Complex Peptide Macrocycle Optimization: Combining NMR Restraints with Conformational Analysis to Guide Structure-Based and Ligand-Based Design

Ajay N. Jain, Alexander C. Brueckner, Christine Jorge, Ann E. Cleves, Purnima Khandelwal, Janet Caceres Cortes, and Luciano Mueller

Supplementary Information on NMR Experimental Data

<u>T_1-relaxation measurements employing inversion recovery</u>

T_1-relaxation values were obtained in a T_1-inversion recovery experiment, sw = 14.8 ppm, the relaxation delay d1 = 10 s, inversion recovery intervals: 0.001, 0.4, 1.0, 2.5, 5.0, 9.8 s

T_1-values in resolved aliphatic peaks range from 1.1 to 0.474 seconds, and T_1-values in resolved low-field peaks range from 2.34 s (indole singlet peak at 10.18 ppm) to 0.66 s (singlet peak at 7.420 ppm)

Figures

Figure S-nmr-1: Pulse sequence of customized HC-HMBC(Cicero, Barbato et al. 2001) with water peak suppression. A 180° sinc-shaped pulse of 2ms duration (green rectangle) is used as a soft water peak inversion pulse. Its purpose is to flip the water magnetization back to +z. Additional water peak suppression is achieved by the application of a CW-on-resonance saturation pulse of 50 Hz amplitude during the relaxation delay d1

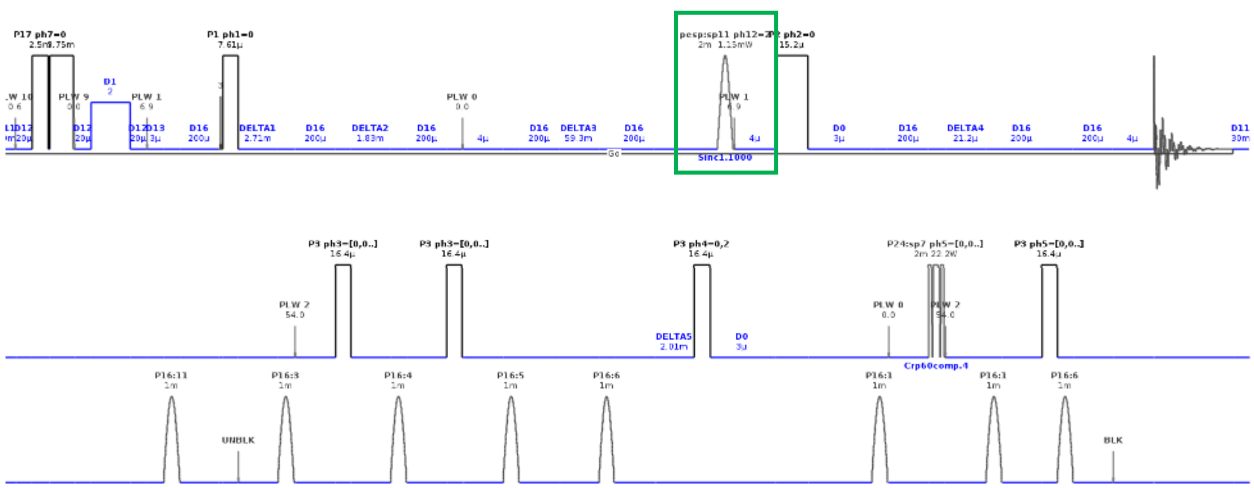

Figure S-nmr-2: HC-HMBC spectrum of compound 1 in 70% H_2O-glycine buffer + 30% CD_3CN. The water-peak was removed by low-frequency deconvolution filter of 0.25 ppm width.

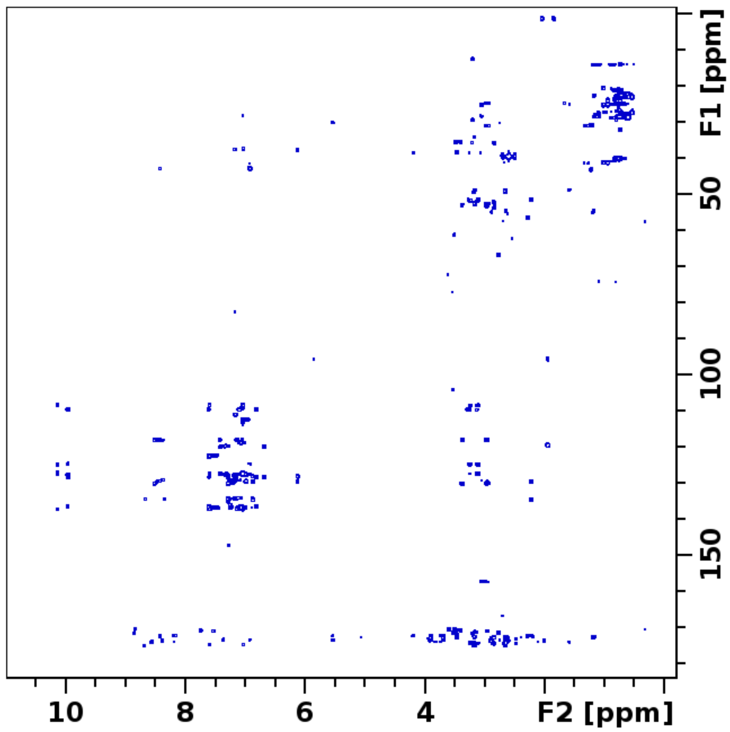

Figure S-nmr-3: Pulse sequence of the COSY experiment with double-quantum filtering (Shaka and Freeman 1983) and Excitation-Sculpting water peak suppression (Hwang and Shaka 1995). Double-quantum filtering is achieved by setting the amplitude of gp2 = 27% and gp3 = 54% (green circles). The low power water inversion pulses preceding the hard proton 180º-pulses were sinc-pulse (sin(x)/x) shapes (green squares) of 2 ms duration each.

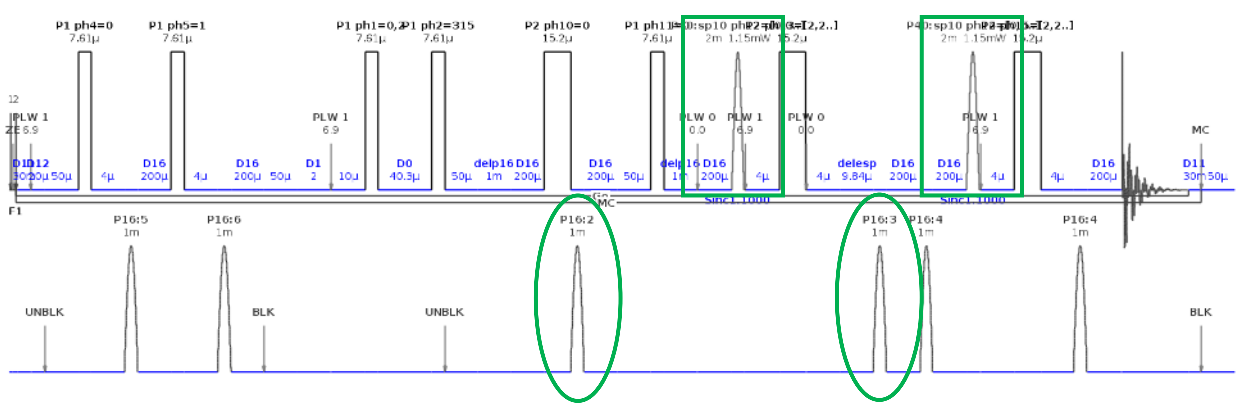

Figure S-nmr-4: DQF-COSY spectrum of compound 1 in 70% H2O glycine buffer, pH 2.5 and 30% acetonitrile-d3, the water-peak was removed by low-frequency deconvolution filter of 0.16 ppm width. The spectrum was processed in phase-sensitive mode followed by a magnitude calculation along F2 delivering enhanced resolution in F1 relative to a 2D-magnitude calculation.

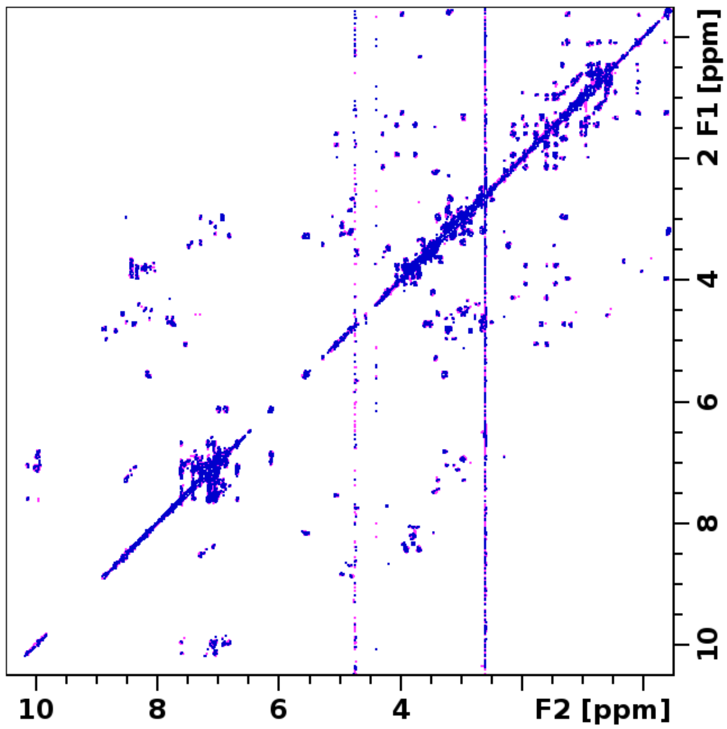

Figure S-nmr-5: NOESY-spectrum, mixing time = 0.2 s, Excitation-Sculpting water peak suppression, t_1-max = 0.04 s, t_2-acquisition time = 0.2 s, water peak suppression by Excitation Sculpting, water peak removal in F2 by a low-frequency deconvolution filter of 0.16 ppm width.

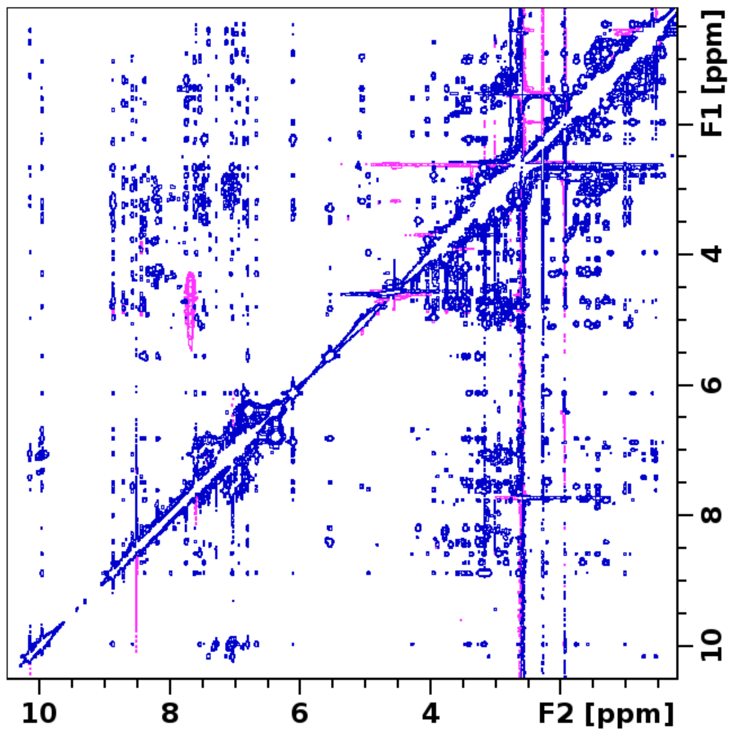

Figure S-nmr-6

Structure with ACD numbering

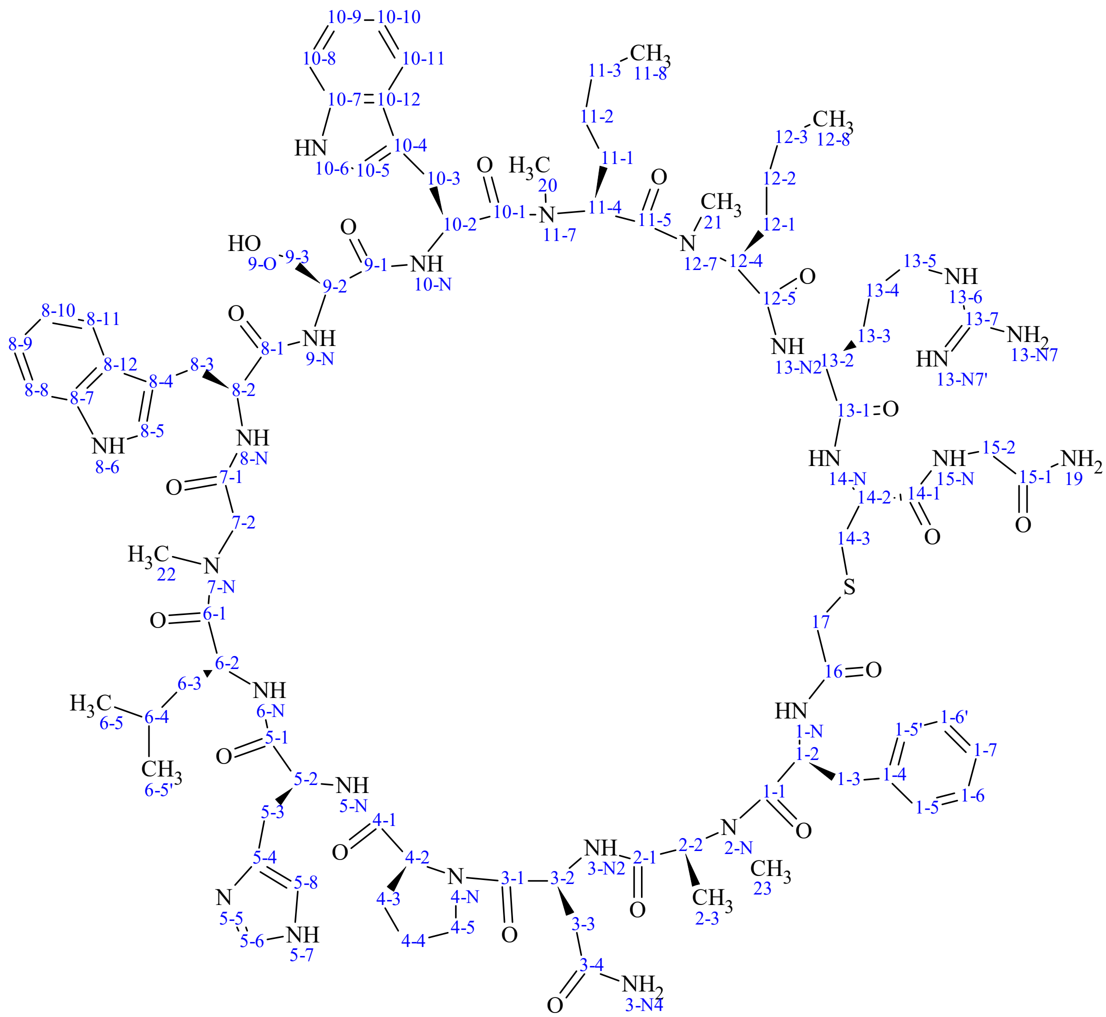

Table 1: Resonance assignments of compound 1 for the 288K and 283K dataset

			288K Dataset		283K Dataset	
ACD Number	Residue	Atom	H Shift (ppm)	C Shift (ppm)	H Shift (ppm)	C Shift (ppm)
1-7	PHS1	HZ	6.988	128.386	6.979	128.307
1-6, 1-6'	PHS1	QE	6.871	129.912	6.868	129.811
1-5, 1-5'	PHS1	QD	6.122	129.745	6.113	129.631
1-4	PHS1	CG		134.744		
1-3	PHS1	QB	2.221	37.812	2.215	37.687
1-2	PHS1	HA	3.430	51.508	3.415	51.475
1-N	PHS1	HN	7.478		7.480	
17	PHS1	HG2	3.408	35.917	3.476	35.839
17	PHS1	HG3	3.478	35.917	3.408	35.839
2-3	ALM2	QB	-0.414	12.538	-0.423	12.441
2-2	ALM2	HA	3.198	56.656	3.186	56.531
23	ALM2	HM	2.279	29.392	2.276	29.342
3-3	ASN3	HB3	2.652	37.419	3.183	37.300
3-3	ASN3	HB2	3.177	37.419	2.649	37.300
3-2	ASN3	HA	4.790	49.220	4.793	49.197
3-N2	ASN3	HN	8.862		8.872	
4-5	PRO4	HD2	3.749	48.821	4.048	48.743
4-5	PRO4	HD3	4.049	48.821	3.751	48.743
4-4	PRO4	HG3	1.444	24.977	1.435	24.875
4-4	PRO4	HG2	1.930	24.977	1.926	24.875
4-3	PRO4	HB3	2.135	30.084	1.582	29.973
4-3	PRO4	HB2	1.590	30.084	2.131	29.973
4-2	PRO4	HA	4.278	62.198	4.278	62.117
5-8	HIS5	HD2	7.287	118.231	7.289	118.040
5-6	HIS5	HE1	8.513	134.744	8.514	134.517
5-4	HIS5	CG		130.233		
5-3	HIS5	HB3	3.371	26.693	3.374	26.587
5-3	HIS5	HB2	2.954	26.693	2.957	26.587
5-2	HIS5	HA	4.544		4.545	
5-N	HIS5	HN	8.564		8.579	
6-5	LEU6	QD1	0.941	20.698	1.015	24.134
6-5'	LEU6	QD2	1.013	24.205	0.941	20.612
6-4	LEU6	HG	1.434	25.167	1.426	25.074
6-3	LEU6	HB3	1.757	41.324	1.583	41.218
6-3	LEU6	HB2	1.579	41.324	1.763	41.218
6-2	LEU6	HA	5.046	48.332	5.049	48.182

6-N	LEU6	HN	7.537		7.541	
7-2	GLM7	HA3	4.191	52.733	3.460	52.657
7-2	GLM7	HA2	3.461	52.733	4.190	52.657
22	GLM7	HM	3.164	38.485	3.166	38.408
8-12	TRP8	CD2		128.386		
8-11	TRP8	HE3	7.608	119.004	7.609	118.906
8-10	TRP8	HZ3	7.157	119.972	7.161	119.984
8-9	TRP8	HH2	7.064	122.710	7.063	122.686
8-8	TRP8	HZ2	6.680	111.227	6.671	111.101
8-7	TRP8	CE2		136.693		
8-6	TRP8	HE1	9.955		9.965	
8-5	TRP8	HD1	6.816	124.827	6.809	124.682
8-4	TRP8	CG		109.727		
8-3	TRP8	QB	3.270	30.242	3.271	30.210
8-2	TRP8	HA	5.532	51.634	5.541	51.475
8-N	TRP8	HN	8.166		8.189	
9-3	SER9	HB2	3.581	63.538	3.497	63.416
9-3	SER9	HB3	3.493	63.538	3.586	63.416
9-2	SER9	HA	4.707	52.042	4.709	
9-N	SER9	HN	8.385		8.395	
10-12	TRP10	CD2		127.475		
10-11	TRP10	HE3	7.590	118.142	7.592	118.040
10-10	TRP10	HZ3	7.043	120.071	7.036	120.091
10-9	TRP10	HH2	7.135	122.796	7.135	122.686
10-8	TRP10	HZ2	7.409	113.228	7.412	113.133
10-7	TRP10	CE2		137.365		
10-6	TRP10	HE1	10.134		10.152	
10-5	TRP10	HD1	7.039	125.067	7.036	124.976
10-4	TRP10	CG		108.501		
10-3	TRP10	HB2	3.107	28.459	3.099	28.297
10-3	MNLE11	HB3	3.236	28.459	3.232	28.297
10-2	MNLE11	HA	4.956	51.779	4.954	51.653
10-N	MNLE11	HN	8.841		8.875	
11-8	MNLE11	QE	0.535	14.038	0.536	13.983
11-4	MNLE11	HA	3.964	54.652	3.953	54.566
11-3	MNLE11	QD	0.731	23.155	0.729	23.073
11-2	MNLE11	HG3	0.066	27.402	0.064	27.275
11-2	MNLE11	HG2	-0.703	27.402	-0.752	27.275
11-1	MNLE11	HB3	-0.383	32.161	-0.406	32.095
11-1	MNLE11	HB2	1.239	32.161	1.241	32.095

20	MNLE11	HM	2.647	30.319	2.645	30.210
12-8	MNLE12	QE	0.598	14.160	0.596	14.090
12-4	MNLE12	HA	1.979	66.935	1.958	66.914
12-3	MNLE12	QD	0.872	23.031	0.860	22.930
12-2	MNLE12	HG3	0.455	28.850	0.445	28.753
12-2	MNLE12	HG2	0.102	28.850	0.091	28.753
12-1	MNLE12	HB3	0.912	27.183	0.897	27.065
12-1	MNLE12	HB2	1.329	27.183	1.322	27.065
21	MNLE12	HM	2.769	41.260	2.770	41.218
13-6	ARG13	HE	7.113		7.133	
13-5	ARG13	HD2	3.047	41.392	2.953	41.324
13-5	ARG13	HD3	2.954	41.392	3.046	41.324
13-4	ARG13	QG	3.133	28.327	3.127	28.221
13-4	ARG13	QG	2.947	28.327		
13-3	ARG13	HB2	1.668	31.197	1.306	31.084
13-3	ARG13	HB3	1.308	31.197	1.666	31.084
13-2	ARG13	HA	4.712	55.027	4.709	
13-N2	ARG13	HN	7.746		7.753	
13-N7	ARG13	HH22			7.626	
13-N7	ARG13	HH21	7.026		7.052	
13-N7'	ARG13	QH1	7.600		6.943	
14-3	CYSS14	HB2	2.839	35.623	3.220	35.484
14-3	CYSS14	HB3	3.214	35.623	2.836	35.484
14-2	CYSS14	HA	4.818	53.557	4.821	53.420
14-N	CYSS14	HN	8.677		8.712	
15-2	GLY15	HA2	3.687	42.918	3.901	42.818
15-2	GLY15	HA3	3.891	42.918	3.678	42.818
15-N	GLY15	HN	8.422		8.442	
19	GLY15	HXT1	6.923		7.389	
19	GLY15	HXT2	7.362		6.952	

Table 2

	intra-res	*i* to *i+1*	long range
single proton pair	13	15	22
pseudo-atom	36	32	47
total	49	47	69

Literature

Cicero, D. O., G. Barbato and R. Bazzo (2001). "Sensitivity Enhancement of a Two-Dimensional Experiment for the Measurement of Heteronuclear Long-Range Coupling Constants, by a New Scheme of Coherence Selection by Gradients." J. Magn. Reson. **148**(1): 209-213.
Hwang, T.-L. and A. J. Shaka (1995). "Water suppression that works. Excitation sculpting using arbitrary waveforms and pulsed field gradients." Journal of Magnetic Resonance, Series A **112**(2): 275-279.
Shaka, A. J. and R. Freeman (1983). "Simplification of NMR Spectra by Filtration through Multiple-Quantum Coherence." **51**: 169-173.