Supporting Information

Ring size of somatostatin analogues (ODT-8) modulates receptor selectivity and binding affinity

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Starting Materials. The Boc-Cys(Mob)-CM resin, Boc-DCys(Mob)-CM, Boc-D/ L-Ncy(Mob)-CM resin and Boc-Lys(2-Cl-Z)-CM resin with a capacity of 0.3-0.5 mequiv/g was obtained according to published procedures.[50] All N^{α}-tert-butoxycarbonyl (BOC) protected amino acids with side chain protection were purchased from Bachem Inc. (Torrance, CA), Chem-Impex Intl. (Wood Dale, IL), Novabiochem (San Diego, CA) or Reanal (Budapest, Hungary). The side chain protecting groups were as follows: Cys(Mob), Lys(2-Cl-Z), and Thr(Bzl). Boc-D/L-Ncy(Mob)-OH was synthesized by a modified procedure reported for the synthesis of ∝-isopropylthiohyppuric acid by Zoller *et al*.[51] in our laboratory. In short, refluxing *tert*-butyl carbamate and glyoxylic acid monohydrate in acetone yielded ∝-hydroxy intermediate which was immediately reacted with 4-methoxybenzylmercaptan (Mob) under Dean-Stark conditions to give the racemic Boc-D/LNcy(Mob)-OH in a 75% yield.[34] To resolve the racemic Boc-D/LNcy(Mob)-OH, first it was converted to Boc-D/LNcy(Mob)-OMe by trimethylsilyldiazomethane, then it was hydrolyzed with Papain[52-54] in a phosphate buffer (pH 6.2) containing 60% acetonitrile at 25 °C. After 24 h, the reaction mixture contained 50% methyl ester and 50% acid according to HPLC. A simple workup and one purification gave Boc-Ncy(Mob)-OH in 90% yield with respect to the L-isomer. The enantiomeric excess was found to be greater than 98% according to chiral HPLC.[34,55] Boc-Hcy-(Mob)-OH and Boc-DHcy-(Mob)-OH were also synthesized in our laboratory. In short: To the stirred mixture of methionine (0.4 mol) in cc. HCl (300 mL), benzylchloride (0.4 mol) was added and refluxed overnight. It was cooled and extracted with diethylether, the aqueous phase was evaporated to dryness; then was dissolved in hot water and neutralized to pH 4.7 with NH_4OH. The precipitate was collected by filtration and dried, yielding S-benzyl-Hcy (0.25 mol; 58%). S-benzyl-Hcy (0.13 mol) was dissolved in liquid NH_3 (500 mL) and treated with Na (7.4 g) in pieces untill the blue color lasted for 10 min. 4-methoxy-benzylchloride (25.4 g) was added to the mixture and reacted by stirring overnight. After acidification to pH 5.5, the precipitate was collected resulting in S-methoxy-benzyl-Hcy (0.1 mol; 77%). The Boc derivative was prepared with Boc_2O, after workup, the product was an oil, which crystallized upon storage, 0.08 mol of Boc-Hcy(Mob)-OH was obtained (80% yield). Mp: 58 °C; $[\alpha]^{22}_{D}$ = +4.8 (c = 1%, EtOAc). Boc-

DHcy(Mob)-OH was synthesized by the same method but starting the synthesis with D-methionine. Boc-DHcy(Mob)-OH could not be crystallized, $[\alpha]^{22}_{D}$ = -5.0 (c = 1%, EtOAc). Carboxypeptidase B was purchased from Roche Molecular Biochemicals (Indianapolis, IN). All reagents and solvents were reagent grade or better and used without further purification.

Peptide Synthesis. Peptides were synthesized by the solid-phase approach with Boc chemistry either manually or on a CS-Bio Peptide Synthesizer Model CS536. The Boc-Cys(Mob)-CM resin, Boc-DCys(Mob)-CM, Boc-D/L-Ncy(Mob)-CM resin and Boc-Lys(2-Cl-Z)-CM resin were used. A 3-equiv excess of Boc-amino acid (1.2 mmol) based on the original substitution of the resin was used for each coupling. Peptide couplings were mediated for 1 h by DIC/HOBt (1.2 mmol/1.8 mmol) in DMF and monitored by the qualitative ninhydrin test.[56] Boc removal was achieved with trifluoroacetic acid (60% in CH_2Cl_2, 1-2% ethanedithiol or m-cresol) for 20 min. An isopropyl alcohol (1% m-cresol) wash followed TFA treatment and then successive washes with triethylamine solution (10% in CH_2Cl_2), methanol, triethylamine solution, methanol and CH_2Cl_2 completed the neutralization sequence.

Peptide cleavage and deprotection with HF, and cyclization. All peptides were cleaved from the resin support with simultaneous side chain deprotection by anhydrous HF containing the scavengers anisole (10% v/v) and methyl sulfide (5% v/v) for 60 min at 0 °C. The diethyl ether precipitated crude peptides were cyclized in 75% acetic acid (200 mL) by addition of iodine (10% solution in methanol) until the appearance of a stable orange color. Forty minutes later, ascorbic acid was added to quench the excess iodine.

Purification and characterization of the analogues. The crude, lyophilized peptides were purified by preparative RP-HPLC[37] on a 5 cm × 30 cm cartridge, packed in the laboratory with reversed-phase Vydac C_{18} silica (15-20 μM particle size, 300 Å) using a Waters Prep LC 4000 preparative chromatograph system, with a Waters 486 tunable absorbance UV detector and Linseis L250E chart recorder. The peptides eluted with a flow rate of 100 mL/min using a linear gradient of 1% B per 3 min increase from the baseline % B. Eluent A = 0.25 N TEAP pH 2.25, eluent B = 60% CH_3CN, 40% A. As a final step, all peptides were rechromatographed in a 0.1% TFA solution and

CH_3CN on the same cartridge at 100 mL/min (gradient of 1% CH_3CN/min). The collected fractions were screened by analytical RP-HPLC on a system using two Waters 501 HPLC pumps, Schimadzu SPD-6A UV detector, Rheodyne Model 7125 injector, Linseis L250E chart recorder and a Vydac C_{18} column (0.46 cm × 25 cm, 5 μm particle size, 300 Å pore size). The fractions containing the product were pooled and subjected to lyophilization. The purity of the final peptide was determined by analytical RP-HPLC performed with a linear gradient using 0.1 M TEAP pH 2.5 as eluent A and 60% CH_3CN/40% A as eluent B on a Hewlett-Packard Series II 1090 Liquid Chromatograph connected to a Vydac C_{18} column (0.21 x 15 cm, 5 μm particle size, 300 Å pore size). The capillary zone electrophoresis (CZE) analysis of the peptides was performed on a Beckman P/ACE System 2050; field strength of 15 kV at 30 °C on an Agilent μSil bare fused-silica capillary (75 μm i.d. x 40 cm length).[38] Mass spectra (MALDI-TOF-MS) were measured on an ABI-Perseptive DE-STR instrument. The instrument employs a nitrogen laser (337 nM) at a repetition rate of 20 Hz. The applied accelerating voltage was 20 kV. Spectra were recorded in delayed extraction mode (300 ns delay). All spectra were recorded in the positive reflector mode. Spectra were sums of 100 laser shots. Matrix alpha-cyano-4-hydroxycinnamic acid was prepared as saturated solutions in 0.3% TFA in 50% CH_3CN. The observed monoisotopic $(M + H)^+$ values of each peptide corresponded with the calculated $(M + H)^+$ values (**Table 1**).

Table S1. Torsion angles ϕ, ψ and χ_1 (in degrees) for the bundle of 20 energy minimized conformers.

Analogue	Angle	Cys[3]	Phe[6]	Phe[7]	DTrp[8]	Lys[9]	Thr[10]	Phe[11]	Cys[14]
1	ϕ		-116 ± 9	67 ± 2	138 ± 5	-94 ± 4	105 ± 12	-111 ± 13	77 ± 5
	ψ		159 ± 5	66 ± 1	-45 ± 6	-65 ± 4	-22 ± 3	-42 ± 13	
	χ_1		-38 ± 9	-159 ± 1	58 ± 1	-80 ± 44	-144 ± 3	-132 ± 20	-103 ± 59
3									**DCys[14]**
	ϕ		-61 ± 9	-38 ± 1	96 ± 3	-152 ± 3	-53 ± 6	-84 ± 12	144 ± 15
	ψ	147 ± 8	-33 ± 1	-39 ± 1	42 ± 5	149 ± 5	-20 ± 4	-143 ± 2	
	χ_1	176 ± 71	-95 ± 1	-112 ± 2	-147 ± 2	-90 ± 10	-92 ± 6	-35 ± 5	131 ± 68
18		**DNcy[3]**							**Ncy[14]**
	ϕ	-63 ± 7	-113 ± 0	-29 ± 0	107 ± 1	-112 ± 0	-161 ± 3	-105 ± 7	-118 ± 28
	ψ	-117 ± 3	46 ± 0	119 ± 1	121 ± 1	-61 ± 0	-174 ± 6	170 ± 2	
	χ_1		-93 ± 1	-167 ± 0	-29 ± 0	-117 ± 1	-170 ± 13	160 ± 12	
19		**Ncy[3]**							**Ncy[14]**
	ϕ	-141 ± 100	147 ± 69	-131 ± 12	-163 ± 4	-162 ± 4	58 ± 9	-101 ± 29	3 ± 62
	ψ	119 ± 53	-158 ± 11	20 ± 5	33 ± 2	-49 ± 5	34 ± 24	-37 ± 16	
	χ_1		-114 ± 34	-91 ± 39	-149 ± 1	-119 ± 2	-55 ± 55	-123 ± 13	
21		**Hcy[3]**							
	ϕ	-92 ± 8	-44 ± 2	-107 ± 2	-71 ± 0	-108 ± 5	-141 ± 7	-140 ± 9	-118 ± 10
	ψ	-28 ± 1	-72 ± 2	-163 ± 1	64 ± 2	-48 ± 7	-136 ± 1	173 ± 6	
	χ_1	-119 ± 1	-110 ± 6	-35 ± 1	31 ± 0	-98 ± 2	-125 ± 70	-60 ± 54	-99 ± 6

Table S2. Proton chemical shifts of the analogues studied by NMR*

Residue	^{1}H	Analogues				
		1	**3**	**18**	**19**	**21**
CH_3				1.93	2.07	1.88
				Ncy	DNcy	Hcy
Cys[3]	NH	-	-	8.35	8.83	8.33
	αH	3.58	3.63	5.61	5.87	4.28
	βCH_2	3.09,2.54	3.17,			1.86, 1.75
	γCH_2		2.29			2.66, 2.52
Phe[6]	NH	-	9.53	8.61	8.63	8.26
	αH	4.36	4.23	4.48	4.82	4.31
	βCH_2	2.90,2.75	2.58,2.48	3.07,2.80	2.86,2.75	2.86, 2.76
	H2,6	7.06	7.08	7.22	7.19	7.11
	H3,5	7.15	7.22	7.28	7.28	7.24
Phe[7]	NH	8.24	9.99	8.37	9.00	7.80
	αH	4.38	4.50	4.32	4.24	4.32
	βCH_2	2.80,2.71	3.04,2.95	2.80,2.80	2.83,2.66	3.06, 2.66
	H2,6	7.18	7.23	7.09	7.01	7.12
	H3,5	7.22	7.16	7.19	7.19	7.24
DTrp[8]	NH	8.49	8.57	8.42	8.43	7.90
	αH	4.34	4.50	4.29	4.07	4.25
	βCH_2	3.14,2.89	3.14,2.87	3.20,2.88	3.07,2.70	3.12, 2.99
	HD1	7.10	7.03	7.01	6.66	7.22
	HE1	10.74	10.76	10.75	10.59	10.77
	HZ2	7.30	7.28	7.32	7.33	7.31

	HH2	7.04	7.02	7.06	7.05	7.06
	HZ3	6.97	6.94	6.99	6.94	6.98
	HE3	7.53	7.54	7.51	7.40	7.50
Lys[9]	NH	7.95	8.19	7.97	8.04	7.79
	αH	4.34	4.04	4.25	4.44	4.16
	βCH_2	1.70,1.47	1.53,1.23	1.76,1.46	1.62,1.46	1.75, 1.52
	γCH_2	1.30,1.14	0.97,0.97	1.19,1.12	1.25,1.17	1.30, 1.22
	δCH_2	1.47,1.47	1.40,1.34	1.44,1.44	1.44,1.44	1.48, 1.48
	εCH_2	2.73,2.73	2.63,2.63	2.70,2.70	2.67,2.67	2.71, 2.71
	εNH	7.60	7.11	7.63	7.60	7.60
Thr[10]	NH	7.77	7.96	7.80	7.72	7.33
	αH	4.02	3.74	4.34	4.57	4.21
	βH	3.89	3.86	4.08	4.07	4.02
	γCH_3	0.85	0.81	1.03	1.15	0.96
	OH		5.09	4.99	4.84	4.89
Phe[11]	NH	8.03	7.56	8.23	9.08	7.99
	αH	4.44	4.39	4.69	4.65	4.67
	βCH_2	3.10,2.85	3.06,2.90	3.10,2.87	3.01,2.93	3.10, 2.78
	H2,6	7.25	7.10	7.33	7.42	7.27
	H3,5					7.20
			DCys	Ncy	DNcy	
Cys[14]	NH	7.90	7.60	8.70	8.36	8.68
	αH	4.11	4.27	5.42	5.59	4.56
	βCH_2	3.06,3.06	3.45,3.35			3.13, 2.87

*The chemical shifts were measured at 298 K in DMSO in ppm with the internal reference of the DMSO signal at 2.49 ppm.