Supporting Information for

Unexpected Relationships Between Structure and Function in a/b-Peptides: Antimicrobial Foldamers with Heterogeneous Backbones

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NMR sample preparation and NOE assignments

Samples were prepared by dissolving lyophilized peptides in methanol-d_3 or 9:1 H_2O:D_2O, 100 mM acetic acid-d_4, pH 3.8. Peptide concentrations were usually 3-4 mM with trace amounts of 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) added as an internal reference. Fully dissolved peptides were syringe-filtered into a 3 mm NMR tube and sonicated to ensure homogeneity. Total sample volume was approximately 250µL.

NMR experiments were acquired on a Varian Inova-600 spectrometer at 4°C, 14°C, or 24°C as required for best spectral resolution. COSY,[S1] TOCSY,[S2] and rotating frame Overhauser spectroscopy (ROESY)[S3] experiments were performed for chemical shift and structure assignment. Mixing times for TOCSY experiments were 80 ms and 200 ms for ROESY experiments. Standard Varian pulse sequences were used, and data were processed using Varian VNMR 5.3 software and Sparky (a PC-based NMR spectra viewing program; T. D. Goddard and D. G. Kneller, SPARKY 3 University of California, San Francisco). Chemical shift assignments were made based upon COSY and TOCSY cross-peaks as well as sequential a-amide NOEs in the ROESY spectrum.

4

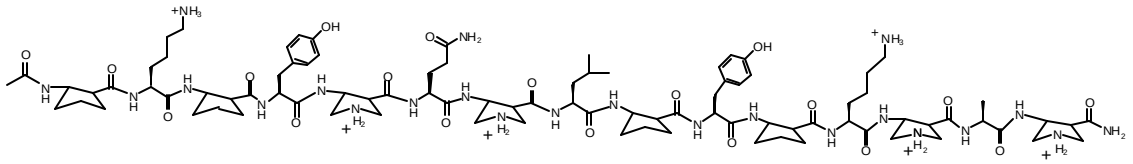


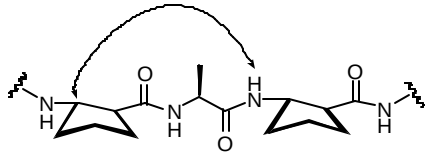
Table S1: NOE Assignments for **4**. Residue numbering is defined above. Graphical definitions of each type of NOE are given after the table.

NOE Type	4 in MeOH-d_3	4 in 9:1 H_2O:D_2O, 100mM acetic acid-d_4, pH 3.8
i_{β} Hβ → i+2$_b$ HN		
1 → 3	Yes	?
3 → 5	No	No
5 → 7	?	No
7 → 9	?	No
9 → 11	No	?
11→ 13	?	?
13→ 15	?	?
i_{β} Hβ → i+2$_b$ Ha		
1 → 3	No	No
3 → 5	No	No
5 → 7	?	No
7 → 9	?	No
9 → 11	No	?
11→ 13	No	No
13→ 15	No	No

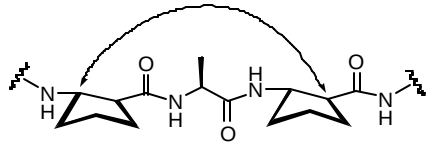
i_a Ha → i+2$_a$ HN		
Ac → 2	No	No
2 → 4	No	?
4 → 6	No	No
6 → 8	?	No
8 → 10	No	No
10→ 12	No	No
12→ 14	?	No
14→ Cterm	No	No
$i_ß$ Hß → i+3$_a$ HN		
1 → 4	Yes	Yes*
3 → 6	Yes	Yes
5 → 8	?	Yes
7 → 10	?	Yes
9 → 12	Yes	?
11→ 14	Yes	?
13→ Cterm	Yes	No
i_a Ha → i+3$_ß$ HN		
Nterm→ 3	Yes	?
2 → 5	Yes	No
4 → 7	Yes	?
6 → 9	?	No
8 → 11	Yes	No
10→ 13	?	No
12→ 15	Yes	?
i_a Ha → i+3$_ß$ Ha		
Nterm→ 3	?	?
2 → 5	Yes	Yes*
4 → 7	Yes	Yes*
6 → 9	Yes	Yes*
8 → 11	Yes	Yes
10→ 13	No	Yes*
12→ 15	Yes	Yes
i_b Hb → i+4$_ß$ HN		
1 → 5	Yes	Yes*
3 → 7	Yes	No
5 → 9	?	No
7 → 11	?	No
9 → 13	No	?
11→ 15	No	?

"Yes" indicates that an NOE was observed that had only one possible assignment.
"Yes*" indicates that an NOE was observed that had two or more possible assignments, one of which was consistent with the indicated nonsequential NOE. Other assignments for this NOE were not consistent with other probable NOEs.
"No" indicates that no NOE was observed, but that the indicated NOE would have been observed if it had been present.
"?" indicates that an NOE was observed, but that it could not be assigned because of overlap with either a sequential or intraresidue NOE, or that more than one probable nonsequential assignment for the NOE was possible.

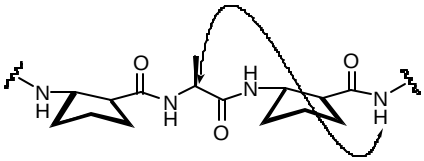
Graphical definitions of NOEs listed in Table S1.



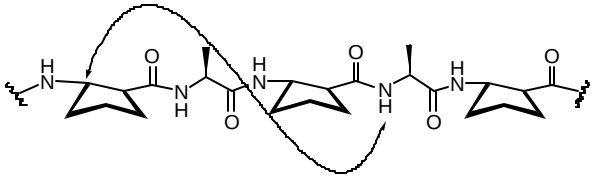
$i_{ß}$ Hß → i+2$_{β}$ HN



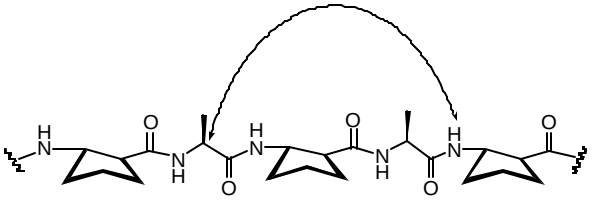
$i_{ß}$ Hß → i+2$_{β}$ Hα



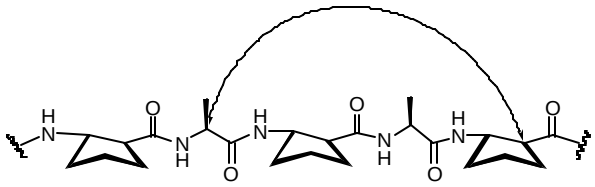
$i_{α}$ Hα → i+2$_{α}$ HN



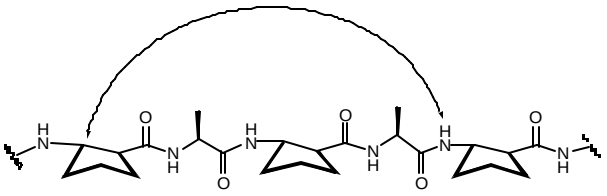
$i_{ß}$ Hß → i+3$_{a}$ HN



i_{a} Ha → i+3$_{ß}$ HN



i_{a} Ha → i+3$_{ß}$ Ha



$i_{β}$ Hβ → i+4$_{ß}$ HN

Table S2 lists the average interproton distances (Å) measured from model helices as reported in Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505. This information is provided to complement the NOE data reported for **4**.

Table S2:

NOE Type and Periodicity	11-Helix	14/15-Helix
$i_{ß}$ Hß → i+3$_a$ HN	3.1	2.3
i_a Ha → i+3$_ß$ Ha	**NONE**	3.0
i_b Hb → i+4$_ß$ HN	**NONE**	3.0
i_b Hb → i+2$_ß$ Ha	2.4	3.7
i_b Hb → i+2$_ß$ HN	3.3	3.9
i_a Ha → i+3$_ß$ HN	3.6	4.3
i_a Ha → i+2$_a$ HN	3.8	**NONE**

Antimicrobial assay:

The bacteria used in these assays were *Escherichia coli* JM109,[S4] *Bacillus subtilis* BR151,[S5] *Staphylococcus aureus* 1206 (methicillin-resistant),[S6] and *Enterococcus faecium* A634 (vancomycin-resistant).[S7] The antibacterial activity of the α/β-peptides was determined in sterile 96-well plates (Falcon 3075 microtiter plate) by a microdilution method. A bacterial suspension of approximately 10^6 CFU/mL BHI medium was added in 50 µL aliquots to 50 µL of medium containing the α/β-peptide in 2-fold serial dilutions for a total volume of 100 µL in each well. The plates were incubated at 37°C for 6 h. Growth inhibition was determined by measuring the OD at wavelengths ranging from 595-650 nm. Each MIC is the result of at least two separate trials; each trial is the result of an assay run in duplicate. Results are accurate within a factor of two.

Hemolysis assay:

Freshly drawn human red blood cells (hRBC, blood type A) were washed several times with Tris buffer (pH 7.2, 150 mM NaCl) and centrifuged until the supernatant was clear. Two-fold serial dilutions of α/β-peptide in Millipore water were added to each well in a sterile 96-well plate (Falcon 3075 microtiter plate), for a total volume of 20 µL in each well. A 1% v/v hRBC suspension (80 µL in Tris buffer) was added to each well. The plate was incubated at 37°C for 1 h and then centrifuged at 3500 rpm for 5 min. The supernatant (80 µL) was diluted with Millipore water (80 µL), and hemoglobin was detected by measuring the OD at 405 nm. The highest concentration of peptide that caused less than 10% hemolysis (melittin at 400 µg/mL defines 100%; Tris buffer defines 0%) is listed as the maximum concentration without significant hemolysis.

References:

S1 Aue, W. P; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.
S2 Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.
S3 Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.
S4 Yanisch-Perron, C.; Viers, J.; Messing, J. *Gene* 1985, *33*, 103.
S5 Young, F. E.; Smith, C.; Reilly, B. E. *J. Bacteriol.* **1969**, *98*, 1087.
S6 Weisblum, B.; Demohn, V. *J. Bacteriol.* 1969, *98*, 447.
S7 Nicas, T. I.; Wu, C. Y. E.; Hobbs, J. N.; Preston, D. A.; Allen, N. E. *Antimicrob. Agents Chemother*. 1989, *33*, 11121.