

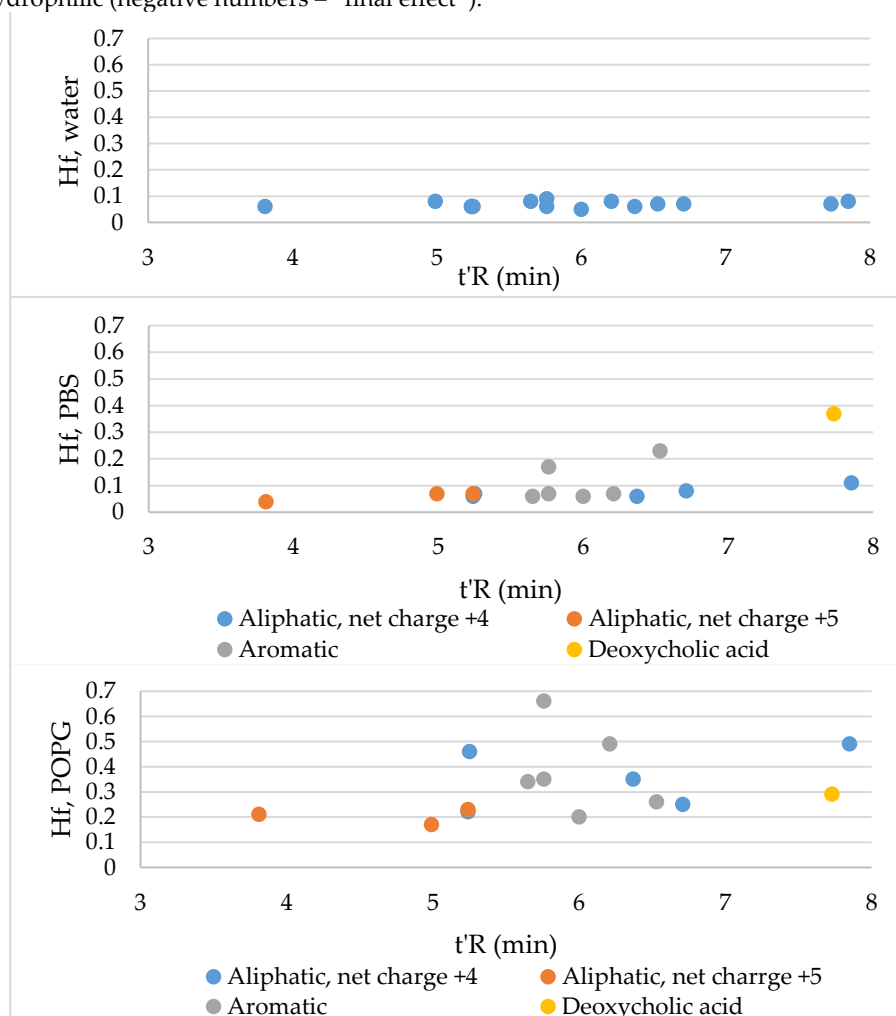
**Table S1.** Results of ESI MS analyses of peptides I-XXII.

No.	Peptide	MS Analysis			No.	Peptide	MS Analysis		
		<i>z</i>	<i>m/z</i> calc.	<i>m/z</i> found			<i>z</i>	<i>m/z</i> calc.	<i>m/z</i> found
<b>I</b>	KR12-NH <sub>2</sub> (KRIVQRIKDFLR-NH <sub>2</sub> )	1	1571.0	-	<b>XII</b>	C <sub>8</sub> <sup>α</sup> -retro-KR12-NH <sub>2</sub>	1	1697.1	-
		2	786.0	786.6			2	849.1	849.5
		3	524.3	824.8			3	566.4	566.7
		4	393.5	-			4	425.0	425.6
							5	340.2	-
<b>II</b>	C <sub>8</sub> <sup>α</sup> -KR12-NH <sub>2</sub>	1	1697.1	-	<b>XIII</b>	2-buthyloctanoic acid-KR12-NH <sub>2</sub>	1	1753.2	-
		2	849.1	849.4			2	877.1	877.6
		3	566.4	566.8			3	585.1	585.5
		4	425.0	-			4	439.0	439.5
<b>III</b>	C <sub>8</sub> <sup>ε</sup> -KR12-NH <sub>2</sub>	1	1697.1	-	<b>XIV</b>	2-ethylhexanoic acid-KR12-NH <sub>2</sub>	1	1697.1	-
		2	849.1	849.6			2	849.1	849.6
		3	566.4	566.8			3	566.4	566.9
		4	425.0	-			4	425.0	425.4
<b>IV</b>	C <sub>8</sub> <sup>α</sup> -Lys-KR12-NH <sub>2</sub>	1	1825.2	-	<b>XV</b>	Benzoic acid-KR12-NH <sub>2</sub>	1	1675.0	-
		2	913.1	913.5			2	838.0	838.5
		3	609.1	609.5			3	559.0	559.5
		4	457.1	457.5			4	419.5	420.3
<b>V</b>	C <sub>8</sub> <sup>ε</sup> -Lys-KR12-NH <sub>2</sub>	1	1825.2	-	<b>XVI</b>	Phenylacetic acid-KR12-NH <sub>2</sub>	1	1689.0	-
		2	913.1	913.6			2	845.0	845.3
		3	609.1	609.7			3	563.7	564.2
		4	457.1	457.6			4	423.0	-
<b>VI</b>	KR12- Lys <sup>ε</sup> (C <sub>8</sub> )-NH <sub>2</sub>	1	1825.2	-	<b>XVII</b>	3-Phenylpropionic acid- KR12-NH <sub>2</sub>	1	1703.1	-
		2	913.1	913.6			2	852.0	852.5
		3	609.1	609.6			3	568.4	569.1
		4	457.1	457.7			4	426.5	427.1
<b>VII</b>	[Lys <sup>ε</sup> (C <sub>8</sub> )] <sup>12</sup> KR12-NH <sub>2</sub>	1	1669.1	-	<b>XVIII</b>	4-Phenylbutanoic acid-KR12-NH <sub>2</sub>	1	1717.1	-
		2	835.0	835.5			2	859.0	859.5
		3	557.0	557.5			3	573.0	573.5
		4	418.0	-			4	430.0	430.6
<b>VIII</b>	[Lys <sup>ε</sup> (C <sub>8</sub> )] <sup>8</sup> KR12-NH <sub>2</sub>	1	1697.1	-	<b>XIX</b>	<i>trans</i> -cinnamic acid-KR12-NH <sub>2</sub>	1	1701.0	-
		2	849.1	849.6			2	851.0	851.5
		3	566.4	566.8			3	567.7	568.1
		4	425.0	-			4	426.0	426.4
<b>IX</b>	C <sub>8</sub> <sup>α</sup> ,C <sub>8</sub> <sup>ε</sup> -KR12-NH <sub>2</sub>	1	1823.2	-	<b>XX</b>	Phenylpropionic acid-KR12-NH <sub>2</sub>	1	1699.0	-
		2	912.1	912.7			2	850.0	850.4
		3	608.4	608.9			3	567.0	567.6
		4	456.6	-			4	425.5	-
<b>X</b>	C <sub>4</sub> <sup>α</sup> , C <sub>4</sub> <sup>ε</sup> -KR12-NH <sub>2</sub>	1	1711.1	-	<b>XXI</b>	4-phenylbenzoic acid-KR12-NH <sub>2</sub>	1	1751.1	-
		2	856.0	856.4			2	876.0	876.50
		3	571.0	571.6			3	584.4	584.8
		4	428.5	429.4			4	438.5	439.1
<b>XI</b>	retro-KR12-C <sub>8</sub> <sup>ε</sup> -NH <sub>2</sub>	1	1697.1	-	<b>XXII</b>	Deoxycholic acid-KR12-NH <sub>2</sub>	1	1945.3	-
		2	849.1	849.5			2	973.1	973.7
		3	566.4	566.9			3	649.1	649.6
		4	425.0	-			4	487.1	487.5

**Table S2.** Calculations of differences between hydrophobicity coefficients of terminal amino acid residues of KR12-NH<sub>2</sub> and its *retro*-analog.

Peptide	Sequence	Hydrophobicity Coefficients of Terminal Amino Acid Residues [1] (min)	Differences in Coefficients Between <i>Retro</i> -Analog and Parent Molecule	Final Effect (min) $\Delta = \Delta\text{Lys} + \Delta\text{Arg}$
KR12-NH <sub>2</sub>	KRIVQRIKDFLR-NH <sub>2</sub>	N-terminal Lys: 1.3		
<i>retro</i> -KR12-NH <sub>2</sub>	RLFDKIRQVIRK-NH <sub>2</sub>	C-terminal Lys: 0.0	$\Delta\text{Lys}$ : -1.3	-2.0
		N-terminal Arg: 3.0	$\Delta\text{Arg}$ : -0.7	
		C-terminal Arg: 3.7		
Ac-KR12-NH <sub>2</sub>	Ac-KRIVQRIKDFLR-NH <sub>2</sub>	N-terminal Lys: -0.3		
Ac- <i>retro</i> -KR12-NH <sub>2</sub>	Ac-RLFDKIRQVIRK-NH <sub>2</sub>	C-terminal Lys: 0.0	$\Delta\text{Lys}$ : 0.3	-1.0
		N-terminal Arg: 2.4	$\Delta\text{Arg}$ : -1.3	
		C-terminal Arg: 3.7		

Hydrophobicity coefficients are from article of Tripet B *et al.* (2006) [1]. Previous studies on *retro*-analogs indicate that terminal amino acid residues are critical for change of retention behaviour and thus hydrophobicity [2]. Calculated differences in hydrophobicity coefficients of lysine and arginine between *retro*-analogs and parent molecules (KR12-NH<sub>2</sub>, Ac-KR12-NH<sub>2</sub>) indicate that both analogs will be more hydrophilic (negative numbers – “final effect”).



**Figure S1.** Helical fraction (Hf) *vs.* adjusted retention time.

## References

1. Tripet, B.; Cepeniene, Dz.; Kovacs, J.M.; Mant, C.T. Oleg V. Krokhin b, Robert S. Hodges Requirements for prediction of peptide retention time in reversed-phase high-performance liquid chromatography: Hydrophilicity/hydrophobicity of side-chains at the N- and C-termini of peptides are dramatically affected by the end-groups and location. *J. Chromatogr. A* **2007**, *1141*, 212–225.

2. Neubauer, D.; Jaśkiewicz, M.; Migoń, D.; Bauer, M.; Sikora, K.; Sikorska, E.; Kamysz, E.; Kamysz, W. Retro analog concept: Comparative study on physico-chemical and biological properties of selected antimicrobial peptides. *Amino Acids* **2017**, *49*, 1755–1771.