

Supporting Information

Transforming cross-linked cyclic dimers of KR-12 into stable and potent antimicrobial drug leads

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Table S1: Sequences, net charge, hydrophobicity, and molecular weight of linear peptides

Peptide	Sequence	Net Charge (at neutral pH) ^a	Grand average of hydrophobicity ^a	Molecular Weight (Expected) ^b	Molecular Weight (Observed) ^b
cd4-CCPP	CFLRGPGGKRIV CRIKAFLRG PGGKRIVK RIK	+11	- 0.178	3581.49	3582.11
cd4-CC	CFLRGAGGKRIV CRIKAFLRG AGGKRIVK RIK	+11	0.034	3529.41	3529.91
KR-12 (Q5K, D9A)	KRIVKRIKAFLR	+6	- 0.300	1527.97	1528.01
KR-12	KRIVQRIKDFLR	+ 4	- 0.708	1571.93	1572.13
LL-37	LLGDFFRKSKEKI GKEFKRIV QRIKDFLR NLVPRTES	+6	- 0.724	4493.32	4493.98

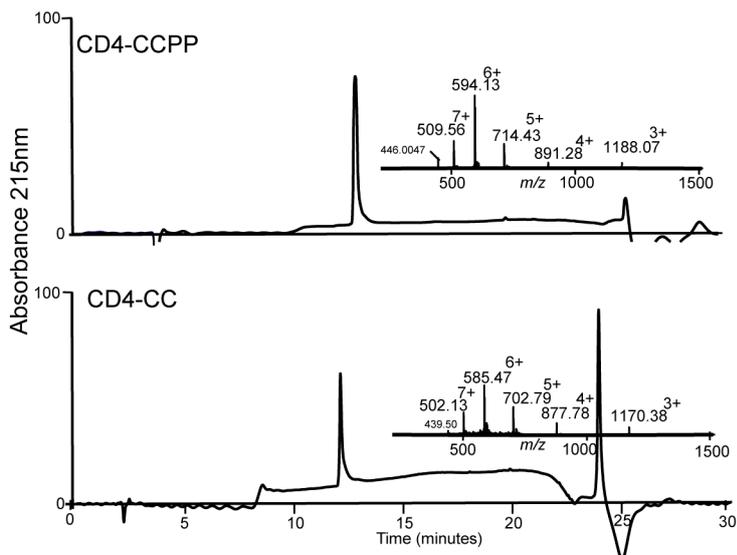
^aExPASy ProtParam tool was used to calculate the molecular weight, net charge, and hydrophobicity index (<http://web.expasy.org/protparam/>)

^bObserved peptides masses presented as (M+1)⁺ have been deconvoluted from (M+2)²⁺, (M+3)³⁺ and (M+4)⁴⁺ masses.

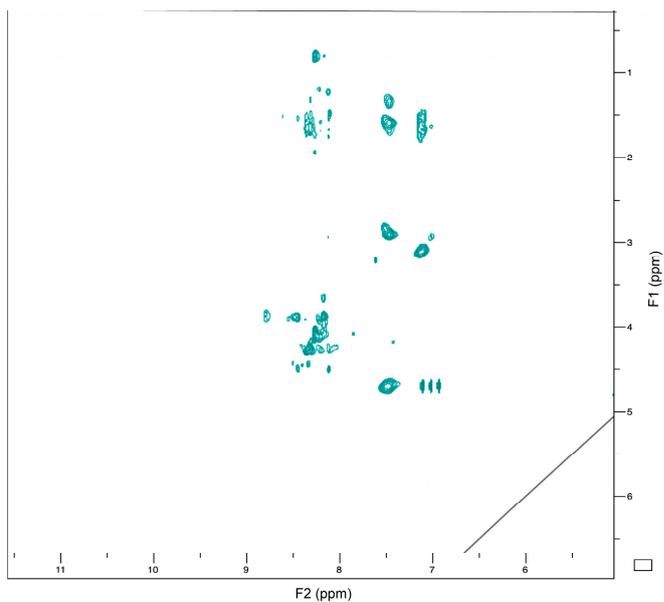
Table S2: MIC values in two-step microdilution assay, MHB, and TSB

Peptides	MIC (μM)					
	Control ¹		MHB ²		TSB ³	
	<i>B. cereus</i>	<i>B. subtilis</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>B. cereus</i>	<i>B. subtilis</i>
LL-37	1.25	1.25	>80	>80	>80	>80
KR-12	2.5	5	>80	>80	>80	>80
KR-12 (Q5K, D9A)	1.25	1.25	>80	>80	> 80	>80
cd4-CCPP	0.312	0.625	>40	>40	>40	>40
cd4-CC	0.625	0.625	>40	>40	>40	>40

¹ The control MICs were determined in a two-step microdilution assay in Tris buffer 10 mM without salts. ² MHB, Muller-Hinton broth (rich media, unbuffered). ³ TSB, Tryptic Soy broth (rich media, unbuffered).



Supplementary Figure S1. Purity and identity analysis of cross-linked cyclic dimers using RP-HPLC and MS. The purity of cross-linked dimers was analyzed at 215 nm and chemical identity was confirmed by LC-MS spectrometer.



Supplementary Figure S2. NMR Spectroscopy. TOCSY spectrum of cd4-CCPP displayed a broadening of signals and overlapping resonances which made it difficult to assign chemical shift assignments.