Supplementary Material for

Therapeutic index of gramicidin S is strongly modulated by D-phenylalanine analogues at the β-turn

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Contents

Tables

Figures

Table ST1. Chemical shifts (δ, ppm) of GS and analogues **1-6** in aqueous solution at pH 3.0 and 5ºC. ^{1}H and ^{15}N chemical shifts were measured in H_2O/D_2O 9:1 v/v and ^{13}C chemical shifts in pure D_2O. ^{1}H, ^{13}C and ^{15}N chemical shifts with significant differences ($\geq$ 0.2 ppm for ^{1}H, $\geq$1.0 ppm for ^{13}C and $\geq$ 2.0 ppm for ^{15}N) relative to GS are highlighted respectively in yellow, cyan and green background.

Residue	Resonance	Peptide						
		GS D-Phe	1 D-Hpa	2 D-1-Nal	3 D-2-Nal	4 D-Dip	5 D-Flg	6 D-Tic
Val 1/1'	HN	7.68	7.63	7.61	7.82	7.79	8.25	7.52
	H^{15}N	121.0	121.1	----	121.4	----	122.2	122.3
	C$_\alpha$H	4.12	4.09	4.10	4.08	4.05	3.79	4.07
	^{13}C$_\alpha$	62.05	62.14	62.01	62.29	62.28	63.33	62.21
	C$_\beta$H	2.14	2.13	2.11	2.10	2.07	1.83	2.20
	^{13}C$_\beta$	33.30	33.21	33.31	33.07	33.06	31.95	33.63
	C$_\gamma$H$_3$	0.88, 0.93	0.88, 0.93	0.86, 0.89	0.88, 0.94	0.85, 0.96	0.72, 0.88	0.93, 1.01
	^{13}C$_\gamma$	21.12, 20.98	21.14, 21.05	21.13, 20.92	21.11, 21.02	21.07, 21.04	20.90, 21.25	20.88, 21.56
Orn 2/2'	HN	8.67	8.67	8.64	8.64	8.64	8.31	8.74
	H^{15}N	125.8	126.0	-----	125.9	126.1	125.5	127.2
	C$_\alpha$H	4.78	4.77	4.79	4.73	4.71	4.36	4.77
	^{13}C$_\alpha$	54.62	54.52	54.61	54.55	54.40	54.12	54.30
	C$_\beta$H	1.71, 1.94	1.68, 1.91	1.73, 1.96	1.70, 1.92	1.69, 1.90	1.49, 1.68	1.49, 1.82
	^{13}C$_\beta$	31.57	31.59	31.57	31.64	31.83	31.92	31.82
	C$_\gamma$H	1.67, 1.67	1.62, 1.62	1.70, 1.70	1.67, 1.67	1.65, 1.65	1.42, 1.47	1.25, 1.25
	^{13}C$_\gamma$	25.83	25.74	25.83	25.71	25.67	25.03	25.97
	C$_\delta$H	3.01, 3.01	2.96, 2.96	3.02, 3.02	3.01, 3.01	3.00, 3.00	2.82, 2.82	2.44, 2.67
	^{13}C$_\delta$	41.65	41.60	41.66	41.63	41.58	41.42	41.28
	N$_\varepsilon$H	7.70	7.63	7.73	7.71	7.72	7.49	7.02
	^{15}N$_\varepsilon$	69.1	69.1	----	69.2	69.1	69.2	68.6
Leu 3/3'	HN	8.71	8.73	8.72	8.59	8.71	8.03	9.02
	H^{15}N	127.9	128.0	----	127.0	127.4	123.8	129.3
	C$_\alpha$H	4.58	4.64	4.62	4.51	4.34	3.79	5.31

	$^{13}C_\alpha$	53.49	53.58	53.51	53.76	53.68	54.63	49.63
	$C_\beta H$	1.39, 1.42	1.46, 1.57	1.35, 1.42	1.26, 1.26	1.14, 1.31	0.32, 0.85	1.51, 1.64
	$^{13}C_\beta$	42.59	42.75	42.57	42.56	42.20	42.00	42.56
	$C_\gamma H$	1.39	1.47	1.42	1.31	1.30	0.81	1.46
	$^{13}C_\gamma$	26.90	27.03	26.93	26.77	26.78	26.38	26.60
	$C_\delta H_3$	0.85, 0.87	0.90, 0.94	0.86, 0.89	0.75, 0.76	0.74, 0.74	0.45, 0.51	0.91, 0.91
	$^{13}C_\delta$	24.25, 24.72	24.39, 24.65	24.31, 24.66	24.55, 24.04	24.60, 23.94	23.46, 24.40	25.68, 24.08
DPhe*4/4'	HN	9.07	9.01	9.21	9.10	8.87	7.83	----
	$H^{15}N$	130.7	129.8	----	129.6	130.0	----	----
	$C_\alpha H$	4.70	4.30	4.81	4.86	5.29	5.32	5.06
	$^{13}C_\alpha$	56.93	54.14	56.16	56.56	58.10	55.87	57.09
	$C_\beta H$	3.01, 3.09	2.06, 2.06	3.49, 3.67	3.23, 3.23	4.40	4.39	3.09, 3.28
	$^{13}C_\beta$	38.43	32.89	35.41	38.77	54.69	49.76	31.53
Pro5/5'	$C_\alpha H$	4.41	4.37	4.17	4.40	4.18	4.53	4.40
	$^{13}C_\alpha$	63.30	63.47	63.30	63.30	63.02	63.26	63.68
	$C_\beta H$	1.88, 1.91	2.01, 2.14	1.48, 1.76	1.73, 1.83	1.68, 1.78	1.86, 2.20	2.11, 2.26
	$^{13}C_\beta$	32.10	32.24	31.88	32.01	31.92	32.07	32.28
	$C_\gamma H$	1.65, 1.73	1.85, 1.98	1.24, 1.48	1.49, 1.69	1.46, 1.67	1.88, 1.92	2.00, 2.14
	$^{13}C_\gamma$	26.19	26.29	25.95	26.24	26.20	27.12	26.61
	$C_\delta H$	2.63, 3.69	3.30, 3.65	3.39, 3.39	2.75, 3.71	2.74, 3.72	3.65, 3.87	3.81, 4.02
	$^{13}C_\delta$	49.97	49.81	49.61	50.03	50.16	50.55	49.91

Table ST2. $^3J_{C\alpha H\text{-}NH}$ coupling constants (Hz) measured for GS and its analogues in 1D ^{1}H NMR spectra recorded in H_2O/D_2O 9:1 v/v at pH 3.0 and 25ºC.

Peptide	D-Phe*[a]	Val[1,1']	Orn[2,2']	Leu[3,3']	D-Phe*[4,4']
GS	D-Phe	9.0	9.2	9.3	3.5
1	D-Hpa	broad	9.2	9.3	4.5
2	D-1-Nal	nd[b]	8.7	8.9	4.3
3	D-2-Nal	broad	9.0	9.1	4.2
4	D-Dip	8.3	9.0	nd	5.4
5	D-Flg	broad	8.2	broad	broad
6	D-Tic	9.2	9.4	9.9	—

[a] D-Phe* stands for D-Phe or the corresponding substitute. [b] Not determined because of signal overlapping.

Table ST3. Structural statistics for the 20 best structures calculated for GS and analogues **1–6**.

	GS (D-Phe)	1 (D-Hpa)	2 (D-1Nal)	3 (D-2Nal)	4 (D-Dip)	5 (D-Flg)	6 (D-Tic)
Distance restraints[a]							
Theoretical	9	9	9	9	9	9	9
Experimental	108	185	78	86	131	145	112
Dihedral angle constraints							
$(\phi + \psi)$[a]	8	8	8	8	8	8	8
H-bond restraints[a]							
NH V1 – O L3'/ NH V1' – O L3	2	2	2	2	2	2	2
NH L3' – O V1/ NH L3 – O V1'	2	2	2	2	2	2	----
RMSD (Å)							
Backbone atoms	0.2±0.1	0.2±0.3	0.4±0.2	0.4±0.2	0.1±0.1	0.1±0.1	0.1±0.1
All heavy atoms	1.2±0.2	1.8±0.3	1.7±0.4	1.4±0.4	0.8±0.2	0.8±0.3	0.5±0.2

[a] See Table ST4.

Table ST4. Characteristic antiparallel β-sheet backbone restraints used in structure calculations of GS and analogues **1-6**. For calculations, residues were renumbered from 1 to 10 starting with Leu 3.

A. List of angle restraints used for structure calculation in GS and analogues **1–6**. Angle restraints for Val1/1' were not included because conformational shifts deviates from those expected for typical β-sheet residues.

```
 1 LEU   PHI    -149.0   -129.0
 1 LEU   PSI     145.0    125.0
 5 ORN   PHI    -149.0   -129.0
 5 ORN   PSI     145.0    125.0
 6 LEU   PHI    -149.0   -129.0
 6 LEU   PSI     145.0    125.0
10 ORN   PHI    -149.0   -129.0
10 ORN   PSI     145.0    125.0
```

B. List of lower limit distance restraints used for structure calculation in GS and analogues **1–6**.

```
10 ORN   C      1 LEU   N      1.36ᵃ
10 ORN   C      1 LEU   CA     2.45ᵃ
10 ORN   O      1 LEU   N      2.29ᵃ
10 ORN   CA     1 LEU   N      2.44ᵃ
 1 LEU   O      4 VAL   N      2.70ᵇ
 1 LEU   O      4 VAL   HN     1.80ᵇ
 4 VAL   O      1 LEU   N      2.70ᵇ
 4 VAL   O      1 LEU   HN     1.80ᵇ
 6 LEU   O      9 VAL   N      2.70ᵇ
 6 LEU   O      9 VAL   HN     1.80ᵇ
 9 VAL   O      6 LEU   N      2.70ᵇ
 9 VAL   O      6 LEU   HN     1.80ᵇ
```
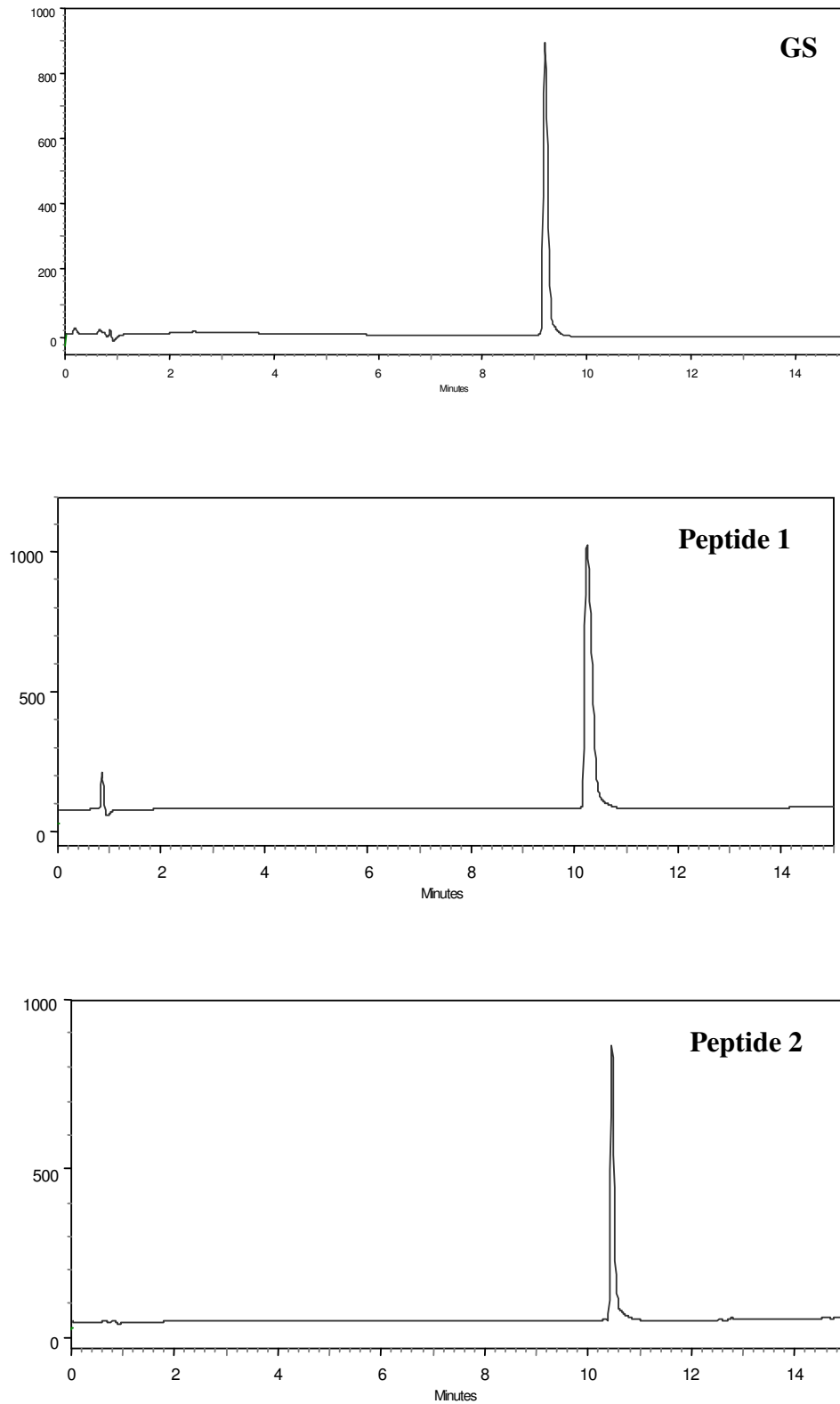
C. List of upper limit distance restraints used for structure calculation in GS and analogues **1–6**.

```
 1 LEU   N     10 ORN   C      1.36ᵃ
 1 LEU   CA    10 ORN   C      2.45ᵃ
 1 LEU   N     10 ORN   O      2.29ᵃ
 1 LEU   N     10 ORN   CA     2.44ᵃ
 1 LEU   O      4 VAL   N      3.00ᵇ
 1 LEU   O      4 VAL   HN     2.00ᵇ
 1 LEU   N      4 VAL   O      3.00ᵇ
 1 LEU   HN     4 VAL   O      2.00ᵇ
 6 LEU   O      9 VAL   N      3.00ᵇ
 6 LEU   O      9 VAL   HN     2.00ᵇ
 6 LEU   N      9 VAL   O      3.00ᵇ
 6 LEU   HN     9 VAL   O      2.00ᵇ
 9 VAL   HA    10 ORN   HN     2.30ᶜ,ᵈ
10 ORN   HA     1 LEU   HN     2.30ᶜ,ᵈ
 4 VAL   HA     5 ORN   HN     2.30ᶜ,ᵈ
 5 ORN   HA     6 LEU   HN     2.30ᶜ,ᵈ
 4 VAL   HN     1 LEU   HN     3.40ᶜ,ᵉ
 9 VAL   HN     6 LEU   HN     3.40ᶜ,ᵉ
10 ORN   HA     5 ORN   HA     2.30ᶜ,ᵉ
10 ORN   HA     6 LEU   HN     3.30ᶜ,ᵉ
 5 ORN   HA     1 LEU   HN     3.30ᶜ,ᵉ
```

ᵃ Required by CYANA for peptide backbone cyclization. ᵇ Restraints corresponding to the four hydrogen-bonds between Val1/1' and Leu3/3'. ᶜ Ideal upper limit restraints for backbone protons in canonical antiparallel β-sheet structures (averaged distances plus 0.1Å). ᵈ Sequential CαH-NH distance restraints in β-strands. ᵉ Cross-strand distance restraints in the antiparallel β-sheet.

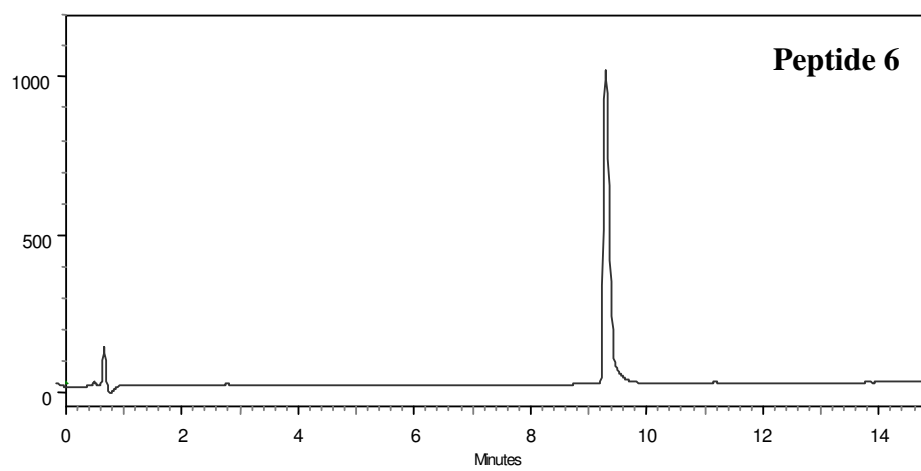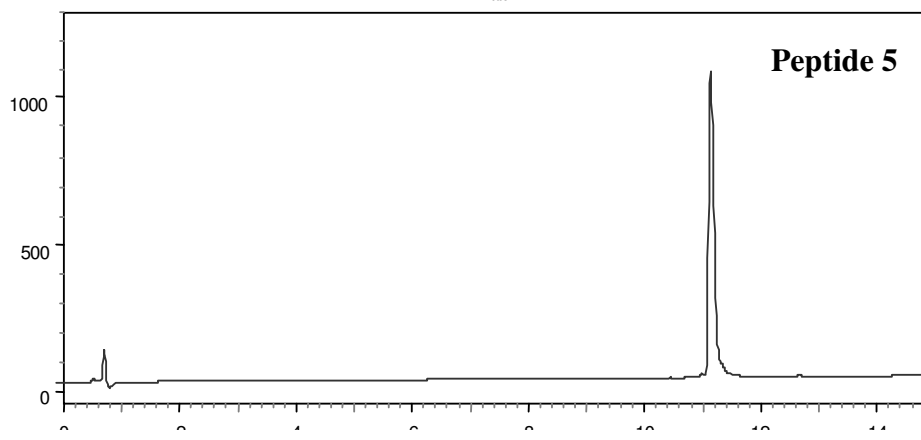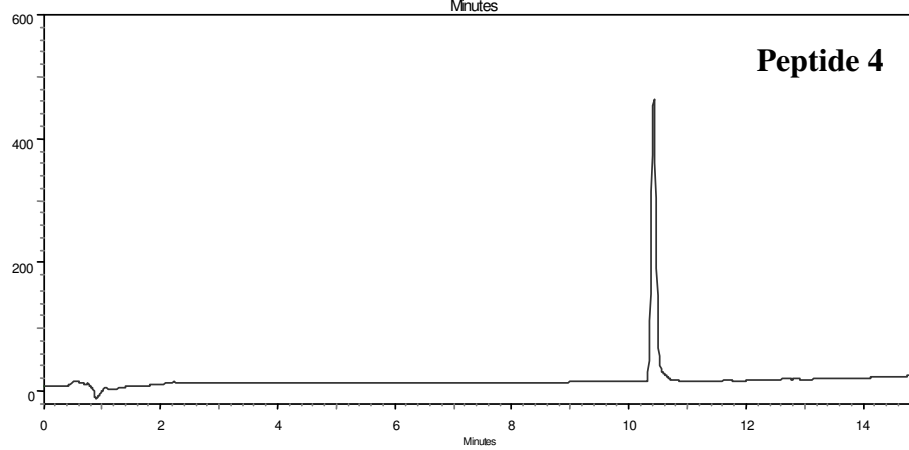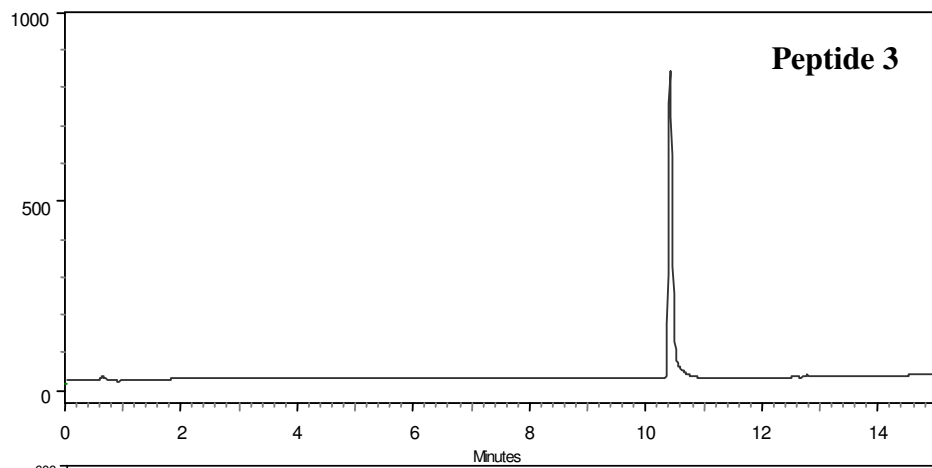| Peptide	D-Phe*	Rt (min)
GS	D-Phe	9.2
1	D-Hpa	10.3
2	D-1-Nal	10.4
3	D-2-Nal	10.4
4	D-Dip	10.6
5	D-Flg	11.3
6	D-Tic	9.5

Figure SF1. Schematic representation of the retention times (Rt) observed for gramicidin S (GS) and analogues **1**–**6** on analytical RP-HPLC. Conditions: C_8 Phenomenex® column (Luna 3 µm, 100 Å, 50 × 4.6 mm); linear gradient of 5–95% CH_3CN (0.036% TFA, v/v) in H_2O (0.045% TFA, v/v) over 15 min; flow rate 1 mL/min. D-Phe* stands for D-Phe or the corresponding replacement.
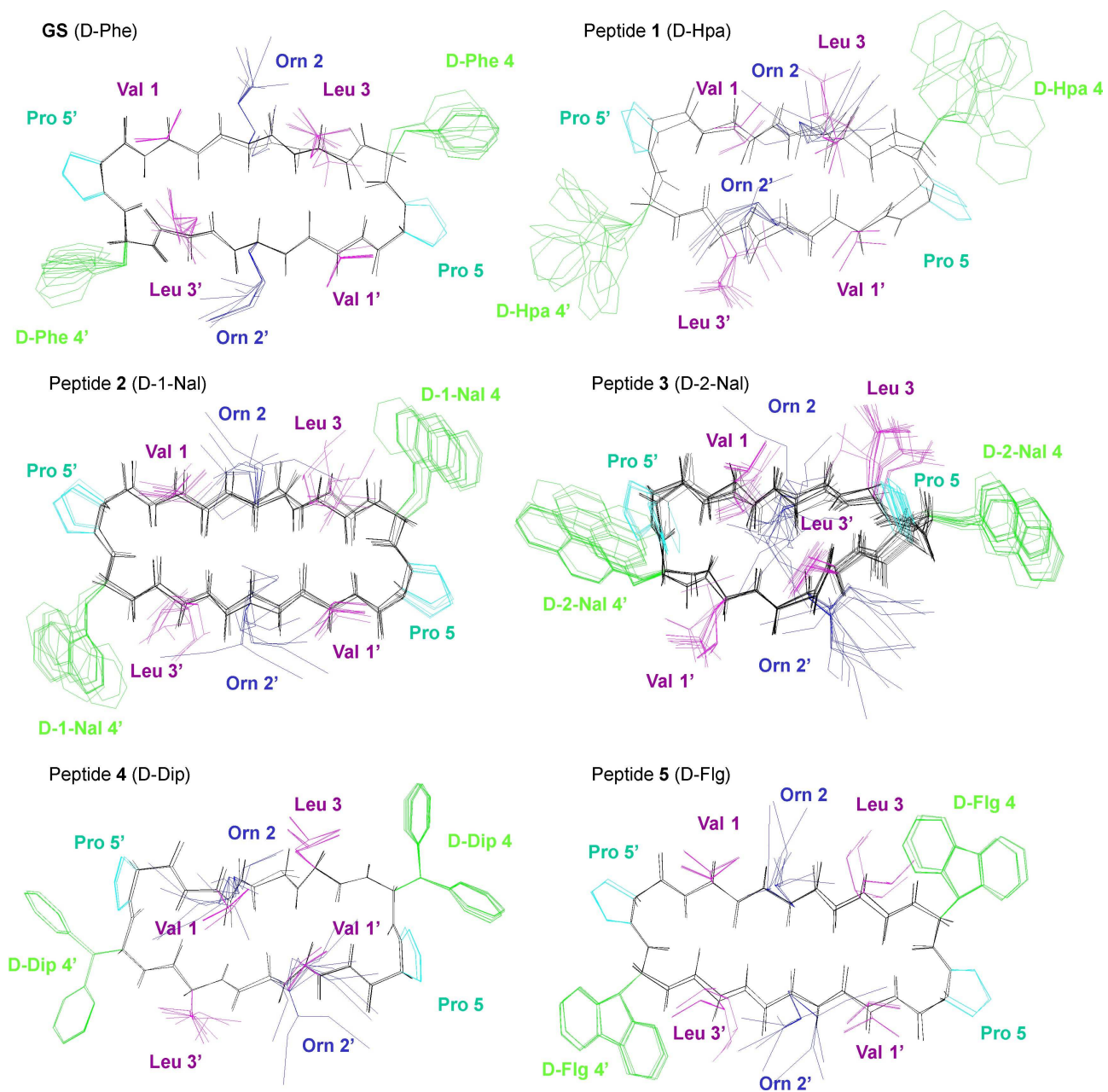
Figure SF2. HPLC profile of purified peptides (GS and analogues **1-6**). See Figure SF1 for elution conditions.

Figure SF2. (Cont.).

Figure SF3. Model structures for GS and analogues **1**–**5**. Backbone atoms are shown in black. Side chains for aromatic residues are colored in green, for Pro in cyan, for Orn in blue, and for Leu and Val in magenta.