Supporting Information

Title:

Discovery of Novel Potent and Selective Agonists at the Melanocortin-3 Receptor

Authors:

Alfonso Carotenuto, Francesco Merlino, Minying Cai, Diego Brancaccio, Ali Munaim Yousif, Ettore Novellino, Victor J. Hruby, Paolo Grieco

Contents of SI

Table S1. Analytical Data for the Studied Peptides

Peptide	Structure	HPLC[a]	HRMS (M+H)	
		k'	Found	Calcd
1 (PG-989)	Ac-Nle-c[Asp-Pro-Pro-DPhe-Arg-Trp-Lys]-NH_2	4.76	1081.5923	1081.5947
2 (PG-990)	Ac-Nle-c[Asp-Pro-Pro-DNal(2)-Arg-Trp-Lys]-NH_2	5.16	1131.6068	1131.6104
3 (PG-991)	Ac-Nle-c[Asp-Trp-Pro-DPhe-Arg-Trp-Lys]-NH_2	5.27	1170.6172	1170.6213
4 (PG-992)	Ac-Nle-c[Asp-Trp-Pro-DNal(2)-Arg-Trp-Lys]-NH_2	5.66	1220.6338	1220.6369

[a]*k'* = [(peptide retention time – solvent retention time)/solvent retention time]. Peptides **1-4** were purified by RP-HPLC (Shimadzu Preparative Liquid Chromatograph LC-8A) on C18-bonded silica column (Phenomenex, Jupiter 4 μm Proteo 90Å, 10x250 mm), by eluting from 10% to 90% acetonitrile (0.1% trifluoroacetic acid) in water (0.1% trifluoroacetic acid) over 20 min with a flow rate of 4.0 mL/min. Peptide retention time and solvent retention time were calculated by analytical UHPLC (Shimadzu Nexera Liquid Chromatograph LC-30AD), from 10% to 90% acetonitrile (0.1% trifluoroacetic acid) in water (0.1% trifluoroacetic acid) over 15 min, eluting with flow rate of 1.0 mL/min on a C18-bonded silica column (Phenomenex Gemini 3 μm 110Å, 4.6x150 mm), at 20 °C.

NMR Spectroscopy Details

Spectra in water solution and micelle were recorded at a temperature of 25 °C. The spectra were calibrated relative to TSP (0.00 ppm) as internal standard. One-dimensional (1D) NMR spectra were recorded in the Fourier mode with quadrature detection. The water signal was suppressed by gradient echo.[1] 2D DQF-COSY, TOCSY, and NOESY spectra were recorded in the phase-sensitive mode. Data block sizes were 2048 addresses in t_2 and 512 equidistant t_1 values. Before Fourier transformation, the time domain data matrices were multiplied by shifted $\sin^2$ functions in both dimensions. A mixing time of 70 ms was used for the TOCSY experiments. NOESY experiments were run with mixing times of 100 ms. $^3J_{\mathrm{HN\text{-}H\alpha}}$ coupling constants were obtained from 1D ^{1}H NMR and 2D DQF-COSY spectra. The temperature coefficients of the amide proton chemical shifts were calculated from 1D ^{1}H NMR and 2D TOCSY experiments performed at different temperatures in the range 25-40 °C by means of linear regression.

1. Hwang, T.L.; Shaka, A.J. Water suppression that works. Excitation sculpting using arbitrary wave-forms and pulsed-field gradients. *J. Magn. Reson.* **1995**, *112*, 275–279.

Table S2. NMR Resonance Assignments[a] of Peptide **1** (PG-989) *trans-trans* state in DPC Solution at 25 °C.

residue	NH ($^3J_{\alpha N}$, -Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Nleu[4]	8.19 (7.1, 7.6)	4.19	1.67, 1.60	0.83(γ); 1.26(δ); 0.83(ε)
Asp[5]	8.32 (7.2, 6.7)	4.99	2.60, 2.53	
Pro[6]		4.57	2.22	1.86, 1.78(γ); 3.36(δ); 3.76(ε)
Pro[7]		4.24	2.01, 1.38	1.86(γ); 3.70, 3.43(δ)
DPhe[8]	8.48 (7.8, 10.1)	4.61	3.16, 2.74	7.23(δ); 7.25 (ε)
Arg[9]	7.88 (6.4, 5.5)	4.37	1.70, 1.59	1.38(γ); 3.07(δ); 7.36(ε)
Trp[10]	8.34 (6.8, 7.3)	4.66	3.34, 3.11	7.21(δ); 10.47, 7.68(ε); 7.42, 7.08(ζ); 7,09(η)
Lys[11]	8.33 (6.5, 7.7)	4.23	1.79, 1.65	1.41, 1.27(γ); 1.53(δ); 3.36, 2.97(ε); 8.09(ζ)

[a] Obtained at pH = 5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm.

[b] $^3J_{\alpha N}$ coupling constants in Hz. -Δδ/ΔT = temperature coefficients (ppb/K) calculated in the range 25-40 °C. Further signals: C$\underline{H_3}$CO, 1.98; CON$\underline{H_2}$, 7.04, 6.20.

Table S3. NMR Resonance Assignments[a] of Peptide **1** (PG-989) *trans-cis* state in DPC Solution at 25 °C.

residue	NH ($^3J_{\alpha N}$, -Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Nleu[4]	7.88 (7.0, 7.1)	5.03	1.54, 1.48	1.14(γ); 0.82(δ); 0.49(ε)
Asp[5]	8.83 (9.3, 2.7)	5.51	2.77, 2.41	
Pro[6]		4.10	2.23, 1.98	2.01, 1.87(γ); 3.98, 3.82(δ)
Pro[7]		4.33	2.43, 2.03	1.97, 1.54(γ); 3.48, 3.26(δ)
DPhe[8]	8.72 (8.6, 1.8)	4.73	2.86, 2.78	6.94(δ); 6.98(ε)
Arg[9]	8.51 (8.6, 1.9)	4.72	1.87, 1.68	1.56, 1.46(γ); 3.24(δ); 7.35(ε)
Trp[10]	9.08 (7.5, 6.4)	5.19	3.38, 3.12	7.26(δ);10.68, 7.61(ε);7.47, 6.93(ζ); 7.05(η)
Lys[11]	8.68 (7.7, 3.6)	4.11	1.68, 1.49	1.26, 1.15(γ); 1.47(δ); 3.39, 2.97(ε); 7.91(ζ)

[a] Obtained at pH = 5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm.

[b] $^3J_{\alpha N}$ coupling constants in Hz. -Δδ/ΔT = temperature coefficients (ppb/K) calculated in the range 25-40 °C. Further signals: $C\underline{H_3}CO$, 1.86; $CON\underline{H_2}$, 7.09, 6.20.

Table S4. NMR Resonance Assignments[a] of Peptide **2** (PG-990) *trans-trans* state in DPC Solution at 25 °C.

residue	NH ($^3J_{\alpha N}$, exc, -Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Nleu[4]	8.17 (6.5, 7.5)	4.21	1.68, 1.62	0.91(γ); 1.27(δ)
Asp[5]	8.33 (7.1, 6.3)	5.01	2.61, 2.55	
Pro[6]		4.54	2.18	1.85, 1.75(γ); 3.76, 3.35(δ)
Pro[7]		4.35	1.55, 1.43	1.30(γ); 3.66, 3.36(δ)
DNal(2')[8]	8.63 (6.7, 9.3)	4.67	3.33, 2.90	7.71, 7.41(δ); 7,77, 7.48(ε); 7.10(ζ)
Arg[9]	7.90 (bs, 4.7)	4.42	1.73, 1.60	1.38(γ) 3.05(δ); 7.36(ε)
Trp[10]	8.33 (6.8, 7.3)	4.68	3.36, 3.11	7.21(δ); 10.51, 7.70(ε); 7.84, 7.42(ζ); 7,10(η)
Lys[11]	8.41 (6.5, 8.3)	4.25	1.81, 1.69	1.43, 1.31(γ); 1.54(δ); 3.36, 2.99(ε); 8.11(ζ)

[a] Obtained at pH = 5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm.

[b] $^3J_{\alpha N}$ coupling constants in Hz. -Δδ/ΔT = temperature coefficients (ppb/K) calculated in the range 25-40 °C. bs: broad signal. Further signals: $C\underline{H_3}CO$, 1.99; $CON\underline{H_2}$, 7.08, 6.01.

Table S5. NMR Resonance Assignments[a] of Peptide **2** (PG-990) *trans-cis* state in DPC Solution at 25 °C.

residue	NH ($^3J_{\alpha N}$, exc, -Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Nleu[4]	7.91 (9.2, 7.1)	5.12	1.63, 1.56	0.85, 0.78(γ); 1.22(δ); 0.41(ε)
Asp[5]	8.84 (8.8, 3.1)	5.56	2.79, 2.42	
Pro[6]		3.82	1.89, 1.71	2.07, 1.62(γ); 3.93, 3.76(δ)
Pro[7]		4.21	2.43, 1.71	1.97, 1.51(γ); 3.46, 3.25(δ)
DNal(2')[8]	8.83 (8.4, 2.7)	4.92	3.11, 3.00	7.42, 7.17(δ); 7.60(ε); 7.77(ζ)
Arg[9]	8.58 (9.0, 3.4)	4.79	1.91, 1.71	1.58, 1.48(γ); 3.24(δ); 7.38(ε)
Trp[10]	9.15 (6.3, 7.5)	5.27	3.40, 3.17	7.27(δ);10.68, 7.70(ε); 7.49, 6.95(ζ); 7,07(η)
Lys[11]	8.73 (7.6, 4.7)	4.11	1.68, 1.48	1.27, 1.17(γ); 1.45(δ); 3.42, 2.96(ε); 7.96(ζ)

[a] Obtained at pH = 5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm.

[b] $^3J_{\alpha N}$ coupling constants in Hz. -Δδ/ΔT = temperature coefficients (ppb/K) calculated in the range 25-40 °C. Further signals: C<u>H</u>$_3$CO, 1.86; CON<u>H</u>$_2$, 7.09, 6.01.

Table S6. NMR Resonance Assignments[a] of Peptide **4** (PG-992) in DPC Solution at 25 °C.

residue	NH ($^3J_{\alpha N}$, -Δδ/ΔT)[b]	$C^{\alpha}H$	$C^{\beta}H$	Others
Nleu[4]	7.98 (7.1, 8.2)	5.02	1.66, 1.52	0.84, 0.64(γ); 1.16(δ); 0.37(ε)
Asp[5]	8.81 (9.2, 2.9)	5.51	2.93, 2.54	
Trp[6]	7.93 (bs, nd)	3.96	3.10, 3.06	6.97 (δ); 10.68, 6.25(ε); 7.42, 6.66(ζ); 7.00(η)
Pro[7]		2.71	1.63, 0.23	1.61, 1.17(γ); 3.31, 3.05(δ)
DNal(2')[8]	8.87 (8.5, 2.7)	4.84	3.06, 2.83	7.38, 7.11(δ); 7.92, 7.55(ε); 7.89, 7.51(ζ)
Arg[9]	8.31 (8.6, 2.7)	4.73	1.88, 1.67	1.53, 1.45(γ); 3.16(δ); 7.15(ε)
Trp[10]	9.08 (6.7, 6.5)	5.17	3.39, 3.15	7.30(δ); 10.67, 7.70(ε); 7.50, 6.96(ζ); 7,08(η)
Lys[11]	8.69 (7.2, 4.0)	4.18	1.74, 1.53	1.31, 1.20(γ); 1.45(δ); 3.38, 3.06(ε); 7.88(ζ)

[a] Obtained at pH = 5, with TSP (δ 0.00 ppm) as reference shift. Chemical shifts are accurate to ±0.02 ppm.

[b] $^3J_{\alpha N}$ coupling constants in Hz. -Δδ/ΔT = temperature coefficients (ppb/K) calculated in the range 25-40 °C. bs: brad signal; nd: not determinable. Further signals: $C\underline{H_3}CO$, 1.91; $CON\underline{H_2}$, 7.12, 6.50.

Table S7. NOE derived upper limit constraints for peptides **1**, **2**, **4**.

Peptide **1**:

3	ACE	QH	11	LYS	HN	5.72
4	NLE	HN	4	NLE	HB2	3.48
4	NLE	HN	4	NLE	HB3	3.48
4	NLE	HN	4	NLE	QB	2.96
4	NLE	HN	4	NLE	QD	6.38
4	NLE	HN	5	ASP	HN	4.29
4	NLE	HA	5	ASP	HN	2.62
4	NLE	HA	10	TRP	HA	3.17
4	NLE	HA	10	TRP	HE3	4.38
4	NLE	HA	11	LYS	HN	3.45
4	NLE	HB2	4	NLE	QE	6.53
4	NLE	HB2	5	ASP	HN	3.73
4	NLE	HB3	4	NLE	QE	6.53
4	NLE	HB3	5	ASP	HN	3.73
4	NLE	QB	4	NLE	QE	5.70
4	NLE	QG	8	DPHE	QE	8.50
4	NLE	QD	5	ASP	HN	6.38
4	NLE	QD	8	DPHE	QE	8.51
4	NLE	QD	10	TRP	HE3	6.38
4	NLE	QE	8	DPHE	QE	8.65
4	NLE	QE	10	TRP	HE3	5.94
5	ASP	HN	5	ASP	HB2	3.39
5	ASP	HN	5	ASP	HB3	3.39
5	ASP	HN	5	ASP	QB	2.89
5	ASP	HN	11	LYS	HN	4.23
5	ASP	HN	11	LYS	HZ1	4.97
5	ASP	HA	6	PRO	HD2	3.39
5	ASP	HA	6	PRO	HD3	3.39
5	ASP	HA	6	PRO	QD	2.93
5	ASP	HA	11	LYS	HZ1	5.13
5	ASP	HB2	11	LYS	HZ1	3.70
5	ASP	HB3	11	LYS	HZ1	3.70
5	ASP	QB	6	PRO	QD	3.79
5	ASP	QB	11	LYS	HZ1	3.00
6	PRO	HA	7	PRO	HA	2.49
6	PRO	HA	8	DPHE	HN	2.83
6	PRO	HA	8	DPHE	QD	7.03
6	PRO	HA	8	DPHE	QE	7.62
6	PRO	HA	9	ARG	HN	4.17
7	PRO	HA	8	DPHE	HN	3.42
7	PRO	HD2	8	DPHE	HN	4.69
7	PRO	HD3	8	DPHE	HN	5.50
8	DPHE	HN	8	DPHE	HB2	3.36
8	DPHE	HN	8	DPHE	HB3	3.36
8	DPHE	HN	8	DPHE	QD	7.62
8	DPHE	HN	8	DPHE	QE	7.62
8	DPHE	HN	9	ARG	HN	2.80
8	DPHE	HA	8	DPHE	QD	6.13
8	DPHE	HA	9	ARG	HN	3.55
8	DPHE	HB2	9	ARG	HN	2.99
8	DPHE	HB3	9	ARG	HN	2.99
8	DPHE	QD	9	ARG	HN	7.34
8	DPHE	QE	9	ARG	HN	7.62

9	ARG	HN	9	ARG	HB2	3.58
9	ARG	HN	9	ARG	HB3	3.58
9	ARG	HN	9	ARG	QB	3.01
9	ARG	HN	9	ARG	HG2	4.88
9	ARG	HN	9	ARG	HG3	4.88
9	ARG	HN	10	TRP	HN	4.32
9	ARG	HA	10	TRP	HN	2.62
9	ARG	HB2	10	TRP	HN	3.95
9	ARG	HB3	10	TRP	HN	3.95
9	ARG	QB	10	TRP	HN	3.23
10	TRP	HN	10	TRP	HB2	3.08
10	TRP	HN	10	TRP	HB3	3.08
10	TRP	HN	10	TRP	HD1	4.32
10	TRP	HN	10	TRP	HE3	5.50
10	TRP	HN	11	LYS	HN	4.35
10	TRP	HA	10	TRP	HD1	4.85
10	TRP	HA	10	TRP	HE3	2.93
10	TRP	HA	11	LYS	HN	2.65
10	TRP	HB2	10	TRP	HD1	3.52
10	TRP	HB2	10	TRP	HE3	4.01
10	TRP	HB2	11	LYS	HN	4.11
10	TRP	HB3	10	TRP	HD1	3.52
10	TRP	HB3	10	TRP	HE3	4.01
10	TRP	HB3	11	LYS	HN	4.11
10	TRP	QB	10	TRP	HE3	3.46
10	TRP	QB	11	LYS	HN	3.54
10	TRP	HD1	11	LYS	HN	5.50
10	TRP	HD1	12	CNH2	HN1	4.63
10	TRP	HE3	11	LYS	HN	4.11
10	TRP	HE1	12	CNH2	HN1	5.25
10	TRP	HZ2	12	CNH2	HN1	5.50
11	LYS	HN	11	LYS	HB2	3.52
11	LYS	HN	11	LYS	HB3	3.52
11	LYS	HN	11	LYS	QB	2.99
11	LYS	HN	11	LYS	HG2	4.32
11	LYS	HN	11	LYS	HG3	4.32
11	LYS	HN	11	LYS	QG	3.73
11	LYS	HN	11	LYS	QD	6.00
11	LYS	HN	12	CNH2	HN1	4.11
11	LYS	HN	12	CNH2	HN2	5.22
11	LYS	HA	11	LYS	QG	3.66
11	LYS	HA	11	LYS	QD	5.07
11	LYS	QB	11	LYS	QG	2.29
11	LYS	HG2	11	LYS	HZ1	4.66
11	LYS	HG3	11	LYS	HZ1	4.66
11	LYS	QG	11	LYS	HZ1	4.08

Peptide **2**:

3	ACE	QH	11	LYS	HN	5.50
4	NLE	HN	4	NLE	HB2	3.52
4	NLE	HN	4	NLE	HB3	3.52
4	NLE	HN	4	NLE	QB	2.99
4	NLE	HN	4	NLE	QD	5.58
4	NLE	HN	5	ASP	HN	4.57
4	NLE	HA	5	ASP	HN	2.71
4	NLE	HA	10	TRP	HE3	4.42
4	NLE	HA	11	LYS	HN	3.42

```
4 NLE  HB2     5 ASP  HN      3.64
4 NLE  HB3     5 ASP  HN      3.64
4 NLE  QB      8 DBNA HD1     5.34
4 NLE  HG2     8 DBNA HD2     5.50
4 NLE  HG3     8 DBNA HD2     5.50
4 NLE  QD      5 ASP  HN      6.38
4 NLE  QD      8 DBNA HD1     6.38
4 NLE  QD      8 DBNA HD2     5.86
4 NLE  QD     10 TRP  HE3     5.39
4 NLE  QD     10 TRP  HZ3     6.23
4 NLE  QE      8 DBNA HZ2     6.00
4 NLE  QE      8 DBNA HD2     5.85
4 NLE  QE     10 TRP  HE3     5.48
4 NLE  QE     10 TRP  HZ3     5.54
5 ASP  HN      5 ASP  HB2     3.42
5 ASP  HN      5 ASP  HB3     3.42
5 ASP  HN      5 ASP  QB      2.92
5 ASP  HN     11 LYS  HN      3.98
5 ASP  HN     11 LYS  HZ1     4.51
5 ASP  HA      6 PRO  HD2     3.36
5 ASP  HA      6 PRO  HD3     3.36
5 ASP  HA     11 LYS  HZ1     4.97
5 ASP  HB2    11 LYS  HZ1     3.52
5 ASP  HB3    11 LYS  HZ1     3.52
5 ASP  QB      6 PRO  QD      3.81
5 ASP  QB     11 LYS  HZ1     3.03
6 PRO  HA      7 PRO  HA      2.65
6 PRO  HA      8 DBNA HN      2.90
6 PRO  HA      8 DBNA HD1     5.00
6 PRO  HA      8 DBNA HD2     5.12
6 PRO  HA      9 ARG  HN      3.79
6 PRO  QD      8 DBNA HZ2     4.87
7 PRO  HA      8 DBNA HN      3.36
7 PRO  HD2     8 DBNA HN      4.66
7 PRO  HD3     8 DBNA HN      5.34
8 DBNA HN      8 DBNA HB2     3.24
8 DBNA HN      8 DBNA HB3     3.24
8 DBNA HN      8 DBNA HD1     4.63
8 DBNA HN      8 DBNA HD2     4.14
8 DBNA HN      9 ARG  HN      3.90
8 DBNA HA      8 DBNA HD1     3.97
8 DBNA HA      8 DBNA HD2     4.34
8 DBNA HA      9 ARG  HN      3.58
8 DBNA HB2     8 DBNA HD2     3.30
8 DBNA HB2     9 ARG  HN      3.08
8 DBNA HB3     8 DBNA HD2     3.30
8 DBNA HB3     9 ARG  HN      3.68
8 DBNA QB      8 DBNA HD1     3.33
8 DBNA QB      8 DBNA HD2     2.88
8 DBNA HD1     9 ARG  HN      4.69
8 DBNA HD2     9 ARG  HN      4.81
9 ARG  HN      9 ARG  HB2     3.52
9 ARG  HN      9 ARG  HB3     3.52
9 ARG  HN      9 ARG  QB      3.04
9 ARG  HN      9 ARG  HG2     4.76
9 ARG  HN      9 ARG  HG3     4.76
9 ARG  HN     10 TRP  HN      4.82
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9	ARG	HA	10	TRP	HN	2.99
9	ARG	HB2	10	TRP	HN	3.67
9	ARG	HB3	10	TRP	HN	3.67
9	ARG	QB	10	TRP	HN	3.16
10	TRP	HN	10	TRP	HB2	3.08
10	TRP	HN	10	TRP	HB3	3.08
10	TRP	HN	10	TRP	HD1	4.26
10	TRP	HN	10	TRP	HE3	5.38
10	TRP	HN	11	LYS	HN	4.14
10	TRP	HA	10	TRP	HD1	4.76
10	TRP	HA	10	TRP	HE3	3.14
10	TRP	HA	11	LYS	HN	2.77
10	TRP	HB2	10	TRP	HD1	3.67
10	TRP	HB2	10	TRP	HE3	4.04
10	TRP	HB2	11	LYS	HN	3.86
10	TRP	HB3	10	TRP	HD1	3.67
10	TRP	HB3	10	TRP	HE3	4.04
10	TRP	HB3	11	LYS	HN	3.86
10	TRP	QB	10	TRP	HD1	3.14
10	TRP	HD1	11	LYS	HN	5.13
10	TRP	HD1	12	CNH2	HN1	4.60
10	TRP	HE3	11	LYS	HN	4.01
10	TRP	HE1	12	CNH2	HN1	4.85
10	TRP	HZ2	12	CNH2	HN1	5.41
11	LYS	HN	11	LYS	HB2	3.39
11	LYS	HN	11	LYS	HB3	3.39
11	LYS	HN	11	LYS	QB	2.93
11	LYS	HN	11	LYS	HG2	4.23
11	LYS	HN	11	LYS	HG3	4.23
11	LYS	HN	11	LYS	QD	6.38
11	LYS	HN	12	CNH2	HN1	3.95
11	LYS	HN	12	CNH2	HN2	4.54
11	LYS	HA	11	LYS	QG	3.72
11	LYS	HA	11	LYS	QD	5.79
11	LYS	HG2	11	LYS	HZ1	4.51
11	LYS	HG3	11	LYS	HZ1	4.51

Peptide **4**:

3	ACE	QH	11	LYS	HN	5.78
4	NLE	HN	4	NLE	HB2	3.58
4	NLE	HN	4	NLE	HB3	3.58
4	NLE	HN	4	NLE	QB	2.99
4	NLE	HN	4	NLE	QD	6.04
4	NLE	HN	5	ASP	HN	4.45
4	NLE	HA	5	ASP	HN	2.65
4	NLE	HA	10	TRP	HA	3.17
4	NLE	HA	10	TRP	HE3	4.17
4	NLE	HA	11	LYS	HN	3.39
4	NLE	HB2	5	ASP	HN	3.58
4	NLE	HB2	8	DBNA	HD1	5.50
4	NLE	HB2	10	TRP	HE3	4.97
4	NLE	HB3	5	ASP	HN	3.58
4	NLE	HB3	8	DBNA	HD1	5.50
4	NLE	HB3	10	TRP	HE3	4.97
4	NLE	QB	8	DBNA	HD2	4.26
4	NLE	QB	10	TRP	HE3	4.29
4	NLE	QB	10	TRP	HZ3	5.19

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4 NLE  QG      8 DBNA HD2     4.53
4 NLE  QG     10 TRP  HE3     4.73
4 NLE  QG     10 TRP  HZ3     4.85
4 NLE  QD      5 ASP  HN      6.38
4 NLE  QD      8 DBNA HD1     6.38
4 NLE  QD      8 DBNA HD2     6.10
4 NLE  QD     10 TRP  HE3     5.76
4 NLE  QD     10 TRP  HZ3     6.38
4 NLE  QE      8 DBNA HE3     6.53
4 NLE  QE      8 DBNA HD2     5.85
4 NLE  QE     10 TRP  HE3     5.66
4 NLE  QE     10 TRP  HZ3     5.60
5 ASP  HN      5 ASP  HB2     3.52
5 ASP  HN      5 ASP  HB3     3.52
5 ASP  HN      5 ASP  QB      2.98
5 ASP  HN     10 TRP  HA      3.89
5 ASP  HN     11 LYS  HN      3.89
5 ASP  HN     11 LYS  HZ1     4.88
5 ASP  HA     11 LYS  HZ1     4.72
5 ASP  HB2    11 LYS  HZ1     3.48
5 ASP  HB3    11 LYS  HZ1     3.48
5 ASP  QB     11 LYS  HZ1     2.90
6 TRP  HN      6 TRP  QB      3.62
6 TRP  HA      6 TRP  HD1     4.97
6 TRP  HA      6 TRP  HE3     3.70
6 TRP  HA      7 PRO  HA      2.59
6 TRP  HA      8 DBNA HN      2.96
6 TRP  HA      8 DBNA HD1     5.31
6 TRP  HA      8 DBNA HD2     3.95
6 TRP  HA      9 ARG  HN      4.17
6 TRP  QB      6 TRP  HD1     3.39
6 TRP  QB      6 TRP  HE3     3.61
6 TRP  HD1     7 PRO  HA      4.66
6 TRP  HD1     7 PRO  HB2     5.04
6 TRP  HD1     7 PRO  HB3     5.04
6 TRP  HD1     7 PRO  QB      4.38
6 TRP  HD1     7 PRO  QD      4.23
6 TRP  HE3     7 PRO  HA      3.92
6 TRP  HE3     8 DBNA HZ2     4.97
6 TRP  HZ3     8 DBNA HE1     5.19
6 TRP  HZ3     8 DBNA HZ2     4.35
7 PRO  HA      8 DBNA HN      3.30
7 PRO  QD      8 DBNA HN      4.96
8 DBNA HN      8 DBNA HB2     3.80
8 DBNA HN      8 DBNA HB3     3.80
8 DBNA HN      8 DBNA HD1     5.10
8 DBNA HN      8 DBNA HD2     4.14
8 DBNA HN      9 ARG  HN      2.77
8 DBNA HA      8 DBNA HD1     2.86
8 DBNA HA      8 DBNA HD2     4.67
8 DBNA HA      9 ARG  HN      3.61
8 DBNA HB2     8 DBNA HD1     3.73
8 DBNA HB2     8 DBNA HD2     3.21
8 DBNA HB2     9 ARG  HN      3.02
8 DBNA HB3     8 DBNA HD1     3.73
8 DBNA HB3     8 DBNA HD2     3.21
8 DBNA HB3     9 ARG  HN      3.02
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8	DBNA	QB	8	DBNA	HD1	3.13
8	DBNA	QB	8	DBNA	HD2	2.73
8	DBNA	HD1	9	ARG	HN	4.85
8	DBNA	HD2	9	ARG	HN	4.38
9	ARG	HN	9	ARG	HB2	3.11
9	ARG	HN	9	ARG	HB3	3.52
9	ARG	HN	9	ARG	HG2	4.72
9	ARG	HN	9	ARG	HG3	4.72
9	ARG	HN	9	ARG	QG	4.15
9	ARG	HN	10	TRP	HN	4.23
9	ARG	HA	10	TRP	HN	2.68
9	ARG	HB2	10	TRP	HN	3.73
9	ARG	HB3	10	TRP	HN	3.48
10	TRP	HN	10	TRP	HB2	3.11
10	TRP	HN	10	TRP	HB3	3.11
10	TRP	HN	10	TRP	HD1	4.42
10	TRP	HN	10	TRP	HE3	5.50
10	TRP	HN	11	LYS	HN	4.57
10	TRP	HA	10	TRP	HD1	4.82
10	TRP	HA	10	TRP	HE3	2.96
10	TRP	HA	11	LYS	HN	2.65
10	TRP	HB2	10	TRP	HD1	3.39
10	TRP	HB2	10	TRP	HE3	3.89
10	TRP	HB2	11	LYS	HN	4.11
10	TRP	HB3	10	TRP	HD1	3.39
10	TRP	HB3	10	TRP	HE3	3.89
10	TRP	HB3	11	LYS	HN	4.11
10	TRP	QB	10	TRP	HD1	2.95
10	TRP	HD1	12	CNH2	HN1	5.50
10	TRP	HE3	11	LYS	HN	4.26
10	TRP	HE1	12	CNH2	HN1	5.50
11	LYS	HN	11	LYS	HB2	3.45
11	LYS	HN	11	LYS	HB3	3.45
11	LYS	HN	11	LYS	QB	2.88
11	LYS	HN	11	LYS	HG2	4.38
11	LYS	HN	11	LYS	HG3	4.38
11	LYS	HN	11	LYS	QG	3.78
11	LYS	HN	12	CNH2	HN1	4.76
11	LYS	HA	11	LYS	HB2	2.96
11	LYS	HA	11	LYS	HB3	2.96
11	LYS	HA	11	LYS	QB	2.57
11	LYS	HA	11	LYS	HG2	4.01
11	LYS	HA	11	LYS	HG3	4.01
11	LYS	HA	11	LYS	QG	3.38
11	LYS	HA	11	LYS	QD	5.91
11	LYS	HG2	11	LYS	HZ1	4.51
11	LYS	HG3	11	LYS	HZ1	4.51
11	LYS	QG	11	LYS	QE	3.24

ACE QH is the methyl group of the N-terminal acetyl funtion. CNH2 HN1 is one of the amide protons of the C-terminal carboxamide function. NLE: Norleucine; DBNA: D(2')Naphthylalanine.

Table S8. Statistical analysis of predicted I-TASSER best scored model.

Properties	*h*MC3R	*h*MC4R
C-score[a]	0.42	0.66
TM-score[b]	0.77 ± 0.10	0.80 ± 0.09
RMSD (Å)	5.4 ± 3.4	4.8 ± 3.1

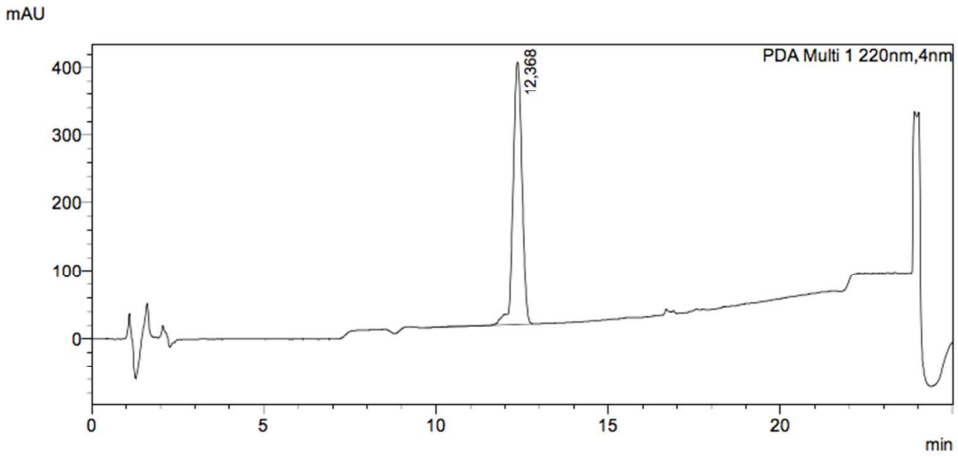
[a] C-score: confidence score; The C-score is computed from the threading alignments for the estimated quality of the models. Ranging from 5 to +2, a C-score > 1.5 implies a model with a correct fold.
[b] TM-score template modelling score; The TM score is a measure of the structural similarity between the model and the native structure. A TM score > +0.5 suggests a model with correct topology.

Table S9. Docking statistics and energy terms.

Complex	Cluster n.	Cluster size	ΔG_{bind}[a]	Electr[b]	H-Bond[b]+VdW[b]+Des	Tors[b]
2/*h*MC3R	3	80	-10.64	-1.39	-15.23	4.47
2/*h*MC4R	11	12	-8.27	-1.52	-13.05	4.77

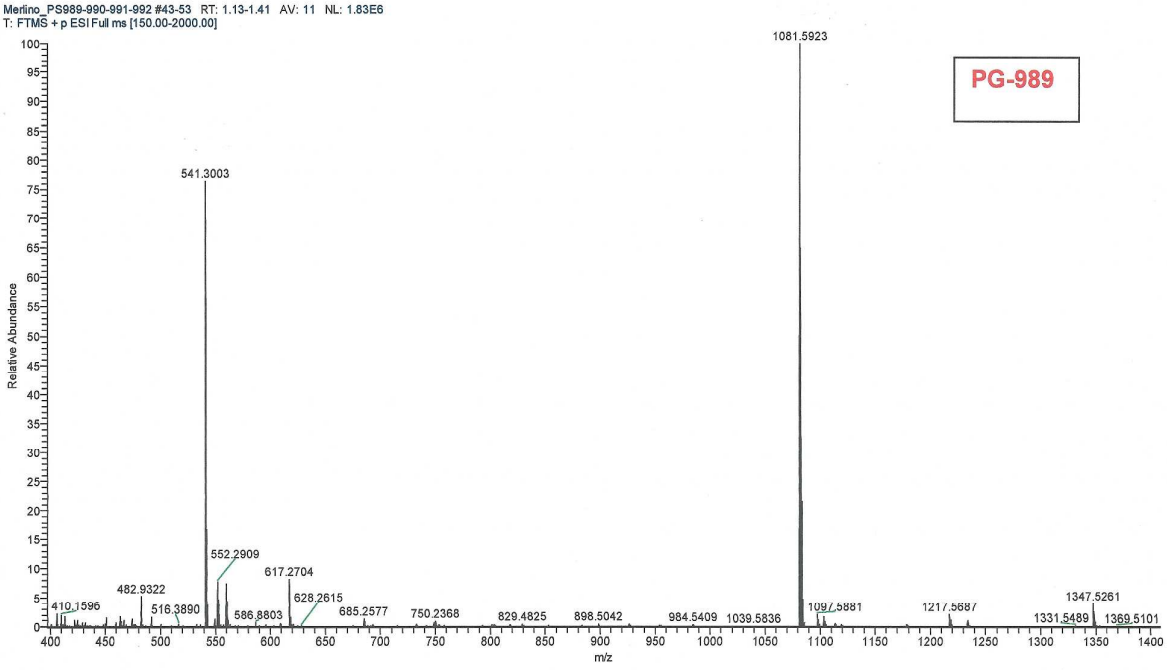
[a]ΔG_{bind}: free energy of binding. [b]Energy terms contributing to the AutoDock4.2 scoring function. Electr: electrostatic; H-Bond: H-Bonding; VdW: Van der Waals; Des: desolvation; Tors: torsional entropy. All terms are given in kcal/mol.

Figure S1. a) RP-UHPLC trace of pure peptide **1**.[a]



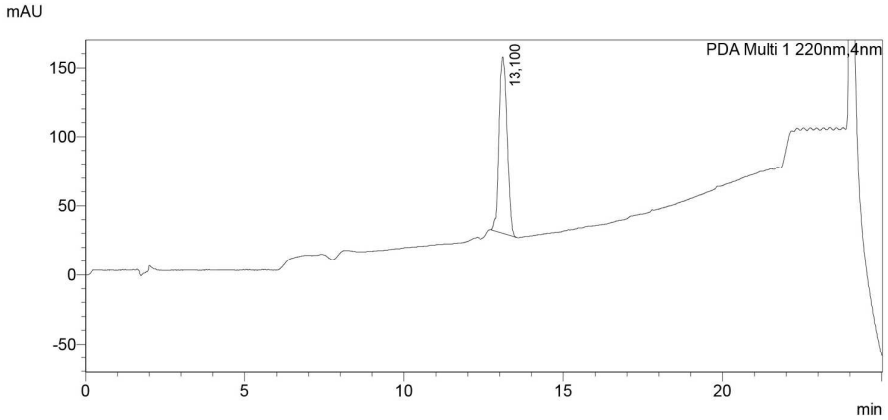
[a] UHPLC chromatogram of Ac-Nle-c[D-P-P-DPhe-R-W-K]-NH_2 (**1**) in a linear gradient of 10-90% of ACN in water containing 0.1% TFA over 15 min on a C18-bonded silica analytical column (Phenomenex Gemini 3 µm 110Å, 4.6x150 mm).

b) HRMS spectrum of peptide **1** (**PG-989**).



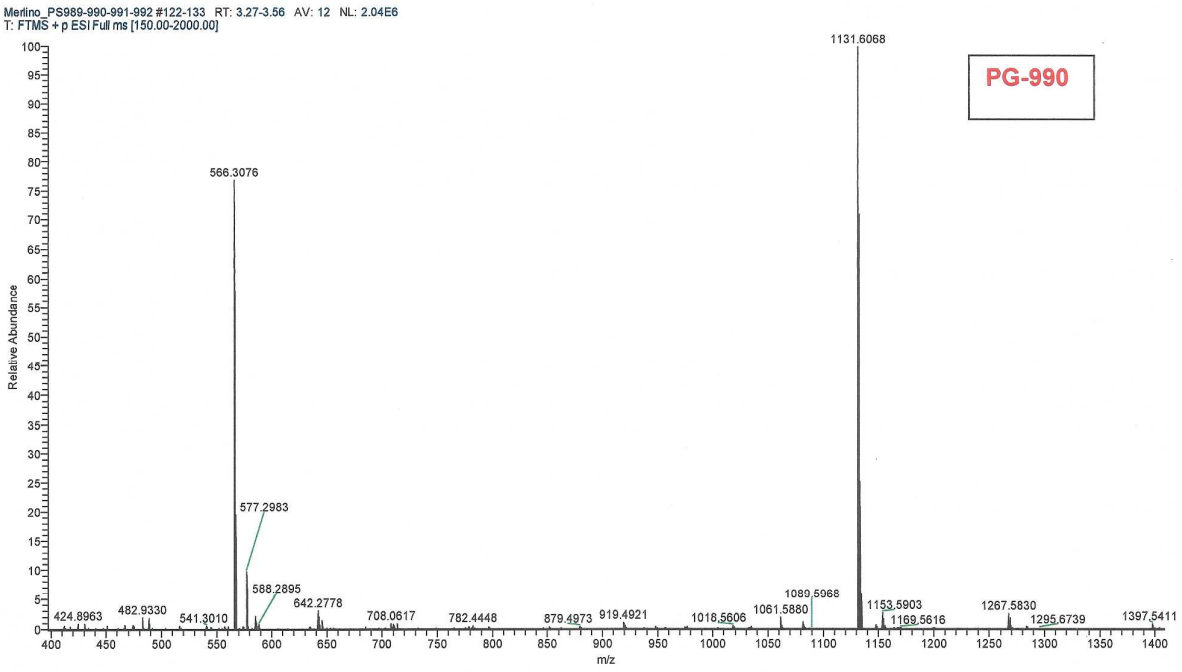
[b] Performed on Thermo Scientific mod LTQ Orbitrap XL.

Figure S2. a) RP-UHPLC trace of pure peptide **2**.[a]



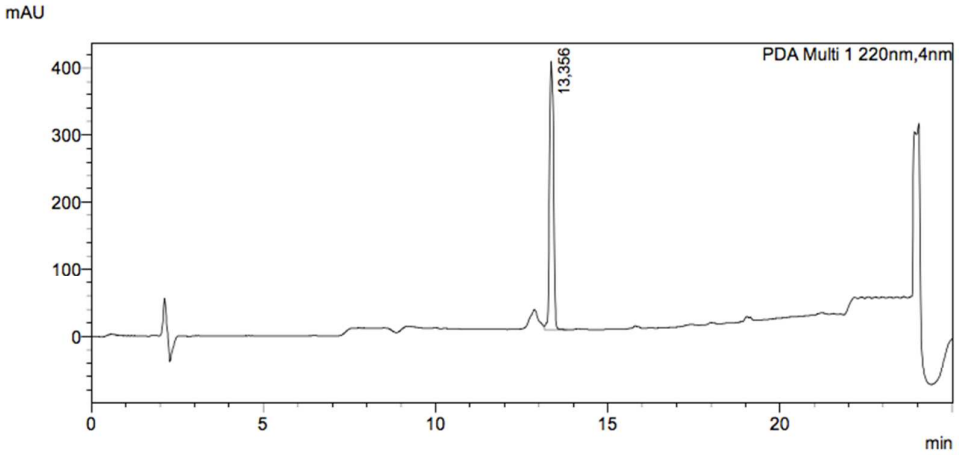
[a] UHPLC chromatogram of Ac-Nle-c[D-P-P-DNal(2')-R-W-K]-NH_2 (**2**) in a linear gradient of 10-90% of ACN in water containing 0.1% TFA over 15 min on a C18-bonded silica analytical column (Phenomenex Gemini 3 µm 110Å, 4.6x150 mm).

b) HRMS spectrum of peptide **2** (**PG-990**).



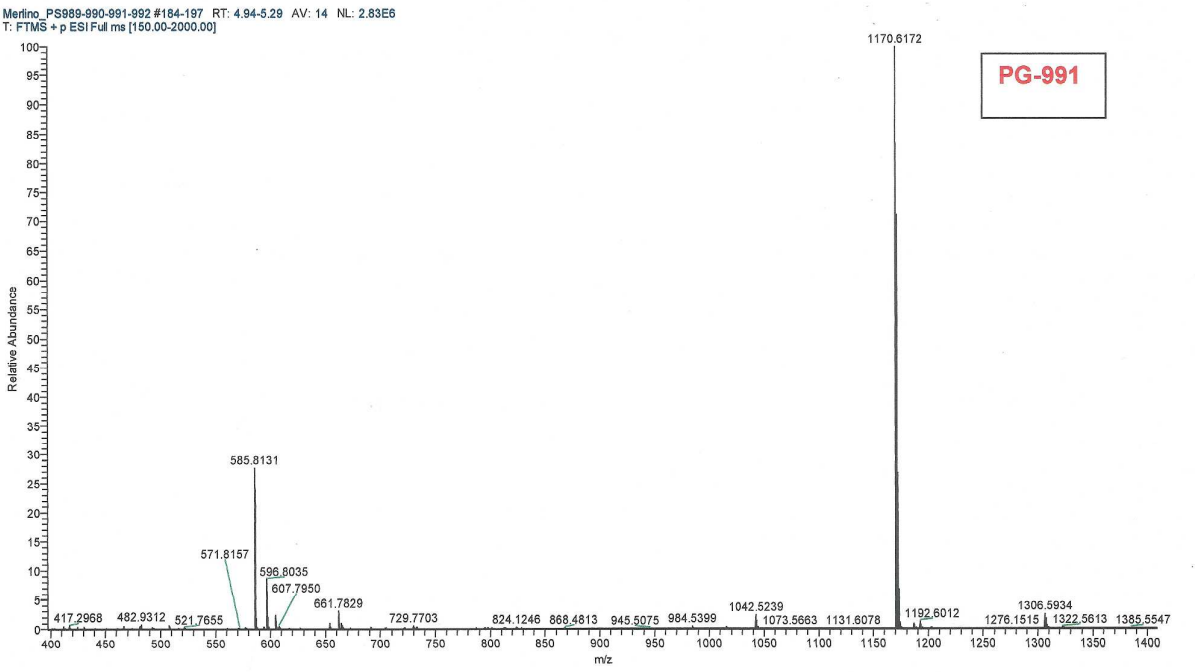
[b] Performed on Thermo Scientific mod LTQ Orbitrap XL.

Figure S3. a) RP-UHPLC trace of pure peptide **3**.[a]



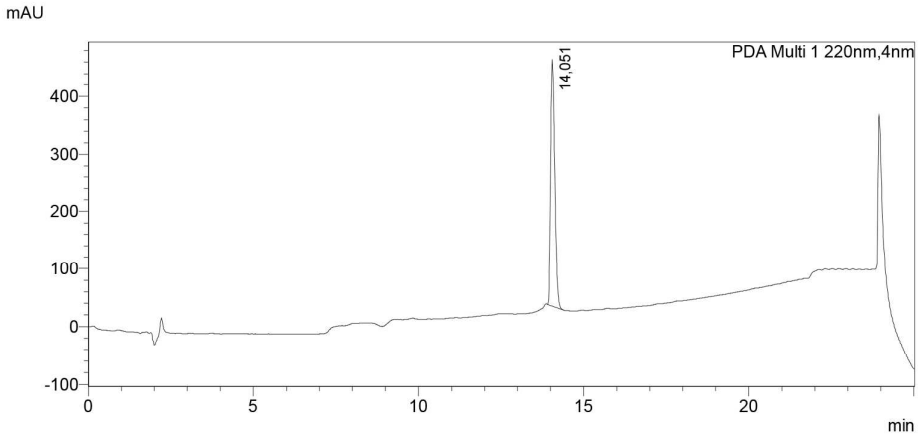
[a] UHPLC chromatogram of Ac-Nle-c[D-W-P-DPhe-R-W-K]-NH_2 (**3**) in a linear gradient of 10-90% of ACN in water containing 0.1% TFA over 15 min on a C18-bonded silica analytical column (Phenomenex Gemini 3 µm 110Å, 4.6x150 mm).

b) HRMS spectrum of peptide **3** (**PG-991**).



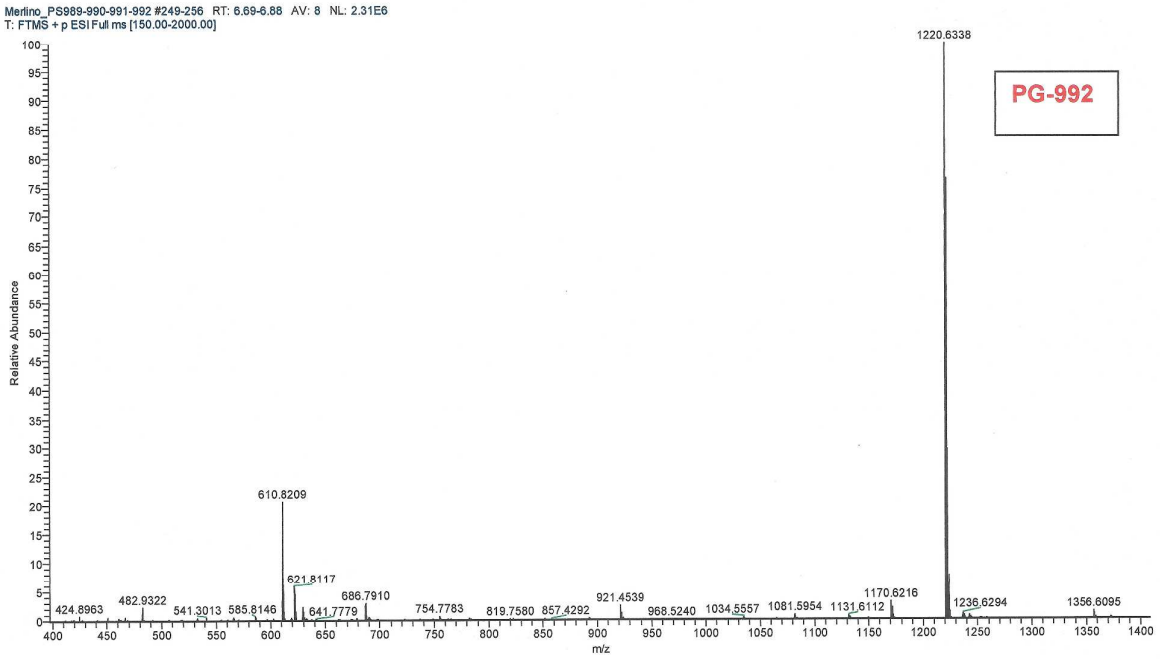
[b] Performed on Thermo Scientific mod LTQ Orbitrap XL.

Figure S4. a) RP-UHPLC trace of pure peptide **4**.[a]



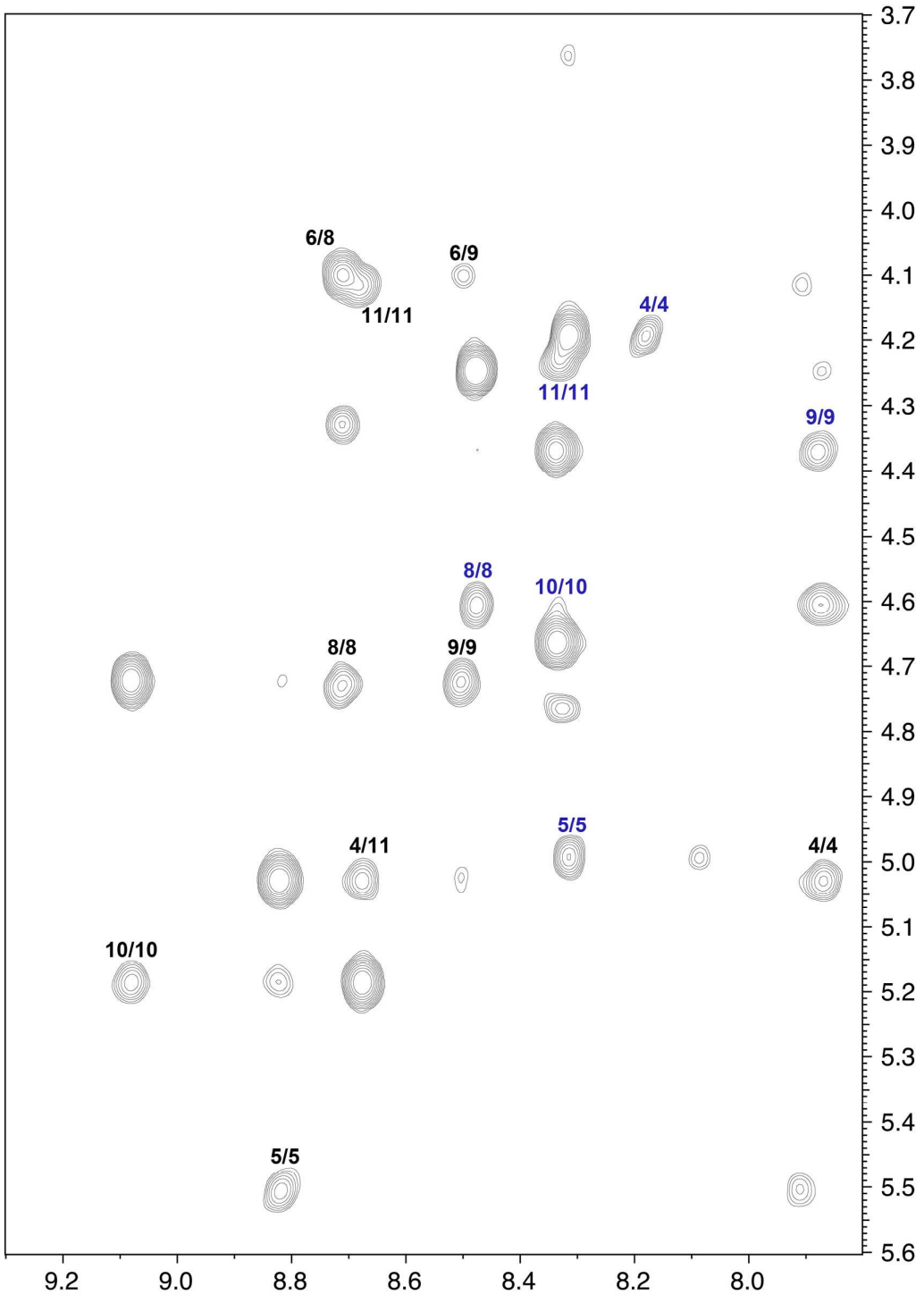
[a] UHPLC chromatogram of Ac-Nle-c[D-W-P-DNal(2')-R-W-K]-NH_2 (**4**) in a linear gradient of 10-90% of ACN in water containing 0.1% TFA over 15 min on a C18-bonded silica analytical column (Phenomenex Gemini 3 µm 110Å, 4.6x150 mm).

b) HRMS spectrum of peptide **4** (**PG-992**).



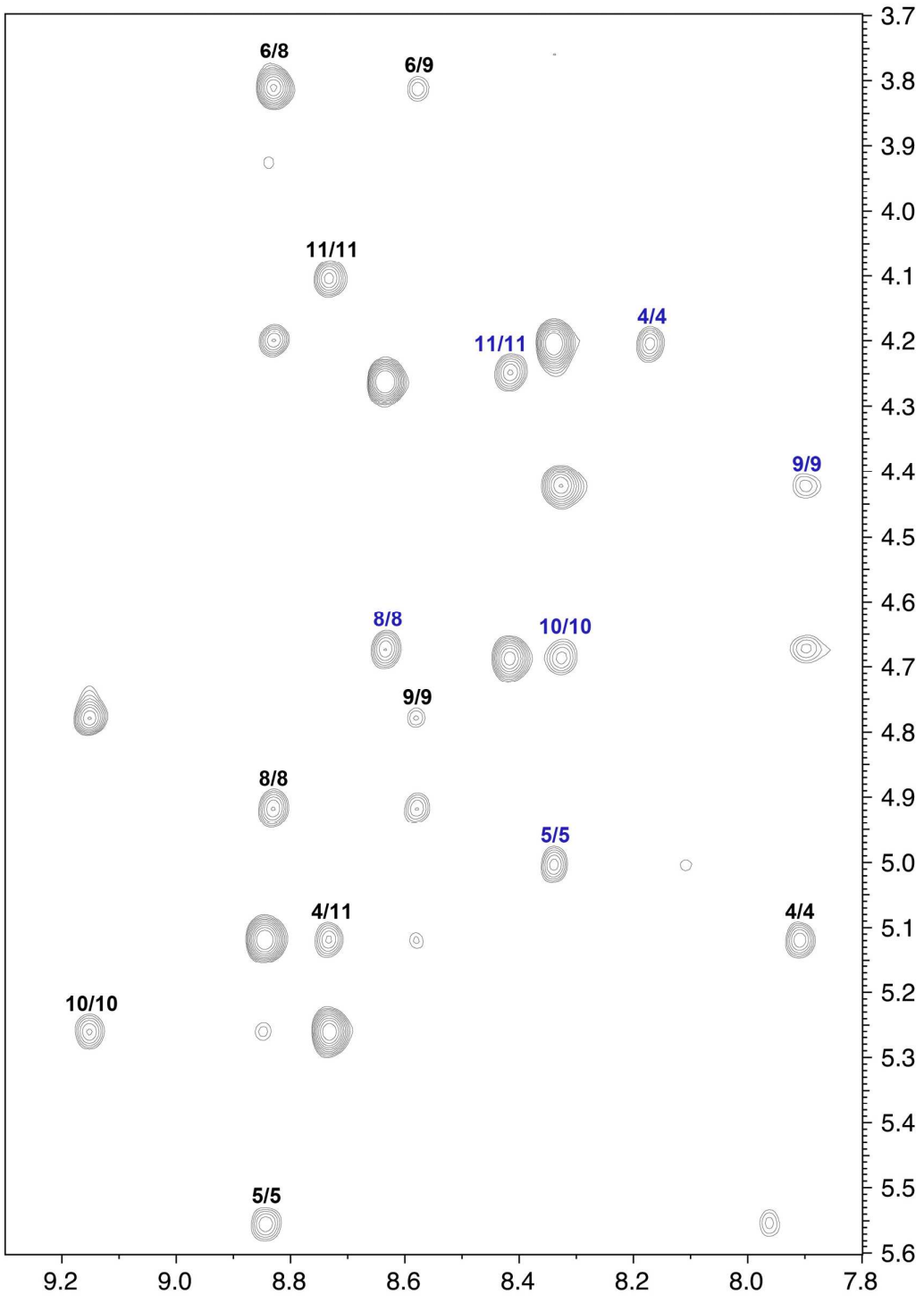
[b] Performed on Thermo Scientific mod LTQ Orbitrap XL.

Figure S5. Zoomed region of the NOESY spectrum of peptide **1**.[a]



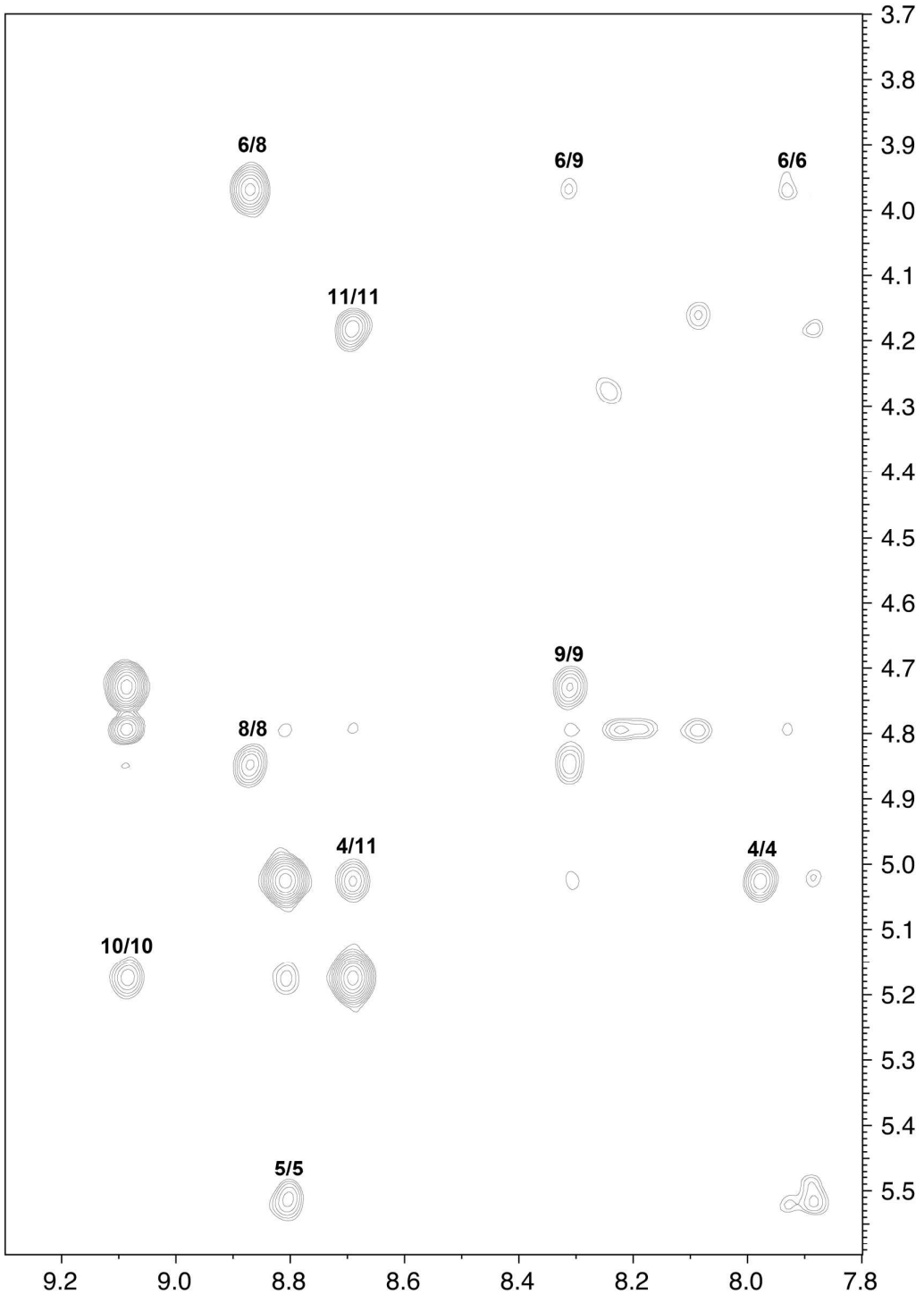
[a] Intraresidual and diagnostic Hα-HN NOEs are labeled. Black: *trans-cis* state; blue: *trans-trans* state.

Figure S6. Zoomed region of the NOESY spectrum of peptide **2**.[a]



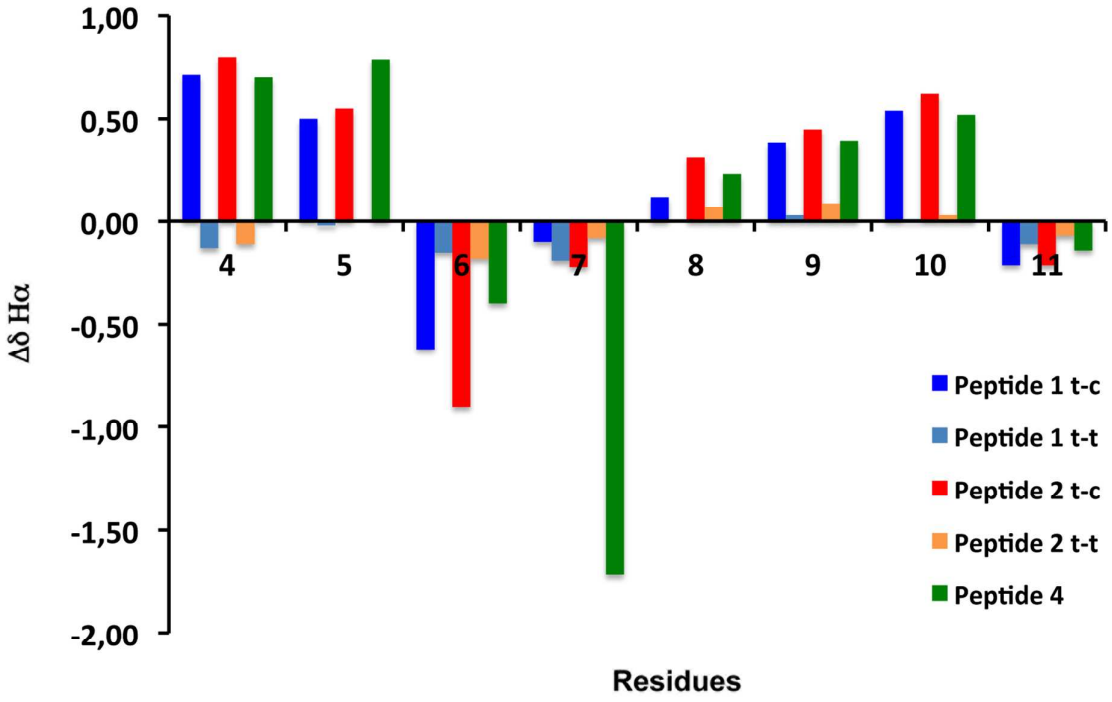
[a] Intraresidual and diagnostic Hα-HN NOEs are labeled. Black: *trans-cis* state; blue: *trans-trans* state.

Figure S7. Zoomed region of the NOESY spectrum of peptide **4**.[a]



[a] Intraresidual and diagnostic Hα-HN NOEs are labeled.

Figure S8. Secondary shifts of the α protons of peptides **1**, **2** and **4** in DPC solution.[a]



[a] t-c: *trans-cis* state; t-t: *trans-trans* state. Reference Hα chemical shift values were taken from Andersen, N. H. et al. FEBS Lett. **1996**, *399*, 47-52.