Structure-Activity Relationships of Peptides Incorporating a Bioactive Reverse-Turn Heterocycle at the Melanocortin Receptors: Identification of a 5,800-fold Mouse Melanocortin-3 Receptor (mMC3R) Selective Antagonist/Partial Agonist versus the Mouse Melanocortin-4 Receptor (mMC4R)

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IV. Figure S2. Illustration of the compound deviation from random coil values[53] (a) control **2** (DPhe), and the analogues modified at the DPhe position (b) **6** (Pro), (c) **8** DNal(1'), (d) **9** DNal(2'), (e) **10** (pI)DPhe, and (f) **11** (DBip). The H^N and H^α protons are represented by solid bars and open bars, respectively. Vertical axes are in parts per million, and the horizontal axes represent the HxRWNA region of each peptide.

V. Table S3. Backbone dihedral angles of the representative structures shown in Figure 4 of the manuscript. The representative structure is the lowest energy conformer of the major family.

Table S1. ^{1}H NMR and chemical shifts assignment (ppm) for the selected analogues.

amino acid	H^N	H^α	H^β	Others
Analogue 6				
Tyr[1]	-	3.97	-	
Cys[2]	9.27	5.67	4.08, 3.98	
His[3]	9.71	6.08	4.26, 4.18	8.26
Pro[4]		5.70	4.21, 4.09	2.28
Cys[5*]	9.2	5.78	4.08, 3.96	
Arg[6]	8.92	5.14	3.38, 2.51	4.08; 2.19, 2.35
Trp[7]		5.18	4.11, 4.04	
Asn[8]	8.47	5.71	4.20, 4.08	
Ala[9]	9.11	5.19	2.28	
Phe[10]	8.98	5.67	4.15, 4.10	8.29
Cys[11]	8.87	5.71	4.18, 3.97	
Tyr[12]	-	-	-	
Ring CH_2 Arg				2.27, 2.54
Ring CH_2 Acetyl				3.69, 3.36
Analogue 8				
Tyr[1]	-	4.04	2.98, 2.76	
Cys[2]	9.00	5.14	2.90, 2.58	
His[3]	8.47	4.88	2.64, 2.54	
DNal(1')[4]	8.89	5.49	3.40, 3.12	7.43
Cys[5*]	8.71	4.77	2.45, 2.45	
Arg[6]	7.97	3.97	2.39, 2.39	3.00, 2.91; 1.12, 1.03; 7.38
Trp[7]		3.85	3.53, 3.14	7.44, 7.11; 10.80
Asn[8]	7.38	4.68	2.66, 2.60	
Ala[9]	8.33	4.92	1.06	
Phe[10]	8.10	4.74	2.70, 2.65	7.38
Cys[11]	8.79	4.97	2.77, 2.77	
Tyr[12]	7.86	4.40	2.78, 2.66	6.96, 7.01
Ring CH_2 Arg				2.40, 3.00
Ring CH_2 Acetyl				3.86, 3.98
Analogue 9				
Tyr[1]	-	5.19	4.08, 3.96	
Cys[2]	9.32	5.91	3.91, 3.82	
His[3]	9.37	5.73	4.03, 3.76	
DNal(2')[4]	9.24	6.21	4.25, 4.10	
Cys[5*]	9.08	5.79	3.91, 3.91	
Arg[6]	8.78	5.03	3.40, 2.37	4.07, 3.98; 2.23, 2.12
Trp[7]	-	-	-	
Asn[8]	9.35	5.93	3.92, 3.82	
Ala[9]	9.04	5.54	2.21	
Phe[10]	9.19	5.66	4.01, 3.89	
Cys[11]	8.96	5.89	3.94, 3.88	
Tyr[12]	8.85	5.54	4.01, 3.87	

Ring CH_2 Arg				2.27, 2.54
Ring CH_2 Acetyl				3.69, 3.36
Analogue 10				
Tyr[1]	-	-	-	
Cys[2]	8.27	5.29	3.07, 2.95	
His[3]	8.63	4.95	3.26, 3.04	
(pI)DPhe[4]	8.17	5.40	3.21, 3.08	
Cys[5*]	8.77	4.97	3.29, 3.06	
Arg[6]	7.69	4.19	2.59, 2.59	3.25, 3.15; 1.46, 1.31
Trp[7]	-	-	-	
Asn[8]	8.37	4.96	3.15, 3.10	
Ala[9]	8.05	4.89	1.38	
Phe[10]	8.44	4.95	3.27, 3.17	
Cys[11]	8.11	5.24	3.07	
Tyr[12]	8.07	4.77	3.19,3.08	
Ring CH_2 Arg				
Analogue 11				
Tyr[1]	-	5.22	4.13, 3.97	
Cys[2]	9.36	5.97	3.97, 3.88	
His[3]	9.48	5.79	4.15, 3.94	9.36, 8.41
DBip[4]	9.24	6.19	4.12 4.06	8.41, 8.61
Cys[5*]	9.03	5.82	3.99, 3.99	
Arg[6]	8.81	5.04	3.41, 2.41	4.09, 4.02; 2.26, 2.15
Trp[7]	-	5.97	4.00, 3.94	8.57, 8.13; 10.78
Asn[8]	9.37	5.78	3.97, 3.97	
Ala[9]	9.06	5.56	2.25	
Phe[10]	9.28	5.72	4.17, 4.02	8.63, 8.48
Cys[11]	9.06	5.97	3.99, 3.99	
Tyr[12]	8.90	5.59	4.06, 3.93	8.13, 7.79
Ring CH_2 Arg				
Ring CH_2 Acetyl				
Ring CH_2 Acetyl				

Analogue 2	**Control**			
Tyr[1]		4.82		
Cys[2]	8.80	5.65	3.53, 3.43	
His[3]	9.02	5.37	3.69, 3.51	8.16, 7.68
DPhe[4]	8.65	5.77	3.70, 3.56	
Cys[5*]	8.62	5.40	3.83, 3.73	
Arg[6]	8.22	4.06	3.02, 3.02	3.71, 3.59; 1.82, 1.74
Trp[7]				
Asn[8]	8.85	5.35	3.73, 3.58	
Ala[9]	8.53	5.22	1.82	
Phe[10]	8.88	5.34	3.74, 3.57	8.18
Cys[11]	8.56	5.59	3.62, 3.53	
Tyr[12]	8.47	5.19	3.64, 3.51	8.23, 7.25
Ring CH_2 Arg				3.74, 3.04
Ring CH_2 Acetyl				4.03, 3.81

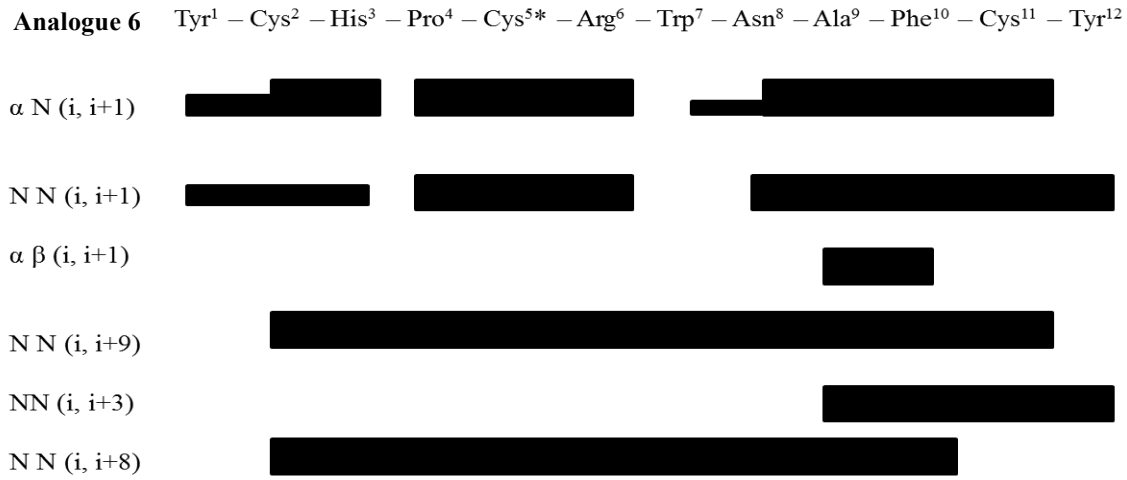
Cys* designates the thioether linkage.

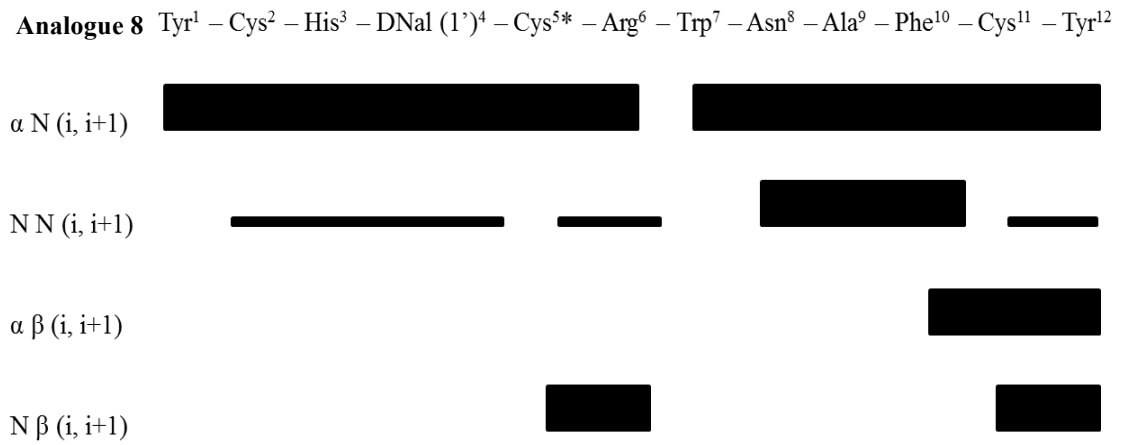
Table S2. Analytical data for the ligands prepared in this study.

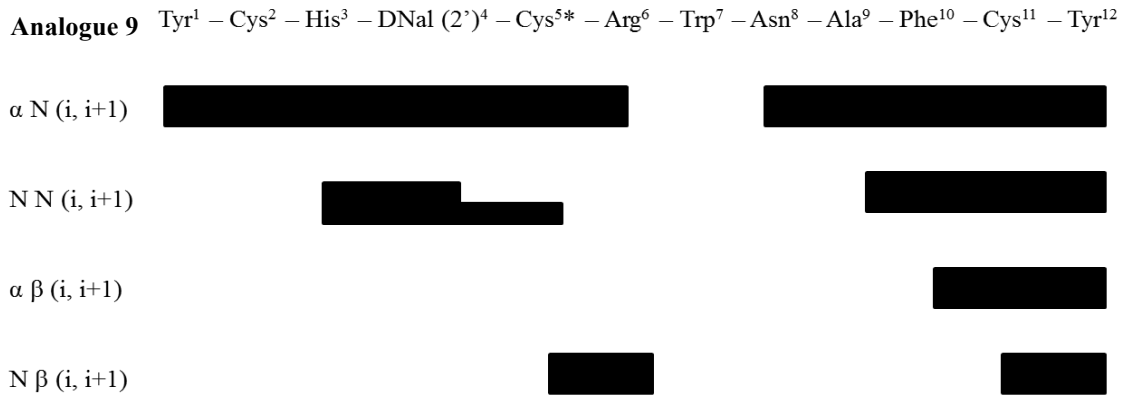
Peptide	R_1	R_2	R_3	R_4	HPLC Purity (%)	HPLC K' MeCN, MeOH	Mol. Wt. Calcd and Observed (mass spec)
1					>99	4.5, 8.0	1506.74 (1506.58)
2	His	DPhe	Arg	Trp	>96	5.5, 10.0	1635.89 (1635.72)
3	**Ala**	DPhe	Arg	Trp	>97	5.5, 8.8	1569.83 (1569.64)
4	**Phe**	DPhe	Arg	Trp	>95	6.3, 10.9	1645.92 (1645.66)
5	**Pro**	DPhe	Arg	Trp	>99	5.8, 11.0	1595.87 (1595.62)
6	His	**Pro**	Arg	Trp	>99	4.9, 7.7	1585.83 (1585.69)
7	His	**Ala**	Arg	Trp	>97	4.3, 7.7	1559.79 (1559.71)
8	His	**DNal(1')**	Arg	Trp	>97	5.4, 9.1	1685.95 (1685.77)
9	His	**DNal(2')**	Arg	Trp	>96	5.9, 10.1	1685.95 (1685.79)
10	His	**(pI)DPhe**	Arg	Trp	>96	6.0, 9.0	1761.79 (1761.70)
11	His	**DBip**	Arg	Trp	>99	6.2, 10.7	1711.99 (1711.73)
12	His	DPhe	**Ala**	Trp	>95	6.1, 10.9	1550.78 (1573.82)
13	His	DPhe	**Lys**	Trp	>95	5.9, 8.4	1607.88 (1630.21)
14	His	DPhe	**hArg**	Trp	>95	5.9, 8.3	1649.92 (1650.58)
15	His	DPhe	**Pro**	Trp	>95	4.6, 8.7	1547.81 (1570.12)
16	His	DPhe	Arg	**Ala**	>99	5.1, 7.2	1520.76 (1520.67)
17	His	DPhe	Arg	**Nal(2')**	>99	6.1, 10.4	1646.91 (1646.80)
18	His	DPhe	Arg	**DNal(2')**	>99	6.2, 10.3	1646.91 (1646.72)
19	His	DPhe	Arg	**Bip**	>96	6.3, 9.3	1672.95 (1672.74)

The HPLC k' value equals [(peptide retention time – solvent retention time)/ solvent retention time]. Two different solvent systems were used. Solvent system 1 is 10% acetonitrile (MeCN) in 0.1% trifluoroacetic acid/H_2O with a gradient to 90% acetonitrile over 35 min. Solvent system 2 is 10% methanol (MeOH) in 0.1% trifluoroacetic acid/ H_2O with a gradient to 90% methanol over 35 min. An analytical Vydac C18 column (Vydac 218TP104) with a flow rate of 1.5 ml/min was used for analytical characterization. The peptide purity was determined by HPLC in both solvent systems at a wavelength of 214 nm.

Figure S1. Summary of the NOE intensities from 400 ms NOESY data observed for compounds **2, 6,** and **8-11**. The height of the bar indicates the strength of the NOE and are categorized as strong (1.8-3.0 Å), medium (1.8-3.5 Å), or weak (1.8-5.0 Å).

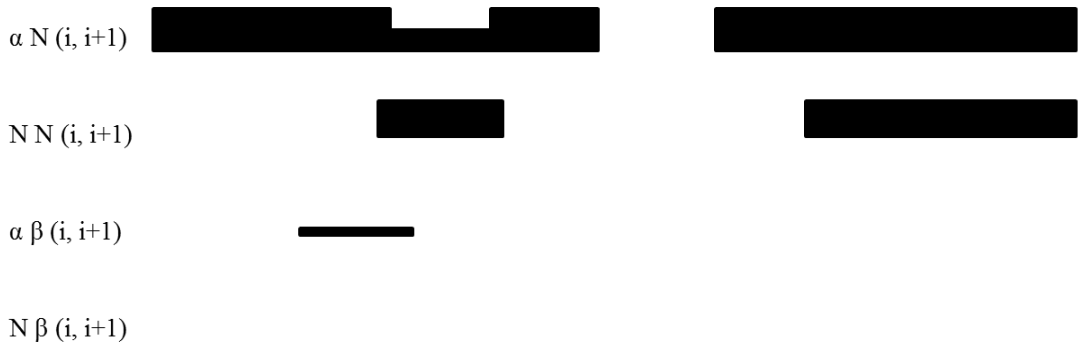






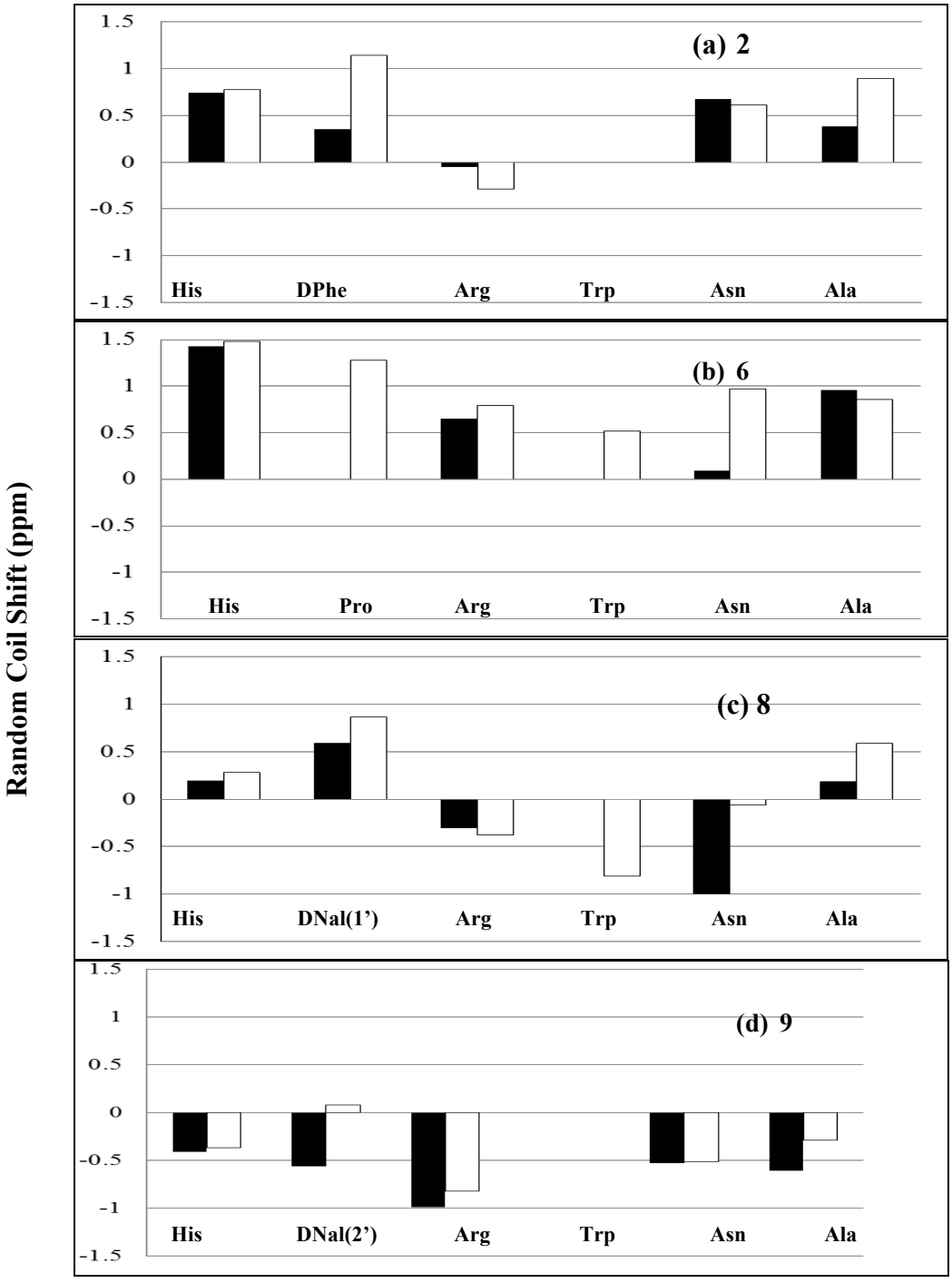
Analogue 10 $Tyr^{1} - Cys^{2} - His^{3} - (pI)DPhe^{4} - Cys^{5}* - Arg^{6} - Trp^{7} - Asn^{8} - Ala^{9} - Phe^{10} - Cys^{11} - Tyr^{12}$

Analogue 11 $Tyr^{1} - Cys^{2} - His^{3} - DBip^{4} - Cys^{5}* - Arg^{6} - Trp^{7} - Asn^{8} - Ala^{9} - Phe^{10} - Cys^{11} - Tyr^{12}$



Analogue 2 Control $Tyr^{1} - Cys^{2} - His^{3} - DPhe^{4} - Cys^{5}* - Arg^{6} - Trp^{7} - Asn^{8} - Ala^{9} - Phe^{10} - Cys^{11} - Tyr^{12}$

Figure S2. Illustration of the compound deviation from random coil values (a) control **2** (DPhe), and the analogues modified at the DPhe position (b) **6** (Pro), (c) **8** DNal(1'), (d) **9** DNal(2'), (e) **10** (pI)DPhe, and (f) **11** (DBip). The H^N and H^α protons are represented by solid bars and open bars, respectively. Vertical axes are in parts per million, and the horizontal axes represent the HxRWNA region of each peptide.



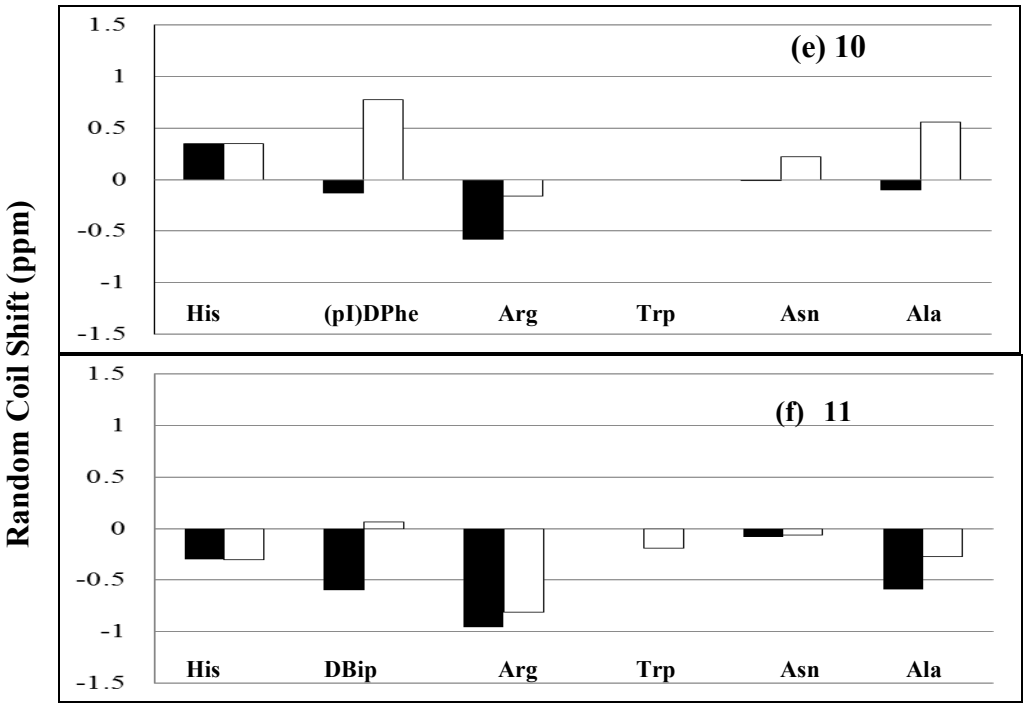

Random Coil Shift (ppm)
1.5
1
0.5
0
-0.5
-1
-1.5
(e) 10
His
(pI)DPhe
Arg
Trp
Asn
Ala
(f) 11
His
DBip
Arg
Trp
Asn
Ala
Amino Acids

Table S3. Backbone dihedral angles of the representative structures shown in Figure 4 of the manuscript. The representative structure is the lowest energy conformer of the major family.

Analogue #	**2** X_{aa}=DPhe	**6** X_{aa}=Pro	**8** X_{aa}=DNal(1')	**9** X_{aa}=DNal(2')	**10** X_{aa}=(pI)DPhe	**11** X_{aa}=DBip
φ Cys2	-139.7943	102.5854	-94.9054	2.1483	-142.46	-137.7133
ψ Cys2	42.1078	-41.7689	48.7695	89.5823	-86.7403	127.3906
φ His3	-110.8693	-153.876	55.3393	-148.9058	-82.4417	-151.3101
ψ His3	37.5965	145.5609	22.159	60.1431	70.0002	48.5147
φ X_{aa}4	-63.8368	85.9529	109.6695	-54.5003	75.0062	158.591
ψ X_{aa}4	-52.19	-51.2365	-44.8168	-50.6896	-72.6511	-44.1138
φ Cys5	-93.4163	-137.304	-170.4402	-149.4562	-82.1177	-57.225
ψ Cys5	173.8435	67.3853	76.0974	178.3109	172.0694	160.3195
φ Arg6	-103.6818	-75.9705	-86.4828	-84.8252	-72.0386	-57.225
ψ Arg6	83.4845	-4.8862	0	-54.6087	-70.5714	160.3195
φ Trp7	46.3122	-176.857	106.5383	102.183	85.0501	-108.4325
ψ Trp7	50.5596	-72.0929	130.0239	-61.9895	-35.1032	-45.7851
φ Asn8	66.2266	-134.977	-147.8068	-111.9809	-133.8701	62.4186
ψ Asn8	48.0761	30.3976	57.8726	166.381	45.2415	49.3589
φ Ala9	-164.9114	-135.487	60.7528	83.8372	-150.8847	-145.95
ψ Ala9	56.111	65.404	51.1402	178.4646	49.7409	46.5598
φ Phe10	176.2256	30.2742	79.5296	54.591	-96.9192	89.3883
ψ Phe10	153.4304	73.1155	31.9244	35.2484	34.8037	19.433
φ Cys11	-78.693	43.1145	130.2803	78.6634	46.7375	-178.3756
ψ Cys11	160.2418	57.8151	-39.3673	29.3244	84.6526	162.9706