Electronic Supplementary Information

Optimization of peptide foldamer-based artificial retro-aldolase

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Table S1. MS and HPLC data of the synthetized peptides

Peptide	Sequence	MS Calculated m/z	MS Experimental m/z	Analytical HPLC t_r [min] (gradient*)
1	Ac-a⏲⏲Kk⏲⏲Ks-NH_2	1046.6726 [M + H^+]	1046.6719 [M + H^+]	8.79 (I)
2	Ac-Ss⏲⏲Ss⏲⏲-GGG-a⏲⏲Ss⏲⏲Ss-NH_2	943.9789 [M + 2 H^+]; 962.8732 [M + K^+H^+]	943.9803 [M + 2 H^+]; 962.8924 [M + K^+H^+]	7.75 (II)
3	Ac-Sk⏲⏲Kk⏲⏲-GGG-a⏲⏲Kk⏲⏲Ks-NH_2	712.1154 [M + 3 H^+]	712.1160 [M + 3 H^+]	8.88 (I)
4	Ac-Sf⏲⏲Ff⏲⏲-GGG-a⏲⏲Kk⏲⏲Ks-NH_2	731.0889 [M + 3 H^+]	731.0884 [M + 3 H^+]	8.87 (II)
5	Ac-Sy⏲⏲Yy⏲⏲-GGG-a⏲⏲Kk⏲⏲Ks-NH_2	1120.1217 [M + 2 H^+]	1120.1213 [M + 2 H^+]	7.89 (II)
6	Ac-Qa⏲⏲Ek⏲⏲-GGG-a⏲⏲Ak⏲⏲As-NH_2	1003.0695 [M + 2 H^+]	1003.0692 [M + 2 H^+]	7.10 (II)
7	Ac-Qa⏲⏲Yk⏲⏲-GGG-a⏲⏲Ak⏲⏲As-NH_2	1020.0799 [M + 2 H^+]	1020.0555 [M + 2 H^+]	7.71 (II)
8	Ac-Ka⏲⏲Yk⏲⏲-GGG-a⏲⏲Ak⏲⏲Ae-NH_2	694.0705 [M + 3 H^+]	694.0716 [M + 3 H^+]	7.94 (II)
9	Ac-Ka⏲⏲Yk⏲⏲-GGG-a⏲⏲Ak⏲⏲Fe-NH_2	1079.1190 [M + 2H+]	1079.1185 [M + 2H+]	7.55 (II)
10	Ac-Ka⏲⏲Yk⏲⏲-GGG-a⏲⏲Ay⏲⏲Ae-NH_2	1058.5875 [M + 2 H^+]	1058.5881 [M + 2 H^+]	8.02 (II)
11	Ac-Ka⏲⏲Yk⏲⏲-GGGG-a⏲⏲Ak⏲⏲Fe-NH_2	1107.6298 [M + 2 H^+]	1107.6996 [M + 2 H^+]	7.85 (II)
12	Ac-Ka⏲⏲Yk⏲⏲-⏲⏲-⏲⏲kA⏲⏲aE-NH_2	1031.1211 [M + 2 H^+]	1031.1221[M + 2 H^+]	7.88 (II)
13	Ac-k⏲⏲Ak⏲⏲Sa-⏲⏲⏲-aS⏲⏲kY⏲⏲e-NH_2	782.6501 [M + 3 H^+]	782.6651 [M + 3 H^+]	7.94 (II)
14	Ac-a⏲⏲Ya⏲⏲Sa-⏲⏲-⏲⏲Ky⏲⏲Sa-NH_2	695.3940 [M + 3 H^+]	695.3954 [M + 3 H^+]	16.41 (III)
15*	Ac-⏲sQ⏲⏲yY⏲⏲-⏲-sE⏲⏲kA⏲-NH_2	1008.5462 [M + 2 H^+]	1008.5677 [M + 2 H^+]	14.56 (III)
16*	Ac-⏲eQ⏲⏲kN⏲⏲-⏲-sY⏲⏲yK⏲-NH_2	1050.5806 [M + 2 H^+]	1050.6244 [M + 2 H^+]	9.00 (III)
17	Ac-a⏲⏲Ya⏲⏲Sa-⏲⏲-⏲⏲Ay⏲⏲Sa-NH_2	1014.0581 [M + 2 H^+]	1014.0566 [M + 2 H^+]	17.46 (III)
18	Ac-a⏲⏲Aa⏲⏲Sa-⏲⏲-⏲⏲Ky⏲⏲Sa-NH_2	996.5739 [M + 2 H^+]	996.5738 [M + 2 H^+]	15.31 (III)
19	Ac-a⏲⏲Ya⏲⏲Sa-⏲⏲-⏲⏲Ka⏲⏲Sa-NH_2	996.5739 [M + 2 H^+]	996.5757 [M + 2 H^+]	15.39 (III)

*Previously published in [1]

(I) H_2O/ACN: 0-3 min – 0% ACN, 3-13 min 90% ACN

(II) H_2O/ACN: 0-2 min – 10% ACN, 2-11 min 90% ACN

(III) H_2O/ACN: 0-2 min – 10% ACN, 2-32 min 90% ACN

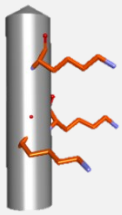
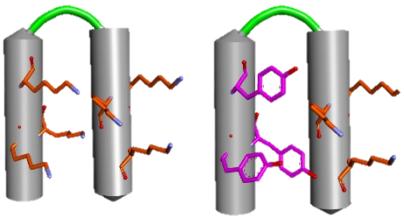
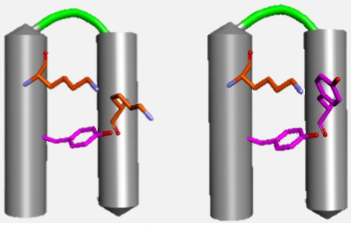
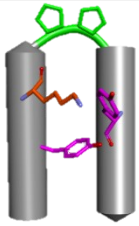
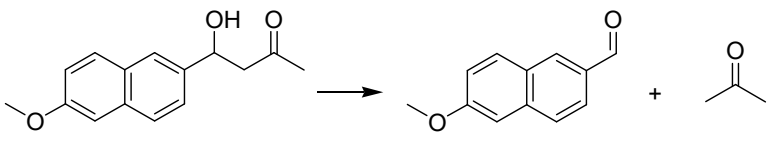
DESIGN ROUND	SCAFFOLD	MODELS	HIGHEST k_{cat} $\cdot 10^6$ [s^{-1}]	COMMENT
1	Helix		6.3 ± 1.4	Single helix with lysine residues organized on one side
2	HLH		11.6 ± 1.4	Helix-loop-helix with minimal active site
3	HLH		22.8 ± 1.2	Helix-loop-helix with native active site
4	HTH		25.0 ± 3.0	Helix-turn-helix with native active site

Figure S1. The design steps leading to optimization of artificial aldolases. In the presented models, the helical structures are represented as grey tubes and the linkers as green wires. The catalytic residues are shown in stick representation.

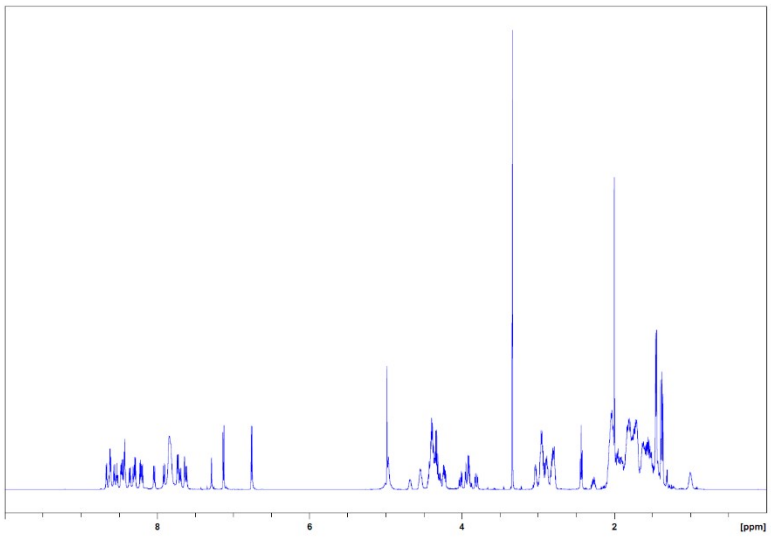


methodol

6-methoxy-2-naphtaldehyde

Figure S2. Retro-aldol reaction of methodol.

A



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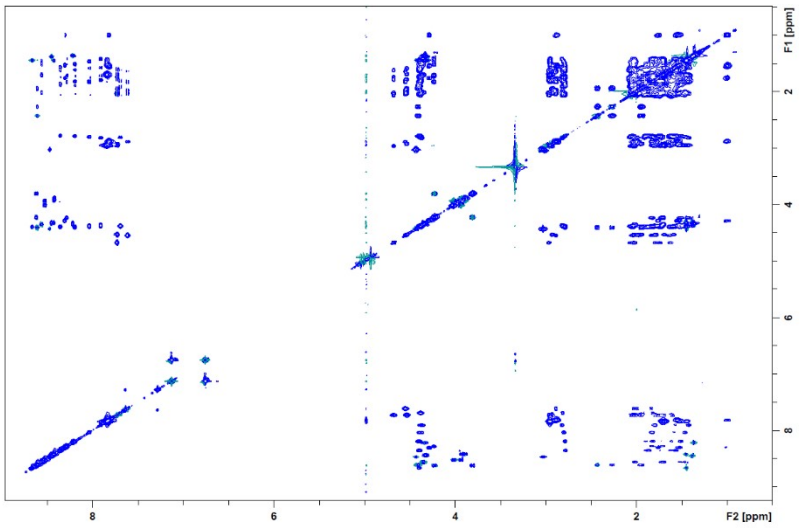

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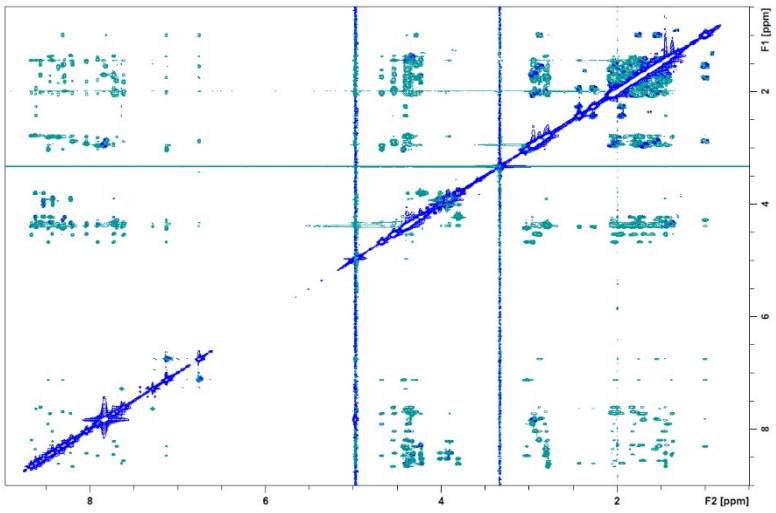
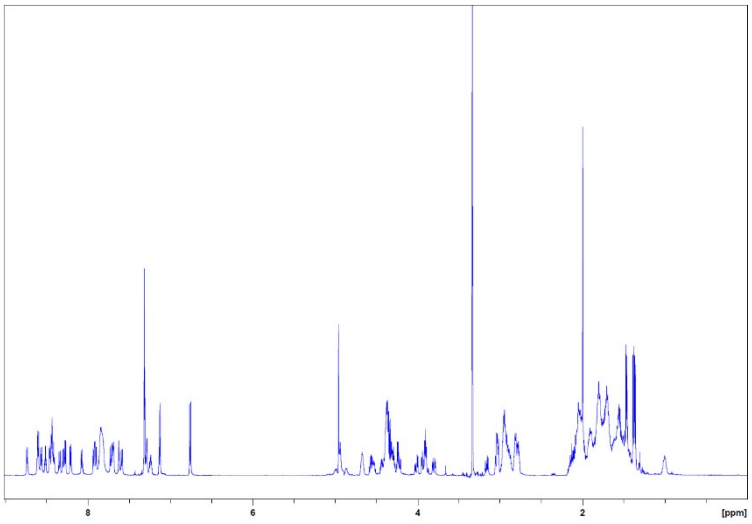


Figure S3.
^{1}H spectrum

(A), TOCSY spectrum (B), and ROESY spectrum (C) recorded for peptide **8** in d^3-MeOH at 291 K.

A



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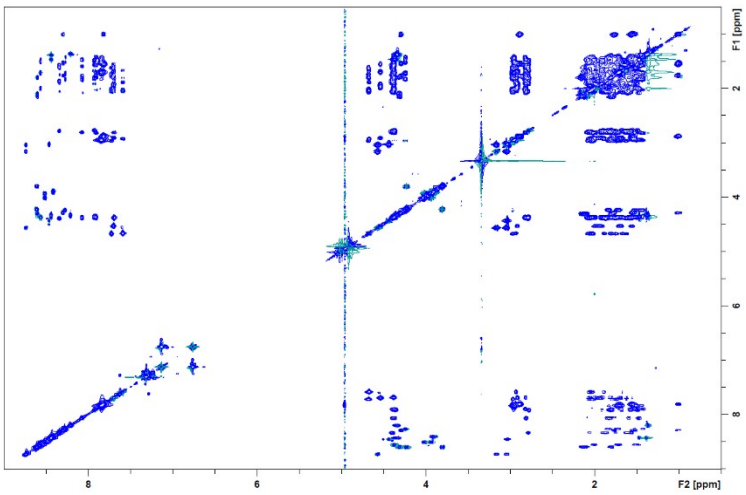


C

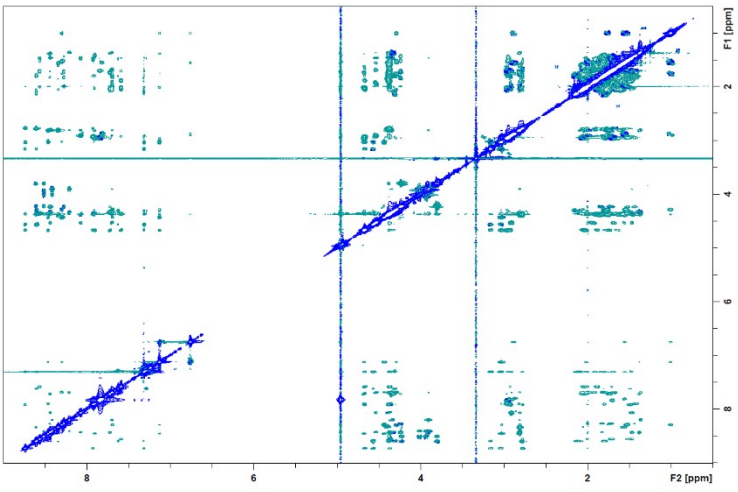
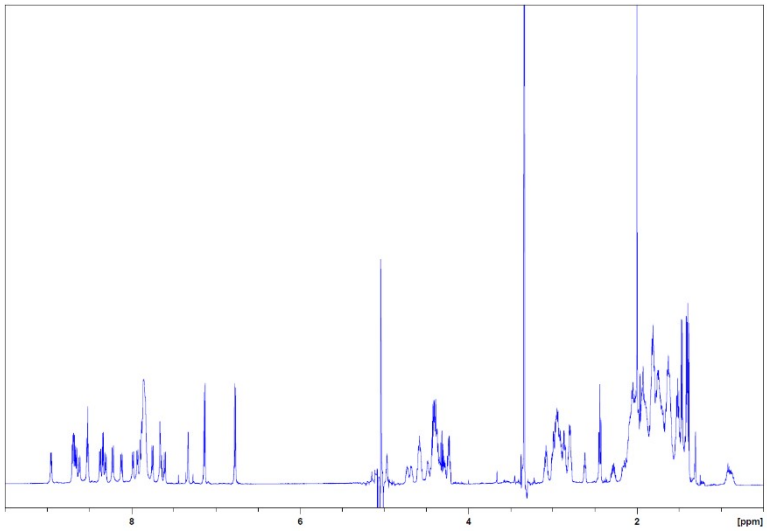
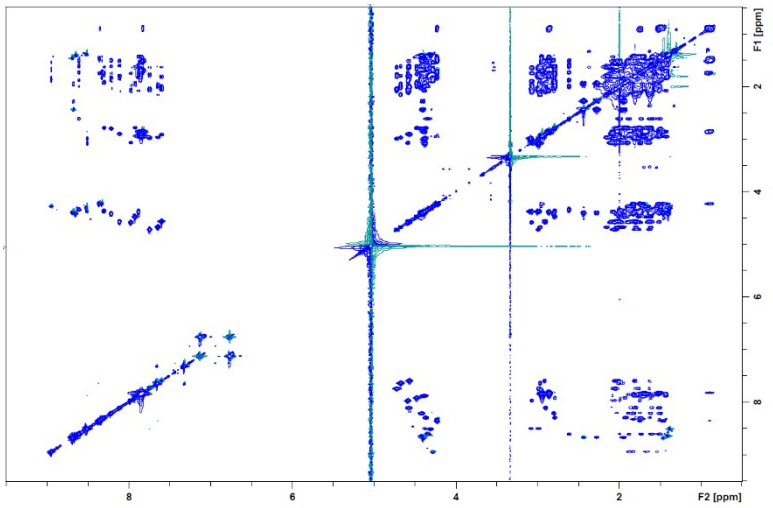


Figure S4. ^{1}H spectrum (A), TOCSY spectrum (B), and ROESY spectrum (C) recorded for peptide **9** in d^{3}-MeOH at 291 K.

A



B



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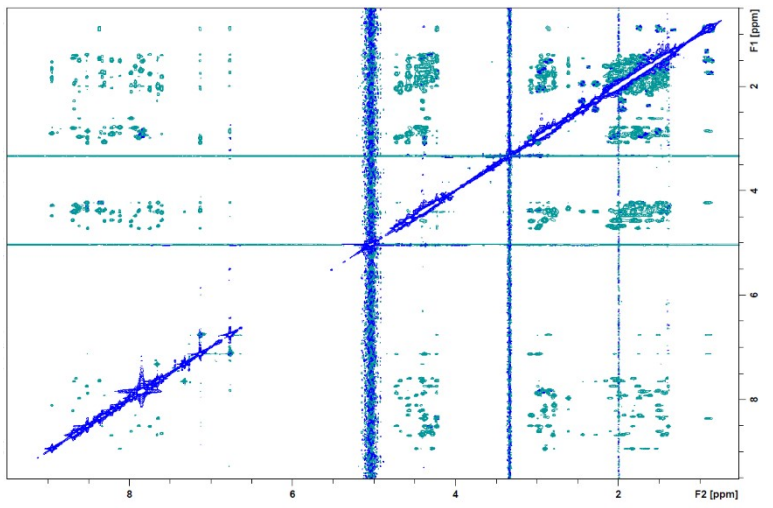


Figure S5. ^{1}H spectrum (A), TOCSY spectrum (B), and ROESY spectrum (C) recorded for peptide **12** in d^{3}-MeOH at 285 K.

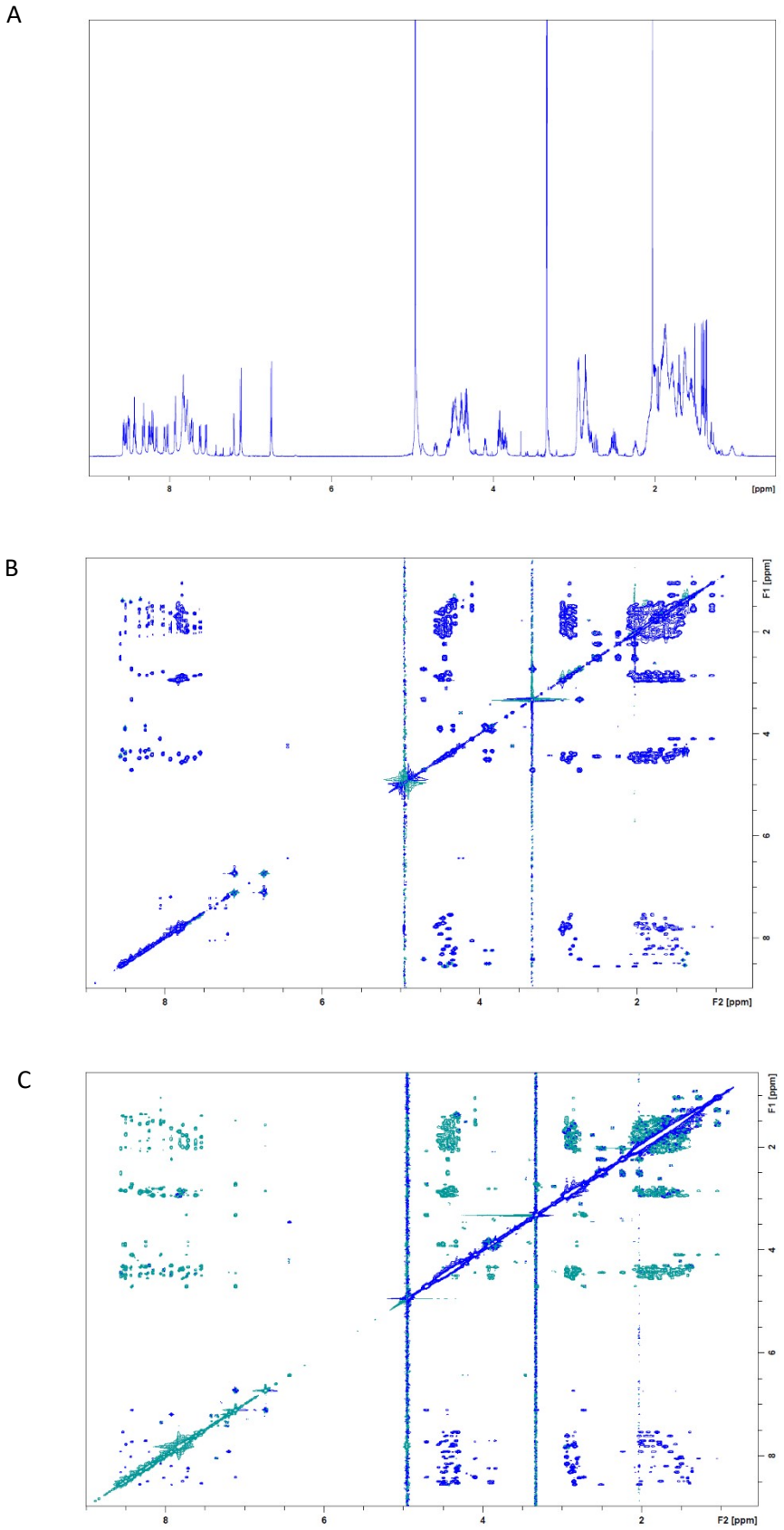
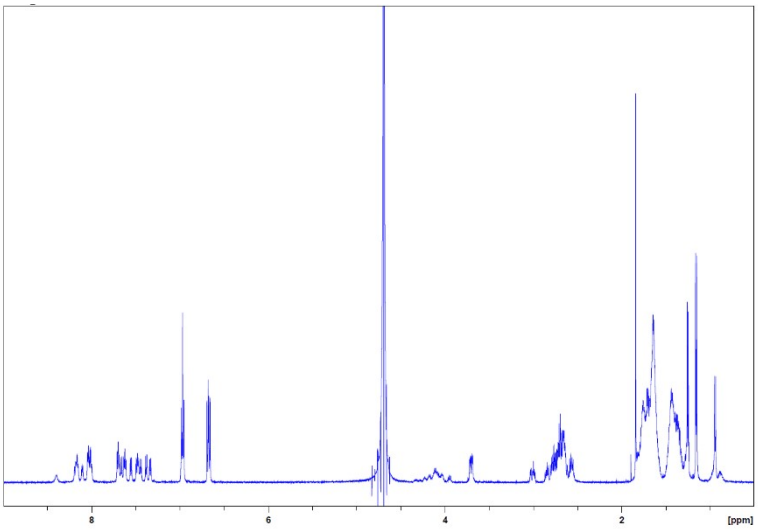
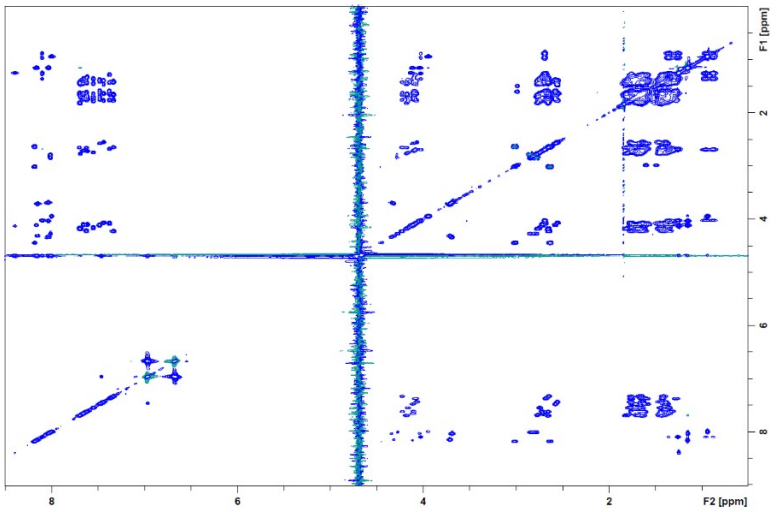


Figure S6. ^{1}H spectrum (A), TOCSY spectrum (B), and ROESY spectrum (C) recorded for peptide **13** in d^{3}-MeOH at 293 K.

A



B



C

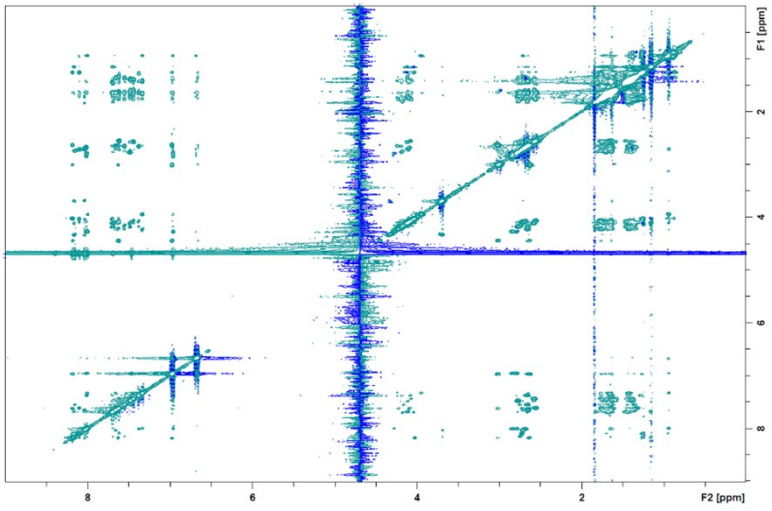


Figure S7. ^{1}H spectrum (A), TOCSY spectrum (B), and ROESY spectrum (C) recorded for peptide **14** in 10 mM phosphate buffer pH 7.5 (10% D_2O) at 293 K.

Table S2. NMR chemical shifts assignments for peptide **8**.

Residue	Proton	Chemical shift [ppm]
Ac0	HA	2.00
Lys1	HN	8.29
	HA	4.24
	CH_2	1.43; 1.52; 1.72; 1.83
ala2	HN	8.46
	HA	4.32
	HB	1.38
CpS3	HN	7.73
	HA	2.97
	HB	4.68
	CH_2	1.60; 1.74; 1.79; 2.03
CpR4	HN	7.91
	HA	2.82
	HB	4.38
	CH_2	1.43; 1.58; 1.72; 1.80; 2.04
Tyr5	HN	8.47
	HA	4.43
	HB	3.03
	HD	7.13
	HE	6.76
	OH	
lys6	HN	8.31
	HA	4.29
	CH_2	1.00; 1.55; 1.53; 1.76
CpS7	HN	7.69
	HA	2.81
	HB	4.38
	CH_2	1.62; 1.82; 1.91; 2.00; 2.07
CpR8	HN	8.36
	HA	2.79
	HB	4.40
	CH_2	1.57; 1.62; 1.75; 1.86; 1.90; 2.06
Gly9	HN	8.62
	HA	4.23; 3.81
Gly10	HN	8.53
	HA	4.02; 3.94
Gly11	HN	8.42
	HA	3.91
ala12	HN	8.22
	HA	4.34
	HB	1.36
CpS13	HN	7.73
	HA	2.93
	HB	4.54
	CH_2	1.61; 1.70; 1.95; 2.03
CpR14	HN	8.04
	HA	2.80
	HB	4.39
	CH_2	1.47; 1.59; 1.76; 1.83; 2.07
Ala15	HN	8.43
	HA	4.32
	HB	1.45
lys16	HN	8.56
	HA	4.36
	CH_2	1.48; 1.52; 1.71; 2.02
CpS17	HN	7.62
	HA	2.89
	HB	4.55
	CH_2	1.64; 1.79; 1.83; 1.99; 2.03
CpR18	HN	8.20
	HA	2.79
	HB	4.40
	CH_2	1.51; 1.61; 1.73; 1.82; 1.90; 2.05
Ala19	HN	8.67
	HA	4.40
	HB	1.45
glu20	HN	8.61
	HA	4.41
	HB	
	HG	2.43
NH_2	HN1	7.64
	HN2	7.29

Table S3. NOESY contacts for peptide **8**.

Sequential (*i*, *i*-1)	**Intensity**
HN1 – HA0	S
HN2 – HA1	S
HN3 – HA2	M
HN4 – HA3	S
HN5 – HA4	S
HN6 – HA5	S
HN7 – HA6	M
HN8 – HA7	S
HN9 – HA8	S
HN10 – HA9	M
HN11 – HA10	M
HN12 – HA11	S
HN13 – HA12	M
HN14 – HA13	S
HN15 – HA14	S
HN16 – HA15	S
HN17 – HA16	M
HN18 – HA17	S
HN19 – HA18	S
HN20 – HA19	S
HN_2 – HA20	M

NH-HN	**Intensity**
HN1 – HN2	M
HN2 – HN3	M
HN4 – HN5	M
HN6 – HN7	M
HN7 – HN8	W
HN8 – HN9	M
HN10 – HN11	M
HN11 – HN12	M
HN12 – HN13	M
HN13 – HN14	W
HN14 – HN15	M
HN16 – HN17	M
HN17 – HN18	W
HN18 – HN19	M
HN20 – HN2(1)	M

Medium range (*i*, *i*-2)	**Intensity**
HN13 – HA11	W
NH12 – HA10	W
HN11 – HA9	W
HN16 – HA14	W
HN19 – HB17	M
HN15 – HB13	M
HN5 – HB3	M
HN14 – HA12	W
HN3 – HA1	W
HN7 – HA5	W
HN17 – HA15	W
HB8 – $CH_2$6	W
HA4 – HB2	W
HN14 – HB12	W
Ar(1)5 – HA3	W

Medium range (*i*, *i*+2)	**Intensity**
HN4 – HA6	W
HB17 – HB19	M
HB3 – HB5	W
HB13 – HB15	M
HA7 – HA9	M

Medium range (*i*, *i*-3)	**Intensity**
HA6 – HA3	W
HA10 – HA7	W
Ar(1)5 – HB2	W
Ar(2)5 – HB2	w

Medium range (*i*, *i*+3)	**Intensity**
HB17 – HE20	W
HA11 – HA14	W
HB13 – HA16	W

Medium range (*i*, *i*-4)	**Intensity**
HD20 – HA16	W

Medium range (*i*, *i*+4)	**Intensity**
HN1 – HA5	W

NH	**3J [Hz]**
HN1	6.37
HN2	7.27
HN3	8.29
HN4	9.37
HN5	5.23
HN6	8.59
HN7	8.41
HN8	9.13
HN10	5.56
HN12	6.91
HN13	8.29
HN14	9.13
HN15	5.28*
HN16	8.23
HN17	8.47
HN18	9.43
HN19	6.31
HN20	8.05

Table S4. NMR chemical shifts assignments for peptide **9**.

Residue	Proton	Chemical shift [ppm]
Ac0	HA	2.00
Lys1	HN	8.27
	HA	4.24
	CH_2	1.43,1.52, 1.73, 1.83
ala2	HN	8.43
	HA	4.34
	HB	1.38
CpS3	HN	7.72
	HA	2.97
	HB	4.68
	CH_2	1.62, 1.74, 2.03
CpR4	HN	7.91
	HA	2.82
	HB	4.38
	CH_2	1.43, 1.58, 1.73, 1.80, 2.03
Tyr5	HN	8.46
	HA	4.44
	HB	3.03
	HD	7.13
	HE	6.67
	OH	
lys6	HN	8.30
	HA	4.29
	CH_2	1.00, 1.53, 1.77
CpS7	HN	7.69
	HA	2.81
	HB	4.38
	CH_2	1.61, 1.69, 1.82, 1.92, 2.07
CpR8	HN	8.34
	HA	2.79
	HB	4.40
	CH_2	1.57, 1.62, 1.75, 1.87, 1.90, 2.06
Gly9	HN	8.61
	HA	3.80, 4.23
Gly10	HN	8.51
	HA	3.94, 4.02
Gly11	HN	8.41
	HA	3.89, 3.92
ala12	HN	8.21
	HA	4.34
	HB	1.36
CpS13	HN	7.70
	HA	2.91
	HB	4.54
	CH_2	1.55, 1.69, 1.93, 1.98
CpR14	HN	8.07
	HA	2.81
	HB	4.38
	CH_2	1.48, 1.59, 1.77, 1.82, 2.09
Ala15	HN	8.44
	HA	4.37
	HB	1.47
lys16	HN	8.56
	HA	4.37
	CH_2	1.50, 1.71, 2.00
CpS17	HN	7.59
	HA	2.93
	HB	4.67
	CH_2	1.67, 1.81, 1.89, 2.05
CpR18	HN	7.93
	HA	2.78
	HB	4.37
	CH_2	1.49, 1.57, 1.68, 1.81, 1.92
Phe19	HN	8.74
	HA	8.73
	HB	3.04, 3.16
	Har	7.22 - 7.33
glu20	HN	8.60
	HA	4.31
	CH_2	1.80, 2.12
NH_2	HN1	7.28
	HN2	7.62

Table S5. NOESY contacts for peptide **9**.

Sequential (*i*, *i*-1)	Intensity
HN1 – HA0	S
HN2 – HA1	S
HN3 – HA2	S
HN4 – HA3	S
HN5 – HA4	S
HN6 – HA5	S
HN7 – HA6	S
HN8 – HA7	S
HN9 – HA8	S
HN10 – HA9	M
HN11 – HA10	M
HN12 – HA11	S
HN13 – HA12	S
HN14 – HA13	S
HN15 – HA14	S
HN16 – HA15	S
HN17 – HA16	S
HN18 – HA17	S
HN19 – HA18	S
HN20 – HA19	S
HN_2 – HA20	S
HN_2 – HA20	W

NH-HN	Intensity
HN1 – HN2	W
HN2 – HN3	M
HN4 – HN5	M
HN6 – HN7	M
HN7 – HN8	W
HN8 – HN9	M
HN10 – HN11	M
HN11 – HN12	M
HN12 – HN13	M
HN13 – HN14	W
HN14 – HN15	M
HN18 – HN19	M
HN19 – HN20	M
HN20 – HN2(1)	M

Medium range (*i*, *i*-2)	Intensity
HN9 – HB7	M
NH19 – HB17	M
HN5 – HB3	M
HN15 – HB19	M
HN12 – HA10	W
HN3 – HA1	W
HN13 – HA11	W
NH2(2) – HA19	W
HAr19 – HB17	W
HD5 – HB3	W
HN14 – HB12	W
HN17 – HB15	M
NH2(2) – HB 19	M
HB4 – HB2	S
HB14 – HB12	S
HB18 – CH2 16	M
Ar(1)5 – HA3	

Medium range (*i*, *i*+2)	Intensity
HN18 – HA20	W?
HN4 – HA6	W?
HB3 – HB5	S
HB17 – HB19 (1)	S
HB17 – HB19 (2)	S

NH	3J [Hz]
HN1	8.39
HN2	7.32*
HN3	8.62
HN4	9.50
HN5	5.28
HN6	8.48
HN7	8.34
HN8	5.09
HN9	6.12*
HN10	5.63
HN11	5.64
HN12	6.97
HN13	8.57
HN14	9.16
HN15	5.28*
HN16	8.18
HN17	8.07
HN18	9.61
HN19	6.37
HN20	7.54*

Table S6. NMR chemical shifts assignments for peptide **12**.

Residue	Proton	Chemical			shift

		[ppm]
Ac0	HA	1.998
Lys1	HN	8.338
	HA	4.234
	CH_2	1.835; 1.724; 1.516; 1.426
ala2	HN	8.526
	HA	4.316
	CH_3	1.388
CpS3	HN	7.752
	HB	2.992
	HA	4.4724
	CH_2	-
CpR4	HN	7.890
	HB	2.862
	HA	4.387
	CH_2	-
Tyr5	HN	8.516
	HA	4.378
	CH_2	2.993 3.084
	CH arom	7.134 (1); 6.772 (2)
lys6	HN	8.372
	HA	4.230
	CH_2	1.755; 1.471; 0.892
CpS7	HN	7.650
	HB	2.969
	HA	4.676
	CH_2	-
CpR8	HN	8.618
	HB	2.616
	HA	4.349
	CH_2	1.998; 1.888; 1.737; 1.605; 1.505
CpS9	HN	7.984
	HB	3.074
	HA	4.590
	CH_2	-
CpS10	HN	8.309
	HB	2.788
	HA	4.374
	CH_2	1.379 1.746 1.629
CpR11	HN	7.932
	HB	2.788
	HA	4.479
	CH_2	-
CpS12	HN	8.119
	HB	2.905
	HA	4.581
	CH_2	-
lys13	HN	8.955
	HA	4.286
	CH_2	1.884; 1.837; 1.672; 1.544
Ala14	HN	8.657
	HA	4.420
	CH_3	1.409
CpR15	HN	7.605
	HB	2.915
	HA	4.567
	CH_2	-
CpS16	HN	8.225
	HB	2.804
	HA	4.424
	CH_2	-
ala17	HN	8.699
	HA	4.379
	CH_3	1.465
Glu18	HN	8.682
	HA	4.409
	CH_2	2.284 1.952
	COOH	2.438
NH_2	HN1	7.662
	HN2	7.328

Table S7. NOESY contacts for peptide **12**.

Sequential (*i*, *i*-1)	**Intensity**
HN1 – HA0	S
HN2 – HA1	S

HN3 – HA2	S
HN4 – HB3	S
HN5 – HB4	S
HN6 – HA5	S
HN7 – HA6	S
HN8 – HB7	S
HN9 – HB8	S
HN10 – HA9	S
HN11 – HA10	S
HN12 – HA11	S
HN13 – HA12	S
HN14 – HB13	S
HN15 – HB14	S
HN16 – HA15	S
HN17 – HA16	S
HN18 – HB17	S
HN – HA20	S (1) W (2)
NH-HN	**Intensity**
HN1 – HN2	W
HN2 – HN3	M
HN4 – HN5	M
HN6 – HN7	M
HN8 – HN9	W
HN10 – HN11	W
HN12 – HN13	W
HN14 – HN15	M
HN16 – HN17	W

HN_2 – HN20	M (1)

Medium range (*i*, *i*-2)	**Intensity**
NH3 - HA1	W
NH5 - HA3	M
NH9 - HA7	M
NH13 - HA11	M
NH15 - HA13	W
NH17 - HA15	M
CH_{arom}5(1) - HA3	W

$^3J_{HN-HA}$ [Hz]	
HN1	6.263
HN3	8.436
HN6	8.778
HN8	9.861
HN9	7.702
HN10	9.117
HN11	7.426
HN12	9.565
HN13	6.036
HN14	7.801
HN15	8.610
HN16	9.433

Table S8. NMR chemical shifts assignments for peptide **13**.

Residue	**Proton**	**Chemical shift [ppm]**
Ac1	HA	2.032
Lys2	HN	8.206
	HA	4.312

	CH_2	1.838; 1.695; 1.516; 1.438
CpS3	HN	7.932
	HB	2.950
	HA	4.502
	CH_2	2.005; 1.938; 1.858 1.634
CpR4	HN	8.026
	HB	2.785
	HA	4.397
	CH_2	2.064; 1.863; 1.750; 1.609; 1.506
Ala5	HN	8.437
	HA	4.344
	HB	1.424
lys6	HN	8.502
	HA	4.385
	CH_2	2.011; 1.763; 1.702; 1.530; 1.466
CpS7	HN	7.784
	HB	2.870
	HA	4.563
	CH_2	1.993; 1.884; 1.775
CpR8	HN	8.231
	HB	2.858
	HA	4.465
	CH_2	2.097; 1.893; 1.625; 1.547
Ser9	HN	8.509
	HA	4.503
	HB	3.921; 3.881
ala10	HN	8.542
	HA	4.322
	HB	1.398
CpS11	HN	7.551
	HB	2.842
	HA	4.377
	CH_2	2.014; 1.923; 1.810; 1.559
CpS12	HN	7.822
	HB	2.947
	HA	4.401
	CH_2	1.924; 1.855;
		1.702
CpS13	HN	7.622
	HB	2.868
	HA	4.472
	CH_2	2.037; 1.938; 1.884; 1.646
ala14	HN	8.312
	HA	4.336
	CH_3	1.364
Ser15	HN	8.253
	HA	4.339
	HB	3.932; 3.847
CpR16	HN	7.717
	HB	2.948
	HA	4.468
	CH_2	1.992; 1.870; 1.639
CpS17	HN	8.163
	HB	2.816
	HA	4.414
	CH_2	1.850; 1.796; 1.721; 7.591; 1.506
lys18	HN	8.063
	HA	4.013
	CH_2	1.558; 1.494
Tyr19	HN	8.425
	HA	4.712
	HB	3.325; 2.736
	Harom	6.744; 7.120
CpR20	HN	7.738
	HB	2.895
	HA	4.524
	CH_2	2.025; 1.786; 1.625
CpR21	HN	8.328
	HB	2.849
	HA	4.482
	CH_2	2.085; 1.890; 1.799; 1.632; 1.557
glu22	HN	8.568
	HA	4.445
	CH_2	2.245; 2.034
	COOH	2.514
NH_2	HN1	7.925
	HN2	7.205

Table S9. NOESY contacts for peptide **13**.

Sequential (*i*, *i*-1)	**Intensity**
HN2 – HA1	S
HN3 – HA2	S
HN4 – HB3	S

HN5 – HB4	S
HN6 – HA5	S
HN7 – HA6	S
HN8 – HB7	S
HN9 – HB8	S
HN10 – HA9	S
HN11 – HA10	S
HN12 – HB11	S
HN13 – HB12	S
HN14 – HB13	S
HN15 – HA14	S
HN16 – HA15	S
HN17 – HB16	S
HN18 – HB17	S
HN19 – HA18	S
HN20 – HA19	S
HN21 – HB20	S
HN22 – HB21	S
HN1 – HA22	S
HN2 - HA22	W

NH-HN	**Intensity**
HN3 – HN2	W
HN5 – HN4	M
HN7 – HN6	M
HN9 – HN8	W
HN11 – HN10	M
HN14 - HN13	W
HN16 – HN15	M
HN18 – HN17	M
HN20 – HN19	M
HN21 – HN20	W
HN22 – HN1	W
HN16 – HN14	W

Medium range (*i*, *i*-2)	**Intensity**
NH4 – HA2	W
$CH_2$4 - HA2	M
NH5 - HA3	M
HB5 - HB3	M
NH7 – HA5	M
HB9 - HB7	M

NH11 – HA9	M
$CH_2$11 - HB9	W
NH12 – HB10	W
NH16 - HB14	W
HB16 - HB14	W
NH17 – HA15	W
HB17 - HB15	M
NH18 – HB16	M
NH19 – HA17	W
NH20 – HA18	M
$CH_2$21 - HA19	M
HA21 - HB19	M
NH22 – HB20	M

Medium range (i, i-3)	**Intensity**
HA12 – HB9	W
HB15 – HA12	W
HB15 - $CH_2$12	W
HN18 – HB15	W
HA19 – HB16	W

Long range (i, i-4)	**Intensity**
HB9 – HA5	W
HN18 – HA14	W
HN22 – HA18	W

$^3J_{HN-HA}$ [Hz]	
HN2	7.387
HN4	8.829
HN7	8.108*
HN8	8.889
HN10	6.847
HN11	7.988
HN12	8.228*
HN13	8.288
HN15	7.868
HN17	9.489
HN18	5.165
HN22	7.567

Table S10. NMR chemical shifts assignments for peptide **14**.

Residue	**Proton**	**Chemical shift [ppm]**
Ac1	HA	1.846
ala2	HN	8.041
	HA	4.043

	HB	1.161
CpS3	HN	7.706
	HB	4.187
	HA	2.663
	CH_2	1.447; 1.653; 1.824
CpR4	HN	7.384
	HB	4.086
	HA	2.668
	CH_2	1.288; 1.441; 1.650; 1.769
Tyr5	HN	8.020
	HA	4 .282
	HB	2.798; 2.846
	HD	6.968; 6.687
	HE	7.476
ala6	HN	8.007
	HA	3.954
	HB	0.949
CpS7	HN	7.341
	HB	4.236
	HA	2.657
	CH_2	1.428; 1.663; 1.772
CpR8	HN	7.666
	HB	4.107
	HA	2.721
	CH_2	1.369; 1.457; 1.639; 1.731
Ser9	HN	8.044
	HA	4.347
	HB	3.696
ala10	HN	8.172
	HA	4.124
	HB	1.163
CpR11	HN	7.695
	HB	4.183
	HA	2.779
	CH_2	1.713
CpR12	HN	7.492
	HB	4.119
	HA	2.583
	CH_2	1.400; 1.611; 1.750
CpS13	HN	7.447
	HB	4.072
	HA	2.562
	CH_2	1.402; 1.475; 1.646; 1.778
CpR14	HN	7.621
	HB	4.110
	HA	2.728
	CH_2	-
Lys15	HN	8.109
	HA	4.031
	HB	1.266
	HD	0.8889; 0.9723
	HG	1.366
	HE	2.693
tyr16	HN	8.191
	HA	4.454
	HB	2.642; 3.018
	HD	6.983; 6.666
	HE	-
CpS17	HN	7.631
	HB	4.234
	HA	2.662
	CH_2	1.342; 1.443; 1.633; 1.753
CpR18	HN	7.558
	HB	4.168
	HA	2.753
	CH_2	1.369; 1.456; 1.650; 1.720; 1.781
Ser19	HN	8.161
	HA	4.324
	HB	3.718
ala20	HN	8.402
	HA	4.139
	HB	1.257
NH_2	HN1	-
	HN2	-

Table S11. NOESY contacts for peptide **14**.

Sequential (*i*, *i*-1)	**Intensity**
2HN – 1HA	M
3HN – 2HA	S
4HN – 3HB	S
4HN – 3HA	S
5HN – 4HB	M

5HN – 4HA	M
5HA – 4HN	M
5HB – 4HN	M
6HN – 5HA	S
6HA – 5HB	S
6HA – 5HD	S
6HB – 5HD	S
6HB – 5HE	S
6HA – 5HE	S
7HN – 6HA	S
7HN – 6HB	S
7HA – 6HB	S
8HN – 7HB	M
8HN – 7HA	S
8HB – 7HN	S
9HN – 8HB	M
9HN – 8HA	S
10HN – 9HA	S
10HN – 9HB	S
10HB – 9HN	W
11HN – 10HA	S
11HN – 10HB	S
12HN – 11HA	S
12HN – 11HB	M
12HG – 11HA	S
13HN – 12HA	S
13HN – 12HB	S
14HN – 13HA	S
14HA – 13HN	S
15HN – 14HB	S
15HN – 14HB	W
15HA – 14HN	W
16HN – 15HA	S
16HB – 15HB	S
16HN – 15HB	W
16HD – 15HB	W
16HD – 15HG	W
16HE – 15HG	W
16HE – 15HE	W
16HD – 15HE	W
17HN – 16HA	S
17HN – 16HB	M
17HG – 16HN	S
17HN – 16HE	M
17HG – 16HE	M
18HN – 17HA	S
18HN – 17HB	W
19HN – 18HA	S
19HN – 18HB	W
20HN – 19HA	W
20HN – 19HB	W

NH-HN	**Intensity**
2HN – 3HN	S
4HN – 5HN	S
6HN – 7HN	S
7HN – 8HN	S
8HN – 9HN	M
9HN – 10HN	M
10HN – 11HN	M
13HN - 14HN	M
14HN – 15HN	M
15HN – 16HN	M
16HN-17HN	S
18HN-19HN	M

Middle range (*i*, *i*-2)	**Intensity**
5HN – 3HB	W
5HD – 3HB	W
7HN – 5HA	W
8HN – 6HB	W
8HA – 6B	W
9HN – 7HB	W
11HN – 9HB	W
12HN – 10HB	M
15HB – 13HA	W
15HD – 13HA	W
16HD – 14HB	S
16HD – 14HG	S
17HN – 15HA	W
17HN – 15HB	W
18HN – 16HA	W
19HN – 17HB	M

Middle range (*i*, *i*-3)	**Intensity**
6HA – HN3	W
8HA – HD5	W
9HN – 6HB	W
14HA – 11HN	W
15HN – 12HA	W

$^{3}J_{HN-HA}$ [Hz]	
HN4	8.17
HN7	8.29
HN8	8.17
HN12	8.53
HN13	7.81
HN15	6.13
HN18	8.35

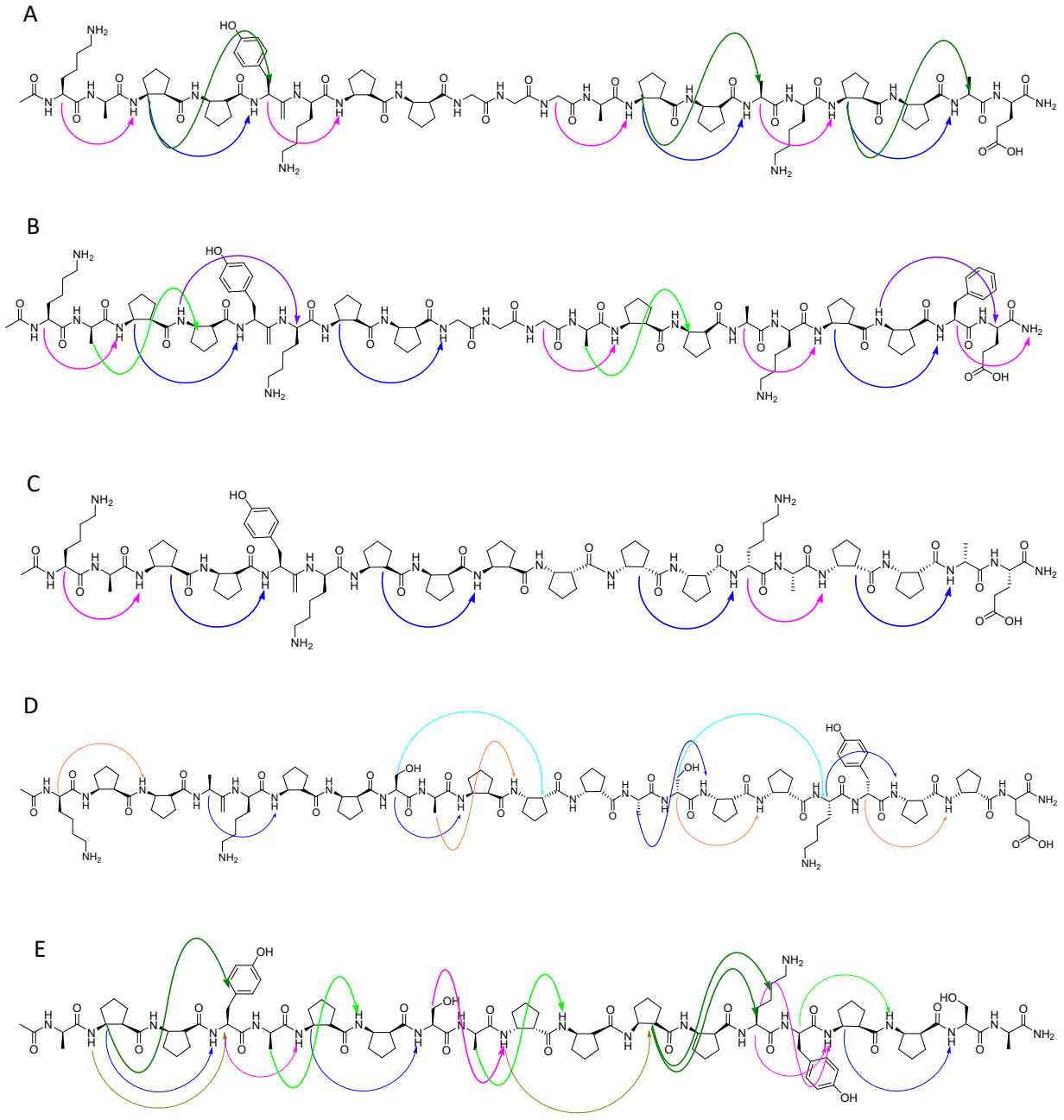


Figure S8. Structural formulas for peptides **8** (A), **9** (B), **12** (C), **13** (D) and **14** (E). The arrows indicate regular non-sequential contacts found in ROESY or NOESY spectra. The coloring of arrows is consistent and indicates contacts between particular atom types, e.g. (1*R*,2*S*)-ACPC-Cα(i) – (*S*)-aa-HN(i+2) is colored dark blue.

Table S12. NMR calculation statistics for the biggest clusters.

Cluster	1	2	3
Total number of NOE restraints	80		
(i, i+1)	60		
(i, i+2)	15		
(i, i+3)	5		
Number of members	25 (25%)	16 (16%)	13 (13%)
Average number of NOE violations per structure	12.1	10.5	9.6
Average amount of NOE violation per structure [Å]	3.3	3.2	2.5
Average RMSD for Cα [Å]	0.256	0.256	0.229

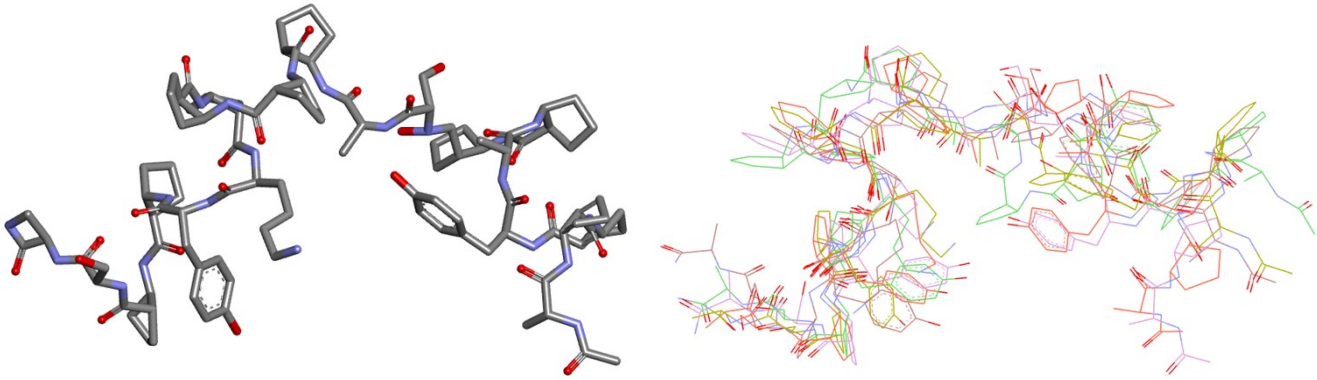


Figure S9. Average structure of cluster 1 (left) and superimposition of the 5 lowest energy structures of cluster 1 (right).

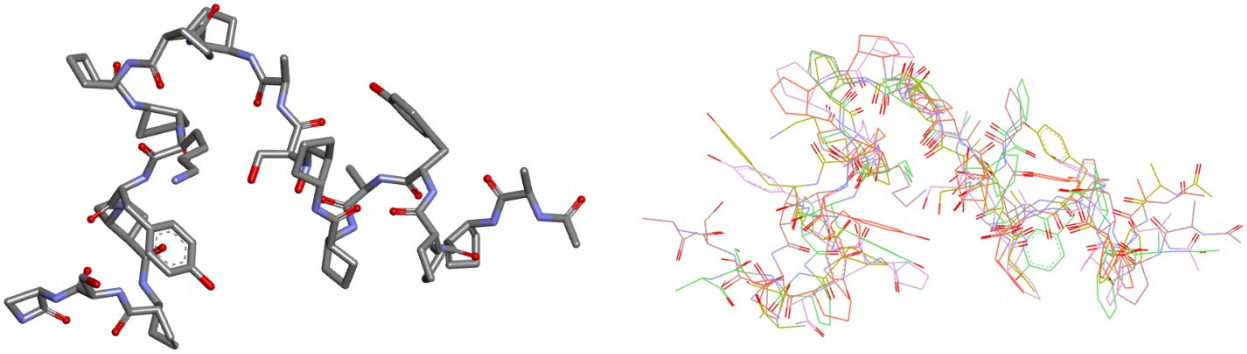


Figure S10. Average structure of cluster 2 (left) and superimposition of the 5 lowest energy structures of cluster 2 (right).

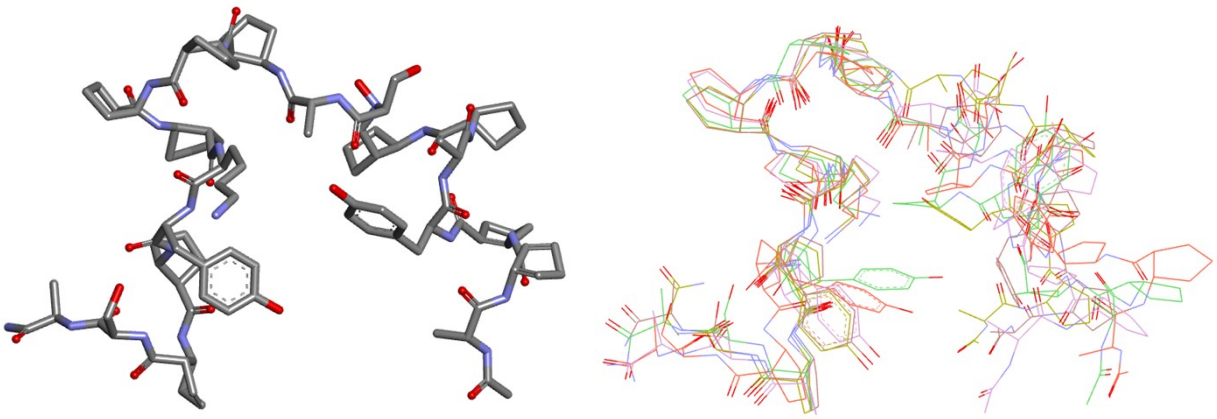
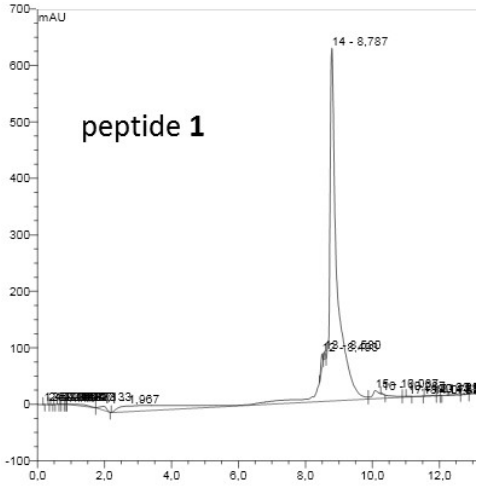
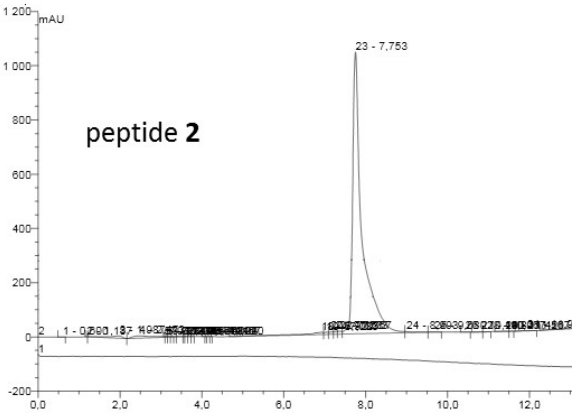


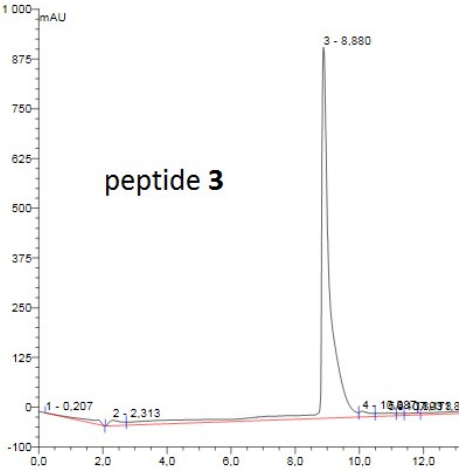
Figure S11. Average structure of cluster 3 (left) and superimposition of the 5 lowest energy structures of cluster 3 (right).

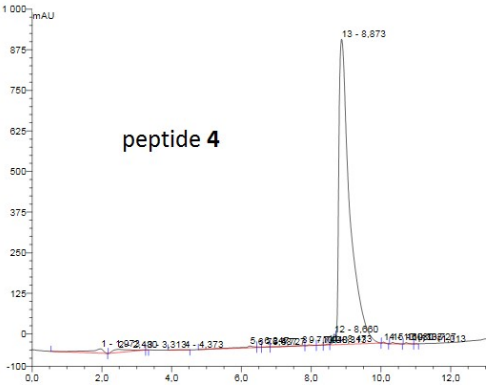
Table S13. Distances between active site residues calculated for 3 clusters (five top energy structures). RMSD are given as errors.

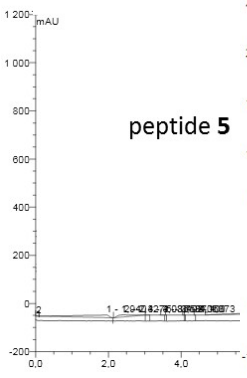
Distance [Å]	**Cluster 1**	**Cluster 2**	**Cluster 3**	**RA95.5-8F (PDB id 5AN7)**
Tyr16(CA)-Tyr5(CA)	14.6 ± 0.8	14.9 ± 1.0	12.2 ± 1.3	12.4
Tyr16(OH)-Tyr5(OH)	10.8 ± 3.1	14.5 ± 4.9	10.1 ± 1.4	2.7
Tyr16(CA)-Lys15(CA)	3.8 ± 0.0	3.7 ± 0.0	3.8 ± 0.0	8.2
Tyr16(OH)-Lys15(NZ)	7.1 ± 1.2	7.7 ± 0.8	7.6 ± 1.4	4.3
Tyr5(CA)-Lys15(CA)	11.9 ± 0.7	12.2 ± 0.9	10.2 ± 0.9	14.7
Tyr5(OH)-Lys15(NZ)	7.9 ± 3.8	14.4 ± 1.3	7.7 ± 2.6	3.2

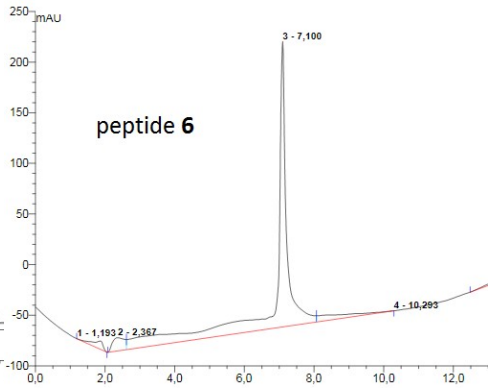

peptide **1**
mAU
14 - 8,787

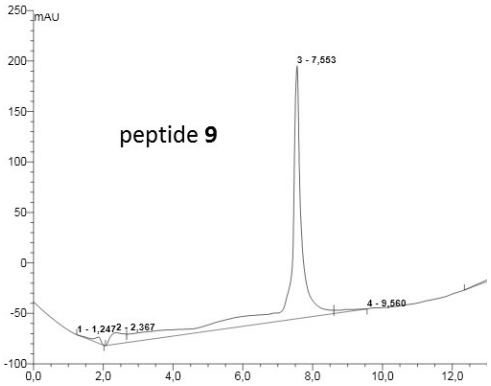

peptide **2**
mAU
23 - 7,753

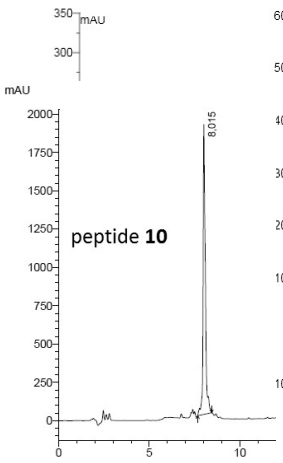

peptide **3**
mAU
3 - 8,880
1 - 0,207
2 - 2,313

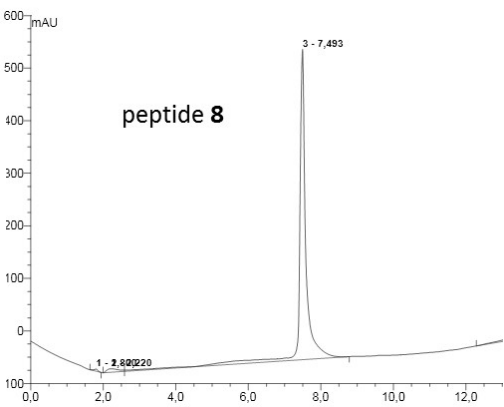

peptide **4**
mAU
13 - 8,873

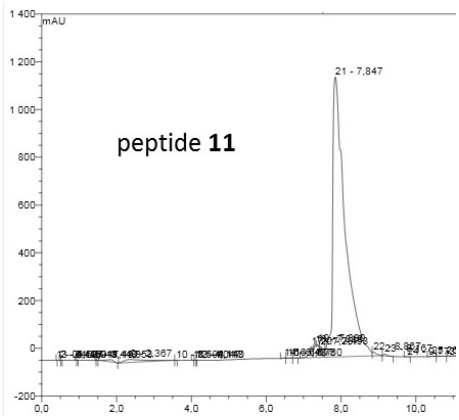

peptide **5**
mAU


peptide **6**
mAU
3 - 7,100
4 - 10,293
1 - 1,193
2 - 2,367


peptide **9**
mAU
3 - 7,553
4 - 9,560
1 - 1,247
2 - 2,367


peptide **10**
mAU
8,015


peptide **8**
mAU
3 - 7,493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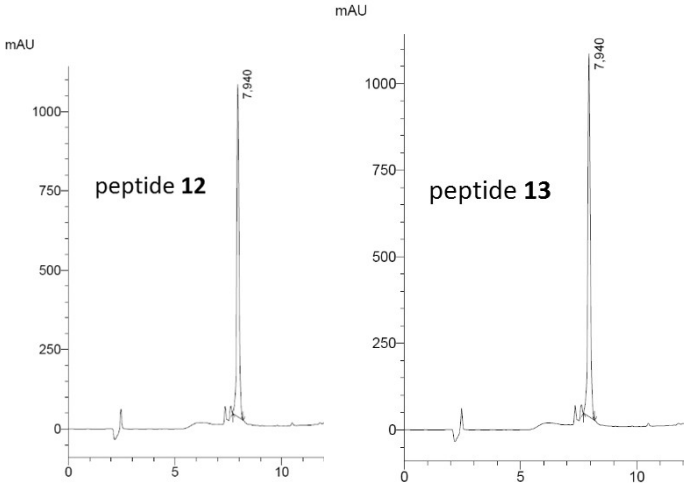
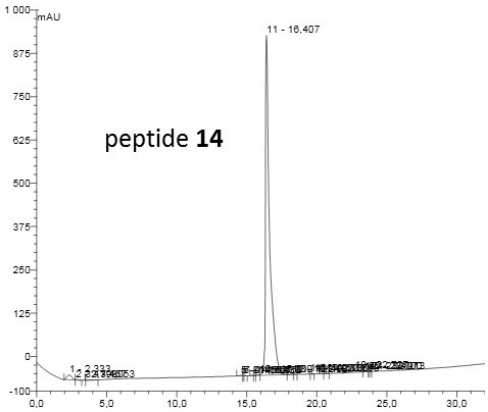
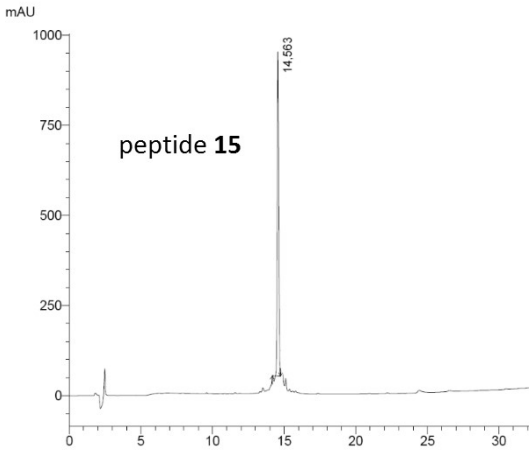
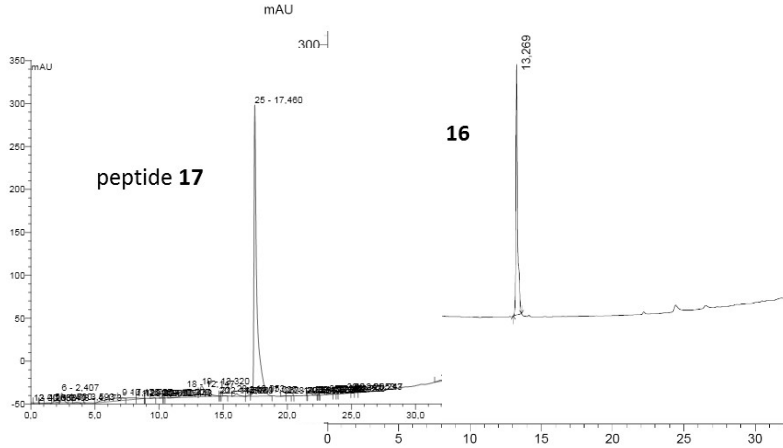
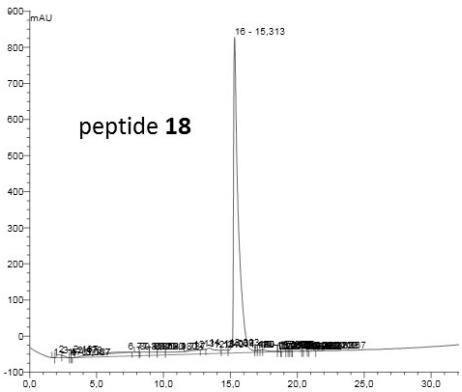
21 - 7,847
peptide **11**


mAU
7,940
peptide **12**
mAU
7,940
peptide **13**


mAU
11 - 16,407
peptide **14**


mAU
14,563
peptide **15**


mAU
25 - 17,460
peptide **17**
13,269
16


mAU
16 - 15,313
peptide **18**

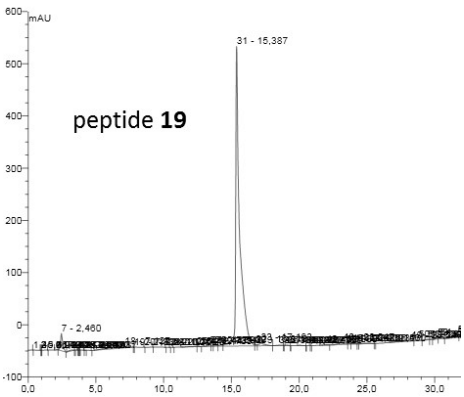

mAU
31 - 15,387
peptide **19**

Figure S12. Analytical HPLC chromatograms for analyzed peptides.