

1 **Structure-activity relationship study of novel peptoids that mimic the**  
2 **structure of antimicrobial peptides**

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9 **Supplementary Material**

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11 **Figure S1.** Schematic presentation of sub-monomeric solid phase peptoid synthesis

12 **Figure S2.** Antimicrobial activity and hydrophobicity correlation in *E. coli*, *S. aureus* and *P.*  
13 *aeruginosa*

14 **Figure S3.** Percentage hemolysis at 128 µg/ml and percentage cell viability at three highest  
15 concentrations for selected peptoids

16 **Figure S4.** Growth inhibition curves for GN-2-Nlys<sub>1-4</sub> Ntrp<sub>5-8</sub> Nile<sub>9</sub> and GN-2-Npm<sub>9</sub> peptoids

17 **Figure S5.** Chemical structures of all peptoids in the present study

18 **Table S1.** Sequence, retention time (Rt) and activity data of peptoids with Mlys substitution

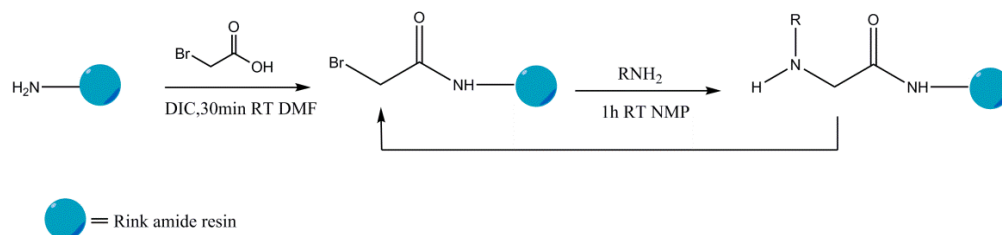
19 **Table S2.** Sequence, retention time (Rt) and activity data of peptoids with Ntrp substitution

20 **Table S3.** Sequence, retention time (Rt) and activity data of peptoids with single monomer  
21 substitution

22 **Table S4.** Sequence, retention time (Rt) and activity data of peptoids with different sequence  
23 rearrangement

24 **Table S5.** Sequence, retention time (Rt) and activity data of peptoids with different chain length

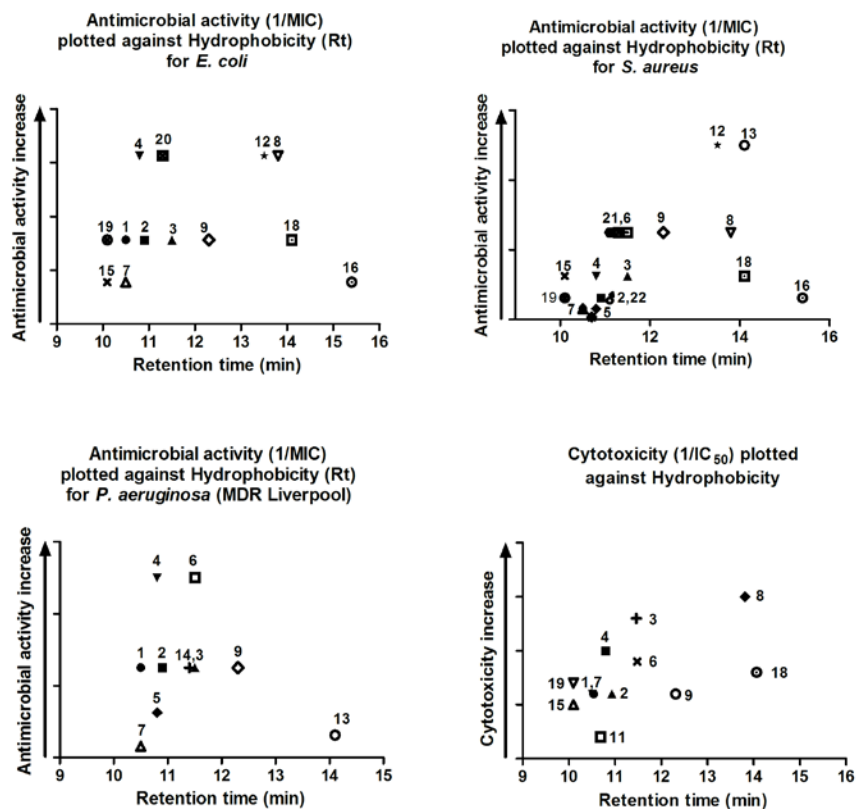
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**Figure S1.** Sub-monomeric solid phase peptoid synthesis.



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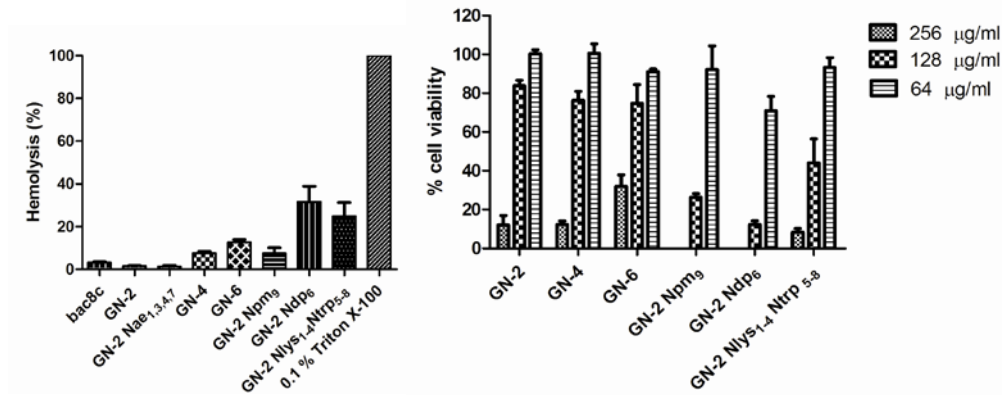
**Figure S2.** Hydrophobicity, reported by retention times in min on RP-HPLC, correlation to the observed antimicrobial activity (1/MIC) against three bacterial strains. *E. coli* (top left), *S. aureus* (top right) and *P. aeruginosa* (bottom left). Hydrophobicity relation to the observed cytotoxicity (1/IC<sub>50</sub>) of selected peptoids (bottom right).

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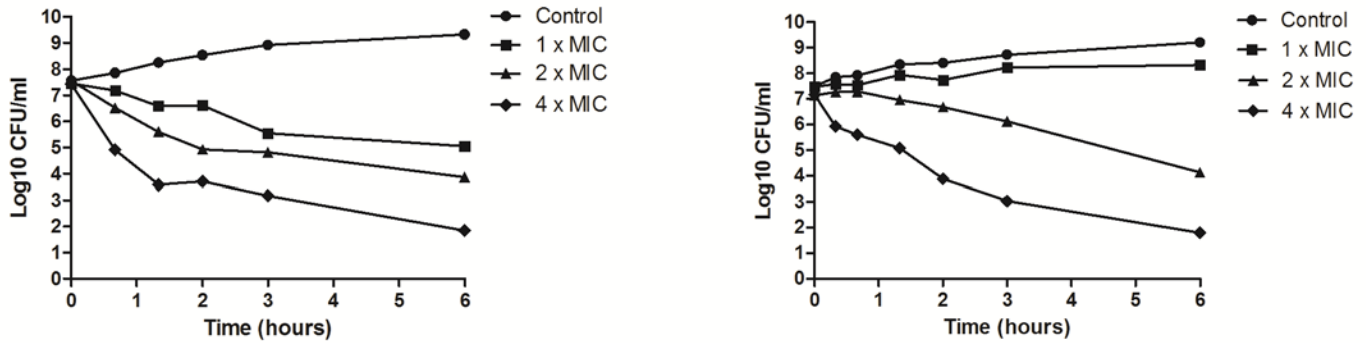
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 2 **Figure S3.** Hemolytic activity shown as percent hemolysis at a concentration of 128 µg/ml. Triton X-100 is used as a positive  
 3 control of 100 % hemolysis (left). Percent cell viability as estimated by MTS assay for the three highest tested concentrations for  
 4 selected peptides (right).

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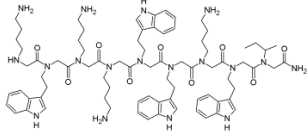


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 7 **Figure S4.** Growth inhibition curves for *E. coli* challenged with 1×, 2× and 4× MIC concentrations of GN-2-Nlys<sub>1-4</sub> Ntrp<sub>5-8</sub> (left)  
 8 and GN-2-Npm<sub>9</sub> peptoid (right) show concentration dependent inhibition. The inhibition with 1× MIC is more pronounced for  
 9 GN-2-Nlys<sub>1-4</sub> Ntrp<sub>5-8</sub> peptoid where significant decrease in CFU/ml is observed. Curves are representative data from three  
 10 individual experiments.

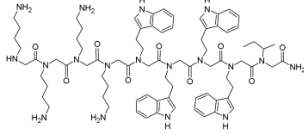
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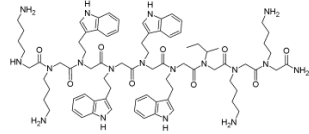
GN-2 peptoid



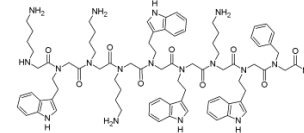
GN-2 NLY<sub>1-2</sub>NIP<sub>2-4</sub>NII<sub>5-9</sub>



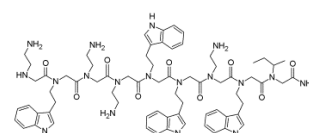
GN 2 NLY<sub>1-2,6,9</sub> NIP<sub>3-4,5,6</sub> NII<sub>7</sub>



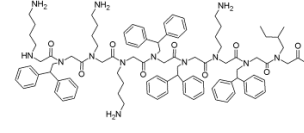
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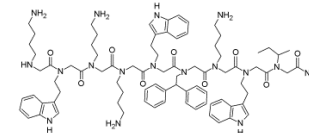
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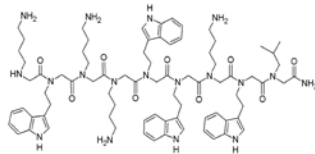
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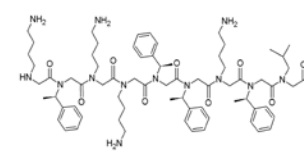
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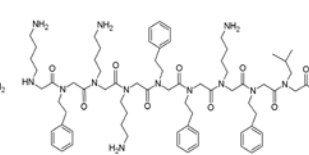
GN-4 peptoid



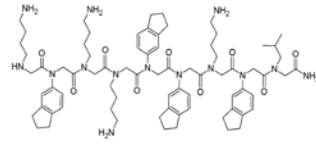
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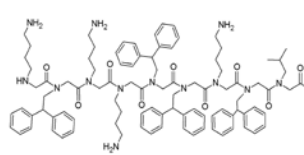
GN-4 NIP<sub>1-2,5,6,8</sub>



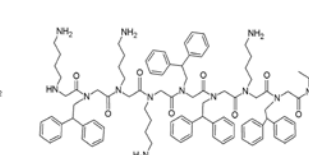
GN-4 NII<sub>1-2,5,8,9</sub>



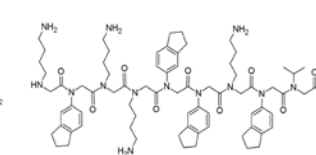
GN-4 NIP<sub>1-2,5,6,8</sub>



GN-4 NIP<sub>1-2,4,6,8</sub> NII<sub>1-9</sub>

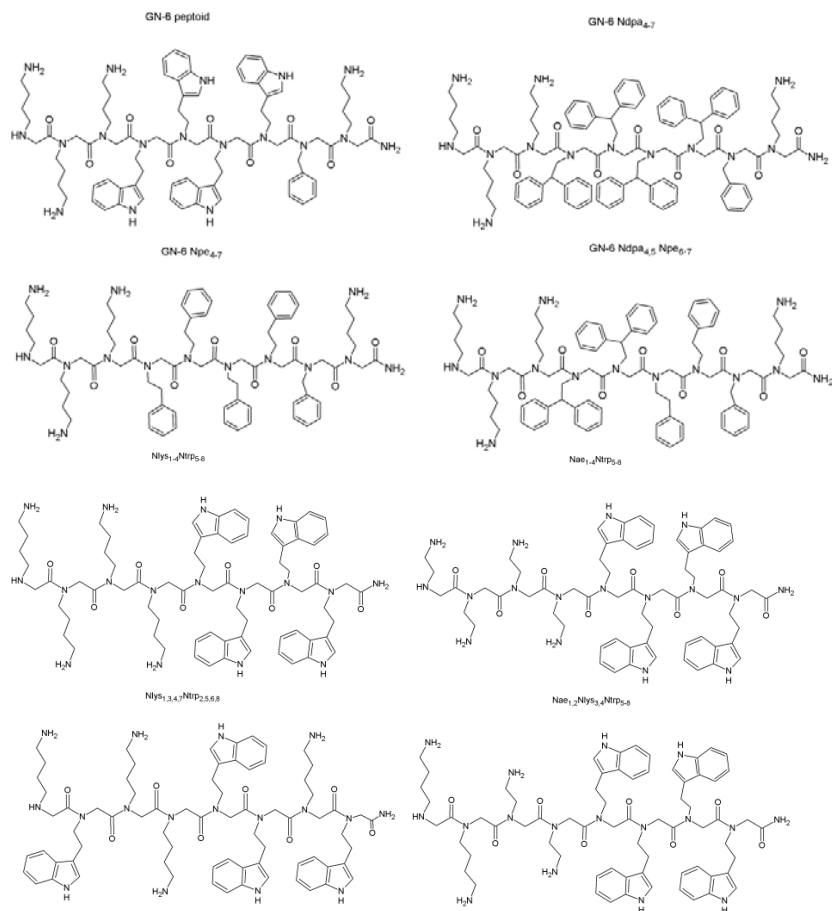


GN-4 NII<sub>1-2,5,8,9</sub> NII<sub>1-9</sub>



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**Figure S5.** Chemical structures of novel peptoids. (A) GN-2 and analogs, (B) GN-4 and analogs, (C) GN-6 and analogs, (D) 8-mers.

1 **Table S1. Sequence, retention time (Rt) and activity data of peptoids with Nlys substitution**

#	Peptoid nomenclature	Sequence (N-C)	R <sub>t</sub> (min) <sup>a</sup>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	HC <sub>10</sub>	Selectivity Ratio	Cytotoxicity
				ATCC 29213	ATCC 25922	PAOI			
20	Nae <sub>1,4</sub> Ntrp <sub>5,8</sub>	H-Nae-NNae-Nae-Nae-Ntrp-Ntrp-Ntrp-Ntrp-NH <sub>2</sub>	11.34	4	16	ND	32	8	
21	Nlys <sub>1,4</sub> Ntrp <sub>5,8</sub>	H-Nlys-Nlys-Nlys-Nlys-Ntrp-Ntrp-Ntrp-Ntrp-NH <sub>2</sub>	11.06	4	16	ND	64	16	
22	Nae <sub>1,2</sub> Nlys <sub>3,4</sub> Ntrp <sub>5,8</sub>	H-Nae-Nae-Nlys-Nlys-Ntrp-Ntrp-Ntrp-Ntrp-NH <sub>2</sub>	11.11	16	16	8	64	4-8	
5	GN-2-Nae <sub>1,3,4,7</sub>	H-Nae-Ntrp-Nae-Nae-Ntrp-Ntrp-Nae-Ntrp-Nile-NH <sub>2</sub>	10.81	32	16	16-32	>128	8	
1	GN-2	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ntrp-Nlys-Ntrp-Nile-NH <sub>2</sub>	10.54	64	32	32	>128	>4	168

<sup>a</sup>Analytical retention time (Rt in minutes) on a reverse phase C<sub>18</sub> Kinetex 100 x 2.1 mm 100A column, 60 °C run on a 15-65 % acetonitrile gradient over 20 min, 0.5 ml/min. ND-Not determined

5 **Table S2. Sequence, retention time (Rt) and activity data of peptoids with Ntrp substitution**

#	Peptoid nomenclature	Sequence (N-C)	R <sub>t</sub> (min) <sup>a</sup>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	HC <sub>10</sub>	Selectivity Ratio	Cytotoxicity
				ATCC 29213	ATCC 25922	PAOI			
16	GN-6-Ndpe <sub>4,7</sub>	H-Nlys-Nlys-Nlys-Ndpe-Ndpe-Ndpe-Ndpe-Npm-Nlys-NH <sub>2</sub>	15.43	16	64	64	16	1	
15	GN-6	H-Nlys-Nlys-Nlys-Ntrp-Ntrp-Ntrp-Ntrp-Npm-Nlys-NH <sub>2</sub>	10.10	8-16	64	64	>128	>16	203
17	GN-6-Npe <sub>4,7</sub>	H-Nlys-Nlys-Nlys-Npe-Npe-Npe-Npe-Npm-Nlys-NH <sub>2</sub>	ND	16	32-64	32-64	32	0.5-2	
8	GN-4-Ndpe <sub>2,5,6,8</sub>	H-Nlys-Ndpe-Nlys-Nlys-Ndpe-Ndpe-Nlys-Ndpe-Nleu-NH <sub>2</sub>	13.81	4	16	16	4	1	67
9	GN-4-Nai <sub>2,5,6,8</sub>	H-Nlys-Nai-Nlys-Nlys-Nai-Nai-Nlys-Nai-Nleu-NH <sub>2</sub>	12.31	4	16-32	16-32	32	8	172
11	GN-4-Nspe <sub>2,5,6,8</sub>	H-Nlys-Nspe-Nlys-Nlys-Nspe-Nspe-Nlys-Nspe-Nleu-NH <sub>2</sub>	10.69	128	32	32	>128	>4	631
7	GN-4	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ntrp-Nlys-Ntrp-Nleu-NH <sub>2</sub>	10.51	32	64	64	>128	>4	166
10	GN-4-Npe <sub>2,5,6,8</sub>	H-Nlys-Npe-Nlys-Nlys-Npe-Npe-Nlys-Npe-Nleu-NH <sub>2</sub>	ND	64	64-128	64-128	>128	>1-2	

<sup>a</sup>Analytical retention time (Rt in minutes) on a reverse phase C<sub>18</sub> Kinetex 100 x 2.1 mm 100A column, 60 °C run on a 15-65 % acetonitrile gradient over 20 min, 0.5 ml/min. ND-Not determined

1 **Table S3. Sequence, retention time (Rt) and activity data of peptoids with single monomer substitution**

#	Peptoid nomenclature	Sequence (N-C)	R <sub>t</sub> (min) <sup>a</sup>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	HC <sub>10</sub>	Selectivity Ratio	Cytotoxicity
				ATCC 29213	ATCC 25922	PAO1			
13	GN-4-Ndpe <sub>2,5,6,8</sub> Nma <sub>9</sub>	H-Nlys-Ndpe-Nlys-Nlys-Ndpe-Ndpe-Nlys-Ndpe-Nma-NH <sub>2</sub>	14.07	2	32	32	4	2	
8	GN-4-Ndpe <sub>2,5,6,8</sub>	H-Nlys-Ndpe-Nlys-Nlys-Ndpe-Ndpe-Nlys-Ndpe-Nleu-NH <sub>2</sub>	13.81	4	16	16	4	1	67
12	GN-4-Ndpe <sub>2,5,6,8</sub> Nval <sub>9</sub>	H-Nlys-Ndpe-Nlys-Nlys-Ndpe-Ndpe-Nlys-Ndpe-Nval-NH <sub>2</sub>	13.5	2	16	16	4-8	2-4	
9	GN-4-Nai <sub>2,5,6,8</sub>	H-Nlys-Nai-Nlys-Nlys-Nai-Nai-Nlys-Nai-Nleu-NH <sub>2</sub>	12.31	4	16-32	16-32	32	8	172
14	GN-4-Nai <sub>2,5,6,8</sub> Nval <sub>9</sub>	H-Nlys-Nai-Nlys-Nlys-Nai-Nai-Nlys-Nai-Nval-NH <sub>2</sub>	11.37	4	32	32	>128	>32	
6	GN-2-Ndpe <sub>6</sub>	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ndpe-Nlys-Ntrp-Nle-NH <sub>2</sub>	11.46	4	32	32	16	4	80
4	GN-2-Npm <sub>9</sub>	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ntrp-Nlys-Ntrp-Npm-NH <sub>2</sub>	10.79	8	16	16	128	16	104
1	GN-2	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ntrp-Nlys-Ntrp-Nle-NH <sub>2</sub>	10.54	64	32	32	>128	>4	168
7	GN-4	H-Nlys-Ntrp-Nlys-Nlys-Ntrp-Ntrp-Nlys-Ntrp-Nleu-NH <sub>2</sub>	10.51	32	64	64	>128	>4	166

<sup>a</sup>Analytical retention time (Rt in minutes) on a reverse phase C<sub>18</sub> Kinetex 100 x 2.1 mm 100A column, 60 °C run on a 15-65 % acetonitrile gradient over 20 min, 0.5 ml/min. ND-Not determined

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2 **Table S4. Sequence, retention time (Rt) and activity data of peptoids with different sequence rearrangement**

#	Peptoid nomenclature	Sequence (N-C)	R <sub>t</sub> (min) <sup>a</sup>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	HC <sub>10</sub>	Selectivity Ratio	Cytotoxicity
				ATCC 29213	ATCC 25922	PAO1			
15	GN-6	H-Mlys-Mlys-Mlys-Ntrp-Ntrp-Ntrp-Ntrp-Npm-Mlys-NH <sub>2</sub>	10.10	8-16	64	64	>128	>16	203
4	GN-2-Npm <sub>9</sub>	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-Npm-NH <sub>2</sub>	10.79	8	16	16	128	16	104
3	GN-2-Mlys <sub>1-4</sub> Ntrp <sub>5-8</sub>	H-Mlys-Mlys-Mlys-Mlys-Ntrp-Ntrp-Ntrp-Ntrp-Nile-NH <sub>2</sub>	11.48	8	32	32	128	4-32	110
2	GN-2	H-Ntrp-Ntrp-Mlys-Mlys-Mlys-Mlys-Nile-Ntrp-Ntrp-NH <sub>2</sub>	10.93	16	32	32	>128	>8	173
1	Ntrp <sub>1,2,8,9</sub> Nile <sub>7</sub> Nlys <sub>3-6</sub> GN-2	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-Nile-NH <sub>2</sub>	10.54	64	32	32	>128	>4	168
19	Mlys <sub>1,3,4,7</sub> Ntrp <sub>2,5,6,8</sub>	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-NH <sub>2</sub>	10.13	16	32	ND	>256	>16	148
21	Mlys <sub>1-4</sub> Ntrp <sub>5-8</sub>	H-Mlys-Mlys-Mlys-Mlys-Ntrp-Ntrp-Ntrp-Ntrp-NH <sub>2</sub>	11.06	4	16	ND	64	16	

3 <sup>a</sup>Analytical retention time (Rt in minutes) on a reverse phase C<sub>18</sub> Kinetex 100 x 2.1 mm 100A column, 60 °C run on a 15-65 % acetonitrile  
4 gradient over 20 min, 0.5 ml/min. ND-Not determined

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6 **Table S5. Sequence, retention time (Rt) and activity data of peptoids with different chain length**

#	Peptoid nomenclature	Sequence (N-C)	R <sub>t</sub> (min) <sup>a</sup>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	HC <sub>10</sub>	Selectivity Ratio	Cytotoxicity
				ATCC 29213	ATCC 25922	PAO1			
1	GN-2	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-Nile-NH <sub>2</sub>	10.54	64	32	32	>128	>4	168
7	GN-4	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-Nleu-NH <sub>2</sub>	10.51	32	64	64	>128	>2-4	166
19	Mlys <sub>1,3,4,7</sub> Ntrp <sub>2,5,6,8</sub>	H-Mlys-Ntrp-Mlys-Mlys-Ntrp-Ntrp-Mlys-Ntrp-NH <sub>2</sub>	10.13	16	32	ND	>256	>16	148
3	GN-2-Mlys <sub>1-4</sub> Ntrp <sub>5-8</sub>	H-Mlys-Mlys-Mlys-Mlys-Ntrp-Ntrp-Ntrp-Ntrp-Nile-NH <sub>2</sub>	11.48	8	32	32	128	4-32	110
21	Mlys <sub>1-4</sub> Ntrp <sub>5-8</sub>	H-Mlys-Mlys-Mlys-Mlys-Ntrp-Ntrp-Ntrp-Ntrp-NH <sub>2</sub>	11.06	4	16	ND	64	16	

7 <sup>a</sup>Analytical retention time (Rt in minutes) on a reverse phase C<sub>18</sub> Kinetex 100 x 2.1 mm 100A column, 60 °C run on a 15-65 % acetonitrile  
8 gradient over 20 min, 0.5 ml/min. ND-Not determined

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