) of **6b**: mp 139–141 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 2.28 (s, 3 H), 3.05 (m, 1 H), 3.19 (m, 1 H), 3.36 (s, 2 H), 4.28 (m, 1 H), 5.40 (d, 1 H, *J* = 12 Hz), 5.50 (d, 1 H, *J* = 12 Hz), 7.41 (d, 2 H, *J* = 8 Hz), 7.47 (d, 2 H, *J* = 8 Hz), 7.57 (m, 4 H), 7.74 (m, 4 H), 8.21 (d, 2 H, *J* = 8 Hz), 8.72 (s, br, 3 H). Anal. $C_{29}H_{35}NO_7S$ (C, H, N).

(±)-4-[1,1-Difluoro-2-(1,1-dimethylethoxy)-2-oxoethyl]-α-aminobenzenepropanoic acid phenylmethyl ester *p*-toluenesulfonate (**6c**) was prepared by using the above procedure for **6a**. Thus from 3.2 g (5.6 mmol) of **5c** there was obtained 2.3 g (70%) of **6c**: mp 145–149 °C. Anal. $C_{29}H_{33}F_2NO_7S$ (C, H, N, F).

(±)-4-[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]-α-aminobenzenepropanoic acid phenylmethyl ester *p*-toluenesulfonate (**6d**) was prepared by using the above procedure for **6a**. Thus from 8.0 g (15 mmol) of **5d** there was obtained 6.6 g (81%) of **6d**: mp 204–205 °C; ^{1}H NMR ($CDCl_3$ plus DMSO) δ 1.80 (s, 9 H), 2.33 (s, 3 H), 3.20 (m, 1 H), 3.37 (m, 1 H), 4.34 (m, 1 H), 5.00 (d, 1 H, *J* = 12 Hz), 5.06 (d, 1 H, *J* = 12 Hz), 7.10 (m, 9 H), 7.75 (d, 2 H, *J* = 8 Hz), 7.95 (d, 2 H, *J* = 8 Hz), 8.61 (s, br, 3 H). Anal. $C_{28}H_{33}N_5O_5S$ (C, H, N).

(±)-4-[[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]methyl]-α-aminobenzenepropanoic acid phenylmethyl ester *p*-toluenesulfonate (**6e**) was prepared by using the above procedure for **6a**. Thus from 4.4 g (7.9 mmol) of **5e** there was obtained 3.0 g (67%) of **6e**: mp 151–154 °C. Anal. $C_{29}H_{35}N_5O_5S\cdot0.5H_2O$ (C, H, N, H_2O).

(±)-α-(Acetylamino)-4-[(1,1-dimethylethoxy)carbonyl]-benzenepropanoic Acid Phenylmethyl Ester (**7a**). Acetic anhydride (1.0 mL, 11 mmol) and triethylamine (2.4 g, 24 mmol) were added sequentially to a solution of 5.0 g (9.0 mmol) of **6a** in CH_2Cl_2 (100 mL) at 0 °C. After 2 h, the reaction mixture was diluted with CH_2Cl_2 (250 mL), washed with 1 N HCl (2 × 20 mL) and saturated aqueous sodium bicarbonate (2 × 20 mL), and dried (Na_2SO_4). Filtration and concentration provided the crude product which was purified by HPLC eluting with a mixture of hexane and ethyl acetate to give 3.3 g (91%) of **7a**. Anal. $C_{23}H_{27}NO_5$ (C, H, N).

(±)-α-(Acetylamino)-4-[2-(1,1-dimethylethoxy)-2-oxoethyl]benzenepropanoic acid phenylmethyl ester (**7b**) was prepared by using the above procedure for **7a**. Thus from 2.0 g (3.4 mmol) of **7b** there was obtained after recrystallization from ethyl acetate–hexane 1.2 g (80%) of **7b**: mp 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 1.92 (s, 3 H), 3.09 (m, 2 H), 3.48 (s, 2 H), 4.90 (m, 1 H), 5.08 (m, 2 H), 6.94 (d, 2 H, *J* = 8 Hz), 7.06 (d, 2 H, *J* = 8 Hz), 7.15 (m, 5 H). Anal. $C_{24}H_{29}NO_5$ (H, N) C: calcd, 70.05; found, 69.62.

(±)-α-(Acetylamino)-4-[1,1-difluoro-2-(1,1-dimethylethoxy)-2-oxoethyl]benzenepropanoic acid phenylmethyl ester (**7c**) was prepared by using the above procedure for **7a**. Thus from 4.3 g (7.4 mmol) of **6c** there was obtained after crystallization from ethyl acetate–hexane 2.0 g (63%) of **7c**: mp 113–114.5 °C. Anal. $C_{24}H_{27}F_2NO_5$ (C, H, N).

(±)-α-(Acetylamino)-4-[2-(1,1-dimethylethyl)-2*H*-tetrazol-5-yl]benzenepropanoic acid phenylmethyl ester (**7d**) was prepared by using the above procedure for **7a**. Thus from 2.0 g (3.8 mmol) of **6d** there was obtained 1.40 g (87%) of **7d** as a glassy solid: ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 9 H), 1.99 (s, 3 H), 3.16 (m, 2 H), 4.96 (m, 1 H), 5.08 (d, 1 H, *J* = 12 Hz), 5.18 (d, 1 H, *J* = 12 Hz), 5.89 (d, 1 H, *J* = 7 Hz, NH), 7.07 (d, 2 H, *J* = 8 Hz), 7.50 (m, 5 H), 7.99 (d, 2 H, *J* = 8 Hz). Anal. $C_{23}H_{27}N_5O_3$ (C, H, N).

(±)-α-(Acetylamino)-4-[[2-(1,1-dimethylethyl)-2*H*-tetrazol-5-yl]methyl]benzeneacetic acid phenylmethyl ester (**7e**) was prepared by using the above procedure for **7a**. Thus from 4.3 g (7.4 mmol) of **6e** there was obtained, after recrystallization from ethyl acetate–hexane, 2.2 g (69%) of **7e**, mp 113–114.5 °C.

(±)-α-(Acetylamino)-4-[(1,1-dimethylethoxy)carbonyl]-benzenepropanoic Acid (**8a**). A solution of 3.3 g (8.0 mmol) of **7a** in 100 mL of ethanol was hydrogenated over 320 mg of 10% Pd/C at room temperature. Upon the consumption of 1 equiv of hydrogen, the mixture was filtered through Celite. The Celite pad was washed with ethanol (50 mL) and the filtrate was concentrated. The resultant solid was recrystallized from ethyl acetate–hexane to give 2.3 g (89%) of **8a**, mp 187–188 °C. Anal. $C_{16}H_{21}NO_5$ (C, H, N).

(±)-α-(Acetylamino)-4-[2-(1,1-dimethylethoxy)-2-oxoethyl]benzenepropanoic acid (**8b**) was prepared by using the above procedure for **8a**. Thus from 2.6 g (6.5 mmol) of **7b** there was obtained 1.9 g (91%) of **8b**, mp 170–172 °C, after recrystallization from ethyl acetate–hexane: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 1.97 (s, 3 H), 3.08 (m, 1 H, β-C*H*H), 3.17 (m, 1 H, β-CH*H*), 3.47 (s, 2 H), 4.80 (m, 1 H), 6.23 (s, br, 1 H), 7.14 (m, 4 H). Anal. $C_{17}H_{23}NO_5$ (C, H, N).

(±)-α-(Acetylamino)-4-[1,1-difluoro-2-(1,1-dimethylethoxy)-2-oxoethyl]benzenepropanoic acid (**8c**) was prepared by using the above procedure for **8a**. Thus from 1.1 g (2.5 mmol) of **7c** there was obtained 0.60 g (69%) of **8c**, mp 149–150.5 °C, after recrystallization from acetonitrile–water. Anal. $C_{17}H_{21}F_2NO_5$ (C, H, F, N).

(±)-α-(Acetylamino)-4-[2-(1,1-dimethylethyl)-2*H*-tetrazol-5-yl]benzenepropanoic acid (**8d**) was prepared by using the above procedure for **8a**. Thus from 3.2 g (7.6 mmol) of **7d** there was obtained 2.2 g (86%) of **8d**, mp 206–207 °C, after recrystallization from ethyl acetate–hexane: ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 9 H), 1.96 (s, 3 H), 3.15 (m, 1 H, β-C*H*H), 3.17 (m, 1 H, β-CH*H*), 4.85 (m, 1 H), 6.29 (s, br, 1 H), 7.28 (d, 2 H, *J* = 8 Hz), 8.04 (d, 2 H, *J* = 8 Hz). Anal. $C_{16}H_{21}N_5O_3$ (C, H, N).

(±)-α-(Acetylamino)-4-[[2-(1,1-dimethylethyl)-2*H*-tetrazol-5-yl]methyl]benzeneacetic acid (**8e**) was prepared by using the above procedure for **8a**. Thus from 2.0 g (4.6 mmol) of **7e** there was obtained 1.2 g (76%) of **8e**, mp 193–194.5 °C, after recrystallization from acetonitrile. Anal. $C_{17}H_{23}N_5O_3$ (C, H, N).

(±)-*N*-Acetyl-3-hydroxyphenylalanine Phenylmethyl Ester (**10**). A 500-mL round-bottom flask fitted with a Dean–Stark water separator was charged with a suspension of 10.0 g (55 mmol) of (±)-3-hydroxyphenylalanine (**9**) and 11.5 g (66 mmol) of *p*-TsOH·H_2O in 60 mL of benzyl alcohol and 250 mL of toluene. The resulting mixture was heated to reflux for 4 h as about 2 mL of water was collected in the trap. The mixture was allowed to cool, was diluted with ether, and was extracted repeatedly with 1 N HCl. The combined extracts were neutralized with excess solid sodium bicarbonate to precipitate 8.72 g of 3-hydroxyphenylalanine phenylmethyl ester, which was used directly in the next step.

A suspension of 8.72 g (32 mmol) of 3-hydroxyphenylalanine phenylmethyl ester in 400 mL of ice-cold dichloromethane was treated dropwise with 1.9 mL (20 mmol) of acetic anhydride. Upon completion of the addition, a solution of 3.70 g of sodium carbonate in 30 mL of water was added simultaneously with an additional 1.9 mL of acetic anhydride. After 1 h, the layers were separated, and the organic layer was washed with water. The combined aqueous layers were extracted with dichloromethane, and the combined extracts were washed with brine and dried. Filtration and concentration afforded a residue which was purified by preparative HPLC, eluting with 20% ethyl acetate–hexane to give 8.49 g (84%) of **10** as a thick oil: ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 3.05 (m, 2 H), 4.91 (m, 1 H), 5.11 (d, 1 H, *J* = 12 Hz), 5.19 (d, 1 H, *J* = 12 Hz), 6.03 (d, 1 H, *J* = 7.5 Hz), 6.22 (s, 1 H), 6.43 (s, 1 H), 6.51 (d, 1 H, *J* = 7 Hz), 6.71 (d, 1 H, *J* = 7 Hz), 7.07 (dd, 1 H, *J* = 7, 7 Hz), 7.33 (m, 5 H). Anal. $C_{18}H_{19}NO_4\cdot0.18H_2O$ (C, H, N, H_2O).

(±)-*N*-Acetyl-3-[[(trifluoromethyl)sulfonyl]oxy]phenylalanine Phenylmethyl Ester (**11**). A solution of 8.30 g (26 mmol) of **10** and 10.0 g (28 mmol) of phenylbis[(trifluoromethyl)sulfonyl]amine in 110 mL of freshly distilled dichloromethane was cooled in an ice bath and 4.10 mL (29 mmol) of triethylamine was added dropwise. The resulting mixture was stirred for 1 h at 0 °C and was allowed to warm to room temperature over 2 h. The mixture was diluted with 250 mL of ethyl acetate, was washed with successive 50 mL portions of water, 1 N NaOH, 1 N HCl, water, and brine, and dried. Filtration and concentration afforded an oil which was purified by preparative HPLC, eluting with 40% ethyl acetate–hexane to give 10.02 g (85%) of **11** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3 H), 3.12 (dd, 1 H, *J* = 11, 5 Hz), 3.21 (dd, 1 H, *J* = 11, 5 Hz), 4.93 (m, 1 H), 5.13 (d, 1 H, *J* = 12 Hz), 5.19 (d, 1 H, *J* = 12 Hz), 5.94 (d, 1 H, *J* = 7 Hz), 6.97 (m, 2 H), 7.12 (dd, 1 H, *J* = 7, 1.6 Hz), 7.25 (s, 1 H), 7.31 (m, 2 H), 7.38 (m, 3 H). Anal. $C_{19}H_{18}F_3NO_6S$

(C, H, N, F, S).

(±)-*N*-Acetyl-3-(2-propenyl)phenylalanine Phenylmethyl Ester (12). Argon was passed through a solution of 9.89 g (22 mmol) of **11**, 2.8 g (66 mmol) of lithium chloride, and 7.00 mL (22.5 mmol) of allyltributyltin in 50 mL of dimethylformamide for 10 min and 0.20 g (0.28 mmol) of bis(triphenylphosphine)-palladium dichloride was added. The bath temperature was raised to 95–100 °C for 2 h at which time a black precipitate was observed to form. The mixture was cooled, diluted with 250 mL of ether, washed with 3 × 50 mL of water and 1 × 50 mL of brine, and dried ($MgSO_4$). Filtration and concentration afforded an oil which was purified by preparative HPLC, eluting with 33% ethyl acetate–hexane to give 6.70 g (85%) of **12** as a white solid, mp 55–56.5 °C. A portion was recrystallized from ether–hexane to give mp 57–58 °C: ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 3.10 (m, 2 H), 3.30 (d, 2 H, J = 6 Hz), 4.92 (m, 1 H), 5.04 (m, 2 H), 5.14 (s, 2 H), 5.90 (m, 2 H, CH=, NH), 6.85 (m, 2 H), 7.05 (d, 1 H, J = 7 Hz), 7.15 (dd, 1 H, J = 7, 7 Hz), 7.31 (m, 2 H), 7.36 (m, 3 H). Anal. $C_{21}H_{23}NO_3$ (C, H, N).

(±)-*N*-Acetyl-3-(carboxymethyl)phenylalanine Phenylmethyl Ester (13). Solutions of 6.25 g (18.5 mmol) of **12** in 120 mL each of acetonitrile and carbon tetrachloride and 11.9 g (55.5 mmol) of sodium metaperiodate in 240 mL of water were combined and stirred mechanically as 0.25 g (1.3 mmol) of ruthenium chloride hydrate was added to the mixture. The mixture darkened immediately and, after 1 h, was diluted with 300 mL of dichloromethane. The layers were separated, and the organic layer was washed with 100 mL of water. The combined aqueous layers were extracted with 200 mL of ether and the combined extracts were dried, filtered, and concentrated.

The residue was dissolved in 180 mL of *tert*-butyl alcohol and 60 mL of 2-methyl-2-butene and a solution of 19.0 g (210 mmol) of sodium chlorite and 19.0 g (137 mmol) of monobasic sodium phosphate in 130 mL of water was added all at once. The mixture was stirred at 0 °C for 2 h and diluted with ether, and the layers were separated. The organic layer was washed with 10% sodium thiosulfate and brine and was dried. Filtration and concentration gave a oily residue which was chromatographed over 200 g of silica gel eluting with 60:40:1 ethyl acetate–hexane–acetic acid to give 4.64 g of a white solid, mp 129–130 °C. Recrystallization from dichloromethane–hexane gave 4.14 g (63%) of **13**: mp 130–131 °C; ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 3.10 (m, 2 H), 3.51 (s, 2 H), 4.87 (m, 1 H), 5.13 (m, 2 H), 6.65 (d, 1 H, J = 8 Hz), 6.93 (d, 1 H, J = 7 Hz), 7.02 (s, 1 H), 7.18 (m, 2 H), 7.30 (m, 2 H), 7.37 (m, 3 H). Anal. $C_{20}H_{21}NO_5$ (H, N) C: calcd, 67.59; found, 66.97.

(±)-*N*-Acetyl-3-[2-(1,1-dimethylethoxy)-2-oxoethyl]-phenylalanine Phenylmethyl Ester (14). A suspension of 4.00 g (11.3 mmol) of **13** in 40 mL of toluene and 15 mL of dimethylformamide di-*tert*-butyl acetal was heated to a bath temperature of 55 °C. After 30 min, a clear solution formed, and after 3 h, the mixture was cooled, diluted with 50 mL of ether, washed with 3 × 25 mL of water and 1 × 25 mL of brine, and dried. Filtration and concentration gave a white solid which was recrystallized from ether–hexane to afford 3.90 g (84%) of **14**: mp 80–83 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 1.99 (s, 3 H), 3.10 (s, 1 H), 3.12 (s, 1 H), 3.44 (s, 2 H), 4.92 (m, 1 H), 5.13 (d, 1 H, J = 12 Hz), 5.17 (d, 1 H, J = 12 Hz), 5.91 (d, 1 H, J = 7 Hz), 6.86 (d, 1 H, J = 7 Hz), 6.96 (s, 1 H), 7.15 (m, 2 H), 7.35 (m, 5 H). Anal. $C_{24}H_{29}NO_5$ (C, H, N).

(±)-α-(Acetylamino)-3-[2-(1,1-dimethylethoxy)-2-oxoethyl]benzenepropanoic Acid (15). A suspension of 3.30 g (8.02 mmol) of **14** in 50 mL of ethanol was hydrogenated over 200 mg of 10% palladium on carbon for 3 h at room temperature under 1 atm of hydrogen. Hydrogen uptake amounted to 220 mL, at which time the mixture was filtered and concentrated to afford 2.54 g of a white solid, mp 167–170 °C dec. Recrystallization from ethanol–hexane afforded 1.77 g (69%) of **15**: mp 169–172 °C dec; ^{1}H NMR (DMSO) δ 1.39 (s, 9 H), 1.77 (s, 3 H), 2.82 (m, 2 H), 3.00 (d, 1 H, J = 5 Hz), 3.03 (d, 1 H, J = 5 Hz), 4.36 (m, 1 H), 7.10 (m, 3 H), 7.22 (m, 1 H), 8.19 (d, 1 H, J = 8 Hz, NH). Anal. $C_{17}H_{23}NO_5$ (C, H, N).

α,α-Difluoro-4-methylbenzeneacetic Acid Ethyl Ester (17). A mixture of 19.1 g (110 mmol) of 4-methyl-α-oxobenzeneacetic acid ethyl ester and 25.0 mL (330 mmol) of (dimethylamido)sulfur trifluoride (DAST) was stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. The excess reagent was quenched by the careful addition of 1 L of ice and water and the product was extracted with hexane (2 × 300 mL). The organic extracts were washed with water (2 × 100 mL) and were dried. Concentration and purification of the residue by HPLC eluting with 30:1 hexane–ethyl acetate afforded 20.0 g (85%) of **17** as a colorless oil. A portion was distilled for analysis: bp 79–80 °C (2.5 mm); ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3 H, J = 7 Hz), 2.38 (s, 3 H), 4.28 (q, 2 H, J = 7 Hz), 7.22 (d, 2 H, J = 8 Hz), 7.46 (d, 2 H, J = 8 Hz). Anal. $C_{11}H_{12}F_2O_2$ (C, H, F).

α,α-Difluoro-4-methylbenzeneacetic Acid 1,1-Dimethylethyl Ester (18). To a solution of 18.9 g (89 mmol) of **17** in 75 mL of acetonitrile was added 17.8 mL (89 mmol) of 5 N KOH. The mixture was stirred at room temperature for 1 h and was concentrated to dryness to give a white solid. This material was dissolved in 75 mL of water and was washed with ether (2 × 75). The aqueous layer was acidified with 1 N HCl (150 mL) and was extracted with ether (3 × 75 mL). The extracts were dried and concentrated to afford 13.9 g (84%) of α,α-difluoro-4-methylbenzeneacetic acid: mp 81–82 °C; ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3 H), 7.2 (d, 2 H, J = 8 Hz), 7.5 (d, 2 H, J = 8 Hz), 12.15 (s, 1 H).

To a solution of 12.8 g (69 mmol) of α,α-difluoro-4-methylbenzeneacetic acid in 120 mL of toluene containing 2 drops of DMF was added 7.0 mL (80 mmol) of oxalyl chloride. The resulting mixture was stirred for 20 h at room temperature and 66 mL of *tert*-butyl alcohol was added. After 24 h, the mixture was concentrated and the residue was distilled, bp 94–96 °C (3 mm). The distillate was crystallized from hexane to give 11.7 g (70%) of **18**, mp 44.5–46 °C. Anal. $C_{13}H_{16}F_2O_2$ (C, H, F).

5-(4-Methylphenyl)-2-(1,1-dimethylethyl)-2*H*-tetrazole (3d). A solution of 5-(4-methylphenyl)-2*H*-tetrazole (**19**;[26] 19.9 g, 120 mmol), *tert*-butyl alcohol (19.5 g, 260 mmol), and concentrated sulfuric acid (5.84 g, 60 mmol) in trifluoroacetic acid (122 mL) was stirred at room temperature (3 h) and diluted with ethyl acetate (250 mL). The mixture was washed sequentially with water (2 × 50 mL), 10% NaOH (until washings were basic), water (2 × 30 mL), and dried (Na_2SO_4). Concentration and purification of the resultant oil by preparative by HPLC eluting with 20:1 hexane–ethyl acetate gave 17.5 g (65%) of **3d** as a clear oil, bp 120–122 °C (0.3 mm). Anal. $C_{12}H_{16}N_4$ (C, H, N).

5-[(4-Bromophenyl)methyl]-2*H*-tetrazole (22). 4-Bromophenylacetonitrile (30.0 g, 150 mmol), sodium azide (10.9 g, 170 mmol), and ammonium chloride (8.9 g, 170 mmol) were heated in DMF (300 mL) at 90 °C for 2 days. The mixture was concentrated, water (200 mL) was added to the residue, and the mixture was basified with 1 M NaOH (170 mL) and washed with ether (2 × 100 mL). Acidification of the aqueous layer with 1 N HCl and collection of the precipitate by filtration afforded the crude product. Recrystallization from ethanol provided 17.2 g (44%) of **22**, mp 173–175 °C. Anal. $C_8H_7BrN_4$ (C, H, Br, N).

5-[(4-Bromophenyl)methyl]-2-(1,1-dimethylethyl)-2*H*-tetrazole (23). A mixture of **22** (18.5 g, 77 mmol) *tert*-butyl alcohol (11.4 g, 150 mmol), trifluoroacetic acid (76 mL, 1.0 mol), and concentrated sulfuric acid (3.8 g, 39 mmol) was stirred for 24 h and then partitioned between ethyl acetate (250 mL) and water (100 mL). The organic layer was washed with water (4 × 100 mL) and 1 M NaOH (2 × 100 mL) and dried (Na_2SO_4). Filtration and concentration produced a solid that was recrystallized from hexane to give 12.3 g (54%) of **23**: mp 69–70 °C; ^{1}H NMR ($CDCl_3$) δ 1.72 (s, 9 H), 4.31 (s, 2 H), 7.43 (d, 2 H, J = 8 Hz), 8.04 (d, 2 H, J = 8 Hz). Anal. $C_{12}H_{15}BrN_4Br$ (C, H, Br, N).

4-[[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]methyl]benzoic Acid (24). To a deoxygenated mixture of **23** (12.0 g, 41 mmol), triphenylphosphine (11.0 g, 42 mmol), tributylamine (8.4 g, 45 mmol), *tert*-butyl alcohol (70 mL), and water (45 mL) in a stainless steel autoclave was added bis(triphenylphosphinepalladium) dichloride (1.0 g, 1.3 mmol). The mixture was heated under carbon monoxide (200 psi) at 100 °C for 2 days. The reaction mixture was diluted with dichloromethane (500 mL), washed with water (3 × 100 mL), and dried. The crude product obtained after concentration was purified by flash chromatography on silica gel eluting with hexane–ethyl acetate (4:1) to remove the less polar

(26) Finnegan, W. G.; Henry, R. A.; Lofquist, R. *J. Am. Chem. Soc.* **1958**, *80*, 3908.

impurities and 5% acetic acid in hexane–ethyl acetate (4:1) to elute 4.2 g (49%) of **24**: mp 139–140 °C; ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 9 H), 4.31 (s, 2 H), 7.40 (d, 2 H, J = 8 Hz), 8.03 (d, 2 H, J = 8 Hz). Anal. $C_{13}H_{16}N_4O_2$ (C, H, N).

4-[[2-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]methyl]benzenemethanol (25). Diisobutylaluminum hydride (48 mL of a 0.8 M solution in toluene, 38 mmol) was added dropwise to a solution of **24** (2.5 g, 9.6 mmol) in toluene (250 mL) at 0 °C, and the mixture was allowed to warm to room temperature overnight. The reaction mixture was cooled to 0 °C and was quenched by careful sequential addition of a mixture of water and THF (8 mL, 5:3), 10% NaOH (5 mL), and a final portion of water (15 mL). After the addition of ether (200 mL) and stirring at room temperature (2 h) the white precipitate was removed by filtration and the filtrate was concentrated. The residue was purified by HPLC on silica gel eluting with hexane–ethyl acetate (7:3) to give 1.6 g (70%) of **25**: mp 65–66 °C; ^{1}H NMR ($CDCl_3$) δ 1.62 (t, 1 H, J = 7 Hz), 1.73 (s, 9 H), 4.26 (s, 2 H), 4.69 (d, 2 H, J = 7 Hz), 7.36 (m, 4 H). Anal. $C_{13}H_{18}N_4O$ (C, H, N).

5-[[4-(Bromomethyl)phenyl]methyl]-2-(1,1-dimethylethyl)-2*H*-tetrazole (4e). Triphenylphosphine (12.9 g, 49 mmol) was added to a solution of **25** (6.0 g, 24 mmol) and carbon tetrabromide (16.5 g, 50 mmol) in diethyl ether (100 mL). After 3 h the reaction mixture was concentrated and the residue purified by preparative HPLC eluting with hexane–ethyl acetate (9:1) to give 4.1 g (54%) of 4e: mp 72–74 °C; ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 9 H), 4.20 (s, 2 H), 4.45 (s, 2 H), 7.36 (m, 4 H).

Method A. Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-NH_2-PAM-Resin. Six grams of Boc-Phe-PAM-resin (substitution 0.36 mmol/g) was suspended and shaken in 50 mL of TFA–CH_2Cl_2 (1:1) 3 × 10 min at room temperature to remove the Boc group. The product was isolated by filtration and washed with CH_2Cl_2 (3 × 50 mL), 8% diisopropylethylamine in CH_2Cl_2 and CH_2Cl_2 to give the free base of Phe-PAM-resin. This was subjected to sequential solid-phase synthesis by using the Fmoc protocol as follows:

step	reagent	time, (repetitions × min)
1	20% piperidine/DMF	1 × 5
2	20% piperidine/DMF	1 × 5
3	DMF	2 × 1
4	CH_2Cl_2	2 × 1
5	2-propanol	2 × 1
6	CH_2Cl_2/DMF	2 × 1
7	3 equiv Fmoc-AA, DCC, HOBt	1 × 60
8	CH_2Cl_2	2 × 1
9	DMF	2 × 1
10–12	repeat steps 7–9 if Kaiser test is positive	
13	CH_2Cl_2	2 × 1
14	DMF	2 × 1
15	2-propanol	2 × 1
16	DMF	2 × 1

At step 7 the appropriate Fmoc-amino acid (6.0 mmol), DCC (1.24 g, 6.0 mmol), and HOBt (1.2 g, 9.0 mmol) were dissolved in 50 mL of 1:1 by volume DMF–CH_2Cl_2 and allowed to couple for 60 min at room temperature. The amino acids were employed in the following order: Fmoc-Asp(O-*t*-Bu)-OH (2.46 g, 6.0 mmol), Fmoc-Met-OH (2.2 g, 6.0 mmol), Fmoc-Trp-OH (2.6 g, 6.0 mmol), Fmoc-Gly-OH (1.8 g, 6.0 mmol), Fmoc-Met-OH (2.2 g, 6.0 mmol). At this point the resin-bound peptide was dried under high vacuum to provide 7.98 g of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin.

Ac-Phe(4-CO_2H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (26) and Ac-(*R*)-Phe(4-CO_2H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (27). Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin (1.76 g, 0.47 mmol) was deprotected with 20% piperidine/DMF (method A, steps 1–6) and coupled to **8a** (700 mg, 1.5 mmol) with DCC (310 mg, 1.5 mmol) and HOBt (270 mg, 2 mmol) in 50 mL of DMF/CH_2Cl_2 (1:1) by volume for 60 min at room temperature. Washing (method A, steps 8–16) and drying gave Ac-(D,L)Phe-(4-CO_2-*t*-Bu)-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-NH_2-PAM-resin. The resin-bound peptide was shaken in 1:1 TFA/CH_2Cl_2 with 1% ethanedithiol (2 × 50 mL) for 10 min each time at room temperature to remove the O-*t*-butyl groups. The peptidyl-resin was then isolated by filtration, washed (3 × 50 mL each) with CH_2Cl_2, DMF, and methanol, and placed in a pressure bottle. It was suspended in 60 mL of methanol, which was cooled to −40 °C, saturated with NH_3, and sealed. The suspension was stirred at room temperature for 3 days. After cooling and venting the excess NH_3, the PAM-resin was filtered and washed with methanol. The combined filtrates were evaporated to dryness to yield 541 mg of crude peptide. Of this material, 80 mg was purified by preparative HPLC and the main peak, which was eluted at 35% modifier, was collected and lyophilized to yield 23 mg (33%) of Ac-(D,L)-Phe(4-CO_2H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, which was homogeneous by HPLC (≥99%): amino acid analysis Asp, 1.00 (1); Gly, 0.98 (1); Met, 2.00 (2); Phe, 1.00 (1); Trp, 0.76 (1); (D,L)Phe(4-CO_2H) not detected; empirical formula $C_{48}H_{59}N_9O_{12}S_2$; MW 1018.18;FAB MS m/z 1018 (M + H).

A 4-mg sample of the material obtained above was separated into two diastereomers as described in the general procedure using a gradient of 10–40% B over 120 min at 5 mL/min. Two peaks were detected eluting at 78 and 84 min. Fractions containing these peaks were each pooled and lyophilized to give 1.5 mg each of a white powder. Analysis by GC on a Chirasil-Val III capillary column as described in the general procedure shows that the compound eluting at a retention time of 78 min is **27** and at 84 min is **26**.

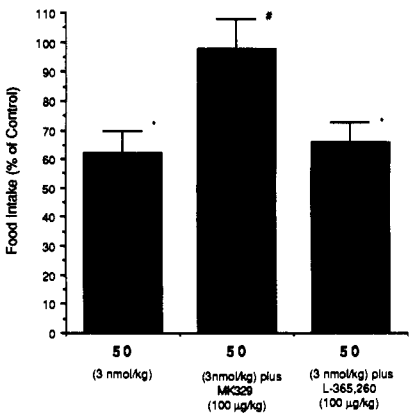


Figure 2. Effects of the selective CCK-A antagonist MK 329 (100 μg/kg) and the CCK-B antagonist L-365,260 (100 μg/kg) on suppression of food intake induced by **50** (3 nmol/kg). Data are mean ± SEM expressed as a percent of the mean intake of control-treated rats. Antagonists and **50** were combined and injected in the same syringe. Food was returned immediately after the injection and measured 1 h later. *p < 0.05 control vs drug treatment, by t test analysis. #p < 0.05, **50** plus MK 329 vs **50** alone or **50** plus L-365,260.

Ac-Phe(4-CH_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (28) and Ac-(*R*)-Phe(4-CH_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (29). Following the procedure used for **26**, a 1.00 g (0.27 mmol) sample of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin was deprotected and coupled to **8b** (280 mg, 0.80 mmol) with the DCC (165 mg, 0.8 mmol) and HOBt (200 mg, 1.5 mmol) in 40 mL of DMF–CH_2Cl_2 (1:1). Deprotection and ammonolysis afforded 392 mg of crude peptide of which an 80-mg sample was purified by HPLC. The main peak, which was eluted at 39% modifier, was collected and lyophilized to yield 13 mg (24%) of Ac-(D,L)-Phe(4-CH_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 which was homogeneous by analytical HPLC: amino acid analysis Asp, 1.02 (1); Gly, 1.00 (1); Met, 1.96 (2); Phe, 1.00 (1); Trp, 0.75 (1); Phe(4-CH_2COOH), 1.00 (1); empirical formula $C_{49}H_{61}N_9O_{12}S_2$; MW 1032.20; FAB MS m/z 1032 (M + H).

A 2-mg sample was separated into two diastereomers as described in the general procedure. The column was equilibrated with 2% B, a gradient of 2–20% B was applied over 5 min, and the column was eluted for 120 min in the isocratic mode at 8 mL/min. Two peaks were detected eluting at 40 and 46 min. Fractions containing these peaks were each pooled and lyophilized to give 0.80 mg of white powder. Analysis by GC on a Chirasil-Val III capillary column as described in the general procedure using **38** as a reference standard shows that the compound eluting at a retention time of 40 min is **29** and at 46 min is **28**.

Ac-Phe(4-CH_2CH_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (30). Following the procedure used for **26**, a 1.76 g (0.47 mmol) portion of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin was deprotected and coupled to **39**[15] (550 mg, 1.5 mmol) with DCC (310 mg, 1.5 mmol) and HOBt (270 mg, 2.0 mmol) dissolved in 50 mL of DMF/CH_2Cl_2 (1:1), allowed to react for 60 min at room temperature and was washed (method A, steps 8–16). Drying afforded Boc-Phe(4-$CH_2CH_2CO_2$-*t*-Bu)-Met-Gly-Trp-Met-Asp-(O-*t*-Bu)-Phe-PAM-resin which was suspended and shaken in 50% TFA–CH_2Cl_2 containing 1% ethanedithiol (2 × 50 mL; 2 × 10 min) at room temperature to remove the Boc and O-*t*-Bu groups. The peptidyl-resin was isolated by filtration, washed (3 × 50 mL each) with CH_2Cl_2, DMF, and methanol, suspended in DMF–CH_2Cl_2 (1:1), and acetylated with 3 equiv each of acetic acid and [(benzotriazol-1-yl)oxy]tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent in the presence of diisopropylethylamine. The acetylated peptidyl-resin was subjected to ammonolysis and lyophilization to yield 831 mg of crude peptide of which a 100-mg sample was purified by HPLC and the main peak was eluted at 36% modifier was collected and lyophilized to give 17 mg (29%) of **30** which was homogeneous by analytical HPLC: amino acid analysis Asp, 1.00 (1); Gly, 1.01 (1); Met, 1.95 (2); Phe, 1.00 (1); Trp, 0.70 (1); Phe(4-CH_2CH_2COOH), 0.97 (1); empirical formula $C_{50}H_{63}N_9O_{12}S_2$; MW 1046.22; FAB MS *m/z* 1046 (M + H).

Ac-Phe(4-CF_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (31) and Ac-(*R*)-Phe(4-CF_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (32). Following the procedure used for **26**, a 1.00 g (0.27 mmol) sample of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin was deprotected and coupled to **8c** (310 mg, 0.80 mmol) with DCC (165 mg, 0.80 mmol) and HOBt (200 mg, 1.5 mmol) in 40 mL DMF–CH_2Cl_2 (1:1). Deprotection and ammonolysis afforded 388 mg of crude peptide of which a 70-mg sample was purified by HPLC. The main peak which was eluted at 30% modifier was collected and lyophilized to yield 14 mg (27%) of Ac-(D,L)-Phe(4-CF_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 which was homogeneous by analytical HPLC. Amino acid analysis: Asp, 1.00 (1); Gly, 1.00 (1); Met, 2.04 (2); Phe, 1.00 (1); Trp, 0.70 (1); Phe(4-CF_2COOH) not detected; empirical formula: $C_{49}H_{59}F_2N_9O_{12}S_2$; MW 1068.20; FAB MS *m/z* 1068 (M + H).

A 2-mg sample was separated into two diastereomers as described in the general procedure. The column was equilibrated with 2% B, a gradient of 2–25% B was applied over 5 min and a gradient of 25–30% B was applied for 120 min at 8 mL/min. Two peaks were detected eluting at 52 and 56 min. Fractions containing these peaks were each pooled and lyophilized to give 0.8 mg of white powder. Analysis by GC on a Chirasil-Val III capillary column as described in the general procedure shows that the compound eluting at a retention time of 52 min is **32** and at 56 min is **31**.

Ac-(*R*,*S*)-Phe(3-CH_2COOH)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (33). Following the procedure used for **26**, a 1.00 g (0.27 mmol) sample of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin was deprotected and coupled to **15** (280 mg, 0.80 mmol) with DCC (165 mg, 0.80 mmol) and HOBt (200 mg, 1.5 mmol) in 40 mL DMF–CH_2Cl_2 (1:1). Deprotection and ammonolysis afforded 456 mg of crude peptide, of which an 80-mg sample was purified by HPLC. The main peak, which was eluted at 41% modifier, was collected and lyophilized to yield 14 mg (25%) of **33**, which was homogeneous by analytical HPLC: amino acid analysis Asp, 1.00 (1); Gly, 0.80 (1); Met, 2.08 (2); Phe, 1.00 (1); Trp, 0.70 (1); Phe(3-CH_2COOH), 1.00 (1); empirical formula $C_{49}H_{61}N_9O_{12}S_2$; MW 1032.20; FAB MS *m/z* 1032 (M + H).

Method B. Ac-Phe(4-tetrazol-5-yl)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (34) and Ac-(*R*)-Phe(4-tetrazol-5-yl)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (35). Boc-Phe (2.6 g, 10 mmol) and HOBt (2.0 g, 15 mmol) were dissolved in 40 mL of DMF–CH_2Cl_2, the solution was chilled to 0 °C, and with stirring (2.06 g, 10 mmol) DCC was added and the mixture was stirred for 60 min at 0 °C. Separately 10 g of benzhydrylamine copolystyrene 1% divinylbenzene crossresin (BHA; 0.56 mmol NH_2/g) was washed with 10% diisopropylethylamine in CH_2Cl_2 for 30 min, filtered, and washed with CH_2Cl_2, DMF, and CH_2Cl_2. The chilled mixture was added to the resin and stirred for 24 h at room temperature. The resin was filtered and washed repeatedly with CH_2Cl_2, DMF, *i*PrOH, and finally with CH_2Cl_2 and was dried under high vacuum. Amino acid analysis showed the resin to contain 0.32 mmol of Phe/g of resin. Unreacted amino groups were capped by shaking the resin with 5 mL of acetic anhydride and 5 mL diisopropylethylamine in CH_2Cl_2 for 60 min. The resin was filtered and washed with CH_2Cl_2, DMF, *i*PrOH, and finally with CH_2Cl_2.

A 1.5 g (0.48 mmol) portion of the Boc-Phe-BHA-resin was subjected to sequential solid-phase synthesis by using the following protocol:

step	reagent	time
1	1% EDT/CH_2Cl_2	1 × 30 s
2	50% TFA/CH_2Cl_2/(w) 1% EDT	1 × 1 min
3	repeat step 1	
4	50% TFA/CH_2Cl_2/(w) 1% EDT	1 × 15 min
5	CH_2Cl_2	1 × 30 s
6	methanol	1 × 30 s
7, 8	repeat steps 5 and 6	
9	CH_2Cl_2	2 × 30 s
10	8% DIPEA	2 × 2 min
11–15	repeat steps 5–9	
16	3 equiv Boc-AA, DCC, HOBt	1 × 60 min
17	1% DIPEA	1 × 30 min
18, 19	repeat steps 6 and 9	
20, 21	repeat steps 16 and 17 if Kaiser test is positive	
22	methanol	1 × 30 s
23, 24	repeat steps 5 and 6	
25	CH_2Cl_2	1 × 30 s
26	methanol	2 × 30 s
27	CH_2Cl_2	3 × 30 s

At step 16 the appropriate Boc-amino acid (1.5 mmol), DCC (310 mg, 1.5 mmol), and HOBt (270 mg, 2.0 mmol) were dissolved in 20 mL of 1:1 by volume DMF–CH_2Cl_2, and allowed to couple for 60 min at room temperature. The amino acids were employed in the following order: Boc-Asp(O-*Bzl*)-OH (485 mg, 1.5 mmol), Boc-Met-OH (380 mg, 1.5 mmol), Boc-Trp(For)-OH (500 mg, 1.5 mmol), Boc-Gly-OH (270 mg, 1.5 mmol), Boc-Met-OH (380 mg, 1.5 mmol), **8d** (500 mg, 1.5 mmol). At this point the resin-bound peptide was dried under high vacuum to provide 2.25 g of Boc-(D,L)-4-Phe(4-tetrazol-5-yl)-Met-Gly-Trp(For)-Met-Asp-(OBzl)-Phe-BHA. Cleavage was achieved by treatment with 5 mL of HF containing 2.0 mL of anisole, 1.0 mL of ethanedithiol, and 15 mL of dimethyl sulfide for 1 h at 0 °C. After evaporation to a small volume, fresh anhydrous HF (20 mL) was distilled into the reaction vessel for a second treatment for 2 h at 0 °C. After thorough evaporation, the resin was washed with 2 volumes of EtOAc, triturated with 4 × 15 mL of 30% acetic acid, filtered, and lyophilized to yield 415 mg of crude peptide.

A 100-mg portion of the crude peptide was purified by HPLC. The main peak, which was eluted at 38% modifier, was collected and lyophilized to yield 8 mg (6.6%) of (D,L)-4-Phe(4-tetrazol-5-yl)-Met-Gly-Trp-Met-Asp-Phe-NH_2 which was homogeneous by HPLC: amino acid analysis Asp, 0.98 (1); Gly, 1.00 (1); Met, 1.92 (2); Phe, 1.00 (1); (D,L)-Phe(4-tetrazol-5-yl), 0.96 (1); Trp, not detected, empirical formula $C_{48}H_{59}N_{13}O_{10}S_2$; MW 1042.19; FAB MS *m/z* 1042 (M + H).

A 2-mg sample was separated into two diastereomers as described in the general procedure. The column was eluted with a gradient of 1–20% B over 5 min then 20–30% B over 120 min at 8 mL/min. Two peaks were detected eluting at 35 min and 39 min. Fractions containing these peaks were each pooled and lyophilized to give 0.8 mg of white powder. Analysis by GC on a Chirasil-Val III capillary column as described in the general procedure shows that the compound eluting at a retention time of 35 min is **35** and at 39 min is **34**.

Ac-Phe[4-(tetrazol-5-yl)methyl]-Met-Gly-Trp-Met-Asp-Phe-NH_2 (36) and Ac-(*R*)-Phe[4-(tetrazol-5-yl)methyl]-Met-Gly-Trp-Met-Asp-Phe-NH_2 (37). Following method B, a 1.50 g (0.48 mmol) sample of Boc-Met-Gly-Trp(For)-Met-Asp-(OBzl)-Phe-BHA-resin was deprotected (method B, steps 1–15) and coupled to **8e** (525 mg, 1.5 mmol) with DCC (310 mg, 1.5 mmol) and HOBt (270 mg, 2.0 mmol) in 50 mL DMF–CH_2Cl_2 (1:1). Deprotection and HF cleavage afforded 456 mg of crude peptide of which an 80 mg sample was purified by HPLC. The main peak, which was eluted at 33% modifier, was collected and lyophilized to yield 18 mg (20%) of Ac-(D,L)-Phe[4-tetrazol-5-

yl)methyl]-Met-Gly-Trp-Met-Asp-Phe-NH_2 which was homogeneous by analytical HPLC: amino acid analysis Asp, 1.00 (1); Gly, 1.06 (1); Met, 1.80 (2); Phe, 1.00 (1); Trp, 0.70 (1); Phe(4-tetrazol-5-yl)methyl], 0.95 (1); empirical formula $C_{49}H_{61}N_{13}O_{10}S_2$; MW 1056.30; FAB MS m/z 1056 (M + H).

A 2-mg sample was separated into two diastereomers as described in the general procedure. The column was eluted with a gradient of 1–20% B over 5 min then 20–30% B over 120 min at 8 mL/min. Two peaks were detected eluting at 51 and 55 min. Fractions containing these peaks were each pooled and lyophilized to give 1.0 mg of white powder. Analysis by GC on a Chirasil-Val III capillary column as described in the general procedure shows that the compound eluting at a retention time of 51 min is **37** and at 55 min is **38**.

4-Bromobenzeneacetic Acid Phenylmethyl Ester (40). A solution of 4-bromobenzeneacetic acid (50 g, 0.23 mol), benzyl alcohol (26 mL, 0.25 mol), and *p*-toluenesulfonic acid (0.3 g) was heated in toluene (200 mL) at reflux with the azeotropic removal of water (15 h). The yellow solution was washed with water (250 mL) and saturated aqueous sodium bicarbonate (250 mL) and dried. Concentration provided a light yellow solid that was recrystallized from hexane to give 48.5 g (69%) of **40**: mp 47.5–48 °C; ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 2 H), 5.14 (s, 2 H), 7.16 (d, 2 H, J = 8 Hz), 7.37 (m, 5 H), 7.45 (d, 2 H, J = 8 Hz). Anal. $C_{15}H_{13}BrO_2$ (C, H, Br).

(*E*)-4-[3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl]benzeneacetic Acid Phenylmethyl Ester (41). A solution of 33.5 g (110 mmol) of **40**, *tert*-butyl acrylate (31.6 mL, 220 mmol), and triethylamine (85 mL, 640 mmol) in DMF (1000 mL) was deoxygenated with argon, whereupon bis(triphenylphosphine)-palladium dichloride (5.1 g, 8.3 mmol) was added. The mixture was heated to a bath temperature of 75 °C for 12 h and was concentrated. The resulting brown residue was purified by flash chromatography eluting with hexane–ethyl acetate (9:1) to give 23.3 g (60%) of **41** as a light yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 9 H), 3.68 (s, 2 H), 5.13 (s, 2 H), 6.35 (d, 2 H, J = 16 Hz), 7.30 (m, 7 H), 7.46 (d, 2 H, J = 8 Hz), 7.56 (d, 1 H, J = 16 Hz). Anal. $C_{22}H_{24}O_4$ (C, H).

4-[3-(1,1-Dimethylethoxy)-3-oxopropyl]benzeneacetic Acid (42). A mixture of 11.6 g (33.0 mmol) of **41** and 0.50 g of 10% Pd/C in 120 mL of ethanol was stirred under a H_2 atmosphere for 3 h. Filtration of the mixture through a pad of Celite and concentration gave a solid that was recrystallized from ethyl acetate–hexane to give 7.5 g (86%) of **42**: mp 55.5–56.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 2.52 (t, 2 H, J = 7.5 Hz), 2.88 (t, 2 H, J = 7.5 Hz), 3.60 (s, 2 H), 7.14 (d, 2 H, J = 8 Hz), 7.18 (d, 2 H, J = 8 Hz). Anal. $C_{15}H_{20}O_4$ (C, H).

4-[3-(1,1-Dimethylethoxy)-3-oxopropyl]benzeneacetic Acid Phenylmethyl Ester (43). To a stirred suspension of 5.3 g (20.0 mmol) of **42** and 2.8 g (20.0 mmol) of K_2CO_3 in 30 mL of DMF was added 3.42 g (20 mmol) of benzyl bromide. After stirring for 24 h, the mixture was concentrated and the residue was extracted with hexane (100 mL). Filtration and concentration gave the product (6.5 g, 92%) **43** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 2.52 (d, 2 H, J = 7.5 Hz), 2.85 (t, 2 H, J = 7.5 Hz), 3.61 (s, 2 H), 5.12 (s, 2 H), 7.15 (d, 2 H, J = 8 Hz), 7.18 (d, 2 H, J = 8 Hz), 7.30 (m, 5 H). Anal. $C_{22}H_{26}O_4$ (C, H).

4-[2-(Phenylmethyl)-2-oxoethyl]benzenepropanoic Acid (44). A solution of 3.0 g (8.5 mmol) of **43** in 10 mL of formic acid was stirred for 8 h. Concentration provided a colorless oil that was shaken vigorously with a mixture of ethyl acetate and hexane (1:3, 20 mL) until a solid formed. The mixture was cooled in ice water and the product was collected to give 2.2 g (87%) of **44** as a white solid: mp 62–64.5 °C; ^{1}H NMR (DMSO) δ 2.53 (t, 2 H, J = 7 Hz), 2.81 (t, 2 H, J = 7 Hz), 3.68 (s, 2 H), 5.11 (s, 2 H), 7.18 (s, 4 H), 7.33 (m, 5 H). Anal. $C_{18}H_{18}O_4$ (C, H).

5-(4-Bromophenyl)-2*H*-tetrazole (45). A solution of 4-bromobenzonitrile (20 g, 110 mmol), sodium azide (7.9 g, 120 mmol), and NH_4Cl (6.5 g, 120 mmol) in DMF (340 mL) was heated at 90 °C under argon. After 2 days the reaction mixture was concentrated and diluted with water (300 mL). After adding enough 1 M NaOH to render the mixture basic to litmus paper, it was washed with ether (4 × 25 mL). The aqueous layer was acidified to pH 3 with 1 N HCl; the precipitated product was collected by filtration and washed with water. The crude product was recrystallized from ethanol to give 19.2 g (76%) of **45**, mp 271–273 °C. Anal. $C_7H_5BrN_4$ (C, H, N).

5-(4-Bromophenyl)-2-(1,1-dimethylethyl)-2*H*-tetrazole (46). A solution of 19.0 g (82 mmol) of **45**, *tert*-butyl alcohol (12.1 g, 164 mmol), trifluoroacetic acid (80 mL), and concentrated sulfuric acid (4.6 g, 41 mmol) was stirred at ambient temperature for 24 h. The mixture was concentrated and dissolved in ethyl acetate (200 mL). After washing with water (3 × 25 mL), 1 M NaOH (3 × 25 mL), and brine (25 mL), the organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting oil was purified by preparative HPLC eluting with hexane–ethyl acetate (9:1) to provide 17.1 g (71%) of **46** as a yellow oil. Anal. $C_{11}H_{13}BrN_4$ (C, H, N).

5-[4-[3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl]phenyl]-2-(1,1-dimethylethyl)-2*H*-tetrazole (47). A solution of 3.0 g (10 mmol) of **46**, *tert*-butyl acrylate (2.9 mL, 20 mmol), and triethylamine (7.9 mL, 59 mmol) in DMF (93 mL) was deoxygenated with argon, whereupon bis(triphenylphosphine)palladium dichloride (0.50 g, 0.80 mmol) was added. After heating at 75 °C for 12 h, the mixture was concentrated and the brown residue was filtered through a short column of silica gel eluting with hexane–ethyl acetate (9:1). The eluent was concentrated and then purified by preparative HPLC eluting with hexane–ethyl acetate (9:1). The fractions containing product were pooled and concentrated, and the residue was recrystallized from hexane to give 1.6 g (55%) of **47**, mp 118.5–120 °C. Anal. $C_{18}H_{24}N_4O_2$ (C, H, N).

4-[5-(1,1-Dimethylethyl)-2*H*-tetrazol-5-yl]benzenepropanoic Acid (48). A solution of 7.5 g (23 mmol) of **47** in 100 mL of ethanol containing 0.50 g of 10% Pd/C was stirred under a blanket of hydrogen (1 atm). After hydrogen uptake ceased, the mixture was filtered through a pad of Celite washing with 30 mL of ethanol. The filtrate was concentrated to give 7.2 g of a colorless oil. This material was stirred for 1 h in a solution of 70 mL of TFA and 70 mL of CH_2Cl_2 and was concentrated. The residue was diluted with 100 mL of water, made basic with enough saturated $NaHCO_3$ to raise the pH to 8, and washed with EtO_2 (2 × 50 mL). The aqueous layer was acidified to pH 3 with 1 N HCl and extracted with EtOAc (2 × 50 mL). The extracts were washed with water (2 × 25 mL) and brine (1 × 25 mL) and were dried. Concentration afforded 4.8 g (76%) of **48**, mp 88–90 °C. Anal. $C_{14}H_{18}N_4O_2$ (C, H, N).

Desamino-Tyr(SO$_3$H)-Met-Gly-Trp-Met-Asp-Phe-NH$_2$ (49). Following method B, a 1.0 g (0.34 mmol) portion of Boc-Met-Gly-Trp(For)-Met-Asp(OBzl)-Phe-BHA-resin was deprotected with TFA–CH_2Cl_2 (1:1) by volume (method B, steps 1–15) and was coupled to 1-[3-(4-hydroxyphenyl)-1-oxopropoxy]-2,5-pyrrolidinedione (396 mg, 1.5 mmol) dissolved in 10 mL of DMF–CH_2Cl_2 (1:1) and allowed to react for 18 h at room temperature. Washing (method B, steps 17–27) and drying gave 1.17 g of desamino-Tyr-Met-Gly-Trp(For)-Met-Asp(OBzl)-Phe-BHA-resin. The resin was cleaved by treatment with HF according to method B to yield 232 mg of crude peptide, a 100-mg sample of which was purified by preparative HPLC. The main peak, which eluted at 38% modifier, was collected and lyophilized to yield 13 mg (9%) of desamino-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2, which was homogeneous by HPLC: amino acid analysis Asp, 1.00 (1); Gly, 1.00 (1); Met, 1.80 (2); Phe, 1.00 (1); Trp, not detected; empirical formula $C_{45}H_{56}N_8O_{10}S_2$; MW 933.12; FAB MS m/z 933 (M + H).

To a suspension of 12 mg of the above peptide in 2 mL of dry pyridine was added 165 mg (0.50 mmol) of acetylpyridinium sulfate. The reaction mixture was stirred for 5 h at room temperature, diluted with 5 mL of 1.5 M NH_4OH, and lyophilized. Purification was achieved by preparative HPLC using a gradient of 15–45% of 0.01 M NH_4OAc–CH_3CN over 60 min at a flow rate of 8 mL/min. The main peak which was eluted at 22% modifier was collected and lyophylized to yield 7 mg (54%) of **49** as the ammonium salt: IR (KBr) 3400 (br, NH), 1660 (br, C=O), 1520 (amide II), 1200 and 1092 (sulfate) cm^{-1}.

3-[4-(Carboxymethyl)phenyl]propanoyl-Met-Gly-Trp-Met-Asp-Phe-NH$_2$ (50). To a solution of 1.10 g (3.68 mmol) of **44** and 0.51 g (4.43 mmol) of *N*-hydroxysuccinimide in 50 mL of CH_2Cl_2 added 3.86 mL of a 1 M solution of DCC in CH_2Cl_2. After 22 h, 1.0 mL of acetic acid was added and the mixture was stirred 1 h. The resulting dicyclohexylurea was removed by filtration, the filtrate was concentrated, the residue was dissolved in 40 mL

of DMF and filtered. The filtrate was diluted to 100 mL with DMF and was hydrogenated over 1.0 g of 10% Pd/C on a Parr apparatus at 50 psi H_2 for 1.5 h. The mixture was filtered and the filtrate diluted to 140 mL with DMF to give a stock solution of 1-[3-[(4-carboxymethyl)phenyl]-1-oxopropoxy]-2,5-pyrrolidinedione.

Following the procedure used for **26**, a 1.00 g (0.27 mmol) sample of Fmoc-Met-Gly-Trp-Met-Asp(O-*t*-Bu)-Phe-PAM-resin was deprotected and reacted with 30 mL (0.80 mmol) of the above active ester solution in 20 mL DMF/CH_2Cl_2 (1:1) for 18 h at room temperature. Deprotection and ammonolysis afforded 733 mg of crude peptide of which an 80 mg sample was purified by HPLC. The main peak, which was eluted at 39% modifier, was collected and lyophilized to yield 8 mg (25%) of **50**, which was homogeneous by analytical HPLC: amino acid analysis Asp, 1.00 (1); Gly, 1.00 (1); Met, 2.00 (2); Phe, 1.00 (1); Trp, 0.71 (1); empirical formula $C_{47}H_{58}N_9O_{11}S_2$; MW 975.20; FAB MS *m/z* 975 (M + H).

3-(4-Tetrazol-5-ylphenyl)propanoyl-Met-Gly-Trp-Met-Asp-Phe-NH_2 (51). Following method B, a 1.4 g (0.48 mmol) portion of Boc-Met-Gly-Trp(For)-Met-Asp(OBzl)-Phe-BHA-resin was deprotected with TFA–CH_2Cl_2 (1:1) by volume (method B, steps 1–15) and was coupled to **48** (475 mg, 1.5 mmol) in 50 mL of DMF–CH_2Cl_2 (1:1) for 60 min at room temperature. Washing (method B, steps 17–27) and drying gave 1.52 g of 3-[4-[2-(1,1-dimethylethyl)tetrazol-5-yl]phenyl]propanoyl-Met-Gly-Trp-(For)-Met-Asp(OBzl)-Phe-BHA-resin. The resin was cleaved by treatment with HF according to method B to yield 340 mg of crude peptide, a 100-mg sample of which was purified by preparative HPLC. The main peak, which eluted at 38% modifier was collected and lyophilized to yield 15 mg (11%) of **51**, which was homogeneous by HPLC: amino acid analysis Asp, 1.07 (1); Gly, 0.97 (1); Met, 2.00 (2); Phe, 1.10 (1); Trp, not detected; empirical formula $C_{46}H_{56}N_{12}O_9S_2$; MW 985.16; FAB MS *m/z* 985 (M + H).

In Vitro Receptor Binding Assay. Frozen bovine striatum (ca. 5 g) or fresh rat pancreas (ca. 5 g) cleaned of fat and extraneous tissue was homogenized in HEPES buffer 1 (10 mM HEPES + 130 mM NaCl + 5 mM $MgCl_2$, pH 7.4) with 35 parts buffer per 1 part tissue on a wet weight/volume basis (ca. 175 mL). The tissue was homogenized two times for ca. 15 s at 0 °C with a Polytron homogenizer at a setting of 6. The tissue was isolated by centrifugation at 48000*g* for 10 min at 0 °C. The resulting tissue pellet was resuspended in HEPES buffer 2 [10 mM HEPES + 130 mM NaCl + 5 mM $MgCl_2$ + 1 mg/L phenylmethanesulfonyl fluoride (PMSF) + 200 mg/L bacitracin] with 1 part striatal tissue (original wet weight) per 80 parts buffer or 1 part pancreas tissue (original wet weight) per 500 to 1000 parts buffer. Incubation was initiated by combining various concentrations of peptide with ([^{3}H]propionyl)-CCK-8 purchased from Amersham (final concentration = 0.15 nM) and tissue homogenate (striatum ca. 0.26 mg of protein in 2 mL final volume; pancreas approximately 0.100 mg of protein in 1 mL final volume). Samples were incubated for 30 min at 25 °C, and the incubation was terminated by pouring the mixture onto a prewetted Whatman GF/B filter on a Sandbeck vacuum filtration manifold. The incubation tubes were washed with 2 × 3 mL of ice-cold HEPES buffer 2 and the wash was filtered through the GF/B filter. The filter was air-dried for 10 min and then placed in a scintillation vial with 12 mL of Du Pont/NEN Aquasol scintillation cocktail. The vials were shaken overnight and then counted with a liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 1 μM native CCK-8 and subtracted from all samples to determine specific binding. The concentration of peptide necessary to inhibit 50% of total specific [^{3}H]CCK-8-(SO_3H) binding (IC_{50} value) was determined by log–probit analysis.

Two-Meal Feeding Assay. Male Sprague–Dawley (CD) rats weighing 180–200 g (Charles River Breeding Laboratories) were acclimated to a 12-h light/dark cycle (6 a.m. to 6 p.m.) in a room kept at 22 °C. They were subsequently fasted for 24 h, weighed, placed in individual cages, and a four-day period of meal training was begun. During this time the rats were given ground laboratory chow (Purina Lab Chow) in jars for 1 h from 9:00 a.m. until 10:00 a.m., the jars were removed from 10:00 a.m. to 12:00 p.m., and placed back in the cages from 12:00 until 1:00 p.m. Under this "1-2-1" meal feeding regime, most rats learn to eat approximately as much per day during the 2 h they have access to food as rats which have food ad libitum over the entire 24-h day. On the fourth day, the rats were weighed again, and any which lost more than 5 g of body weight were excluded from the test. The animals were then distributed into experimental (n = 5 or 6) and control groups (n = 6–12), but not matched for body weight. Peptides either in saline or in 0.5% DMSO/saline, if insoluble, at concentrations of 0–320 μg/mL per kg of body weight were administered intraperitoneally 15 min before the first meal on day 5 of meal feeding. The rats were then given their meals as they had been during the previous four days, and the food cups were weighed both before and after each meal to determine food consumption. Food intake was expressed as a mean and standard error of the mean in percent of control values for the various groups. The treated groups were compared to the control groups by t test analysis.

Overnight Food Deprivation Assay. Male Sprague–Dawley rats weighing an average of 260 g (Charles River Breeding Laboratories) were used. Animals were housed and tested in individual hanging wire-mesh cages in a temperature-controlled environment (22 °C) with a 12-h light/dark cyclic (lights on at 6:00 a.m.). A maintenance diet of ground Purina laboratory chow and tap water was provided ad libitium except where noted below. Rats were adapted to these conditions for a minimum of 1 week before feeding tests were conducted.

For feeding tests, rats were divided into groups of five to seven and matched for body weight. They were tested in the morning after a single 17-h overnight fast. Rats received a 1 mL/kg ip injection of vehicle (0.5% DMSO in saline) or peptide and preweighed food cups were returned immediately after the injection. Food cups were again weighed 1 h later. Food intake is expressed as a mean ± SEM percent of control values. Differences between drug- and vehicle-treated groups were determined by post hoc t tests. ED_{50}s were determined by log–probit analysis.

To evaluate the ability of the CCK-A antagonist MK-329 or the CCK-B antagonist L-365,260[9] to block the anorectic effect of compounds, a modified protocol was used. In these studies rats were given ip injections of either vehicle, peptide at the ED_{50} dose, or peptide plus 100 μg/kg of MK 329 or L-365,260, and food intake was measured over 1 h as above. These doses of MK 329 and L-365,260 have been shown to have no effect on food intake when given alone in this feeding paradigm. Data were analyzed as above.

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Registry No. **3a**, 13756-42-8; **3b**, 33155-60-1; **3d**, 127152-61-8; **4a**, 108052-76-2; **4b**, 77292-91-2; **4c**, 127152-63-0; **4e**, 127152-68-5; **5a**, 127153-49-5; **5b**, 127152-70-9; **5c**, 127152-73-2; **5d**, 127152-71-0; **5e**, 127152-72-1; **6a**, 127152-74-3; **6b**, 127152-78-7; **6c**, 127152-82-3; **6d**, 127152-80-1; **6e**, 127152-84-5; **7a**, 127152-86-7; **7b**, 127152-88-9; **7c**, 127152-90-3; **7d**, 127152-89-0; **7e**, 127152-91-4; **8a**, 127152-92-5; **8b**, 127152-94-7; **8c**, 127152-96-9; **8d**, 127152-95-8; **8e**, 127152-97-0; **9**, 775-06-4; **9** (benzyl ester), 127153-04-2; **10**, 127153-05-3; **11**, 127153-06-4; **12**, 127153-07-5; **13**, 127153-08-6; **14**, 127153-09-7; **15**, 127153-10-0; **16**, 5524-56-1; **17**, 131323-06-3; **18**, 131323-07-4; **19**, 24994-04-5; **21**, 16532-79-9; **22**, 127152-64-1; **23**, 127152-65-2; **24**, 127152-66-3; **25**, 127152-67-4; **26**, 127153-21-3; **27**, 127153-20-2; **28**, 127153-24-6; **29**, 127153-23-5; **30**, 127153-27-9; **31**, 131323-08-5; **32**, 131323-09-6; **33**, 127153-34-8; **34**, 127153-31-5; **35**, 127153-30-4; **36**, 127153-38-2; **37**, 127153-37-1; **39**, 123993-23-7; **40**, 127152-98-1; **41**, 127152-99-2; **42**, 127153-00-8; **43**, 127153-01-9; **44**, 127153-02-0; **45**, 50907-23-8; **46**, 127153-14-4; **47**, 131351-94-5; **48**, 127153-17-7; **49**, 72163-38-3; **50**, 127153-35-9; **51**, 127153-43-9; $Ph_2C{=}NCH_2COOCH_2Ph$, 81477-91-0; $Bu_3SnCH_2CH{=}CH_2$, 24850-33-7; $4\text{-}MeC_6H_4CF_2COOH$, 131323-10-9; *t*-BuOH, 75-65-0; $4\text{-}BrC_6H_4CH_2COOH$, 1878-68-8; $CH_2{=}CHCOOBu\text{-}t$, 1663-39-4; $PhCH_2Br$, 100-39-0; $4\text{-}BrC_6H_4CN$, 623-00-7; Fmoc-Asp(OBu-*t*)-OH, 71989-14-5; Fmoc-Met-OH, 71989-28-1; Fmoc-Trp-OH, 35737-15-6; Fmoc-Gly-OH, 29022-11-5; Boc-Phe-OH, 13734-34-4; Boc-Asp(OBzl)-OH, 7536-58-5; Boc-Met-OH, 2488-15-5; Boc-Trp(For)-OH, 47355-10-2; Boc-Gly-OH, 4530-20-5; 1-[3-(4-hydroxyphenyl)-1-oxopropoxy]-2,5-pyrrolidinedione, 34071-95-9.