Supporting Information

Lactam-stabilized helical analogues of the analgesic μ-conotoxin KIIIA

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Running title: *Lactam analogues of the analgesic μ-conotoxin KIIIA*

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Table SI-1 Chemical shifts (NH and CαH) and $^3J_{NHC\alpha H}$ coupling constants for Peptides **1** - **8** at 5 ºC, pH 2.7. $^3J_{NHC\alpha H}$ coupling constants were measured from 1D spectra except in the case of overlapped peaks ([a]) which were measured from a DQF-COSY spectrum. Single peak annotation denotes coupling constants too small to be measured from 1D spectra. [n.d.] The Asp7 resonance in D7K11 was not observed

Peptide **1** (K5D9)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys5*	8.47	4.26	single peak
Ser6	8.75	4.26	2.2
Lys7	8.10	4.03	5.6
Trp8	8.34	4.35	4.2
Asp9*	9.35	4.65	3.6
Arg10	7.94	4.16	5.6
Asp11	7.97	4.53	6.9
His12	7.70	4.51	9.1 [a]
Ser13	8.00	4.47	6.8
Arg14	8.56	4.34	7.5

Peptide **2** (D5K9)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Asp5*	8.89	4.55	3.5
Ser6	8.95	4.09	single peak
Lys7	8.00	4.06	5.6 [a]
Trp8	7.88	4.38	4.9
Lys9*	9.24	3.96	4.1
Arg10	7.72	4.11	5.6
Asp11	7.96	4.53	7.4 [a]
His12	7.77	4.45	8.6
Ser13	7.98	4.43	6.3 [a]
Arg14	8.50	4.35	7.3

Peptide **3** (K7D11)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys7*	8.54	4.01	single peak
Trp8	8.86	4.22	single peak
Ala9	8.70	4.12	4.2
Arg10	7.88	4.06	5.3
Asp11*	8.35	4.55	6.5
His12	7.61	4.46	9.2
Ser13	7.84	4.44	6.6
Arg14	8.45	4.33	7.3

Peptide **4** (D7K11)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Asp7*	-	-	- [n.d.]
Trp8	9.75	4.61	5.9
Ala9	8.64	4.14	5.5
Arg10	8.11	4.14	6.3
Lys11*	8.06	4.14	6.5
His12	8.46	4.63	7.1 [a]
Ser13	8.36	4.42	6.6
Arg14	8.62	4.31	7.1

Peptide **5** (K9D13)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys7	8.56	3.91	4.1
Trp8	8.52	4.32	3.9
Lys9*	8.38	3.99	2.9
Arg10	8.10	4.00	2.9
Asp11	8.24	4.40	5.8
His12	8.20	4.17	7.2
Asp13*	8.29	4.71	6.7 [a]
Arg14	7.76	4.23	7.0

Peptide **6** (D9K13)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys7	8.51	4.01	4.9
Trp8	8.48	4.51	4.6
Asp9*	8.64	4.41	4.6
Arg10	8.30	3.96	5.4 [a]
Asp11	8.13	4.53	6.7
His12	8.09	4.45	7.7 [a]
Lys13*	8.08	4.31	5.9 [a]
Arg14	8.18	4.24	7.3

Peptide **7** (K9D13 linear)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys7	8.47	4.00	overlap
Trp8	8.09	4.60	6.3
Lys9	7.93	4.08	6.5
Arg10	8.18	4.18	6.4
Asp11	8.40	4.58	6.9
His12	8.51	4.58	7.6
Asp13	8.46	4.64	overlap
Arg14	8.45	4.26	overlap

Peptide **8** (D9K13 linear)

	NH	CαH	$^3J_{NHC\alpha H}$ (Hz)
Lys7	8.35	4.06	5.8
Trp8	8.25	4.68	6.8
Asp9	8.28	4.60	overlap
Arg10	8.28	4.12	overlap
Asp11	8.28	4.57	overlap
His12	8.28	4.59	overlap
Lys13	8.41	4.29	6.7
Arg14	8.57	4.24	7.0

Table SI-2 Characterization of Peptides **1** – **8** by Mass Spectroscopy, Capillary Electrophoresis and RP-HPLC.

Peptide	Calculated [M+H]+	Observed [M+H]+ [a]	% Purity by HPLC [b]	% Purity by CE [c]	Yield [d] (mg)
1	1337.68	1337.56	99	96	4.9
2	1337.68	1337.59	99	97	17.8
3	1078.56	1078.46	98	98	9.8
4	1078.56	1077.65	NA	77	18.8
5	1163.62	1163.52	98	97	13.5
6	1163.62	1163.77	NA	86	10.6
7*	1181.33	1181.50	99	NA	NA
8*	1181.33	1181.78	99	NA	NA

*Commercially available. NA, not available.

[a] Mass spectra (MALDI-MS) were measured on an ABI-Perseptive DE-STR instrument in positive reflector mode.

[b] Quantitative RP-HPLC was performed using a HP1090 instrument and a Grace Vydac C18 column (2 x 150 mm, 5 μm particle size, 300 Å pore size). The solvent system was comprised of eluent A = TEAP at pH 2.25, eluent B = 60% CH_3CN, 40% A. A gradient was performed from 5% B to 45% B in 30 min at a flow rate of 0.2 mL/min.

[c] Capillary electrophoresis (CE) was performed using a Beckman PACE 5500 instrument. The electrophoresis buffer was 0.1 M sodium phosphate (15% acetonitrile), pH 2.5. Separation was accomplished by application of 20 kV to the capillary (0.75 μm x 50 cm). Detection was at 214 nm. Impurities in peptides **4** and **6**, which were not analysed by RP-HPLC, were detected by CE; accordingly, the potencies of these analogues could be slightly higher than reported.

[d] Yield obtained based on 0.2 mmol scale synthesis as described in Experimental procedures.

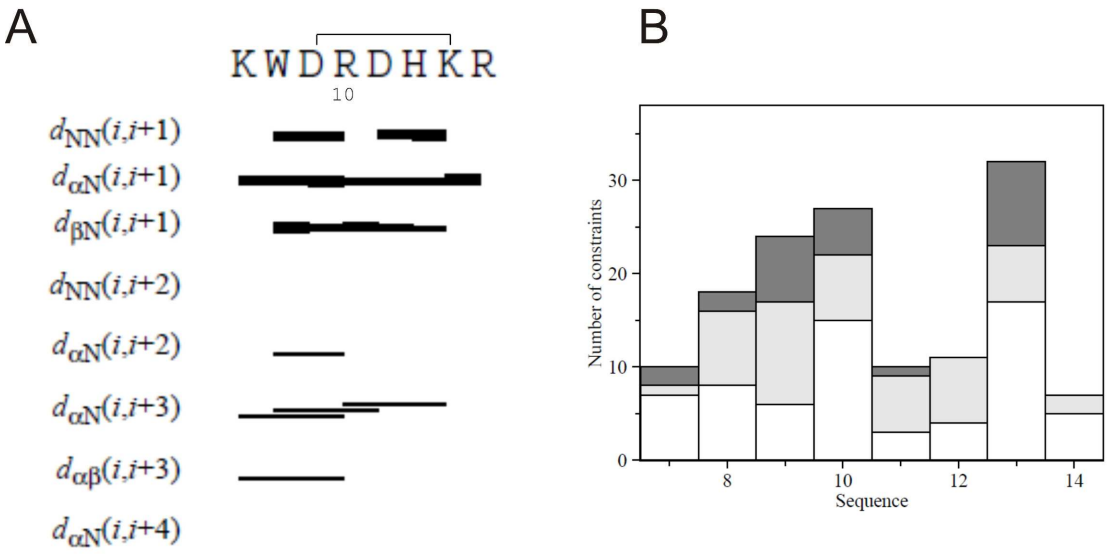

Fig. SI-1: Summary of (A) sequential and short-range NOE connectivities and (B) number of short-range (dark grey), sequential (light grey) and intra-residue (white) NOE restraints plotted as a function of residue number following preliminary structure calculations in CYANA for D9K13 lactam-stabilized peptide (**6**). Intensities of sequential d_{NN}, $d_{\alpha N}$ and $d_{\beta N}$ connectivities are indicated by the height of the bars.

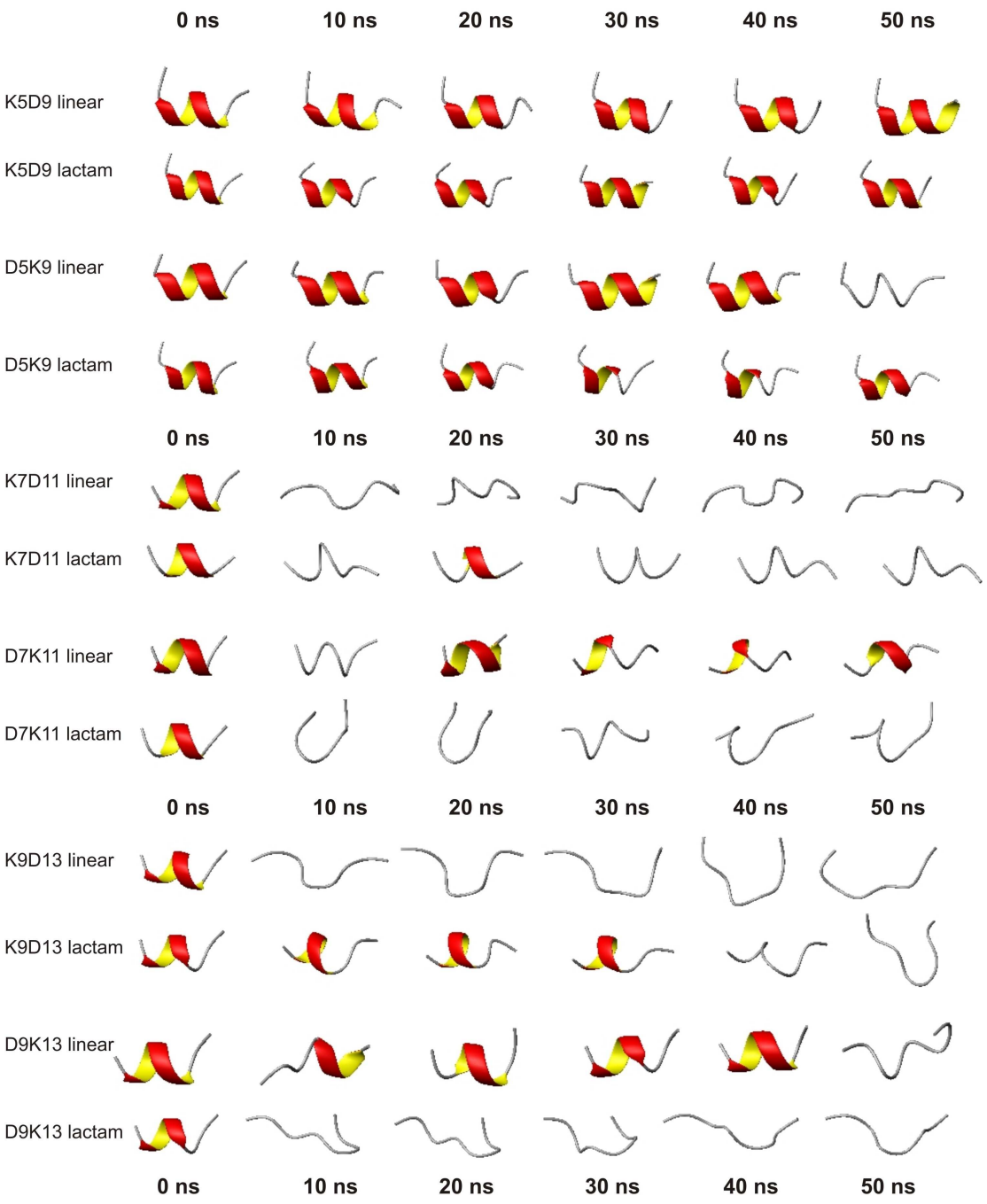

Fig. SI-2: Trajectory snapshots of MD simulation (50 ns) for each lactam analogue and its linear counterpart, taken at 10 ns time intervals.

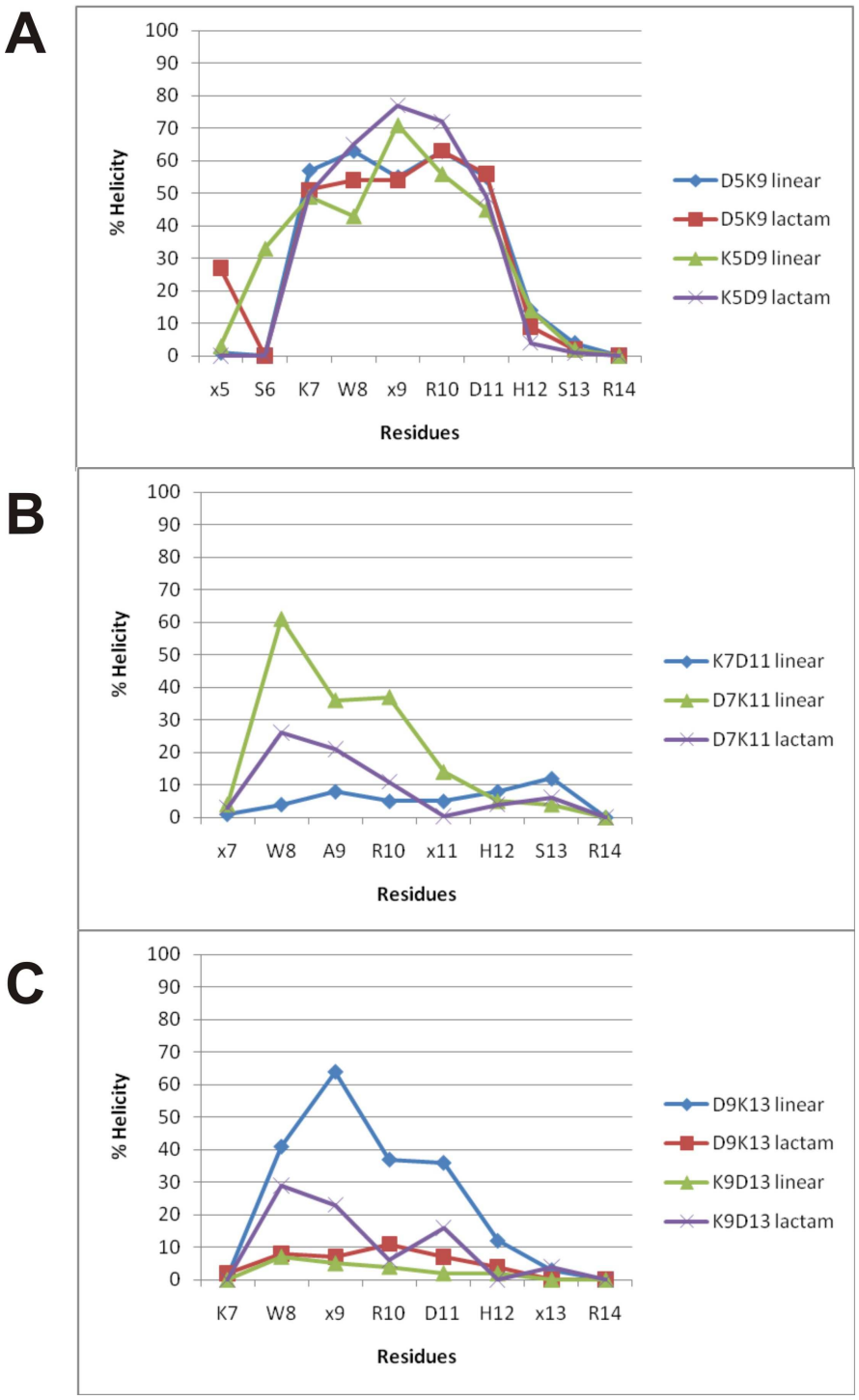

Fig. SI-3: Percent helical occupancy for each residue of the minimized linear and lactam stabilized analogues of μ-KIIIA for (A) 5-9 analogues, (B) 7-11 analogues and (C) 9-13 analogues through 50 ns of MD simulation. Percentage helicity was calculated using the g_helix function in GROMACS.