Supplementary data

Impact of the amino-acid sequence on the conformation of side-chain lactam-bridged octapeptides

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Table S1. Amino-acid sequence and analytical data of the synthesized and purified cyclopeptides (B = Aib; Z = Nle).

cyclopeptide no.	KD-*(i,i+4)*-lactam-bridged sequence[a]	$M_{calc.d}$ (Da)	$[M+H/Na]^+_{found}$[b] (Da)	$[M-H]^-_{found}$[c] (Da)	t_R[d]
1	**K**RLK**D**LVP	991.27	992.28 (H^+)		25.3
3	SR**K**KEL**D**P	995.17	996.50 (H^+)		18.9
4	V**K**KVE**D**LQ	981.18	1004.93 (Na^+)	980.88	23.5
5	VS**K**VEI**D**Q	940.09	941.11 (H^+)		22.5
7	SR**K**KEL**D**A	969.13	970.03 (H^+)		18.0
8	SR**K**KLE**D**P	995.17	995.93 (H^+)		18.4
9	SR**K**KLE**D**A	969.13	970.30 (H^+)		18.7
10	V**K**RBQ**D**LQ	994.18	995.18 (H^+)		21.4
11	V**K**RLQ**D**LQ	1022.24	1023.15 (H^+)		24.4
12	V**K**RZQ**D**LQ	1022.24	1023.78 (H^+)		24.3
13	V**K**RWQ**D**LQ	1095.29	1096.41 (H^+)		24.9
14	V**K**RVQ**D**LQ	1008.21	1009.12 (H^+)		22.6
15	V**K**QLQ**D**LQ	994.18	1016.97 (Na^+)	993.33	26.4
16	V**K**QZQ**D**LQ	994.18	1017.30 (Na^+)	992.86	26.3
17	V**K**QWQ**D**LQ	1067.24	1090.15 (Na^+)	1066.13	26.5
18	V**K**KEL**D**LQ	994.19	996.04 (H^+)		24.7
19	V**K**KEV**D**LQ	981.18	1004.07 (Na^+)	980.02	23.3

[a]All cyclopeptides are N-terminally acetylated and C-terminally amidated. Bold residues are side-chain cyclized. [b]Mass obtained by MALDI-TOF-MS in positive mode. [c]Mass obtained by MALDI-TOF-MS in negative mode. [d]Retention time t_R obtained by using the elution system and gradient specified in the section Methods.

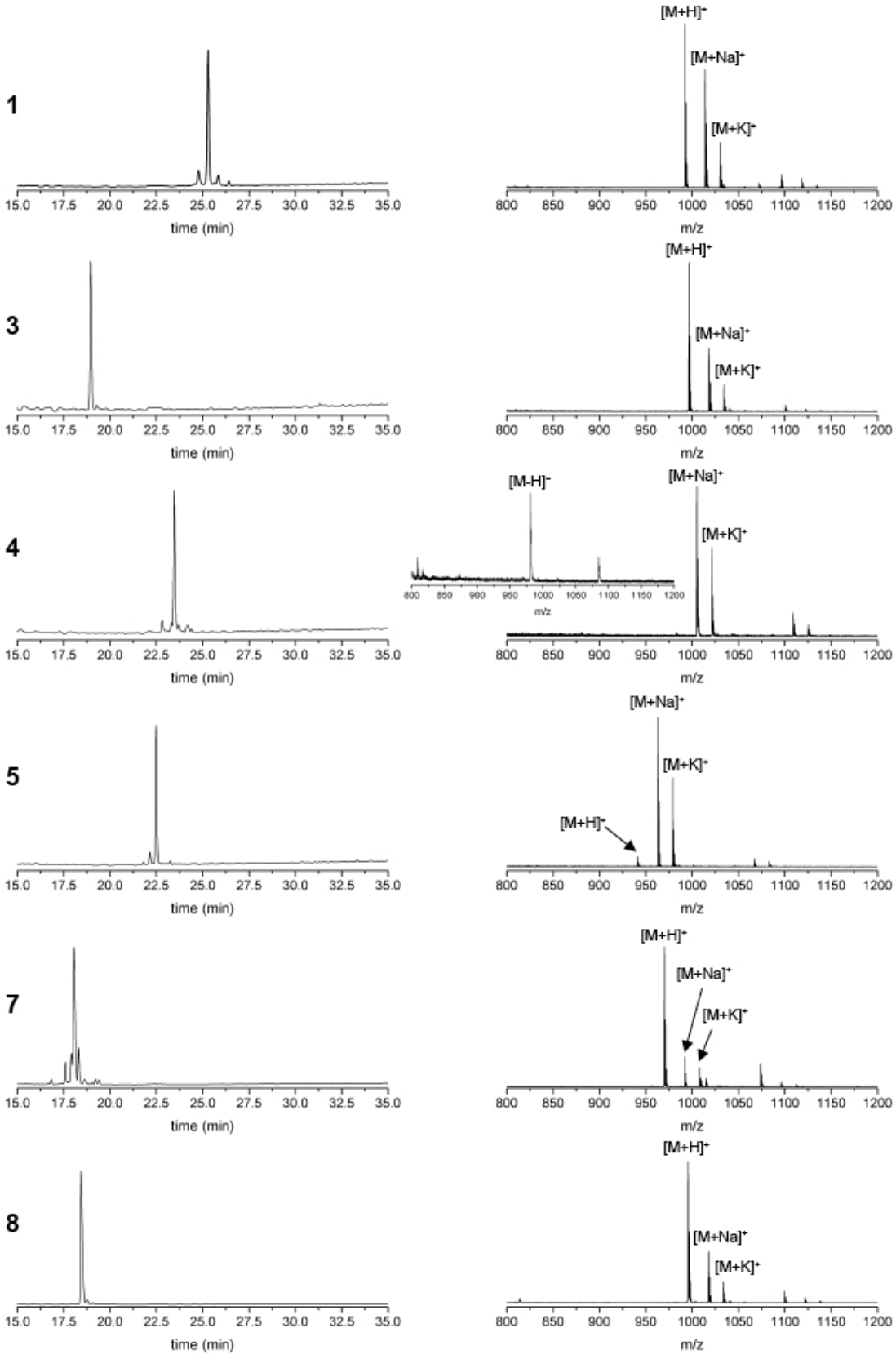

Figure S1. RP-HPLC and MALDI-TOF-MS characterization of purified cyclopeptides **1**, **3**, **4**, **5**, **7**, **8**.

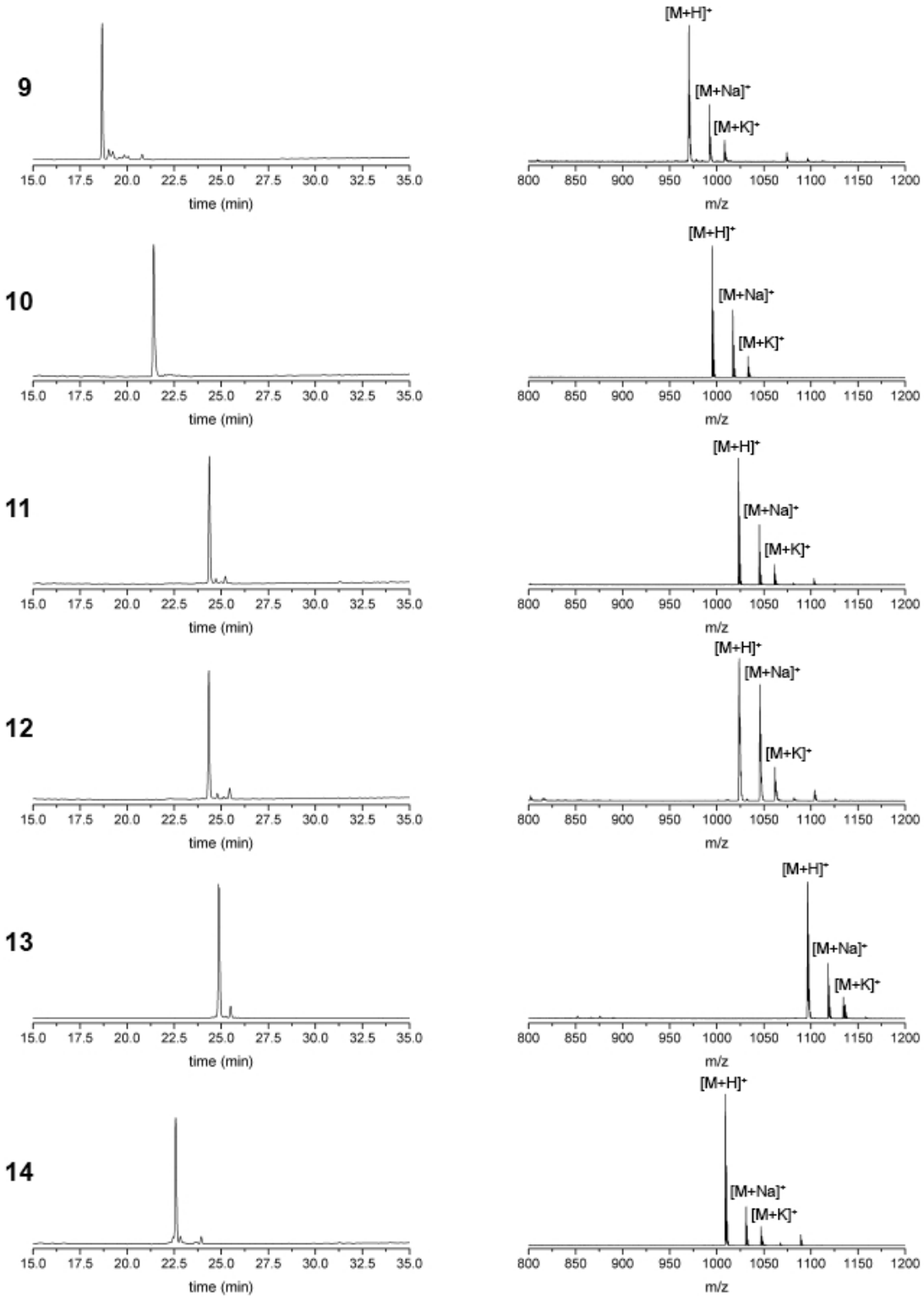

Figure S2. RP-HPLC and MALDI-TOF-MS characterization of purified cyclopeptides **9**-**14**.

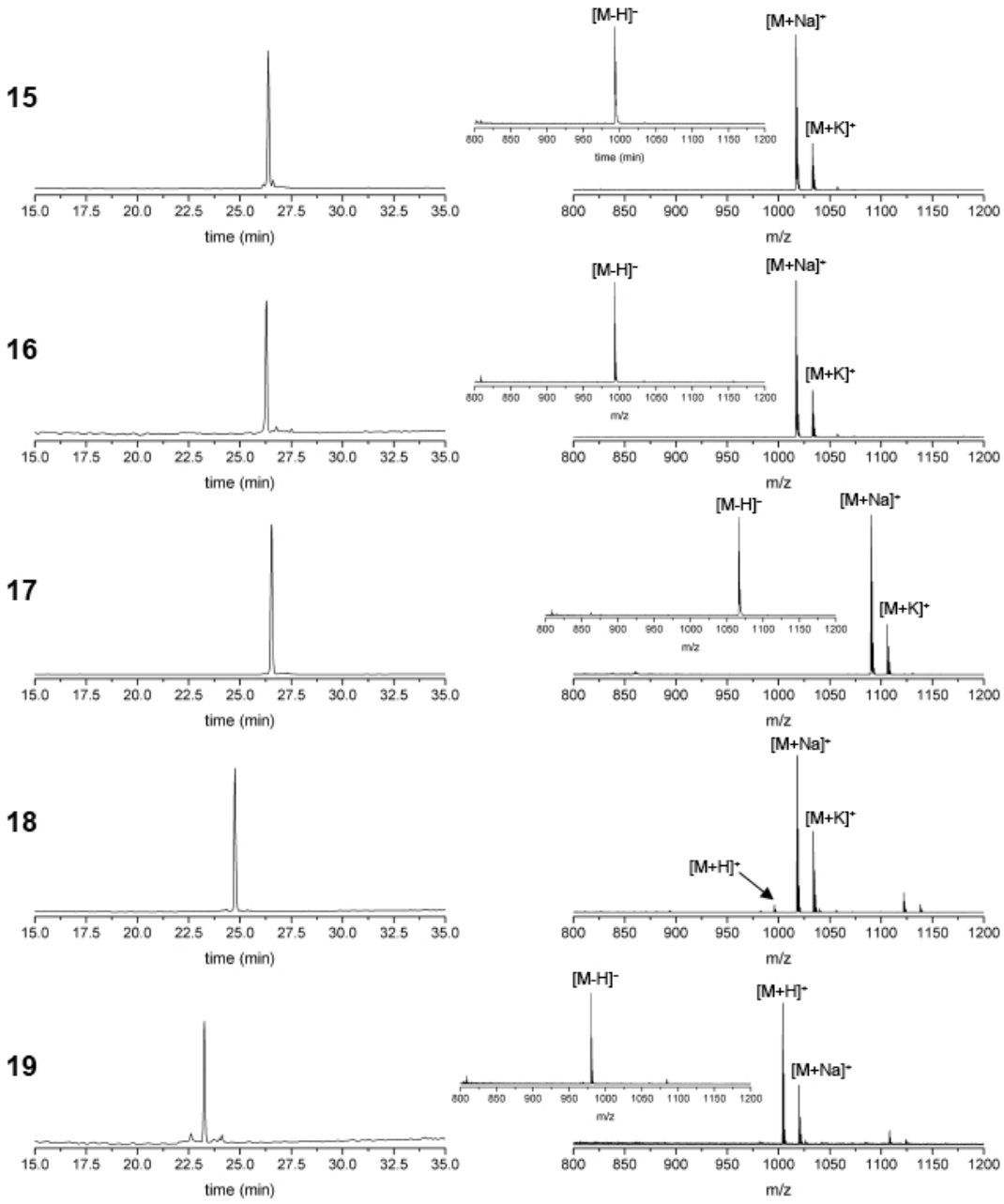

Figure S3. RP-HPLC and MALDI-TOF-MS characterization of purified cyclopeptides **15**-**19**.

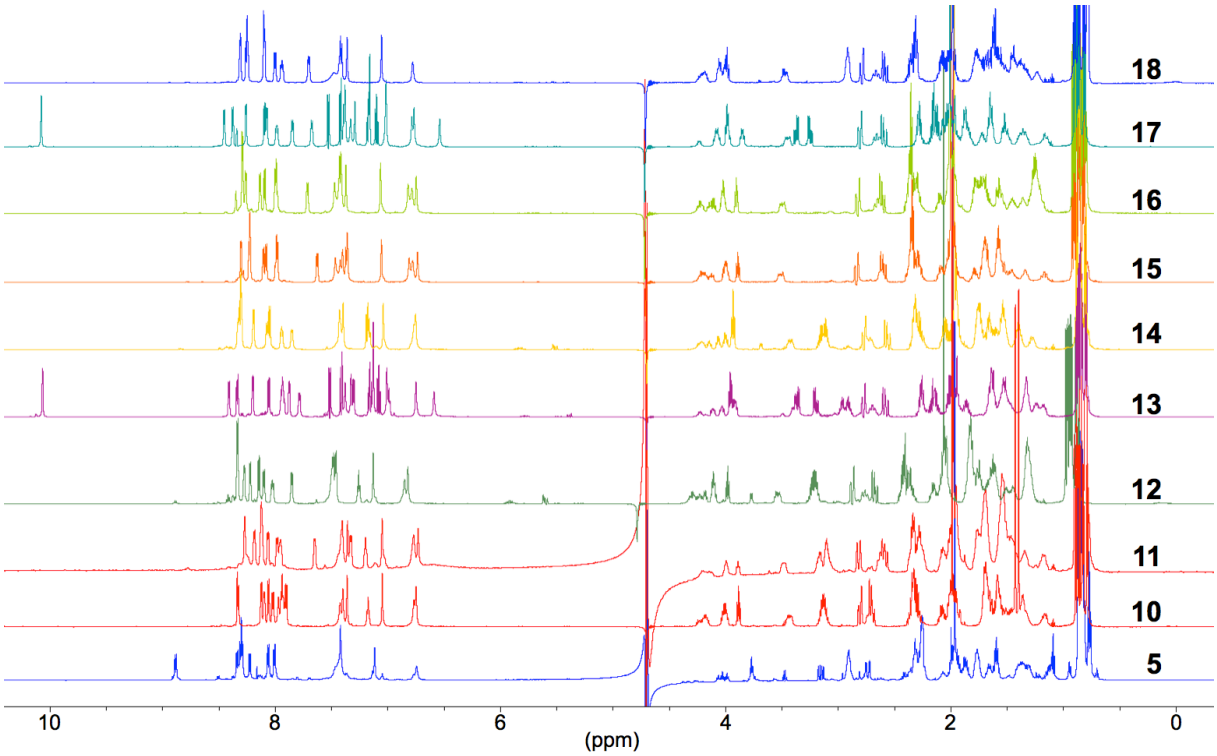

Figure S4. 1D ^{1}H-NMR spectra of purified cyclopeptides **5, 10-18** in H_2O/D_2O (12:1, v/v) at 298 K (the peptides were 0.2-0.9 mg in 500 µl solvent).

Table S2. ^{1}H and ^{13}C chemical shifts (ppm) of cyclopeptide **10**, ~1 mM in H_2O/D_2O (12:1, v/v) at 303 K.

Residue	α-NH	α-CH	β-CH	γ-CH	δ-CH	ε-CH	ε-NH	ζ-NH (bridge)
V1	8.152	3.982 63.47	2.071 32.35	0.981/0.958 20.67/20.98	-	-	-	-
K2	8.216	4.267 57.00	1.788 31.53	1.479/1.260 24.85	1.622/1.439 29.26	2.818/3.526 41.66	-	8.016
R3	8.188	4.096 58.14	1.796 30.38	1.668/1.556 27.16	3.227 43.21	-	7.265	-
Aib4	8.066	- 59.22	1.494/1.526 26.18/27.26	-	-	-	-	-
Q5	8.038	4.117 57.48	2.104 29.00	2.447/2.389 34.02	-	-	6.844/ 7.494	-
D6	8.421	4.656 54.16	2.894/2.800 38.43	-	-	-	-	-
L7	7.995	4.336 55.84	1.755/1.686 42.12	1.682 26.89	0.886/0.925 23.42/24.75	-	-	-
Q8	8.114	4.292 55.80	2.177/2.052 29.33	2.426 33.94	-	-	6.863/ 7.519	-

Table S3. NOE constraints of cyclopeptide **10** (LYL and ASL are LYS and ASP in the lactam bridge).

NOE type	Residue i	Residue i+n	NOE	Upper limit
Sequential	1 VAL HA	2 LYL H	vs	2.5
Intra-residue	1 VAL HB	1 VAL H	vs	2.5
Sequential	2 LYL HA	3 ARG H	ms	3.5
Intra-residue	3 ARG HA	3 ARG H	mw	4.5
Sequential	3 ARG HA	4 AIB H	mw	4.5
Intra-residue	3 ARG QB	3 ARG H	ms	3.5
Intra-residue	5 GLN HA	5 GLN H	mw	4.5
Intra-residue	5 GLN HA	5 GLN QB	mw	4.5
Sequential	5 GLN HA	6 ASL H	mw	4.5
Sequential	6 ASL H	5 GLN H	mw	4.5
Intra-residue	5 GLN H	5 GLN QB	mw	4.5
Inter-residue	6 ASL HA	2 LYL HE3	mw	4.5
Intra-residue	6 ASL HA	6 ASL H	mw	4.5
Sequential	6 ASL HA	7 LEU H	mw	4.5
Sequential	6 ASL H	7 LEU H	mw	4.5
Sequential	7 LEU HA	8 GLN H	mw	4.5
Intra-residue	1 VAL HA	1 VAL QG2	ms	3.5
Intra-residue	2 LYL HA	2 LYL QB	ms	3.5
Intra-residue	2 LYL HA	2 LYL H	mw	4.5
Inter-residue	6 ASL HB3	2 LYL HE2	vs	2.5
Intra-residue	2 LYL HN	2 LYL HE3	ms	3.5
Intra-residue	7 LEU HA	7 LEU H	mw	4.5
Intra-residue	6 ASL H	6 ASL HB3	mw	4.5

Table S4. NMR statistics for the obtained structural ensemble of cyclopeptide **10**.

Parameter	Value/Number
NOE constraints	23
intraresidue	13
sequential/interresidue	10
NOE constraints below upper limits	
very-strong/medium-strong/medium-weak/weak (2.5/3.5/4.5/5.5 Å)	23
Force field energies (kJ/mol)	
total	-2.83716×10^4
van der Waals	$6.92961E \times 10^3$
electrostatic	-2.82470×10^5
RMSD to the mean coordinates (Å) all atoms of ten structures	1.44
Cα atoms (residues 1-8) (Å)	0.543
Cα and Cβ atoms (residues 1-8) (Å)	0.653

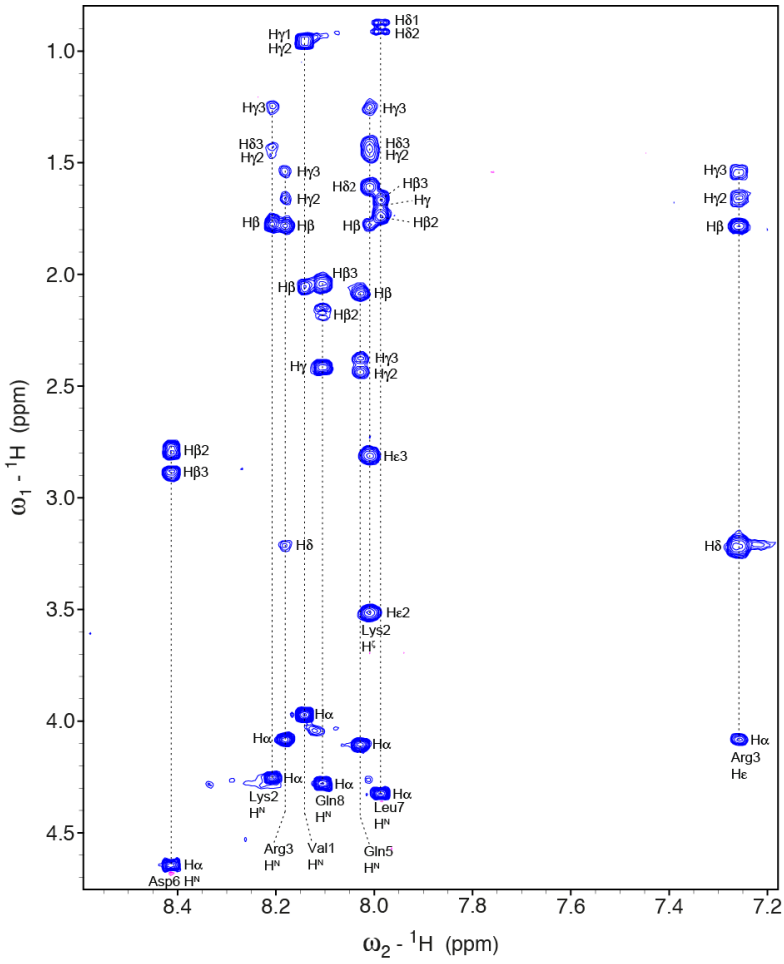

Figure S5. 2D ^{1}H-^{1}H TOCSY spectrum of **10**, ~1 mM in H_2O/D_2O (12:1, v/v), at 303 K (mixing time of 80 ms).

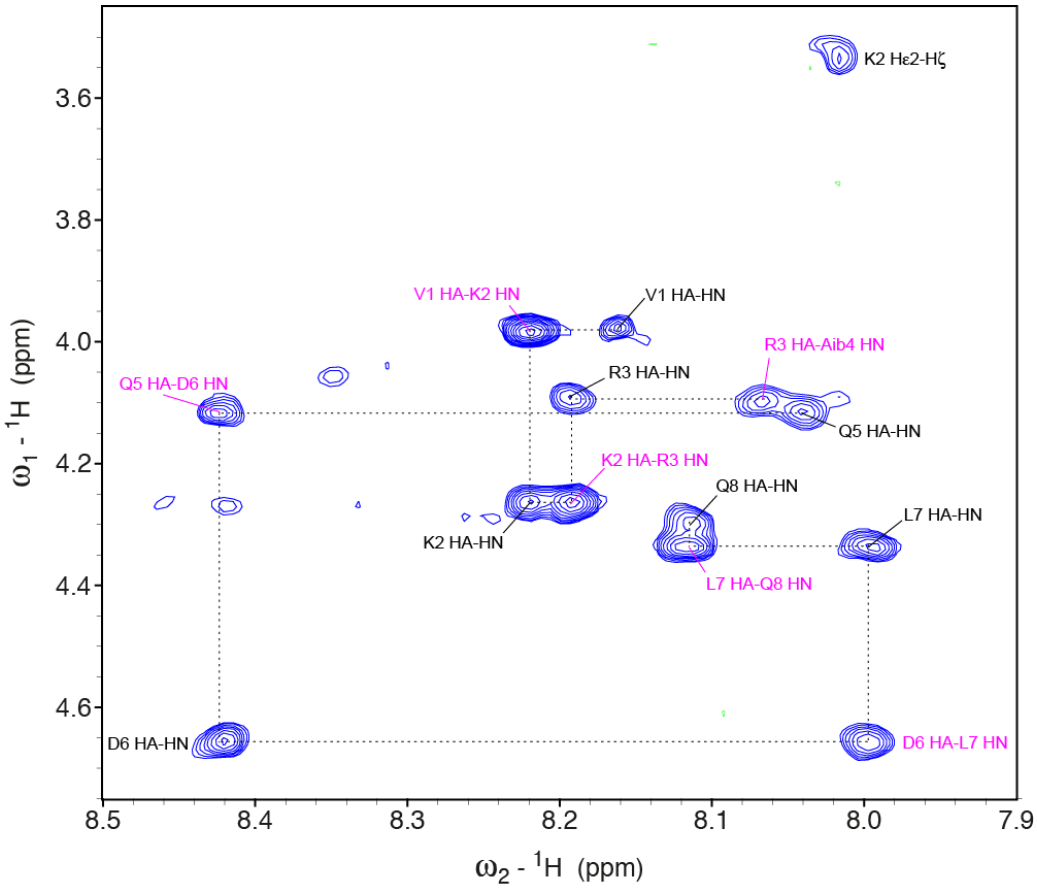

Figure S6. NMR assignment walk illustrated with a 2D ROESY spectrum of **10**, ~1 mM in H_2O/D_2O (12:1, v/v), at 303 K (mixing time 200 ms).

Table S5. Sequence alignment of the cyclopeptides based on the core triad (B = Aib, Z = Nle).

	Group A									
1			K	R	L	K	D	L	V	P
3	S	R	K	K	E	L	D	P		
8	S	R	K	K	L	E	D	P		
17		V	K	Q	W	Q	D	L	Q	
	Group B									
4		V	K	K	V	E	D	L	Q	
5	V	S	K	V	E	I	D	Q		
19		V	K	K	E	V	D	L	Q	
	Group C									
7	S	R	K	K	E	L	D	A		
9	S	R	K	K	L	E	D	A		
	Group D									
10		V	K	R	B	Q	D	L	Q	
11		V	K	R	L	Q	D	L	Q	
12		V	K	R	Z	Q	D	L	Q	
13		V	K	R	W	Q	D	L	Q	
14		V	K	R	V	Q	D	L	Q	
15		V	K	Q	L	Q	D	L	Q	
16		V	K	Q	Z	Q	D	L	Q	
18		V	K	K	E	L	D	L	Q	

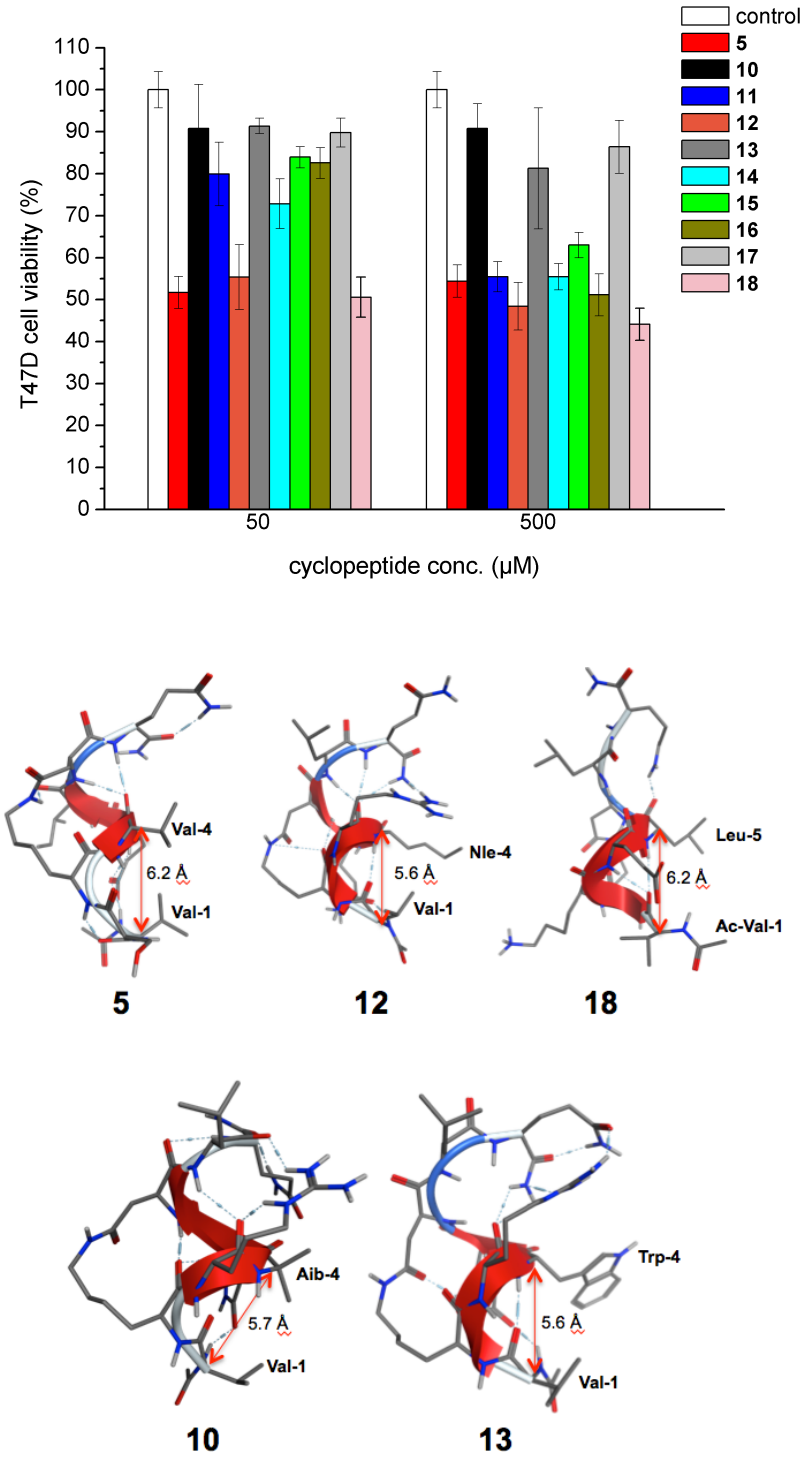

Figure S7. (Top) T47D cell viability upon 24 h incubation with the indicated cyclopeptides at the concentration of 50 μM and 500 μM. Cyclopeptides **5** (group B), **12** and **18** (group D) were the most active. Cyclopeptides **10** and **13** (group D) and **17** (group A) were inactive. (Bottom) Arrangement of the side chains at positions 1 and 4/5 in the active cyclopeptides **5**, **12**, **18** and in the inactive cyclopeptides **10** and **13**.