Tetrapeptide CCK-A Agonists: Effect of Backbone N-Methylations on *in Vitro* and *in Vivo* CCK Activity

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N-Methylation of backbone amide bonds was conducted on a tetrapeptide that had been identified previously (Shiosaki, K.; et al. *J. Med. Chem.* **1991**, *34*, 2387–2842) as a potent and selective CCK-A agonist. N^{α}-Methylation at the position corresponding to Asp^{32} (CCK-33 numbering) was consistent with high affinity, efficacy, and selectivity for the CCK-A receptor. Combination of this (*N*-Me)Asp with the (*N*-Me)Phe modification also provided a highly active analogue. The observation of parallel structure–binding affinity profiles with respect to sites of N-methylation in the C-terminal regions of tetrapeptide vs heptapeptide CCK analogues suggests that the two series interact similarly with the CCK-A receptor.

Introduction

Previous reports from these laboratories have disclosed a novel series of potent and selective CCK-A agonists, typified by the tetrapeptide Boc-Trp-Lys(CONH-Ph-*o*-Me)-Asp-(*N*-Me)Phe-NH_2 (A-71623).[1-4] We also have reported on the sulfated heptapeptide des-NH_2-Tyr(SO_3^-)-Nle-Gly-Trp-Nle-(*N*-Me)Asp-Phe-NH_2 (A-71378) as a potent and selective CCK-A agonist,[5-7] in which the (*N*-Me)Asp residue not only is responsible for imparting CCK-A receptor selectivity, but also appears to contribute to increased *in vivo* potency and duration of action.[7] It was therefore of interest to investigate whether N-methylation of the Asp residue in the tetrapeptide series, either alone or in combination with (*N*-Me)Phe, would be beneficial to the activity of these compounds as CCK-A agonists, since a favorable outcome would offer an additional avenue for increasing enzymatic stability, decreasing polarity, and restricting conformational freedom. Furthermore, previous studies conducted in several related CCK heptapeptide series had demonstrated a distinct pattern of biological activity as a function of N-methylation of residues in the C-terminal tetrapeptide region, i.e., N-methylation at Asp^{32} or at Phe^{33} (CCK-33 numbering) was found to be quite favorable to interactions with the CCK-A receptor,[7] whereas N-methylation at position 31[7-9] and at the C-terminal carboxamide moiety[10] were detrimental to activity at this receptor subtype. Therefore, a determination of whether similar structure–activity relationships would be maintained for corresponding modifications in the tetrapeptide series would permit a preliminary assessment of the extent to which corresponding regions of the two series may interact similarly with the CCK-A receptor.

Methods

Peptide synthesis was carried out in solution using known peptide coupling methods. The N-methylated amino acids H-(*N*-Me)Phe-NH_2[4] and Fmoc(*N*-Me)Asp-(OBn)-OH[7] were prepared by literature methods as described previously. Cbz-(N^{α}-Me)Lys(phthaloyl)-OH (28) was prepared by the method of Freidinger[11] and converted to Boc-(N^{α}-Me)Lys(NHCO-Ph-*o*-Me)-OH (29) by selective deprotections and acylations under standard conditions. Boc-(*N*-Me)Trp-OH (31) was prepared by conventional *N*-Boc protection of commercially available L-abrine. Other protected amino acids and reagents were commercially available. Physical data for final compounds are collected in Table 1, except for 1, which is described in ref 4.

In vitro and *in vivo* assays were conducted as described previously.[7] Briefly, *in vitro* binding data were determined as IC_{50} for half maximal inhibition of ^{125}I-labeled Bolton-Hunter-CCK-8 in guinea pig pancreas (CCK-A receptors) or cortex (CCK-B receptors), and functional activity was determined as percent maximal stimulation of phosphatidylinositol hydrolysis in guinea pig pancreatic acini (CCK-A receptors) relative to that elicited by CCK-8. Appetite suppression data are expressed as ED_{50}, the dose that caused the half-maximal inhibition of intake of a liquid diet by food-deprived rats during a 1-h time period following intraperitoneal injection of the drug in a distilled water vehicle.

Table 1. Physical Data for Compounds 1–7

compd	formula	anal.	FAB⁺ MS *m/e*	FAB⁺ MS adduct
2	$C_{44}H_{58}N_8O_9 \cdot 1.5H_2O$	CHN	863	M + Na^+
3	$C_{45}H_{58}N_8O_9 \cdot 1.5H_2O$	CHN	893	M + K^+
			877	M + Na^+
			855	M + H^+
4	$C_{44}H_{58}N_8O_9 \cdot 0.8\ HOAc \cdot 1.2H_2O$	CHN	841	M + H^+
5	$C_{45}H_{58}N_8O_9 \cdot 1.7H_2O$	CH[a]	855	M + H^+
6	$C_{44}H_{56}N_8O_9 \cdot 2H_2O$	CHN	863	M + Na^+
			841	M + H^+
7	$C_{44}H_{56}N_8O_9 \cdot 0.6H_2O \cdot 0.6HOAc$	CHN	863	M + Na^+
			841	M + H^+
8	$C_{39}H_{48}N_8O_7 \cdot HCl \cdot 0.2HOAc$	*b*	741	M + H^+
9	$C_{45}H_{58}N_8O_9 \cdot 2H_2O \cdot HOAc$	CHN	899	M + $2Na^+$
			877	M + Na^+
10	$C_{40}H_{50}N_8O_7 \cdot 2H_2O \cdot 2HCl$	CHN	777	M + Na^+
			755	M + H^+

[a] For 5. N: calcd, 12.65; found, 12.17. [b] See the Experimental Section.

Results and Discussion

In vitro biological data for new compounds 2–10 are presented in Table 2, together with data for reference

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Table 2. Biological Data for N-Methylated Analogues of A-71294[a,b]

no.	structure					radioligand binding IC_{50} (nM)			PI hydrolysis
						pancreas	cortex	C/P[c]	% max[d]
1	Boc Trp	Lys(CONH-Ph-o-Me)	Asp	Phe	NH_2	3.8 ± 0.49[e]	1400 ± 490[e]	370	101[e]
2					NHMe	170 ± 26 (3)	4250 ± 81 (3)	25	9 ± 2 (4)
3					NMe_2	117 ± 36 (3)	>10,000	>85	20 ± 2 (4)
A-71623				(*N*-Me)Phe		3.7 ± 0.85[f]	4500 ± 770[f]	1180	100[e]
4			(*N*-Me)Asp			4.3 ± 0.5 (3)	>10000	>2300	101 ± 3 (4)
5			(*N*-Me)Asp	(*N*-Me)Phe		5.3 ± 1.0 (4)	427 ± 80 (3)	80	93 ± 2 (4)
6		(N^{α}-Me)Lys(CONH-Ph-o-Me)				67.8 ± 27.9 (3)	775 ± 67 (3)	11	80 ± 2 (4)
7	(*N*-Me)Trp					567 ± 253 (3)	>10000	>18	46 ± 5 (4)
8	H (*N*-Me)Trp					16.3 ± 4.3 (3)	3720 ± 577 (3)	228	90 ± 3 (3)
9	(*N*-Me)Trp		(*N*-Me)Asp			707 ± 215 (3)	>10000	>14	8 ± 1 (3)
10	H (*N*-Me)Trp		(*N*-Me)Asp			14.3 ± 3.1 (5)	69200 ± 1500 (3)	4840	97 (2)

[a] Entries beneath 1 represent point modifications of 1. [b] Number of determinations for each assay is indicated in parentheses. [c] IC_{50} cortex/IC_{50} pancreas. [d] Indicates percent stimulation at 10^{-4} M relative to maximum stimulation by CCK-8. [e] Data from ref 4. [f] Data from ref 3.

Table 3. Activity of Selected Analogues in Suppression of Food Intake in Rat

compd	ED_{50} (nmol/kg)[a]	compd	ED_{50} (nmol/kg)[a]
1	12.5 (8.4 – 17.7)	5	2.7 (1.8 – 3.9)
A-71623	3.6[b]	8	226.0 (131.7 – 387.9)
4	1.5 (0.9 – 2.6)	10	7.0 (4.0 – 12.3)

[a] Data in parentheses indicate 95% confidence intervals. [b] Data from ref 14.

Table 4. Binding of N-Methylated Analogues in Sulfated Heptapeptide Series to Guinea Pig Pancreas

no.	structure	IC_{50} (nM)[a]
11	X-Trp-Nle-Asp-Phe-NH_2	0.77 ± 0.05
12	X-Trp-Nle-Asp-(*N*-Me)Phe-NH_2	0.99 ± 0.28
13	X-Trp-Nle-(*N*-Me)Asp-Phe-NH_2	0.50 ± 0.04
14	X-Trp-Leu-Asp-Phe-NH_2	4.7 ± 1.0
15	X-Trp-(*N*-Me)Leu-Asp-Phe-NH_2	140 ± 15
16	Y-Trp-Nle-Asp-Phe-NH_2[b]	0.93 ± 0.10
17	Y-Trp-Nle-Asp-Phe-NHMe[b]	18.8 ± 2.8

[a] Except where noted, data are from ref 7; X = (des-NH_2)Tyr(SO_3^-)-Nle-Gly-; radioligand is [^{125}I]Bolton-Hunter-CCK-8. [b] Selected data from ref 10; Y = Boc-Tyr(SO_3^-)-Nle-Gly-; radioligand is [^{3}H]propionyl-CCK-8.

compounds 1 (the unmethylated parent) and A-71623, the corresponding (*N*-Me)Phe analogue. Mono- or di-N-methylation of the C-terminal carboxamide (compounds 2 and 3) caused respectively 45- and 30-fold decreases in affinity for the CCK-A receptor relative to 1, accompanied by substantial reductions in functional efficacy. In contrast, analogues incorporating either (*N*-Me)Asp (4, A-74498) or (*N*-Me)Asp-(N-Me)Phe (5) showed high affinity for pancreatic CCK-A receptors, with binding affinity roughly equivalent to that of 1 and A-71623.[12] In addition, enhanced CCK-A selectivity relative to A-71623 was observed for 4 (>2300-fold), whereas the receptor selectivity of compound 5 was decreased. Compounds 4 and 5 were full agonists in stimulation of PI turnover.

Relative to the unmethylated parent 1, the (N^{α}-Me)Lys analogue 6 possessed ca. 20-fold lower binding affinity to CCK-A receptors, but 80% of the functional efficacy in PI hydrolysis was maintained. The (*N*-Me)Trp analogue 7 had 150-fold lower CCK-A binding affinity and a substantial decrease in functional efficacy compared to the parent. On the other hand, removal of the Boc group from 7 resulted in compound 8, which regained high affinity and nearly full efficacy at CCK-A receptors. The combination of either Boc(*N*-Me)Trp (9) or H-(*N*-Me)Trp (10) with (*N*-Me)Asp had little effect on the binding activity of these analogues compared to 7 and 8, respectively, although compound 9 did suffer a further loss of efficacy in PI hydrolysis compared to 7.

Analogues with favorable *in vitro* profiles were evaluated for activity in suppression of food intake in food-deprived rats. The results are presented in Table 3. The compounds containing (*N*-Me)Phe (A-71623), (*N*-Me)Asp (4), or (*N*-Me)Asp-(*N*-Me)Phe (5) showed 3–8-fold improvements in *in vivo* activity compared to 1. Replacement of the N-terminal Boc group of 1 with a methyl group (compound 8) resulted in ca. 25-fold weaker activity in the feeding assay; however, potent suppression of food intake was recovered in the corresponding (*N*-Me)Asp analogue (compound 10). Thus, in contrast to the inconsequential effect of the (*N*-Me)Asp residue on *in vitro* activity at pancreatic CCK-A receptors, this modification has a pronounced beneficial effect *in vivo* when the N-terminus is not protected by acylation. A possible interpretation is that the (*N*-Me)Asp residue influences the pharmacokinetic properties of the molecule, perhaps by conferring stability against degradation at or near the N-terminus.

Comparison with Sulfated CCK Series. To allow ready comparison with data in Table 2, selected data from previous studies on N-methylated CCK heptapeptide analogues are reproduced in Table 4. In both series of compounds, analogues with comparable affinity for CCK-A receptors relative to the unmethylated parent peptides were obtained after N-methylation at Phe (A-71623 vs 1 and 12 vs. 11) or at Asp (4 vs 1 and 13 vs 11). Similarly, N^{α}-methylation at Lys in the tetrapeptide series (6 vs 1) or at Leu^{31} in the heptapeptide (15 vs 14) had comparable effects on CCK-A binding, i.e., ca. 20- and 30-fold lower affinity, respectively. Compared to the ca. 40-fold lower affinity resulting from mono-N-methylation of the C-terminal carboxamide in the tetrapeptide series (2 vs 1), the corresponding modification in a heptapeptide series (17 vs 16) was found to cause a comparable reduction (ca. 20-fold) in binding affinity for the pancreatic CCK receptor relative to the unmethylated parent. Thus, N-methylations at positions 31, 32, and 33 and the C-terminal carboxamide within the tetrapeptide and heptapeptide series had comparable effects on CCK-A binding affinity with respect to both direction and magnitude. These results lend support to the hypothesis that the C-terminal regions of the two distinct series of peptides are interacting in similar fashions with the CCK-A receptor.[15]

Conclusion

N-Methylated analogues of a previously identified series of potent and selective CCK-A tetrapeptide agonists were prepared. Methylation at the position corresponding to Asp^{32} was consistent with high affinity, efficacy, and selectivity for the CCK-A receptor, and the combination

of this modification with (*N*-Me)Phe also produced a highly active analogue. Parallel structure–activity profiles of the tetrapeptide and heptapeptide series with respect to N-methylation at positions 31, 32, and 33 suggest that the C-terminal regions of the two series are interacting similarly with the CCK-A receptor.

Experimental Section

Reagents, standard reaction workup conditions, and standard analytical procedures were as described previously.[7] NMR spectra were obtained at 300 MHz unless otherwise noted. Purity of final compounds is based on satisfactory agreement (±0.4%) of results from combustion analyses with theoretical values calculated from the formulas in Table 1, except where noted. In the case of compound 8, purity was determined to be >98% based on ^{1}H-NMR (500 MHz) and analytical HPLC data.

General Synthetic Procedures. A. Boc Deprotection with Trifluoroacetic Acid (TFA). The Boc-protected amine is allowed to stand at room temperature in the presence of 1:1 TFA/CH_2Cl_2 for ca. 1 h, the volatile components are evaporated, and the residue is treated with dry Et_2O. If a filterable solid is obtained, the precipitate is collected by filtration, washed with dry Et_2O, and dried under vacuum. Otherwise the ether is evaporated and the residue is dried under high vacuum and used directly in the subsequent step.

B. Boc Deprotection with HCl/Dioxane. The Boc-protected amine is allowed to stand at room temperature in the presence of 4 N HCl/dioxane for ca. 1 h, the volatile components are evaporated, and the residue is subsequently processed as described in general procedure A.

C. Peptide Coupling Using 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide Hydrochloride (EDC) and 1-Hydroxybenzotriazole Hydrate (HOBt). A solution of the acid component (1 mmol), the amine (1 mmol) (or amine salt (1 mmol) plus tertiary amine base (1 mmol)), and HOBt (1 mmol) in the specified solvent (2-4 mL) at 0 °C is treated with EDC (1.1 mmol), and the solution is allowed to warm slowly to ambient temperature and stir overnight (or until complete by TLC), and then it worked up as is specified.

D. Formation of Symmetrical Anhydrides. A solution of the acid component (2 mmol) in CH_2Cl_2 (10–20 mL) at 0 °C is treated with EDC (1 mmol). After being stirred 0.5-1 h, the solution is used directly in the coupling step.

E. Hydrogenolysis of Benzyl Esters. A solution of the peptide benzyl ester (100-200 mg) in MeOH (25 mL) is shaken in the presence of 10% Pd/C under 4 atm of H_2. When the reaction is judged complete by TLC, the catalyst is removed by filtration and the solvent is evaporated to afford the crude product.

H-Phe-NHMe Trifluoroacetate (18). Boc-Phe-OH (1.0 g, 3.8 mmol) and methylamine hydrochloride (0.26 g, 4.0 mmol) were coupled in CH_2Cl_2 using general procedure C with DIEA as base. Standard extractive workup provided 964 mg of the crude amide. A portion (931 mg) was deprotected according to general procedure A to afford 681 mg (64% overall) of a white powder: ^{1}H NMR (DMSO-d_6) δ 2.60 (d, J = 4 Hz, 3H), 2.98 (m, 2H), 3.90 (t, J = 7.5 Hz, 1H), 7.21 (m, 2H), 7.32 (m, 3H), 8.19 (br m, 3H), 8.30 (m, 1H); MS (CI) m/e 179 (M + H$^+$), 196 (M + NH_4^+).

Boc-Trp-Lys(CONH-Ph-*o*-Me)-Asp(OBn)-OH (19). A solution of compound **22** (21.5 g, 38 mmol) and *N*-hydroxysuccinimide (4.6 g, 39.9 mmol) in DMF (100 mL) at 0 °C was treated with *N*,*N*-dicyclohexylcarbodiimide (8.23 g, 39.9 mmol). The reaction was stirred for 1 h at 0 °C and 1 h at ambient temperature. The solution was filtered, and the filtrate was added to a suspension of H-Asp(OBn)-OH (11.0 g, 49.4 mmol) and NEt_3 (10.6 mL, 76 mmol) in DMF (40 mL). The reaction was stirred for 1 h, filtered, and concentrated. The residue in EtOAc was washed with 10% citric acid and brine, and the organic layer was dried (Na_2SO_4). The product was crystallized from EtOAc/heptane to afford 32.5 g of impure product. Further purification was effected by chromatography (silica gel, CH_2Cl_2/MeOH (19:1 to 3:1)) followed by crystallization from EtOAc/hexane to afford 24.2 g (83%) of the title compound: NMR (DMSO-d_6) δ 1.10 (m, 2H), 1.28 (s, 9H), 1.40 (m, 2H), 1.62 (m, 2H), 2.20 (m, 1H), 2.21 (s, 3H), 2.53 (dd, J = 5.5, 15 Hz, 1H), 2.82 (dd, J = 6, 15 Hz, 1H), 3.0 (m, 1H), 3.13 (m, 2H), 4.20 (m, 1H), 4.32 (m, 1H), 4.45 (m, 1H), 5.20 (s, 2H), 6.82 (m, 1H), 6.92–7.13 (m, 8H) 7.26–7.49 (m, 6H), 7.60 (d, J = 7.5 Hz, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 7.5 Hz, 1H), 8.35 (br s, 1H), 10.80 (br s, 1H); MS (FAB$^+$) m/e 809 (M + K$^+$), 709 (M − Boc + K$^+$), 671 (M − Boc + H$^+$).

Boc-Trp-Lys(CONH-Ph-*o*-Me)-Asp-Phe-NHMe (2). To a solution of amine salt 18 (50 mg, 0.17 mmol), tripeptide acid **19** (132 mg, 0.17 mmol), diisopropylethylamine (DIEA) (0.089 mL, 0.51 mmol), and CH_2Cl_2 (3 mL) at 0 °C was added bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) (43 mg, 0.17 mmol). After being stirred for 4 h at 0 °C, the mixture was allowed to warm to room temperature and stir overnight. After standard acid–base workup, the crude product was chromatographed (silica gel, 4% MeOH/$CHCl_3$) to afford 56 mg of protected tetrapeptide. A 52-mg sample was subjected to catalytic transfer hydrogenolysis[16] with ammonium formate in MeOH in the presence of 10% Pd–C. The mixture was filtered and evaporated and then purified by reverse-phase HPLC (C_{18}, gradient mixtures of CH_3CN and 50 mM NH_4OAc, pH 4.5). Pure fractions were combined and lyophilized twice to afford 17 mg of pure product: ^{1}H NMR (DMSO-d_6) δ 1.1–1.7 (m, 15H), 2.17 (s, 3H), 2.55 (d, J = 4 Hz, 3H), 2.60–3.15 (m, 8H), 4.25 (m, 2H), 4.35 (m, 1H), 4.49 (m, 1H), 6.61 (br s, 1 H), 6.84 (m, 2H), 6.95 (m, 1H), 7.01–7.13 (m, 4H), 7.15–7.28 (m, 6H), 7.31 (d, J = 9 Hz, 1H), 7.60 (d, J = 9 Hz, 1H), 7.82 (m, 2H), 7.96 (m, 2H), 8.23 (m, 1H), 10.81 (s, 1H).

H-Phe-NMe_2 Trifluoroacetate (20). This compound was prepared from Boc-Phe-OH and dimethylamine hydrochloride by procedures analogous to those described for preparation of 18: ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H), 2.79 (s, 3H), 2.99 (m, 2H), 4.58 (t, J = 7.5 Hz, 1H), 7.20 (m, 2H), 7.32 (m, 3H), 8.15 (br s, 3H); MS (CI) m/e 193 (M + H$^+$).

H-Asp(OBn)-Phe-NMe_2 Hydrochloride (21). Boc-Asp(OBn)-OH (158 mg, 0.49 mmol) was coupled to **20** (150 mg, 0.49 mmol) in CH_2Cl_2 according to general procedure C using *N*-methylmorpholine (NMM) as base. The crude product from acid–base workup was subjected to general procedure A, which did not afford a solid product. A solution of HCl in dioxane was added to provide the HCl salt which solidified upon addition of anhydrous Et_2O. Collection by filtration afforded 185 mg of white solid: ^{1}H NMR (DMSO-d_6) δ 2.75 (s, 3H), 2.78–2.90 (m, 5H, includes 2.81, s, 3H), 2.97 (m, 2H), 4.12 (m, 1H), 4.89 (m, 1H), 5.15 (m, 1H), 7.25 (m, 5H), 7.40 (m, 5H), 8.22 (br m, 3H), 8.88 (d, J = 8 Hz, 1H); MS (CI) m/e 398 (M + H$^+$).

Boc-Trp-Lys(CONH-Ph-*o*-Me)-OH (22). A solution of H-Lys(Cbz)-OH (3.84 g, 13.7 mmol) in MeOH (200 mL) and H_2O (50 mL) was treated with *N*-benzyltrimethylammonium hydroxide (5.75 g of 40 wt % solution in MeOH, 13.7 mmol). The mixture was stirred until the solution became clear, and then the solvents were evaporated. The residue was dissolved in DMF (120 mL), and Boc-L-tryptophan succinimidyl ester (Boc-Trp-OSu, 5.0 g, 12.46 mmol) was added. After 18 h at ambient temperature, the solvent was evaporated in vacuo, 10% aqueous citric acid (100 mL) was added, and the aqueous layer was extracted with EtOAc (150 mL, then 75 mL). The combined organic layers were washed with brine, dried, and concentrated to afford the crude product as a yellow oil. Recrystallization from EtOAc/heptane afforded 6.2 g (88%) of Boc-Trp-Lys(Cbz)-OH as white solid. This didpeptide (6.05 g, 10.7 mmol) was dissolved in DMF (100 mL) and stirred under an atmosphere of H_2 in the presence of 10% Pd/C (1.21 g) for 3 h, after which the catalyst was removed by filtration. The filtrate was then cooled to 0 °C and treated with NMM (1.29 mL, 11.8 mmol) and o-tolyl isocyanate (1.46 mL, 11.8 mmol), and then the reaction mixture was allowed to warm to ambient temperature. After stirring overnight the solvent was evaporated and the residue was partitioned between EtOAc (500 mL) and H_2O (200 mL). The organic layer was washed with 10% citric acid, H_2O, and brine, dried ($MgSO_4$), and concentrated. The residue was recrystallized from EtOAc/heptane to afford 4.77 (79%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 1.11–1.52 (m, 6H), 1.30 (s, 9H), 1.57–1.85 (m, 2H), 2.16 (s, 3H), 2.84–2.96 (m, 1H), 3.02–3.14 (m, 3H), 4.19–4.30 (m, 2H), 6.25 (br d, 1H, J = 9 Hz), 6.54 (t, 1H, J = 4.5 Hz), 6.74 (d, 1H, J = 9 Hz), 6.84 (t, 1H, J = 7.5 Hz), 6.97 (t, 1H, J = 7.5 Hz), 7.02–7.20 (m, 4H), 7.32 (d, 1H, J = 9 Hz), 7.56 (s, 1H), 7.61 (d, 1H, J = 9 Hz),

7.82 (d, 1H, J = 7.5 Hz), 8.11 (d, 1H, J = 7.5 Hz), 10.81 (br s, 1H), 12.66 (br s, 1H); MS (FAB$^+$) m/e 566 (M + H$^+$), 588 (M + Na$^+$). Anal. Calcd for $C_{50}H_{61}N_8O_9 \cdot 1.5H_2O$: C, H, N.

Boc-Trp-Lys(CONH-Ph-*o*-Me)-Asp-Phe-NMe$_2$ (3). A solution of amine salt **21** (90 mg, 0.21 mmol), dipeptide acid **22** (117.3 mg 0.21 mmol), HOBt.H_2O (32 mg, 0.21 mmol), and DIEA (0.037 mL, 0.21 mmol) in 1:1 THF/CH_2Cl_2 (5 mL) at −20 °C was treated with EDC (40 mg, 0.21 mmol). After being kept at −20 °C for 2 days, the solution was allowed to warm to room temperature. The solvent was evaporated, and the mixture was subjected to standard acid–base extractive workup to afford 197 mg of the crude protected tetrapeptide. Hydrogenolysis of a 155-mg sample according to general procedure E was followed by chromatography (silica gel, 56:3:2:1 EtOAc/pyridine/H_2O/HOAc). Pure fractions were combined, concentrated, diluted with H_2O, and lyophilized twice to afford 65 mg of the title compound: ^{1}H NMR (DMSO-d_6) δ 1.12 (m, 1H), 1.30 (s, 9H), 1.42 (m, 3H), 1.54 (m, 1H), 1.68 (m, 1H), 2.15 (s, 3H) 2.48 (1H, obscured), 2.62 (dd, J = 3, 9 Hz, 1H), 2.8 (dd, J = 4, 8 Hz, 1H), 2.92 (dd, J = 4, 9 Hz, 1H), 3.02–3.18 (m, 3H), 3.30 (1H, obscured), 4.23 (m, 1H), 4.3 (m, 1H), 4.54 (q, J = 3 Hz, 1H), 4.83 (q, J = 4 Hz, 1H), 6.59 (br s, 1H), 6.79 (d, 5 Hz, 1H), 6.85 (t, J = 4.5 Hz, 1H), 7.02–7.13 (m, 5H), 7.15–7.21 (m, 4H), 7.23 (m, 2H), 7.31 (d, J = 5 Hz, 1H), 7.59 (d, J = 5 Hz, 1H), 7.65 (br s, 1H), 7.80 (m, 1H), 7.95 (m, 2H), 8.22 (d, J = 4.5 Hz, 1H), 10.78 (s, 1H).

Fmoc-(*N*-Me)Asp(OBn)-Phe-NH$_2$ (23). A solution of Fmoc-(*N*-Me)Asp(OBn)-OH[7] (4.45 g, 9.69 mmol), H-Phe-NH_2 (1.6 g, 9.69 mmol), and NMM (1.1 mL, 10 mmol) in DMF at 0 °C was treated with benzotriazol-1-yltris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent),[17] and the mixture was allowed to warm to ambient temperature and stir overnight. The mixture was diluted with EtOAc and subjected to standard acid–base workup to afford 2.88 g (49%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 2.13 (s) and 2.15 (s) (N-CH_3), 2.45 (m, 1H), 2.85 (m, 2H), 3.04 (dd, J = 4.5, 13.5 Hz, 1H), 4.15–4.35 (m, 3H), 4.46 (m, 1H), 4.95 (m, 1H), 5.06 (m, 2H), 7.05–7.50 (m, 16H), 7.63 (m, 2H), 7.83 (d, J = 7.5 Hz, 0.5H), 7.91 (m, 1.5H), 8.08 (d, J = 7.5 Hz, 0.5H), 8.19 (d, J = 8 Hz, 0.5H); MS (FAB) m/e 606 (M + H$^+$).

Boc-Lys(CONH-Ph-*o*-Me)-OH (24). To a solution of N^α-Boc-Lys-OH (1.0 g, 4.1 mmol) in H_2O (5 mL), dioxane (7 mL), and 2 N NaOH (2 mL) at 0 °C were added in portions o-tolyl isocyanate (1.12 g, 8.4 mmol) and additional 2 N NaOH (4.2 mL, 8.4 mmol). The mixture was stirred, allowed to warm to ambient temperature, and then made basic with additional aqueous NaOH. The solution was washed with EtOAc, then acidified with aqueous $KHSO_4$. The solution was again extracted with EtOAc, and the latter organic phase was dried (Na_2SO_4) and concentrated. The residue was crystallized from Et_2O/hexane to afford 1.2 g of product: NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.30–1.55 (m, 3H), 1.70–1.90 (m, 3H), 2.28 (s, 3H), 3.22 (m, 2H), 4.29 (m, 1H), 4.90 (br m, 2H), 5.23 (br d, J = 7.5 Hz, 1H), 7.10–7.31 (m, 4H); MS (CI) m/e 380 (M + H)$^+$.

Boc-Lys(CONH-Ph-*o*-Me)-(*N*-Me)Asp(OBn)-Phe-NH$_2$ (25). Compound **23** (510 mg, 0.84 mmol) was treated with a solution of 50% diethylamine in CH_3CN for 40 min, and then the volatile components were evaporated under reduced pressure. Additional CH_3CN was added and evaporated to afford 438 mg of crude free amine. A 225-mg portion of the crude product in 2 mL of DMF at 0 °C was treated with the symmetrical anhydride prepared from **24** (407 mg, 1.075 mmol) according to general procedure C. The solution was allowed to warm to room temperature and stir for 18 h. After extractive workup, the crude product was chromatographed (silica gel, 5% MeOH/$CHCl_3$) to afford 160 mg (50%) of pure protected tripeptide. NMR ($CDCl_3$) showed a mixture of conformers, ca. 2:1; selected resonances are provided: δ 1.32 and 1.42 (Boc), 2.08 and 2.48 (N-Me), 2.15 and 2.16 (Ar-CH_3), 4.15, 4.38, 4.48, 4.53, 5.22 (α-protons), 5.05 (m, 2H, Ph-CH_2); MS (FAB$^+$) m/e 745 (M + H$^+$), 767 (M + Na$^+$).

Boc-Trp-Lys(CONH-Ph-*o*-Me)-(*N*-Me)Asp-Phe-NH$_2$ (4). Compound **25** (86 mg, 0.115 mmol) was Boc-deprotected according to general procedure A. The residue in CH_2Cl_2 (2 mL) at 0 °C was treated with DIEA (0.022 mL, 0.126 mmol) and Boc-Trp-OSu (51 mg, 0.126 mmol). Additional base was added until the solution caused moist pH paper to turn green, and then the mixture was allowed to warm to ambient temperature and stir overnight. After standard extractive workup, the crude product was subjected to hydrogenolysis according to general procedure E, followed by chromatography (silica gel, 56:3:2:1 EtOAc/pyridine/H_2O/HOAc). Pure fractions were combined, concentrated, diluted with H_2O, and lyophilized to afford 42 mg of pure product; ^{1}H NMR (DMSO-d_6) (two conformers) δ 1.05–1.9 (m, 15H, includes 1.30 (s) and 1.31 (s), total 9H), 2.10–2.25 (m, 5H, includes 2.15 (s) and 2.20 (s)), 2.58 (m, 1.5H), 2.70 (dd, J = 6, 9 Hz, 0.5H), 2.75–3.2 (m, 3H), 3.41 (0.5H, obscured), 4.21 (m, 0.5H), 4.32 (m, 1H), 4.40 (m, 0.5H), 4.59 (br m, 1H), 4.90 (m, 0.5H), 5.08 (m, 0.5H), 6.67 (m, 0.5H), 6.82 (m, 1H), 6.87 (m, 0.5H), 6.95 (m, 1H), 7.05 (m, 4H), 7.11–7.28 (m, 5.5H), 7.30 (d, J = 5 Hz, 1H), 7.45 (s, 0.5H), 7.50 (s, 0.5H), 7.57 (t, J = 4 Hz, 1H), 7.18 (d, J = 5 Hz, 0.5H), 7.81 (d, J = 5 Hz, 0.5H), 8.02 (d, J = 5 Hz, 0.5H), 8.10 (br m, 0.5H), 8.26 (br m, 0.5H), 8.35 (m, 1H), 8.45 (br m, 0.5H), 8.59 (br m, 0.5H), 8.81 (br m, 0.5H), 10.8 (s, 0.5H), 10.97 (br s, 0.5H); MS (FAB$^+$) m/e 841 (M + H$^+$), 741, 577.

H-(*N*-Me)Phe-NH$_2$ Trifluoroacetate (26). A solution of Boc-(*N*-Me)Phe-OH (3.0 g, 10.75 mmol) and NMM (1.2 mL, 10.75 mmol) in THF (25 mL) at −20 °C was treated with isobutyl chloroformate (1.4 mL, 10.75 mmol) dropwise over 10 min. After the mixture was stirred an additional 5 min, concentrated NH_4OH (5 mL, prechilled to −15 °C) was added in one portion. The mixture was allowed to warm to ambient temperature and then was concentrated to remove THF. The residual mixture was subjected to standard acid–base workup to provide a crude product which was deprotected according to general procedure A. A solid product was obtained (quantitative yield) after treatment with Et_2O/hexane: NMR (CD_3OD) δ 2.65 (s, 3H), 3.18 (d, J = 7 Hz, 2H), 4.01 (t, J = 7 Hz, 1H), 7.31 (m, 5H); MS (CI) m/e 179 (M + H$^+$).

Fmoc-(*N*-Me)Asp(OBn)-(*N*-Me)Phe-NH$_2$ (27). Compound **26** (344 mg, 1.18 mmol), Fmoc-(*N*-Me)Asp(OBn)-OH[7] (540 mg, 1.18 mmol), and NEt_3 (538 mL, 3.89 mmol) were combined in CH_2Cl_2 at 0 °C, and then BOP-Cl was added. The mixture was allowed to warm to room temperature and stir overnight. The solution was diluted with EtOAc and subjected to standard acid–base extractive workup. The crude product was chromatographed (silica gel, 2:1 hexane/acetone) to afford 360 mg (49%) of the title compound: ^{1}H NMR ($CDCl_3$) (multiple conformers) δ 1.9–3.0 (m, 11H, methyl singlets at 1.92, 2.09, 2.21, and 2.82), 3.12 (m, 1H), 3.40 (m, 1H), 4.19 (m, 1H), 4.35–4.45 (m, 2H), 4.6–4.8 (m, 1H), 4.98–5.18 (m, 2H), 5.32–5.68 (m, 2H), 6.08–6.29 (m, 1H), 6.98–7.62 (m, 15H), 7.65–7.87 (m, 3H); MS (FAB$^+$) m/e 642 (M + Na$^+$).

Boc-Trp-Lys(CONH-Ph-*o*-Me)-(*N*-Me)Asp-(*N*-Me)Phe-NH$_2$ (5). Dipeptide **27** was converted to the title compound by a series of reactions analogous to those described for preparation of **4** from didpeptide **23**: ^{1}H NMR (DMSO-d_6) δ 1.15 (m, 1H), 1.20–1.35 (m, 10H, includes 1.30 (s, 9H)) 1.35–1.52 (m, 4H), 2.0 (dd, J = 2, 10 Hz, 1H), 2.06 (s, 3H), 2.17 (m, 4H, includes s, 3H), 2.75 (m, 1H and s, 3H), 2.90 (m, 2H), 3.04 (dd, J = 3, 9 Hz, 1H), 3.1 (m, 1H), 3.21 (dd, J = 3, 9 Hz, 1H), 4.2 (m, 1H), 4.42 (q, J = 4 Hz, 1H), 5.33 (dd, J = 2, 7 Hz, 1H), 5.51 (dd, J = 3, 9 Hz, 1H), 6.58 (t, J = 3 Hz, 1), 6.73 (d, J = 5 Hz, 1H), 6.85 (t, J = 4 Hz, 1H), 6.95 (t, J = 5 Hz, 1H), 7.08 (m, 6H), 7.15–7.40 (m, 9H), 7.59 (m, 2H), 7.83 (d, J = 5 Hz, 1H), 7.95 (d, J = 5 Hz, 1H), 10.80 (s, 1H).

Cbz-(*N*-Me)Lys(phthaloyl)-OH (28). A solution of N^α-Cbz-Lys-OH (2.02 g, 7.2 mmol), *N*-carbethoxyphthalimide (1.73 g, 7.9 mmol), and NEt_3 (1.1 mL, 7.9 mmol) in 20 mL of CH_2Cl_2 was stirred at ambient temperature for 3 days. The solvent was evaporated, and the residue was chromatographed (silica gel, 550:20:11:6 EtOAc/pyridine/HOAc/H_2O). Pooled fractions were concentrated, diluted with H_2O, and lyophilized to afford 2.8 g of Cbz-Lys(phthaloyl)-OH:MS (CI) m/e 411 (M + H$^+$), 428. Via the literature procedure,[11] a solution of the bis-N-protected lysine (2.1 g, 5.3 mmol), *p*-toluenesulfonic acid (0.1 g), and paraformaldehyde (1.0 g) in toluene was heated under reflux with azeotropic removal of H_2O. After 24 h, additional paraformaldehyde (1.0 g) was added, and heating was continued for an additional 24 h. The mixture was allowed to cool, diluted with EtOAc, washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated to a residue (1.38 g), which was

dissolved in $CHCl_3$ (15 mL) and treated with triethylsilane (1.53 mL, 9.6 mmol) and trifluoroacetic acid (5 mL). After standing at ambient temperature for 44 h, the volatile components were evaporated, and the acidic component was isolated by normal extractive procedures to afford 1.32 (61%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 1.39 (m, 2H), 1.62–1.90 (m, 3H), 2.02 (m, 1H), 2.90 (s, 3H), 3.68 (m, 2H), 4.62 (m, 0.5H) and 4.77 (m, 0.5H), 5.15 (s, 2H), 7.32 (m, 5H), 7.71 (m, 2H), 7.83 (m, 2H); MS (CI) *m/e* 442 (M + NH_4^+).

Boc-(*N*-Me)Lys(CONH-Ph-*o*-Me)-OH (29). A solution of **28** (1.25 g, 3.1 mmol) in 4 mL of MeOH was added to a suspension of 100 mg of 10% Pd/C in 4 mL of MeOH, and the mixture was stirred under an atmosphere of H_2 for 18 h. The resultant mixture was diluted with 8 mL of H_2O, treated with NEt_3 (457 mL, 3.3 mmol) and di-*tert*-butyl dicarbonate (720 mg, 3.3 mmol), and stirred overnight. The mixture was diluted with aqueous HOAc and filtered, and the filtrate was concentrated. The remaining aqueous solution was extracted with EtOAc, and the combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to 280 mg of oily residue. A 239-mg (0.64-mmol) sample of the product was dissolved in absolute EtOH and treated with 34 mL (0.7 mmol) of hydrazine hydrate. The solution was heated under reflux for 1 h, whereupon an additional 34 mL of hydrazine hydrate was added and heating under reflux was continued for 2 h. The mixture was concentrated under vacuum, diluted with aqueous HOAc, and filtered to remove precipitated phthalhydrazide. The filtrate was washed with EtOAc and lyophilized to afford 190 mg of white powder, which was dried under reduced pressure at 50 °C. A 185-mg (0.76-mmol) portion of the product was suspended in dry DMF and treated with NEt_3 (115 mL, 0.83 mmol) and *o*-tolyl isocyanate (104 mL, 0.83 mmol). The mixture was stirred for 1 h, treated with additional NEt_3 (115 mL, 0.83 mmol) and *o*-tolyl isocyanate (104 mL, 0.83 mmol), and stirred for an additional 0.25 h, whereupon the mixture was diluted with EtOAc and aqueous $NaHCO_3$. The layers were mixed and separated, and then the aqueous layer was acidified with aqueous $KHSO_4$ and extracted with EtOAc. The organic layer containing acidic product was dried over Na_2SO_4 and evaporated to 258 mg of crude product, which was chromatographed (silica gel, 10:9:1 hexane/EtOAc/HOAc) to afford 125 mg of pure product: ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 2H), 1.45 (s, 9H), 1.52 (m, 1H), 1.75 (m, 1H), 1.97 (m, 1H), 2.29 (s, 3H), 2.80 (br s, 3H), 3.22 (m, 2H), 4.43 (m, 0.4 H), 4.70 (m, 1.6H), 7.0 (s, 1H), 7.07–7.35 (m, 4H); MS (CI/NH_3) *m/e* 394 (M + H)$^+$, 411.

H-(*N*-Me)Lys(CONH-Ph-*o*-Me)-Asp(OBn)-Phe-NH_2 Trifluoroacetate (30). A solution of **29** (53 mg, 0.135 mmol) and NMM (0.015 mL, 0.135 mmol) in THF (0.7 mL) at −15 °C was treated with isobutyl chloroformate (0.017 mL, 0.132 mmol), and the mixture was stirred for 4 min before a chilled solution of the trifluoroacetate salt of H-Asp(OBn)-Phe-NH_2[18] (98 mg, 0.2 mmol) in DMF (0.5 mL) was added, followed by addition of DIEA (0.035 mL, 0.2 mmol). The mixture was allowed to warm to ambient temperature and stir overnight and was then subjected to standard acid–base workup to afford the crude product in quantitative yield. Treatment with 1:1 TFA/CH_2Cl_2 at room temperature for 1 h, followed by evaporation of volatile components and precipitation of the product with anhydrous Et_2O, provided 85 mg (85%) of the title compound: ^{1}H NMR (DMSO-d_6) δ 1.25 (m, 2H), 1.40 (m, 2H), 1.68 (m, 2H), 2.18 (s, 3H), 2.38 (s, 3H), 2.63 (dd, J = 9, 16.5 Hz, 1H), 2.80 (dd, J = 9, 14 Hz, 1H), 2.90 (dd, J = 4.5, 16.5 Hz, 1H), 3.01 (m, 2H), 3.61 (m, 1H), 4.42 (m, 1H), 4.78 (m, 1H), 5.10 (s, 2H), 6.52 (m, 1H), 6.86 (t, J = 15 Hz, 1H), 7.05–7.30 (m, 8H), 7.38 (m, 5H), 7.45 (s, 1H), 7.60 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 8.88 (d, J = 8 Hz, 1H); MS (FAB$^+$) *m/e* 659 (M + H)$^+$, 681 (M + Na)$^+$.

Boc-Trp-(*N*-Me)Lys(CONH-Ph-*o*-Me)-Asp-Phe-NH_2 (6). According to general procedure D, Boc-Trp-OH (69 mg, 0.228 mmol) was converted to the symmetrical anhydride, which was added to compound **30** (43 mg, 0.057 mmol) and DIEA (0.010 mL, 0.06 mmol) in a solution of 1:1 CH_2Cl_2/DMF. Extractive workup and chromatography (silica gel, 4:96 MeOH/$CHCl_3$) afforded 40 mg of protected tetrapeptide. Catalytic hydrogenolysis (H_2, 10% Pd–C, MeOH) of 36 mg of the above product, followed by recrystallization of the crude product from MeOH/H_2O, afforded 22 mg of the title compound: ^{1}H NMR (DMSO-d_6, 100 °C) δ 1.15–1.40 (m, 11H, includes d 1.30, s, 9H), 1.45 (br s, 2H), 1.60 (br s, 1H), 1.80 (br s, 1H), 2.18 (s, 3H), 2.5 (1H, obscured), 2.65 (m, 1H), 2.8–3.5 (9H, obscured), 4.45 (q, J = 3 Hz, 1H), 4.55 (q, J = 4.5 Hz, 1H), 4.66 (q, 4 Hz, 1H), 4.90 (br s, 1H), 6.22 (br s, 1H), 6.38 (br s, 1H), 6.87 (t, J = 4.5 Hz, 1H), 6.93 (t, J = 4.5 Hz), 7.05–7.15 (m, 4H), 7.20 (m, 4H), 7.33 (d, J = 5 Hz, 1H), 7.39 (s, 1H), 7.55 (m, 2H), 7.71 (d, J = 4.5 Hz, 1H); ^{1}H NMR (DMSO-d_6, 65 °C) reveals N-CH_3 at δ 2.87; MS (FAB$^+$) *m/e* 863, 841, 741.

Boc-(*N*-Me)Trp-OH (31). To a solution of L-abrine (5.0 g, 23 mmol) in water/dioxane (1:1) was added DIEA (9 mL) and di-*tert*-butyl dicarbonate (6 g, 27.6 mmol) and the mixture was stirred overnight at ambient temperature. The solvents were removed under reduced pressure, and resulting residue was diluted with water and acidified to pH 2 using 1 N HCl. The mixture was extracted with ethyl acetate, and the combined ethyl acetate extracts were washed successively with water and brine and then dried ($MgSO_4$) to give 7.26 g of the title compound as a white foamy product which was carried on to the next step without purification: ^{1}H NMR (DMSO-d_6) δ 1.06 (s, 9H), 2.65 (br s, 3H), 3.02–3.30 (m, 2H), 4.28 (m, 2H), 6.98 (t, J = 9 Hz, 1H), 7.1 (m, 3H), 7.32 (d, J = 12 Hz, 1H), 7.53 (d, J = 12 Hz, 1H), 10.82 (br s, 1H), 12.85 (br s, 1H); MS (FAB$^+$) *m/e* 319 (M + H)$^+$.

Boc-(*N*-Me)Trp-Lys(Cbz)-Asp(OBn)-Phe-NH_2 (32). A solution of acid **31** (0.88 g, 2.76 mmol), H-Lys(Cbz)-Asp(OBn)-Phe-NH_2 hydrochloride[4] (1.74 g, 2.76 mmol), and BOP-Cl (0.9 g, 3.5 mmol) in methylene chloride (20 mL) and DMF (5 mL) was cooled to 0 °C, and NMM (0.66 mL, 6 mmol) was added. The reaction mixture was allowed to warm to ambient temperature and stir for 48 h. Another portion of BOP-Cl (0.65 g, 1.0 mmol) was added, and stirring was continued for 18 h. The solvents were removed *in vacuo*, and the residue was taken up in EtOAc (300 mL) and washed with 1 M H_3PO_4, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was flash chromatographed (silica gel, 2:98 MeOH/$CHCl_3$) to give 1.03 g (37%) of the title compound: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.29 (s, 9H), 1.38–1.8 (m, 6H), 2.74 (m, 3H), 2.98–3.15 (m, 6H), 3.16–3.40 (m, 2H), 4.30 (m, 1H), 4.68 (m, 1H), 4.84–4.93 (m, 2H), 5.04 (s, 2H), 5.11 (s, 2H), 6.60 (br s, 1H), 6.98 (m, 2H), 7.51 (m, 2H), 7.10–7.40 (m, 18H), 7.57 (d, J = 7 Hz, 1H), 7.89 (d, J = 7 Hz, 1H), 8.37 (d, J = 7 Hz, 1H), 10.43 (br s, 1H); MS (FAB$^+$) *m/e* 832 (M + H − Boc)$^+$.

Boc-(*N*-Me)Trp-Lys(CONH-Ph-*o*-Me)-Asp-Phe-NH_2 (7). A solution of **32** (90 mg, 0.097 mmol) in glacial acetic acid (5 mL) was stirred in the presence of 10% Pd–C (0.06 g) under 1 atm of hydrogen for 3.5 h. The reaction mixture was filtered through Celite, and the solvent was reduced in vacuo to a volume of 1–2 mL. Diethyl ether (100 mL) was added to the acetic acid solution to precipitate Boc-(*N*-Me)Trp-Lys-Asp-Phe-NH2 (72 mg, 100%) as a slightly pink powder: MS (FAB$^+$) *m/e* 707 (M + H)$^+$. A solution of this tetrapeptide (62 mg, 0.09 mmoL) and NMM (0.017 mL, 0.15 mmol) in DMF (5 mL) was treated with o-tolyl isocyanate (0.15 mL, 0.15 mmol) and stirred at ambient temperature for 18 h. The DMF was evaporated in vacuo, and the residue was directly applied to a silica gel column and eluted with ethyl acetate–pyridine–acetic acid–water (260:20:6:1). After evaporation of solvents, the residue was dissolved in water–acetone (10:1, v:v), lyophilized, and further dried (50 °C) to yield 34 mg (45%) of the title compound as a white solid: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.04–1.73 (m, 6H), 1.30 (br s, 9H), 2.17 (s, 3H), 2.42–2.68 (m, 1H), 2.80–2.90 (m, 1H), 2.98–3.31 (m, 6H), 2.69 (s, 3H), 4.28 (m, 1H), 4.36 (m, 1H), 4.48 (m, 1H), 4.92 (m, 1H), 6.68 (br s, 1H), 6.85 (t, J = 7 Hz, 1H), 6.95 (t, J = 7 Hz, 1H), 7.02–7.15 (m, 5H), 7.17–7.28 (m, 5H), 7.33 (m, 3H), 7.52–7.64 (m, 2H), 7.79–7.92 (m, 3H), 8.22 (br s, 1H), 10.78 (s, 1H).

H-(*N*-Me)Trp-Lys(CONH-Ph-*o*-Me)-Asp-Phe-NH_2 Hydrochloride (8). Compound **7** (40 mg, 0.048 mmol) was treated with 4 N HCl/dioxane for 1.5 h, the solvent was evaporated, and the residue was titurated with ethyl ether. The crude product was purified by preparative reverse-phase HPLC (C_{18}; A = 50 mM NH_4OAc (pH 4.5), B = CH_3CN; gradient 10% to 50% B over 50 min). Pure fractions were lyophilized to afford 8 mg of the title compound: partial ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.15 (s, 3H), 2.20 (s, 3H); α-protons 4.25 (m, 1H), 4.35 (m, 1H), 4.45 (m, 1H); the NMR spectrum was consistent with a pure compound containing 0.2 mol of HOAc; MS (FAB$^+$) *m/e* 741 (M + H)$^+$. Analytical HPLC showed a single peak (C_{18}, isocratic

elution with 35% CH_3CN/50 mM aqueous NH_4OAc, monitored at 254 and 280 nm).

Boc-(*N*-Me)Trp-Lys(CONH-Ph-*o*-Me)-(*N*-Me)Asp-Phe-NH_2 (9). Compound **25** (135 mg, 0.19 mmol) was N-deprotected according to general procedure B. The resultant hydrochloride salt (100 mg, 0.15 mmol) was coupled to Boc-(*N*-Me)Trp-OH (50 mg, 0.15 mmol) according to general procedure A using NMM as the base and DMF as the solvent. Following standard acid–base workup, the product was precipitated from acetone/H_2O to afford 115 mg of the protected tetrapeptide. To a stirred suspension of 10% Pd/C (100 mg) in HOAc (5 mL) were added the above tetrapeptide (100 mg) and cyclohexadiene (0.5 mL). After 4 h of continued stirring and monitoring by TLC, the catalyst was removed by filtration through Celite, and the filtrate was concentrated. The residue was crystallized from acetone/H_2O to afford 62 mg (48%) of the title compound. ^1H NMR (DMSO-d_6, 500 MHz) showed a mixture of several conformers; selected resonances from the apparent major conformer are provided: δ 1.04 (Boc), 1.89 (N-Me), 2.15 (Ar-Me), 2.77 (N-Me), 4.29, 4.90, 5.01, and 5.23 (α-protons).

H-(*N*-Me)Trp-Lys(CONH-Ph-*o*-Me)-(*N*-Me)Asp-Phe-NH_2 Hydrochloride (10). A solution of compound **9** (0.05 g, 0.0585 mmol) in 8 mL of 1.5 M HCl in acetic acid was stirred at ambient temperature for 1.5 h. The product was precipitated with Et_2O, and the solid was collected, washed with fresh Et_2O, and dried to yield 35 mg (79%) of the title compound: mp 169–171 °C; ^1H NMR (DMSO-d_6, 500 MHz) showed a mixture of conformers; selected resonances from the apparent major conformer are provided: δ 2.10 and 2.11 (N-Me and Ar-Me), 2.20 (N-Me), 4.03 (α-H), 4.40 (2 α-H), 5.0 (α-H).

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(1) Peptide nomenclature throughout this paper follows the guidelines of the IUPAC–IUB Joint Commission on Biochemical Nomenclature *Eur. J. Biochem.* 1984, *138*, 9–37. Other standard abbreviations are as defined in: Guidelines for Authors. *J. Org. Chem.* 1991, *56*, 9A–13A.

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