**Figure S1.** Examples of LC-MS of some peptides used in this study.

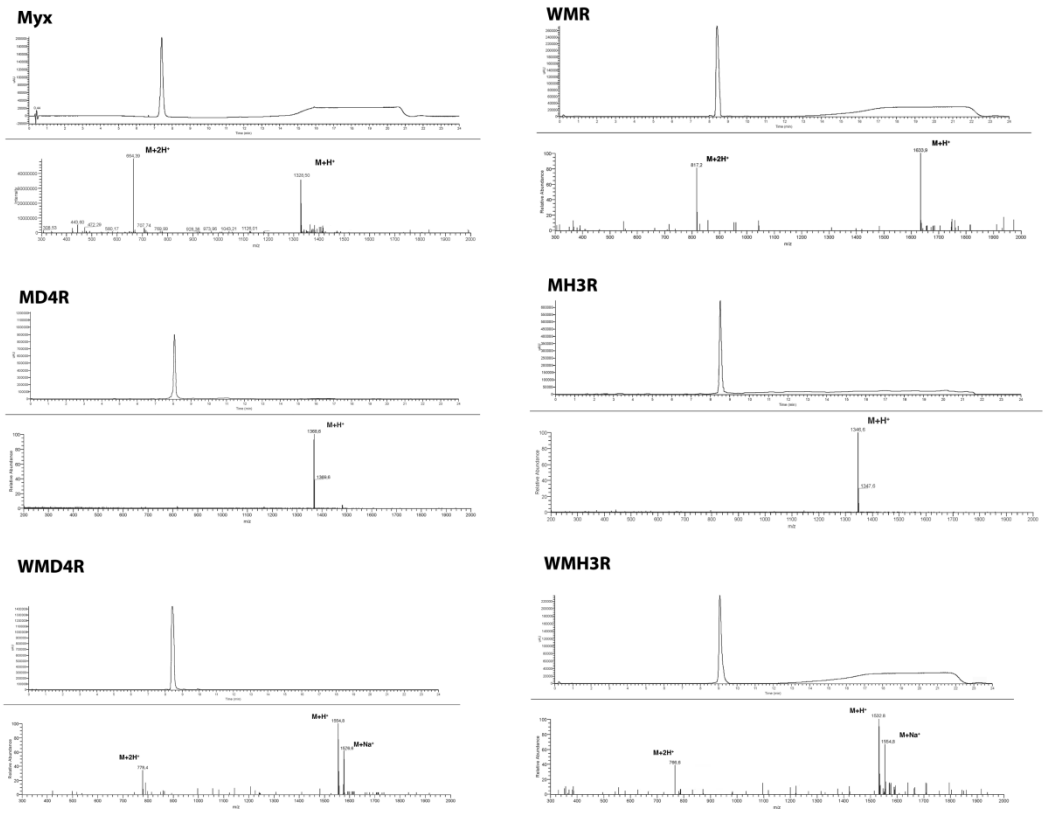


**Figure S2. A)** 2D [$^1$H, $^1$H] NOESY spectrum of Myxinidin acquired in 10 mM sodium phosphate buffer pH=7.4; **B)** One dimensional proton spectra of Myxinidin in phosphate buffer (red) and in presence of TFE (50/50 v/v) (black). The $H_N$ and aromatic proton region is reported in panel B.

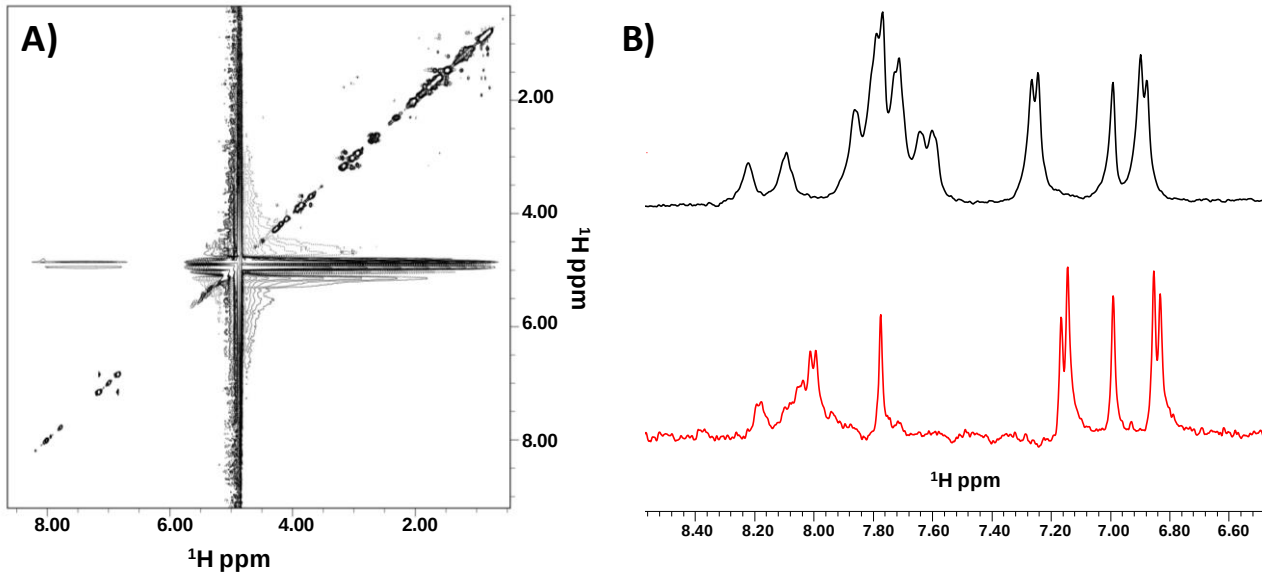

**Figure S3.** Comparison of 2D [$^{1}$H, $^{1}$H] TOCSY (right side) and 2D [$^{1}$H, $^{1}$H] NOESY (left side) of Myxinidin in phosphate buffer/TFE (50/50 v/v).

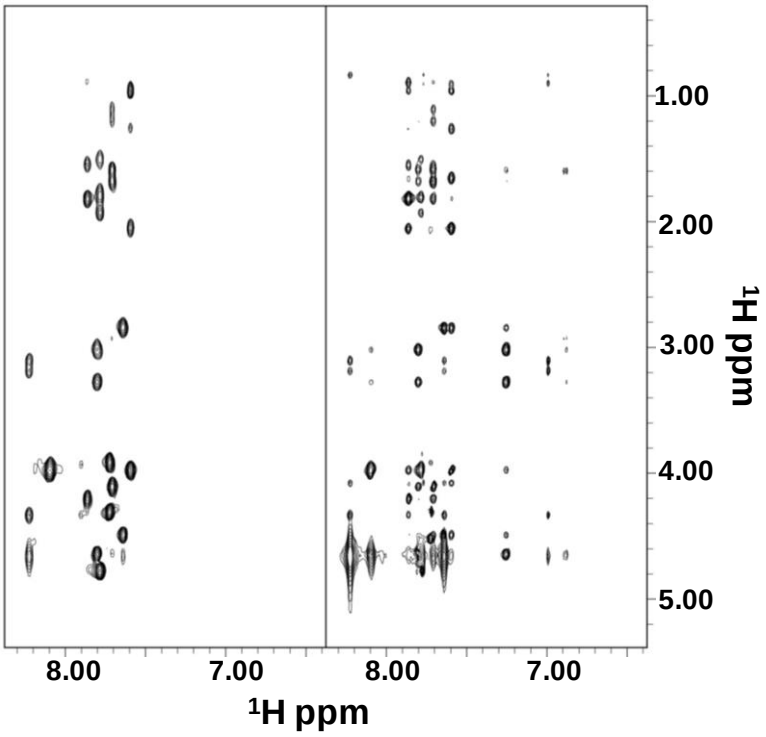

**Figure S4.** Hα chemical shift deviations from random coil values ($\delta_{obs}$-$\delta_{rc}$) evaluated in a solution containing 50% TFE. Data are set equal to zero for the unassigned Hα chemical shift of Gly1.

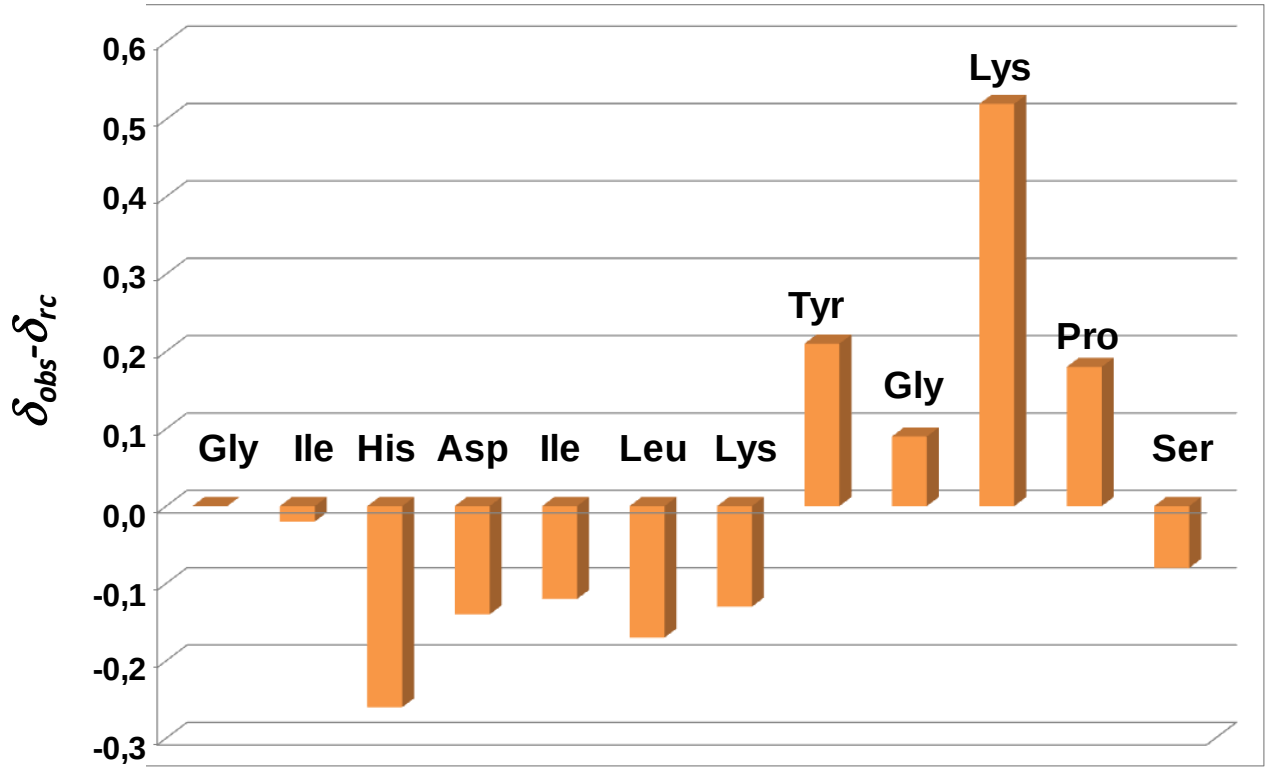

**Table S1.** Chemical shifts ppm of Myxinidin evaluated at 298 K in 10 mM sodium phosphate buffer pH=7.4/TFE (50/50 v/v).

| Residue | $H_N$ | Hα | Hβ | Hγ | Others |
|---|---|---|---|---|---|
| **1Gly** | | | | | |
| **2Ile** | | 4.07 | 1.84 | $H\gamma_{CH2}$ 1.28-1.42 $H_{\gamma CH3}$ 0.84 | $H\delta_{CH3}$ 0.92 |
| **3His** | 8.22 | 4.33 | 3.10-3.19 | | Hδ2 6.99 Hε1 7.78 |
| **4Asp** | 7.64 | 4.49 | 2.83-2.85 | | |
| **5Ile** | 7.59 | 3.97 | 2.05 | $H\gamma_{CH2}$ 1.26-1.64 $H\gamma_{CH3}$ 0.98 | $H\delta_{CH3}$ 0.88 |
| **6Leu** | 7.86 | 4.18 | 1.56-1.81 | 1.82 | 0.88-0.92 |
| **7Lys** | 7.70 | 4.10 | 1.59-1.68 | 1.12-1.20 | Hδ 1.61 Hε 2.93 |
| **8Tyr** | 7.80 | 4.64 | 3.01-3.27 | | Hδ 7.25 Hε 6.90 |
| **9Gly** | 8.09 | 3.97 | | | |
| **10Lys** | 7.78 | 4.75 | 1.80-1.93 | 1.51 | Hδ 1.75 Hε 3.06 |
| **11Pro** | | 4.51 | 2.32 | 2.06-2.10 | Hδ 3.74-3.84 |
| **12Ser** | 7.72 | 4.30 | 3.91 | | |

**Table S2.** Statistics for Myxinidin NMR ensemble of structures.

| | |
|---|---|
| **NOE upper distance limits** | 142 |
| **Angle constraints** | 42 |
| **Residual target function, Å$^2$** | 0.35 |
| **Residual NOE violations** | |
| Total Number | 2 |
| Number >0.2 Å | 2 |
| Maximum violation, Å | 0.27 |
| **Residual angle violations** | |
| Total Number | 0 |
| **Atomic pairwise RMSD, Å** | |
| Backbone atoms (a.a. 2-9) | 0.24±0.06 |
| **Ramachandran statistics[&]** | |
| Residues in core regions | 92.2% |
| Residues in allowed regions | 6.1% |
| Residues in generous regions | 1.7% |
| Residues in disallowed regions | 0.0% |

[&]Procheck NMR analysis (residues 1-12) *(Laskowski RA et al., (1996) AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. J Biomol NMR, 8 (4): 477-486).*

| **Table 3S.** Myxinidin analogues MIC on different microbial strains | | | | | |
|---|---|---|---|---|---|
| MIC value (μM) | | | | | |
| **Peptide** | ***E. coli*** | ***P. aeruginosa*** | **S. *typhimurium*** | ***K. pneumonia*** | ***S. aureus*** |
| **Myxinidin** | 5 | 30 | 20 | 10 | 10 |
| **G 1** | >50 | 10 | 3 | >50 | 10 |
| **I 2** | >50 | >50 | >50 | >50 | 30 |
| **H 3** | >50 | 15 | 10 | 30 | 20 |
| **D 4** | >50 | >50 | >50 | 30 | 5 |
| **I 5** | 20 | 10 | 4 | 20 | 20 |
| **L 6** | >50 | >50 | >50 | 20 | 5 |
| **K 7** | 15 | 15 | 15 | 20 | 10 |
| **Y 8** | 20 | >50 | >50 | 20 | 10 |
| **G 9** | 5 | 15 | 10 | 10 | 10 |
| **K 10** | 15 | >50 | >50 | 10 | 20 |
| **P 11** | >50 | 30 | 10 | 30 | 10 |
| **S 12** | >50 | >50 | >50 | 30 | >50 |

| **Table 4S.** Myxinidin analogues MIC on different microbial strains | | | | | |
|---|---|---|---|---|---|
| MIC value (μM) | | | | | |
| **Peptide** | ***E. coli*** | ***P. aeruginosa*** | **S. *typhimurium*** | ***K. pneumonia*** | ***S. aureus*** |
| **Myxinidin *** | 5 | 30 | 20 | 10 | 10 |
| **WMR**-$NH_2$ * | 2 | 2 | 1.2 | 3 | 2.2 |
| **WMR**-COOH | 10 | 4 | 10 | 20 | 4 |
| **MH3R**-$NH_2$ * | 5 | 20 | 5 | 5 | 10 |
| **WMH3R**-$NH_2$ | 5 | 30 | 25 | 15 | 5 |
| **WMH3R**-COOH | 15 | 5 | 8 | 30 | 15 |
| **MD4R**-$NH_2$ * | 2.2 | 10 | 3 | 3 | 10 |

| | | | | | |
|---|---|---|---|---|---|
| **WMD4R-**$NH_2$ | 8 | 35 | 12 | 6 | 4 |
| **WMD4R-C**OOH | 8 | 12 | 10 | 12 | 8 |

* The data relative to these peptides have been obtained in a previous work (16).

**Table 5S.** Lipid composition of different bacteria

| **lipid** | ***E. coli*** | ***P. aeruginosa*** | **S. *typhimurium*** | ***K. pneumonia*** | ***S. aureus*** |
|---|---|---|---|---|---|
| **PE** | 85 | 60 | 76 | 82 | 0 |
| **PG** | 10 | 21 | 17 | 5 | 57 |
| **CL** | 5 | 11 | 7 | 6 | 19 |