Analogs of Ac-CCK-7 Incorporating Dipeptide Mimics in Place of Met[28]-Gly[29] [1]

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A series of analogs of Ac-CCK-7 [Ac-Tyr(SO_3H)-Met[28]-Gly[29]-Trp-Met-Asp-Phe-NH_2, (1)] were prepared in which the Met[28]-Gly[29] dipeptide was replaced by ω-aminoalkanoic acids. Compounds were assessed in binding assays using homogenated rat pancreatic membranes and bovine striatum as the source of CCK-A and CCK-B receptors, respectively, and for anorectic activity after intraperitoneal administration to rats. The analog incorporating 4-aminobutanoic acid (**5**) was only 8 times less potent than 1 in the pancreatic binding assay, was more potent in the striatal binding assay, and was more potent than 1 in reducing food intake in rats. Using a bioactive cyclic analog of Ac-CCK-7 as a template, several rigid spacers were designed and tested as substitutes for the Met[28]-Gly[29] dipeptide. The analogs incorporating 3-aminobenzoic acid (**20**) and (1*S*)-*trans*-2-aminocyclopentanecarboxylic acid (**26**) proved highly effective in the binding assays and as anorectic agents. We hypothesize that for stimulation of CCK-A receptors, the main function of the N-terminal tripeptide of Ac-CCK-7 is to orient the tyrosine sulfate with respect to Trp[30] and that the bioactive arrangement of these elements lies among those which are readily available to both **20** and **26**. NOESY and distance-constrained molecular dynamics experiments carried out on **20** and **26** identified conformations in which the relative orientation of the tyrosine hydroxide and the α-carbon atom of tryptophan were similar, providing the basis for further drug design efforts.

Cholecystokinin (CCK) represents a family of brain-gut peptides which are released from gut endocrine cells in response to nutrient ingestion[2] and participates in the regulation of digestive processes. Peripheral activities of CCK are mediated through CCK-A receptors[3] and include stimulation of enzyme secretion from pancreatic acinar cells,[4] inhibition of gastric emptying,[5] and stimulation of gall bladder contraction.[6] Both peripheral type (CCK-A) and brain (CCK-B) CCK receptors are found in the CNS and among other activities, may modulate dopaminergic and opiate-mediated neural transmission.[7-9] Exogenous administration of CCK decreases meal size in a number of species including rats[10] and humans,[11] and recent evidence suggests that this effect is mediated by CCK-A receptors.[12,13] As part of our overall program to develop orally active CCK mimetics for use as appetite suppressants, we require detailed knowledge of the role played by each structural element in CCK at its receptors.

Previous structure–activity work with CCK analogs indicates that the C-terminal tetrapeptide of Ac-CCK-7 (1) contains all of the functionality necessary for signal transduction at either CCK-A or CCK-B receptors;[14] however, the N-terminal tripeptide of 1 makes an important contribution to potency at peripheral (CCK-A type) receptors. Requirements for agonist activity at these receptors include an intact C-terminal carboxamide,[15-17] an aryl-, lower alkyl-, or cycloalkylalanine in position 33,[18-20] and tryptophan or a close analog in position 30.[21,22] Furthermore, a sulfated tyrosine or a suitable acidic isosteric replacement at the N-terminus markedly en-

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hances potency.[23-25] Application of this knowledge of necessary pharmacophores to the design of mimetics of CCK-7, demands an appreciation of their three-dimensional relationship. Since the methionine in position 28 can be substituted by norleucine[26] or a substituted lysine[27] and the Met-Gly amide bond has recently been shown to play little or no direct role in receptor binding,[28] it seems likely that the Met-Gly portion of Ac-CCK-7 serves as a spacer. In the present work, we set out to confirm this suspicion with the synthesis of the analogs shown in Table I in which this dipeptide was substituted by simple ω-aminoalkanoic acids and thence to further define the relationship between the N-terminal acidic group and the crucial Trp[30] moiety with the synthesis of analogs incorporating conformationally defined amino acids in place of Met-Gly. To guide this latter endeavor, we employed conformational models of the previously described cyclic derivative of Ac-CCK-7 **2** as templates.[27]

Ac-Tyr(SO_3H)^{27}-Met^{28}-Gly^{29}-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2

1

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

2

Chemistry

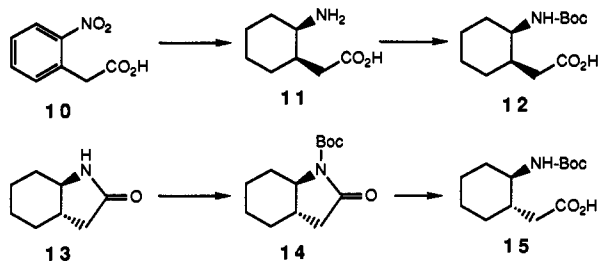
The unsulfated peptides were prepared by solid phase methodology utilizing either the Boc/Bzl strategy, employing BHA resin and HF cleavage[29] (method A) or the Boc/OFm strategy employing PAM resin followed by ammonolysis (method B). Coupling reactions were mediated by DIC/HOBt and monitored by the ninhydrin test.[30] The crude peptides were purified to homogeneity by preparative HPLC on a μ bondapack C-18 column in a 5–65% 0.022% TFA–acetonitrile system, and their purity was verified by analytical HPLC. They were characterized by FAB MS and amino acid analysis after acid hydrolysis.

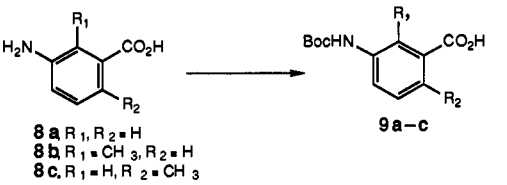
Sulfation to give the peptides shown in Tables I and II was accomplished using pyridinium acetyl sulfate.[31] The products were purified by preparative HPLC on a μ bondapack C-18 column in a 10–40% 0.01 M NH_4OAc–acetonitrile system, and their purity was verified by analytical HPLC. The IR of each gave the 1050 cm^{-1} peak characteristic of a tyrosine sulfate ester.[32]

The Boc-protected *m*-aminobenzoic acid derivatives **9a–c** were prepared from the corresponding amines by treatment with di-*tert*-butyl dicarbonate. As shown in Scheme I, catalytic hydrogenation of 2-nitrobenzeneacetic acid (**10**) over rhodium on carbon gave a 49% yield of the known *cis*-2-aminocyclohexaneacetic acid (**11**)[33] which was readily converted to its Boc derivative, **12**, for use in peptide synthesis. The corresponding trans isomer was prepared from the lactam **13**.[33] In order to avoid the use of forcing conditions, ring opening was accomplished by transformation to the imide **14** by treatment with sodium hydride and di-*tert*-butyl dicarbonate followed by hydrolysis with lithium hydroxide to give the Boc-protected amino acid **15** in good yield.

A quantity of ethyl (1*S*)-cis-2-hydroxycyclopentanecarboxylate (**16**), $[\alpha]^{25}_D$ −14.3° (*c* 1.0, $CHCl_3$), was available as a side product from the ruthenium BINAP-catalyzed reduction of 2-(ethoxycarbonyl)cyclopentanone[34,35] and was freed of contaminating *trans* isomer by silica gel chromatography. The corresponding (1*R*)-*cis* isomer had

Scheme I





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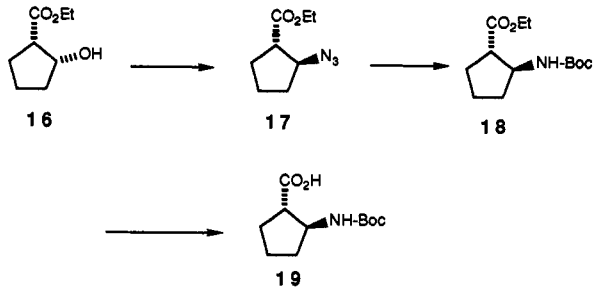
Scheme II



previously been prepared by the yeast-mediated reduction of 2-(ethoxycarbonyl)cyclopentanone and gave the opposite rotation, $[\alpha]^{25}_D$ +14.5°,[36] thus confirming our stereochemical assignment. Treatment with hydrazoic acid under Mitsunobu conditions[37] gave a 63% yield of the azide 17 which was hydrogenated over palladium on carbon in a Parr shaker in DMF in the presence of excess di-*tert*-butyl dicarbonate to give the Boc-protected (1*S*)-*trans*-amino ester 18 (Scheme II). Ester hydrolysis to the acid 19 was effected with lithium hydroxide.

Results and Discussion

Receptor binding activity for the CCK-A and CCK-B receptor subtypes was determined using solubilized membranes prepared from fresh pancreatic tissue obtained from fasted rats or bovine striatum, respectively, as previously described by Van Dijk[38] and detailed in the Experimental Section. 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 the peptides listed in Table I necessary to inhibit 50% of total specific[^{3}H]propanoyl-CCK-8 binding (IC_{50} value) was determined by log-probit analysis, and data for active compounds were confirmed by duplicate experiments. The IC_{50} for CCK-8 under these conditions was approximately 1 nM for both tissue preparations.

Test peptides were evaluated for their ability to suppress food intake in one of two meal-fed rat models. In the "1-2-1" protocol, 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 intraperitoneal injection 15 min before the first meal to groups of 5–6 rats, and the amount of food eaten during each meal was determined. Since the compounds prepared during the course of this work were not optimized to resist proteolysis, the simpler "overnight fasted protocol" was also employed. In this assay, groups of 5–7 rats were fasted overnight, administered vehicle or drug by ip injection, and presented with food cups for 1 hour. The average food consumed during the test period was compared with vehicle treated controls. In both models, the treated groups were compared to the control groups using the *t*-test. Data are expressed as percent of saline-treated control food intake during the first hour of the experiment and as the dose which caused 50% inhibition of the control intake (ED_{50}) as determined from log-probit analysis. The ED_{50} values for CCK-8 and several analogs at the 1-h time point were found to be similar using either protocol.[23]

Analysis of both the receptor binding and food intake suppression data for the Ac-CCK-7 analogs incorporating ω-aminoalkanoic acids in place of the Met-Gly dipeptide shown in Table I indicates that substitution by either glycine or β-alanine resulted in a loss of activity. However, substitution by γ-aminobutyric acid gave **5** which has only 8-fold lower affinity than Ac-CCK-7 in the rat pancreatic binding assay and is twice as potent in the striatal binding assay. Furthermore, it is equipotent as an anorectic agent. Substitution of longer ω-aminoalkanoic acids such as δ-aminovaleric acid or ϵ-aminohexanoic acid to give **6** and **7**, respectively, revealed a gradual decrease in potency with increasing chain length. The γ-aminobutyric acid portion of **5** possesses one less atom than the dipeptide which it replaces suggesting that the bioactive conformation of Ac-CCK-7 at peripheral receptors has a bend in this region consistent with previous conformational proposals.[39-42]

Having established that the Met-Gly dipeptide of Ac-CCK-7 could be replaced with a simple spacer lacking both the internal amide bond and side chain of the parent without loss of CCK-A receptor agonist activity, we were interested in determining the three-dimensional relationship between the tyrosine sulfate and the tryptophan moieties in the bioactive conformation. Previously, we have described the synthesis and characterization of the cyclic CCK-A receptor agonist **2** together with NMR and molecular dynamic studies leading to the proposal of six distinct conformational families, elements of which may approximate the bioactive conformation.[27] These families served as templates for the next step in the process.

In order for rigid amino acids to successfully serve as Met-Gly replacements in analogs of **1**, they would have to form amide bonds with tyrosine[27] and tryptophan[30] and should be able to accommodate not only the appropriate separation, but also the amide bond orientation found in the cyclic templates. As a means to rapidly test a variety of cyclic amino acids, *N*-methylacetamides of candidate spacers were built within the molecular modeling program SYBYL in order to simulate these amide bonds. To approximate the appropriate amide bond orientations, the distances D_1–D_4 shown in Figure 1a were measured for

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Table I. Biological Activity of Met[28]-Gly[29] Substituted Analogs of Ac-CCK-7

Ac-Tyr(SO_3H)-HN-**X**-CO-Trp-Met-Asp-Phe-NH_2

compd	X	CCK binding: IC_{50}, nM pancreas	CCK binding: IC_{50}, nM striatum	dose, μg/kg, ip	food intake, % of control	ED_{50}, μM/kg, ip
1	Met-Gly (Ac-CCK-7)	0.60	4.4	32	6 ± 1**	7.0
				10	36 ± 2**	
				3	81 ± 8	
3	$-(CH_2)-$	1200	1300	32	69 ± 2**	
4	$-(CH_2)_2-$	10000	3600	32	108 ± 9	
5	$-(CH_2)_3-$	4.9	1.8	320	1 ± 3**	
				32	11 ± 7**	
				20	6 ± 2**	4.7
				3	69 ± 5**	
				0.3	104 ± 5	
6	$-(CH_2)_4-$	22	9	320	9 ± 7**	32
				32	54 ± 10**	
				3	102 ± 5	
				0.3		
7	$-(CH_2)_5-$	360	13	320	80 ± 5	

*$p < 0.05$; **$p < 0.01$.

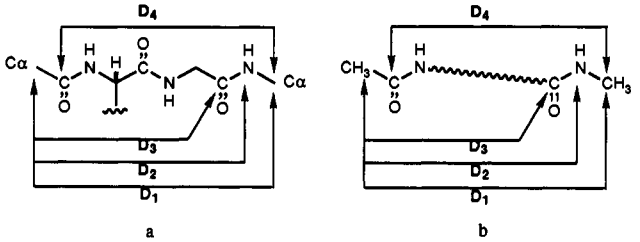

Figure 1. (a) Elements of the N-terminal Tyr-Lys-Gly-Trp of **2** between which intramolecular distances were determined. (b) Corresponding distance constraints employed in conformational search of *N*-acetyl-ω-aminocycloalkanoic acid *N*-methylamides intended to serve as potential Met-Gly replacements in Ac-CCK-7 analogs.

each of the cyclic conformational families and were employed as distance constraints (±0.1 Å) in conformational searches as illustrated in Figure 1b, in which each rotatable bond of the potential spacers was rotated in 5° increments while maintaining planar *trans*-amides. Conformers of molecules which emerged from this effort were inspected visually for reasonableness before annealing and minimization. Those conformers which still fit one or more of the cyclic templates after this process were considered for incorporation into CCK-7 analogs.

The compounds which were synthesized as part of this effort are shown in Table II. Use of one of the first molecules considered, *m*-aminobenzoic acid (**8a**), led to the preparation of **20** whose overall profile is similar to that of the lead compound **5**. In an effort to add further constraints, the *o*-methyl derivatives **8b** and **8c** were also incorporated, giving **21** and **22**, respectively. While **21** was nearly equipotent in the binding assays, it was 8-fold less potent in the feeding assay, and **22** was markedly less potent in all assays. In order to verify the specificity of the spacer geometry, the corresponding peptide **23**, derived from *p*-aminobenzoic, was synthesized. As expected, **23** was found to be markedly less potent than **20** in the pancreatic receptor binding assay and as an anorectic agent. Surprisingly, however, this compound was highly potent in the striatal receptor binding assay. While the C-terminal tetrapeptide CCK-4 is sufficient for potent binding to the CCK-B receptor subclass,[43] others have also observed marked effects on introduction of conformation constraint in the N-terminal portion of longer homologs,[44-46] suggesting that there are auxiliary binding sites for elements of the N-terminal portion of these homologs at CCK-B receptors.

The peptides **24** and **25** which incorporated racemic *cis*- and *trans*-2-aminocyclohexaneacetic acid, respectively, did not have interesting activity, but **26**, prepared from the 2-aminocyclopentanecarboxylic acid **19** was only 10-fold less potent than **5** in peripheral binding and meal feeding experiments. These results suggest that both **20** and **26** can attain the relationship between the N-terminal acidic group and the C-terminal tetrapeptide required for stimulus of CCK-A receptors and consequently, they were chosen for study by NMR on the premise that one of the conformations available to both should represent the bioactive conformation. Since available evidence suggests that the N-terminal tripeptide of CCK-7 serves mainly to orient the tyrosine sulfate group, we were particularly interested in employing **20** and **26** to determine plausible relationships between the tyrosine hydroxyl group and the α-carbon atom of Trp[30]. Such information could serve as a prelude to the design of CCK analogs in which an acidic moiety intended to mimic the tyrosine sulfate is incorporated into a tryptophan surrogate by a suitable linker.

The observed NOE-derived distances were used as constraints in an optimization procedure based on molecular dynamics and energy minimization using the CHARMM program package.[47] This procedure readily folds a peptide from its fully-extended conformation to an ensemble of 30 low-energy conformers which satisfy the experimental distance constraints as has been de-

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(46) Charpentier, B.; Pelaprat, D.; Durieux, C.; Dor, A.; Reibaud, M.; Blanchard, J.-C.; Roques, B. P. Cyclic cholecystokinin analogues with high selectivity for central receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1968–1972.

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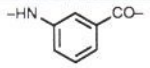
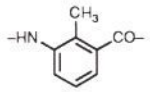
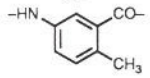
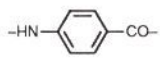
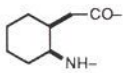
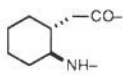
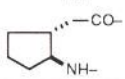
Table II. Biological Activity of Constrained Met[28]-Gly[29] Substituted Analogs of Ac-CCK-7

Ac-Tyr(SO_3H)-**X**-Trp-Met-Asp-Phe-NH_2

compd	X	CCK binding: IC_{50}, nM pancreas	CCK binding: IC_{50}, nM striatum	dose, μg/kg, ip	food intake % of control	ED_{50}, μM/kg, ip
20		2.1	0.48	320 32 3	4 ± 2** 14 ± 6** 52 ± 7*	3
21		2.7	0.91	320 32 14 3.2	5.9 ± 1.8** 44 ± 6.9** 58 ± 16 104 ± 14	24
22		73	5.0	320	95 ± 11	>320
23		1600	0.085	1240	75 ± 9	>1240
24		161	3.4	320	80 ± 9	>320
25		165	ND	320	90 ± 10	>320
26		25	0.029	320 32	9 ± 3.5** 58 ± 11*	40

[a] ND = not determined. *$p < 0.05$; **$p < 0.01$.

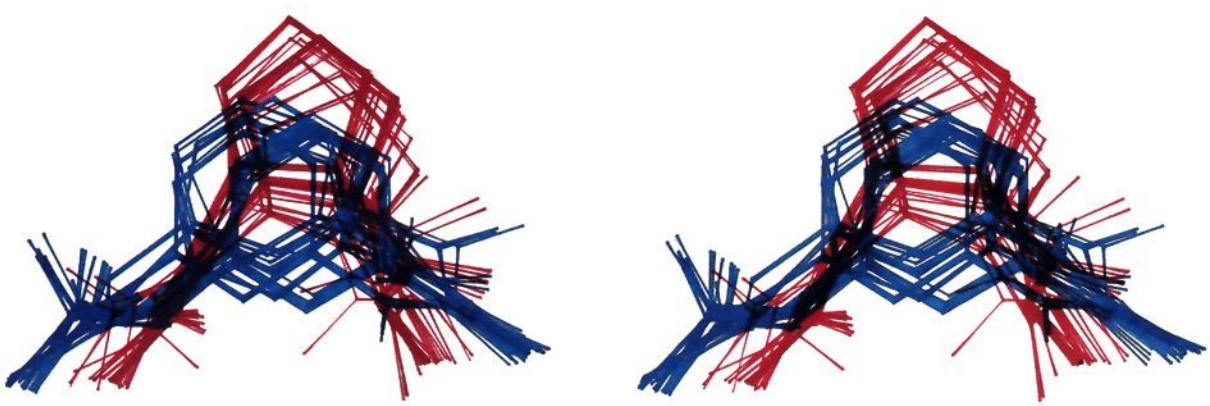


Figure 2. Stereoview of the backbones of the peptide spacers from tyrosine Cα to tryptophan Cα. Thirty optimized conformers for each peptide were alined by overlapping the Tyr Cα–C′ and Trp N–Cα bonds. *m*-Aminobenzoyl is shown in blue, cyclopentanoyl in red.

scribed.[48,49] Figure 2 depicts a stereoview of 30 conformers optimized to have low energy and fit NOE-derived interproton distances for the spacer moieties of **20** and **26**. The stereoviews in Figure 3 show the orientation of the tyrosine side chain for each of these 30 conformers. It is apparent from this view that the tyrosine side chain of the cyclopentyl derivative **26**, shown in red, adopts a limited range of conformations. The conformers of **20** for which the orientation of the tyrosine hydroxyl group are similar to observed conformers of **26** are shown in blue, while those for which there is no corresponding conformer of **26** are shown in green. We speculate that the bioactive conformation of the N-terminal portion of Ac-CCK-7 at CCK-A receptors is represented among this group of related conformers.

Figure 4 shows the observed orientations of the tryptophan β- and γ-carbon atoms for the 30 conformations relative to the rigid spacers and illustrates the comparative mobility of these atoms indicating that the present constraints do little to fix the relationship between the tyrosine sulfate and the tryptophan indole ring itself.

In conclusion, we hypothesize that for stimulation of CCK-A receptors, the main function of the N-terminal tripeptide of Ac-CCK-7 is to orient the tyrosine sulfate with respect to Trp[30] and that the bioactive arrangement of these elements lies among those which are readily available to both **20** and **26**. Using these conformations as templates, we are in the process of designing analogs of CCK-4 in which an acidic group capable of mimicking the tyrosine sulfate is linked directly to a tryptophan analog via a hydrocarbon chain.

Experimental Section

General. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. ^{1}H-NMR

(48) Williamson, M. P.; Madison, V. S. Three-Dimensional Structure of Procine C5adesArg from ^{1}H Nuclear Magnetic Resonance Data. *Biochemistry* **1990**, *29*, 2895–2905.

(49) Fry, D. C.; Madison, V. S.; Bolin, D. R.; Greeley, D. N.; Toome, V.; Wegrzynski, B. B. Solution Structure of an Analogue of Vasoactive Intestinal Peptide as Determined by Two-dimensional NMR and Circular Dichroism. *Biochemistry* **1989**, *28*, 2399–2409.

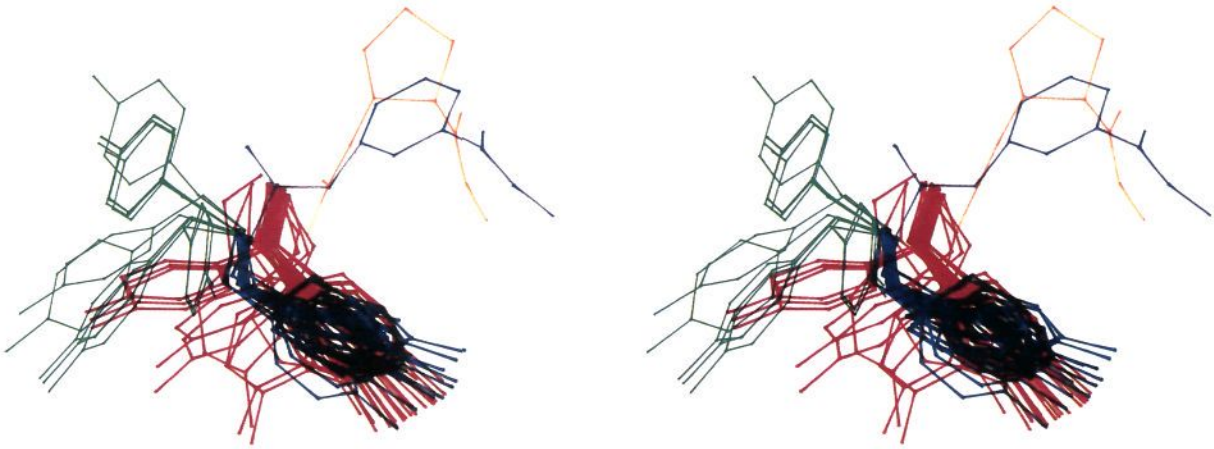


Figure 3. Stereoview of tyrosine ring conformers for 30 optimized structures for each peptide: cyclopentyl, red; matching *m*-aminobenzoyl, blue; nonmatching *m*-aminobenzoyl, green. The spacer backbones for the minimum-energy conformers are also shown: cyclopentanoyl, orange; *m*-aminobenzoyl, violet.

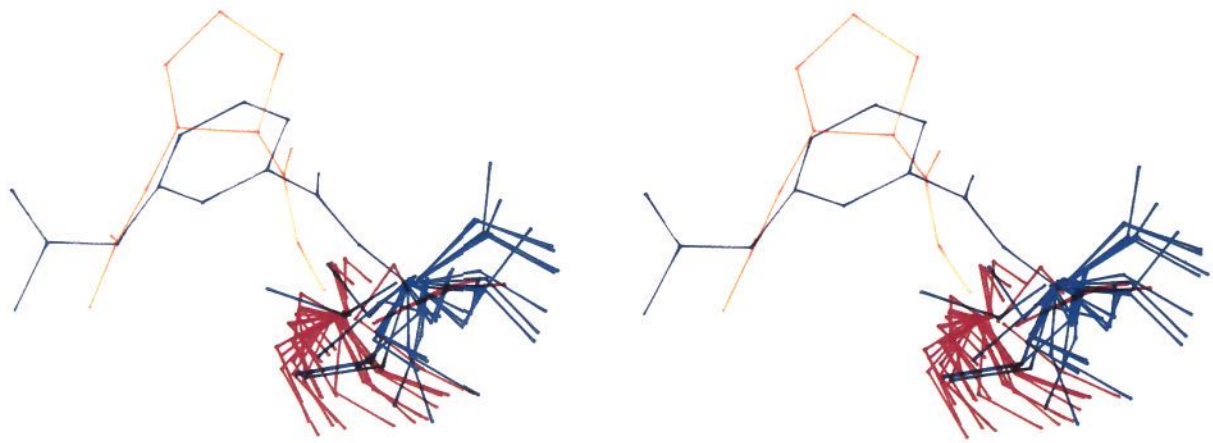


Figure 4. Stereoview for 30 optimized conformers of tryptophan Cα, Cβ, and Cγ: cyclopentanoyl, red; *m*-aminobenzoyl, blue. The spacer backbones for the minimum-energy conformers are also shown: cyclopentanoyl, orange; *m*-aminobenzoyl, violet.

spectra were recorded with Varian XL-200 and XL-400 spectrometers, using tetramethylsilane (TMS) as internal standard. IR spectra were recorded on a Beckman IR-9 or IR-12 spectrophotometer. Electron impact (EI, 70 ev) and fast atom bombardment (FAB) mass spectra were taken on VG ZAB-1F or VG 70E-HF mass spectrometers.

All solvents used in the preparation of peptides, e.g. methylene chloride (CH_2Cl_2), 2-propanol, DMF, and methanol, were Burdick and Jackson "distilled in glass" grade and used without additional distillation. TFA, DIEA, PIP, DIC, and HOBt, were purchased from Fluka Chemical Corp.; EDT was purchased from Sigma Chemical Co. and all were used without further purification. All protected amino acids were of the L-configuration unless otherwise indicated and were obtained from Bachem or prepared as described below. For amino acid analyses, peptides were hydrolyzed in 6 N HCl containing phenol at 115 °C for 24 h in evacuated Reacti-Therm hydrolysis tubes. Analyses were performed on a Beckman 121M amino acid analyzer. Preparative HPLC separations were run on a 2.3 × 30 cm μ bondapack C-18 column (ES Industries); a precolumn of Whatman Co:Pell ODS pellicular packing was used. The peptides were assembled in a stepwise manner using a Vega 1000 peptide synthesizer.

Ac-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH_2 (3) was prepared using method A. From Boc-Phe-BHA-Resin (1.0 g, 0.325 mmol/g substitution), 1.62 g Ac-Tyr(2,6-Cl_2-Bzl)-Gly-Trp(For)-Met-Asp(OBzl)-Phe-BHA Resin was obtained and was deportected with HF to give 258 mg of crude peptide. A 130-mg portion was purified by preparative HPLC, with the product eluting at 38% modifier to yield 20 mg (15.6%) of Ac-Tyr-Gly-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.10 (1), Gly 1.00 (1), Met 0.90 (1), Tyr 0.90 (1), Phe 1.20 (1), Trp not determined (ND). Empirical formula: $C_{42}H_{50}N_8O_{10}S$, MW 858.96. FAB MS: *m/z* 858 (M + H). Sulfation of 18 mg gave 14 mg (70%) of **3** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula $C_{42}H_{49}N_8O_{13}S_2$ 1:1 NH_4^+, MW 956.05.

Ac-Tyr(SO_3H)-β-Ala-Trp-Met-Asp-Phe-NH_2 (4) was prepared using method A. From Boc-Phe-BHA-Resin (1.0 g, 0.325 mmol/g substitution), 1.52 g Ac-Tyr(2,6-Cl_2-Bzl)-β-Ala-Trp(For)-Met-Asp(OBzl)-Phe-BHA resin was obtained and deprotection with HF gave 262 mg of crude peptide. A 130-mg portion was purified by preparative HPLC, with the product eluting at 35% modifier to yield 16 mg (11%) of Ac-Tyr-β-Ala-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), β-Ala 1.00 (1), Met 0.91 (1), Tyr 0.96 (1), Phe 1.00 (1), Trp ND. Empirical formula: $C_{43}H_{52}N_8O_{10}S$, MW 873.00. FAB MS: *m/z* 873 (M + H). Sulfation of 14 mg gave 12 mg (77%) of **4** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{43}H_{51}N_8O_{13}S$ 1:1 NH_4^+, MW 970.10.

Method A. Ac-Tyr(SO_3H)-(4-aminobutanoyl)-Trp-Met-Asp-Phe-NH_2 (5). Boc-Phe-OH (530 mg, 2.00 mmol) and HOBt (405 mg, 3.00 mmol) were dissolved in CH_2Cl_2 (20 mL) and DMF (20 mL) and chilled to 0 °C, and DIC (255 mg, 2.00 mmol) was added. The mixture was stirred for 60 min at 0 °C. Separately, 1 g of benzhydrylamine copolysterene 1% divinylbenzene crosslinked resin (0.36 mmol N/g) was washed with 10% diisopropylethylamine in methylene chloride for 30 min, filtered, and washed with CH_2Cl_2, DMF, and CH_2Cl_2. The above mixture was

added to the resin and stirred for 24 h at room temperature. The resin was filtered and washed with CH_2Cl_2, DMF, methanol, and CH_2Cl_2 and dried under high vacuum. Amino acid analysis showed the resin to contain 0.325 mmol of phenylalanine per gram of resin.

Boc-Phe-BHA resin (1.0 g, 0.325 mmol) was then subjected to sequential solid phase synthesis using the following protocol:

Step	Reagent	Time
1	1% EDT/CH_2Cl_2	1x30 sec.
2	50% TFA/CH_2Cl_2/ with 1%EDT	1x1 min.
3	Repeat Step 1	
4	50% TFA/CH_2Cl_2/w1%EDT	1x15 min.
5	CH_2Cl_2	1x30 sec.
6	Methanol	1x30 sec.
7-8	Repeat steps 5 and 6	
9	CH_2Cl_2	2x30 sec.
10	8% DIEA	2x2 min
11-15	Repeat step 5-9	
16	2.00 mmol. of Boc-AA,DIC (255 mg, 2.00 mmol) HOBt(405 mg, 3.00 mmol) in 20 mL of 1:1 DMF/CH_2Cl_2, room temperature	1x60 min.
17	1% DIEA	1x30 min
18 -19	Repeat Steps 6 and 9	
20-21	Repeat steps 16 and 17 if Kaiser test is positive	
22	Methanol	1x30 sec.
23-24	Repeat steps 5 and 6	
25	CH_2Cl_2	1x30 sec.
26	Methanol	2x30 sec.
27	CH_2Cl_2	3x30 sec.

Removal of the Boc-protecting group and acetylation of the resin with 10 mL of acetic anhydride and 10 mL of pyridine in CH_2Cl_2 for 60 min yielded 1.45 g of Ac-Tyr(2,6-Cl_2-Bzl)-(4-aminobutanoyl)-Trp(For)-Met-Asp(OBzl)-Phe-BHA resin which was cleaved by treatment with 9 mL of HF containing 4 mL of anisole, 1.0 mL of EDT, and 25 mL of dimethyl sulfide for 1 h at 0 °C. After evaporation to a low volume, fresh anhydrous HF (32 mL) was distilled into the reaction vessel for a second treatment for 2 h at 0 °C. After through evaporation, the resin was washed with 2 volumes of ethyl acetate, triturated with 30% acetic acid (4 × 20 mL), filtered, and lyophilized to yield 253 mg of crude peptide. An 80-mg portion of the crude peptide was purified by preparative HPLC, eluting with a linear gradient (4 h) of 5–65% of 0.022% TFA–CH_3CN at a flow rate of 8 mL/min with detection at 280 nm. The main peak, which eluted at 35% modifier, was collected and lyophilized to yield 13 mg (14%) of Ac-Tyr-(4-aminobutanoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 0.98 (1), Phe 1.00 (1), 4-aminobutanoic acid 1.20 (1), Trp ND. Empirical formula: $C_{44}H_{54}N_8O_{10}S$, MW 887.02. FAB MS: m/z 887 (M + H).

A suspension of 12 mg unsulfated peptide in 2 mL of dry pyridine was treated with 355 mg of pyridinium acetyl sulfate. The reaction mixture was stirred for 5 h at room temperature, diluted with 5 mL of 1.5 M NH_4OH, and lyophilized. Preparative HPLC using a linear gradient of NH_4OAc–CH_3CN (10–40% 0.01 M) over 60 min at a flow rate of 6 mL/min and detection at 290 nm afforded 9 mg (65%) of **5** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{44}H_{53}N_8O_{13}S_2$ 1:1 NH_4^+, MW 984.13.

Ac-Tyr(SO_3H)-(5-aminopentanoyl)-Trp-Met-Asp-Phe-NH_2 (6) was prepared using method A. From Boc-Phe-BHA resin (1.0 g, 0.325 mmol/g substitution), 1.50 g of Ac-Tyr(2,6-Cl_2Bzl)-(5-aminopentanoyl)-Trp(For)-Met-Asp(OBzl)-Phe-BHA resin was obtained. Deprotection with HF gave 217 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 34% modifier) to yield 17 mg (12.5%) of Ac-Tyr-(5-aminopentanoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), 5-aminopentanoic acid 1.20 (1), Met 1.00 (1), Tyr 0.98 (1), Phe 1.00 (1), Trp ND. Empirical formula: $C_{45}H_{58}N_8O_{10}S$, MW 901.06. FAB MS: m/z 901 (M + H). Sulfation of 16 mg gave 13 mg (72%) of **6** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{45}H_{55}N_8O_{13}S_2$ 1:1 NH_4^+, MW 998.13.

Ac-Tyr(SO_3H)-(6-aminohexanoyl)-Trp-Met-Asp-Phe-NH_2 (7) was prepared using method A. From Boc-Phe-BHA resin (1.00 g, 0.325 mmol/g substitution), 1.55 g of Ac-Tyr(2,6-Cl_2-Bzl)-(6-aminohexanoyl)-Trp(For)-Met-Asp(OBzl)-Phe-BHA resin was obtained. Deprotection with HF gave 301 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 36% modifier) to yield 12 mg (12%) of Ac-Tyr-(6-aminohexanoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), 6-aminohexanoic acid 1.12 (1), Met 1.00 (1), Tyr 0.98 (1), Phe 1.00 (1), Trp ND. Empirical formula: $C_{46}H_{58}N_8O_{10}S$, MW 915.06. FAB MS: m/z 915 (M + H). Sulfation of 11 mg gave 8 mg (67%) of **7** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{57}N_8O_{13}S_2$ 1:1 NH_4^+, MW 1012.

3-[[(1,1-Dimethylethoxy)carbonyl]amino]benzoic Acid (9a). To a solution of 3-aminobenzoic acid (**8a**) (1.00 g, 7.15 mmol, 98% pure) in dioxane (7 mL) and water (7 mL) was added 1 N NaOH (7.2 mL, 7.2 mmol) dropwise. After cooling at 0 °C, di-*tert*-butyl dicarbonate (2.58 g, 11.8 mmol) was added and the mixture was stirred at room temperature for 4.5 h. The mixture was evaporated to remove most of the solvent and acidified at 0 °C with saturated $KHSO_4$ to a pH of 2–3. The mixture was extracted with EtOAc (3×). The combined extracts were washed with H_2O (2×), dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized from MeOH–CH_2Cl_2 to give **9a** (1.02 g, 4.28 mmol) in 60% yield as crystals: mp 196–197 °C. IR (KBr): 3355, 1695 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.48 (s, 9 H), 7.26 (m, 1 H) 7.53 (m, 1 H), 7.62 (m, 1 H), 8.14 (s, 1 H), 9.54 (s, 1 H). MS: m/z 237 (M^+), 181, 137, 57. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.44; H, 6.29; N, 5.75.

3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methylbenzoic Acid (9b). A mixture of 2-methyl-3-nitrobenzoic acid (1.00 g, 5.41 mmol), 10% Pd(C) (0.30 g) and di-*tert*-butyl dicarbonate (4.49 g, 20.5 mmol) in DMF (56 mL) was hydrogenated at 50 psi H_2 for 2.5 h and was filtered. The residue obtained after concentration was chromatographed over silica gel, eluting with EtOAc–hexane–HOAc 39:60:1 to give **9b** (1.06 g, 86%), mp 170–171 °C. IR (KBr): 3245, 1680, 1528 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.46 (s, 9 H), 2.32 (s, 3 H) 7.21 (m, 1 H), 7.41 (m, 1 H), 7.51 (m, 1 H), 8.70 (s, 1 H). MS: m/z 251 (M^+). Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 61.84; H, 6.86; N, 5.54.

5-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-methylbenzoic Acid (9c) was prepared as above for **9b**. Mp: 200–202 °C. IR (KBr): 3355, 1695 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.47 (s, 9 H), 2.42 (s, 3 H), 7.16 (m, 1 H), 7.46 (m, 1 H), 7.99 (s, 1 H), 9.41 (s, 1 H), 12.77 (br s, 1 H). MS: m/z 251 (M^+). Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 61.98; H, 6.80; N, 5.59.

***(rac)-cis*-2-Aminocyclohexaneacetic Acid (11).** A mixture of 2-nitrophenylacetic acid (**10**) (15.0 g, 82.8 mmol), 5% Rh/C (6.75 g), and EtOH (450 mL) was hydrogenated at 60 °C under 100 psi for 6 h and filtered through Celite. After evaporation, the residue was recrystallized from MeOH–CH_2Cl_2 to give **11** (6.40 g, 40.7 mmol) in 49% yield as crystals: mp 185–187 °C (lit.[33] mp, 185–187 °C).

***(rac)-cis*-2-[[(1,1-Dimethylethoxy)carbonyl]amino]cyclohexaneacetic Acid (12).** To a solution of **11** (900 mg, 5.72 mmol) in dioxane (18 mL) and water (9 mL) was added 1 N NaOH (5.76 mL, 5.76 mmol) dropwise. After cooling at 0 °C, di-*tert*-butyl dicarbonate (1.37 g, 6.28 mmol) was added and the mixture was stirred at room temperature for 5 h. The mixture was evaporated to remove most of the solvent and acidified at 0 °C with saturated $KHSO_4$ to a pH of 2–3. The mixture was extracted with EtOAc (3×). The combined extracts were washed with H_2O (2×) and brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by silica gel chromatography eluting with HOAc–EtOAc–hexane (1:49:50) to give **12** (1.42 g, 5.52 mmol) in 96% yield as a glass. IR (KBr): 3445, 1712, 1685, 1652 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.04–1.40 (m, 4 H), 1.46 (s, 9 H), 1.50–1.81 (m, 4 H), 1.92–2.40 (m, 3 H), 3.78 and 3.93 (br s, total 1 H), 4.47 and 4.82 (br d, J = 8 Hz, total 1 H), 10.98 (br s, 1 H). MS: m/z 257 (M^+),

201, 198, 183, 156, 57. Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.53; H, 9.05; N, 5.18.

(*rac*)-*trans*-Octahydro-2-oxo-1*H*-indole-1-carboxylic Acid 1,1-Dimethylethyl Ester (14). To a suspension of sodium hydride (99 mg, 4.13 mmol) in dry 1,2-dimethoxyethane (DME) was added a solution of (*rac*)-*trans*-octahydro-2*H*-indol-2-one (13)[33] (550 mg, 3.94 mmol) in DME (10 mL) dropwise. The mixture was then heated at reflux for 1 h. After cooling to room temperature, di-*tert*-butyl dicarbonate (1.73 g, 7.93 mmol) and 4-(dimethylamino)pyridine (35 mg) were added and the mixture was stirred at room temperature for 3.5 h. The reaction was quenched by addition of saturated NH_4Cl (18 mL), and the mixture was extracted with EtOAc (3×). The combined extracts were washed with brine (2×), dried ($MgSO_4$), and evaporated to dryness. The residue was purified by silica gel chromatography, eluting with EtOAc/hexane (1:2) to give 14 (800 mg, 3.34 mmol) in 85% yield as a solid: mp 78–80 °C. IR (KBr): 1782, 1772, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.18–1.48 (m, 4 H), 1.55 (s, 9 H), 1.64–1.97 (m, 4 H), 2.14 (dd, J_{gem} = 16 Hz, J_{vic} = 14 Hz, 1 H), 2.43 (dd, J_{gem} = 16 Hz, J_{vic} = 7 Hz, 1 H), 2.60 (m, 1 H), 3.26 (m, 1 H). MS: *m/z* 239 (M^+), 224, 184, 166, 140. Anal. Calcd for $C_{13}H_{21}NO_3$: C, 65.25; H, 8.85; N, 5.85. Found: C, 65.26; H, 8.96; N, 5.81.

(*rac*)-*trans*-2-[[(1,1-Dimethylethyl)oxy]carboxamido]cyclohexylacetic Acid (15). To a solution of (*rac*)-*trans*-octahydro-2-oxo-1*H*-indole-1-carboxylic acid 1,1-dimethylethyl ester (14) (960 mg, 4.01 mmol) in THF (48 mL) and H_2O (24 mL) was added at room temperature $LiOH \cdot H_2O$ (830 mg, 19.8 mmol). The slightly cloudy mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of 10% HOAc–H_2O (60 mL), and the mixture was evaporated to remove most of the THF. The residue was extracted with Et_2O (3×). The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated to give 15 (1.02 g, 3.97 mmol) in 99% yield as an amorphous solid. IR (KBr): 3370, 1702, 1680 cm^{-1}. ^1H NMR ($DMSO-d_6$): δ 0.88–1.32 (m, 4 H), 1.37 (s, 9 H), 1.42–1.94 (m, 6 H), 2.43 (m, 1 H), 2.98 (m, 1 H), 6.69 and 6.32 (d, J = 9 Hz, total 1 H), 11.98 (br s, 1 H). MS: *m/z* 257 (M^+), 201, 184, 166, 156, 142. Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.36; H, 9.08; N, 5.35.

(1*S*)-*trans*-2-Azidocyclopentanecarboxylic Acid Ethyl Ester (17). To a solution of (1*S*)-*cis*-2-hydroxycyclopentanecarboxylic acid ethyl ester (16)[35] (1.00 g, 6.32 mmol) and triphenylphosphine (1.88 g, 6.95 mmol) in dry benzene (31 mL) at room temperature under argon were added hydrazoic acid (1.3 M in benzene, 5.35 mL, 6.95 mmol) and diethyl azodicarboxylate (1.15 mL, 6.95 mmol). The mixture was stirred at room temperature for 3 h and evaporated to dryness. The residue was purified by silica gel chromatography, eluting with CH_2Cl_2 to give 17 (734 mg, 4.01 mmol) in 63% yield as a glass. Ir ($CHCl_3$): 2105, 1728 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (t, J = 8 Hz, 3 H), 1.62–1.92 (m 4 H), 1.97–2.14 (m, 2 H), 2.73 (m, 1 H), 4.08–4.25 (m, 3 H). MS: *m/z* 155 ($M^+ - N_2$), 138, 1.09.

(1*S*)-*trans*-2-[[(1,1-Dimethylethoxy)carbonyl]amino]cyclopentanecarboxylic Acid Ethyl Ester (18). A mixture of 17 (606 mg, 3.31 mmol) and di-*tert*-butyl dicarbonate (2.71 g, 12.4 mmol) in dry DMF (50 mL) was hydrogenated over 10% Pd/C (404 mg) in a Parr hydrogenation flask at 50 psi for 3 h. The mixture was filtered through a pad of Celite washing thoroughly with CH_2Cl_2. The combined filtrates were concentrated, and the residue was partitioned between CH_2Cl_2 and brine. The aqueous layer was extracted with CH_2Cl_2 (3×). The combined extracts were dried (Na_2SO_4) and evaporated to dryness. The residue was purified by a silica gel chromatography, eluting with 10% EtOAc/hexane to give 18 (795 mg, 3.09 mmol) in 93% yield as solids: mp 56–58 °C; $[\alpha]^{25}_D$ + 41.4° (*c* 0.96, $CHCl_3$). IR ($CHCl_3$): 3345, 1712, 1702 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.26 (t, J = 8 Hz, 3 H), 1.44 (s, 9 H), 1.72 (m, 2 H), 1.81–2.05 (m, 2 H), 2.12 (m, 1 H), 2.56 (m, 1 H), 4.05–4.20 (m, 3 H), 4.57 (br s, 1 H). MS: *m/z* 200 ($M^+ - C_4H_9$), 184, 156, 138, 128, 57. Anal. Calcd for $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.75; H, 9.05; N, 5.41.

(1*S*)-*trans*-2-[[(1,1-Dimethylethoxy)carbonyl]amino]cyclopentanecarboxylic Acid (19). To a solution of 18 (755 mg, 2.93 mmol) in MeOH (46 mL) and H_2O (14 mL) were added 30% H_2O_2 (1.51 mL) and solid $LiOH \cdot H_2O$ (1.23 g, 29.3 mmol). The mixture was stirred at 0 °C for 17 h and was quenched by the addition of Na_2SO_3 solution (5.45 g in 30 mL of H_2O) followed by the addition of saturated aqueous $NaHCO_3$ (30 mL) at 0 °C. After concentration to remove most of the MeOH, the residue was extracted with CH_2Cl_2. The aqueous layer was acidified with 3 N HCl to a pH of 2–3, saturated with solid NaCl, and extracted with CH_2Cl_2 (3×). The combined extracts were dried (Na_2SO_4) and evaporated to dryness. The residue was recrystallized from CH_2Cl_2–hexane to give 19 (574 mg, 2.50 mmol) in 85% yield as solids: mp 117–120 °C; $[\alpha]^{25}_D$ + 2.69° (*c* 1.02, $CHCl_3$). IR ($CHCl_3$): 3375, 1722, 1678 cm^{-1}. ^1H NMR ($DMSO-d_6$): δ 1.37 (s, 9 H), 1.49–1.70 (m, 3 H), 1.85 (m, 2 H), 3.95 (m, 1 H), 6.94 and 6.60 (br d and br s, rotamers, 1 H). MS: *m/z* 189 ($M^+ - CO_2H$), 127, 73, 57. Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.63; H, 8.35; N, 6.11. Found: C, 57.34; H, 8.36; N, 5.99.

Method B. Ac-Tyr(SO_3H)-(3-aminobenzoyl)-Trp-Met-Asp-Phe-NH_2 (20). Boc-Phe-PAM resin (1.00 g, 0.36 mmol/g substitution) was suspended and shaken in 10 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, 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 using the same protocol as described in method A. At step 16 the appropriate Boc-amino acid, diisopropylcarbodiimide, and HOBt were added. The amino acids were employed in the following order: Boc-Asp(OFm)-OH, Boc-Met-OH, Boc-Trp-OH, Boc-3-aminobenzoic acid, Boc-Tyr-OH. At this point the resin-bound peptide was acetylated with acetic anhydride in pyridine to yield Ac-Tyr-(3-aminobenzoyl)-Trp-Met-Asp(OFm)-Phe-PAM resin. The peptidyl resin was treated with 20% piperidine–DMF for 10 min at room temperature, filtered, and washed with CH_2Cl_2, DMF, and methanol. The resulting material (1.55 g) was placed in a pressure bottle, suspended in 40 mL of methanol, 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 resin was filtered and washed with methanol. The combined filtrates were evaporated to dryness to yield 424 mg of crude peptide. A 150-mg portion was purified by preparative HPLC using the same conditions as method A. The main peak, which was eluted at 36% modifier, was collected and lyophilized to yield 32 mg (27%) of Ac-Tyr-(3-aminobenzoyl)-Trp-Met-Asp-Phe-NH_2, which was homogeneous by HPLC. Amino acid analysis: Asp 1.00 (1), Met 1.20 (1), Tyr 1.00 (1), Phe 1.01 (1); Trp ND, 3-aminobenzoic acid ND. Empirical formula: $C_{47}H_{52}N_8O_{10}S$, MW 921.05. FAB MS *m/z* 921 (M + H). Sulfation of 30 mg as described in method A gave 27 mg (82%) of **20** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{47}H_{51}N_8O_{13}S_2$ 1:1 NH_4, MW 1018.14.

Ac-Tyr(SO_3H)-(2-methyl-3-aminobenzoyl)-Tyr-Met-Asp-Phe-NH_2 (21) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.52 g of Ac-Tyr-(3-amino-2-methylbenzoyl)-Trp-Met-Asp-Phe-PAM resin was obtained. Treatment with NH_3 gave 312 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 36% modifier) to yield 20 mg (19%) of Ac-Tyr-(3-amino-2-methylbenzoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.10 (1), Met 1.00 (1), Tyr 0.90 (1), Phe 1.00 (1); Trp and 3-amino-2-methylbenzoic acid ND. Empirical formula: $C_{48}H_{54}N_8O_{10}S$ MW 935.07. FAB MS: *m/z* 935 (M + H). Sulfation of 18 mg gave 14 mg (70%) of **21** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{53}N_8O_{13}S_2$ 1:1 NH_4^+, MW 1032.17.

Ac-Tyr(SO_3H)-(5-amino-2-methylbenzoyl)-Trp-Met-Asp-Phe-NH_2 (22) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.50 g of Ac-Tyr-(5-amino-2-methylbenzoyl)-Trp-Asp-Phe-PAM resin was obtained. Treatment with NH_3 give 415 mg of crude peptide. A 100-mg portion was purified by preparative HPLC (product eluted at 36% modifier) to yield 26 mg (32%) of Ac-Tyr-(5-amino-2-methylbenzoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 1.00 (1), Phe 0.98 (1); Trp and 5-amino-2-methylbenzoic acid ND. Empirical formula: $C_{48}H_{54}N_8O_{10}S$, MW 935.07. FAB MS: *m/z* 935 (M + H). Sulfonation of 13 mg gave 8 mg (55%) of **22** monoammonium salt which was

homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{53}N_8O_{13}S_2$ 1:1 NH_4^+, MW 1032.17.

Ac-Tyr(SO$_3$H)-(4-aminobenzoyl)-Trp-Met-Asp-Phe-NH$_2$ (23) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.52 g of Ac-Tyr-(4-aminobenzoyl)-Trp-Met-Asp-Phe-PAM resin was obtained. Treatment with NH_3 gave 312 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 38% modifier) to yield 12 mg (11.5%) of Ac-Tyr-(4-aminobenzoyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 0.90 (1), Phe 1.06 (1); Trp and 4-aminobenzoic acid ND. Empirical formula: $C_{47}H_{52}N_8O_{10}S$, MW 921.05. FAB MS: m/z 921 (M + H). Sulfonation of 10 mg gave 7 mg (64%) of **23** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{47}H_{51}N_8O_{13}S_2$ 1:1 NH_4, MW 1018.14.

Ac-Tyr(SO$_3$H)-[(±)-*cis*-2-aminocyclohexaneacetyl]-Trp-Met-Asp-Phe-NH$_2$ (24) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.50 g of Ac-Tyr-((±)-*cis*-2-aminocyclohexaneacetyl)-Trp-Met-Asp-Phe-PAM resin was obtained. Treatment with NH_3 give 471 mg crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 39% modifier) to yield 25 mg (35%) of Ac-Tyr-((±)-*cis*-2-aminocyclohexaneacetyl)-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 0.90 (1), Phe 1.00 (1), Trp and (±)-*cis*-2-aminocyclohexaneacetic acid ND. Empirical formula: $C_{48}H_{60}N_8O_{10}S$, MW 941.12. FAB MS: m/z 941 (M + H). Sulfonation of 20 mg gave 17 mg (77%) of **24** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{59}N_8O_{13}S_2$ 1:1 NH_4, MW 1038.22.

Ac-Tyr(SO$_3$H)-[(±)-*trans*-2-aminocyclohexaneacetyl]-Trp-Met-Asp-Phe-NH$_2$ (25) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.50 g Ac-Tyr-[(±)-*trans*-2-aminocyclohexaneacetyl]-Trp-Met-Asp-Phe-PAM resin was obtained. Treatment with NH_3 give 521 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 39% modifier) to yield 24 mg (37%) of Ac-Tyr-[(±)-*trans*-2-aminocyclohexaneacetyl]-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 0.90 (1), Phe 1.00 (1); Trp and (±)-*trans*-2-aminocyclohexaneacetic acid ND. Empirical formula: $C_{48}H_{60}N_8O_{10}S$, MW 941.12. FAB MS: m/z 941 (M + H). Sulfonation of 22 mg gave 17 mg (70%) of **25** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{59}N_8O_{13}S_2$ 1:1 NH_4, MW 1038.22.

Ac-Tyr(SO$_3$H)-[(1*S*)-*trans*-2-aminocyclopentanoyl]-Trp-Met-Asp-Phe-NH$_2$ (26) was prepared using method B. From Boc-Phe-PAM resin (1.0 g, 0.36 mmol/g substitution), 1.56 g Ac-Tyr-[(1*S*)-*trans*-2-aminocyclopentanoyl]-Trp-Met-Asp-Phe-PAM resin was obtained. Treatment with NH_3 give 387 mg of crude peptide, a 100-mg portion of which was purified by preparative HPLC (product eluted at 38% modifier) to yield 19 mg (22%) of Ac-Tyr-[(1*S*)-*trans*)-2-aminocyclopentanoyl]-Trp-Met-Asp-Phe-NH_2. Amino acid analysis: Asp 1.00 (1), Met 1.00 (1), Tyr 0.95 (1), Phe 1.00 (1); Trp and (1*S*)-*trans*)-2-aminocyclopentanecarboxylic acid ND. Empirical formula: $C_{46}H_{58}N_8O_{10}S$, MW 913.07. FAB MS: m/z 913 (M + H). Sulfation of 18 mg gave 16 mg (80%) of **26** monoammonium salt which was homogeneous by HPLC. IR (KBr): 1050 cm^{-1}. Empirical formula: $C_{48}H_{55}N_8O_{13}S_2$ 1:1 NH_4, MW 1010.16.

NMR Experiments. The samples for NMR spectroscopy consisted of 4–7 mM solutions of **20** and **26** in fully deuterated DMSO (99.9%; MSD Isotopes). NMR experiments were performed on a Varian VXR-500S spectrometer, operating at 499.843 MHz for the observation of protons, using 16 bit A/D conversion and quadrature phase detection. Samples were maintained at 22.5 °C. The two-dimensional experiments performed were COSY[50-52] and NOESY.[51,53] They were acquired in phase-sensitive mode[54] and were collected as 512 t_1 increments of 2048 points per FID, with spectral widths of 6000–7000 Hz and acquistion times of 0.146–0.171 s per transient. A relaxation delay of 1 s preceded each transient. The total number of transients per FID was 48–160. NOESY experiments utilized a mixing time of 0.4 s. No secondary effects were observed at this mixing time. Data from two-dimensional experiments were transformed by zero-filling to a final size of 2K × 2K real points, multiplying by a sine bell function in t_2 extending to 600 points, and a sine bell function in t_1 extending to 256 points and shifted by 0° (COSY) or 50° (NOESY).

The proton resonances of the peptides were assigned from COSY and NOESY spectra using standard procedures.[55] All nonoverlapping NOEs were measured and converted into distance constraints, using assumptions commonly used in the derivation of peptide structures from NOE data[55] and outlined previously.[49]

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 no. 1 (10 mM HEPES + 130 mM NaCl + 5 mM $MgCl_2$, pH 7.4) using 35 parts buffer per 1 part tissue on a wet weight/volume basis (ca. 175 mL). The tissue was homogenized 2 × for ca. 15 sec at 0 °C using a Polytron homogenizer at a setting of 6. The tissue was isolated by centrifugation at 48000g for 10 min at 0 °C. The resulting tissue pellet was resuspended in HEPES buffer no. 2 (10 mM HEPES + 130 mM NaCl + 5 mM $MgCl_2$ + 1 mg/L phenylmethanesulfonyl fluoride (PMSF) + 200 mg/L Bacitracin): 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 [*propionyl*-^{3}H]CCK-8 purchased from Amersham (final concentration = 0.15 nM) and tissue homogenate (striatum approximately 0.26 mg protein in 2 mL final volume; pancreas approximately 0.100 mg 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 no. 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 DuPont/NEN Aquasol scintillation cocktail. The vials were shaken overnight and then counted using 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, and this was found to be approximately 90% of total binding in pancreatic tissue and about 80% in striatal tissue. The concentration of peptide necessary to inhibit 50% of total specific [*propionyl*-^{3}H]CCK-8 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. Rats were then weighed and placed in individual cages for a 4-day period of meal training. 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., and the jars were removed from 10:00 a.m. to 12:00 p.m. and placed back in the cages from 12:00 p.m. 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 that 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 body weight were excluded from the test. The animals were then distributed into experimental (n = 5 to 6) and control groups (n = 6 to 12)

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matched for body weight. Peptides were dissolved in saline or 0.5% DMSO/saline at concentrations of 0 to 320 μg/mL per kg body weight and 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 4 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 12:12 light/dark cycle (lights on at 6:00 a.m.). A maintenance diet of ground Purina laboratory chow and tap water was provided ad libitum 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 0.33 mL/kg, ip injection of vehicle (100% DMSO) 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.

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