Structural and functional characterization of a modified legionaminic acid involved in glycosylation of a bacterial lipopolysaccharide

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Supporting Figure S1

Supporting Figure S2

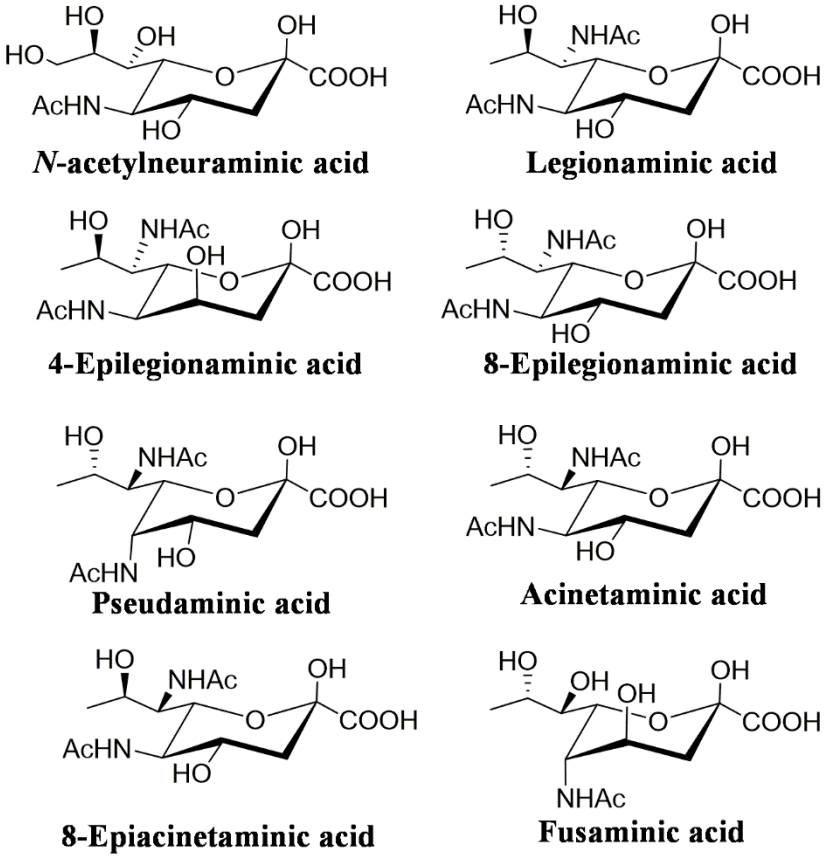
Supporting Figure S3

Supporting Figure S4

Supporting Figure S5

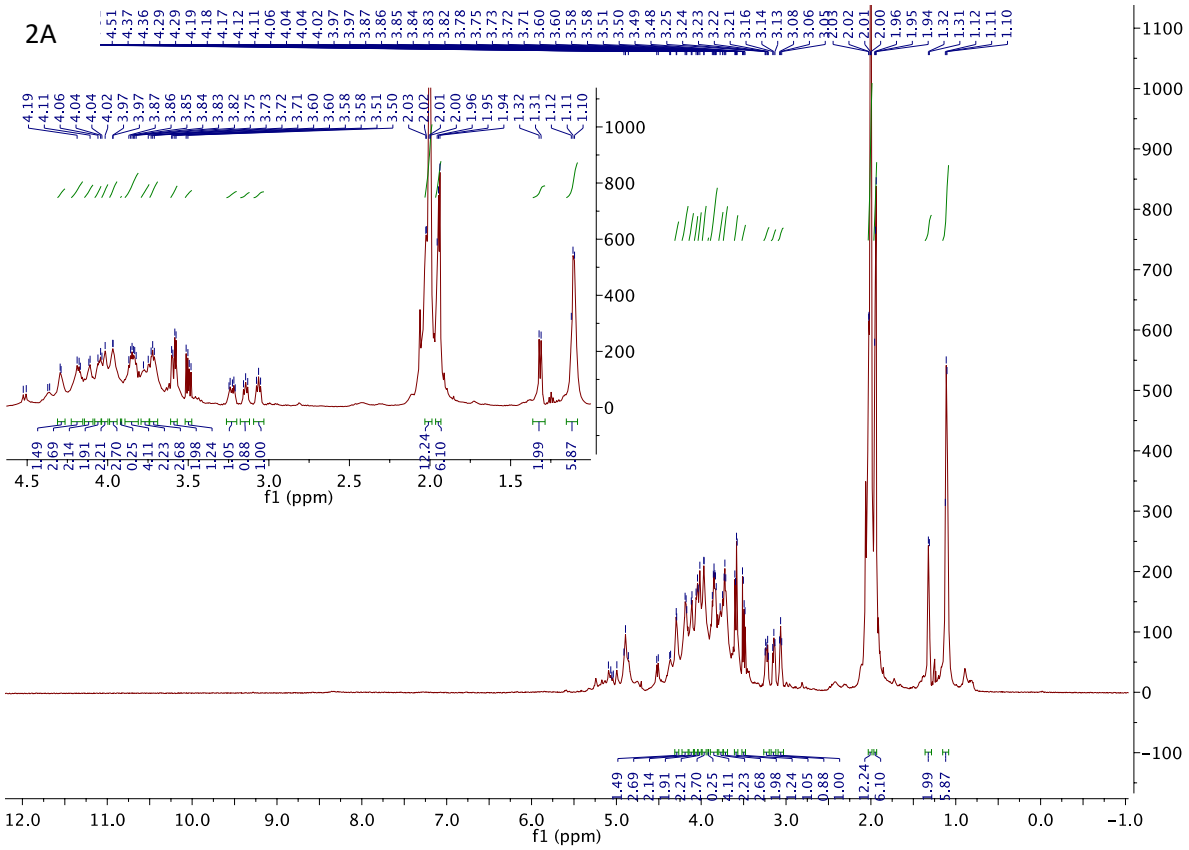
Supporting Figure S6

Supporting Figure S7

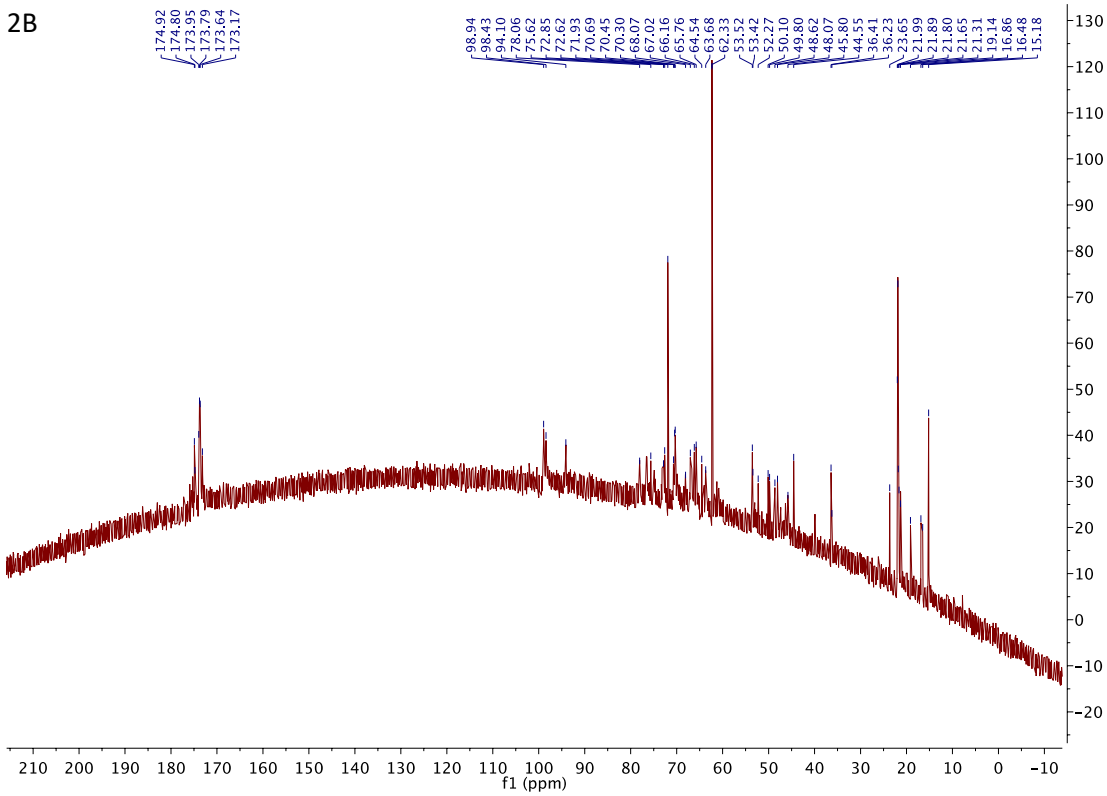


Supporting information Figure S1: Diversity of nonulosonic acids biosynthesized by bacteria. Many bacterial species have been shown to produce *N*-acetylneuraminic acid (Sialic acid) or the di-*N*-acetylated bacterial specific NulOs of which there are seven described isomers.

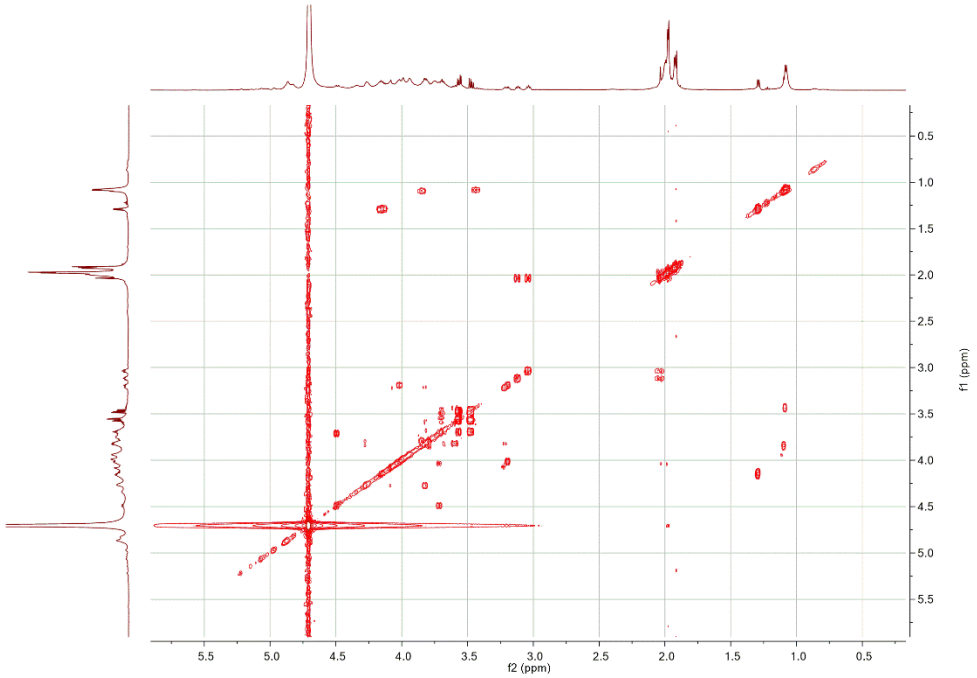
2A



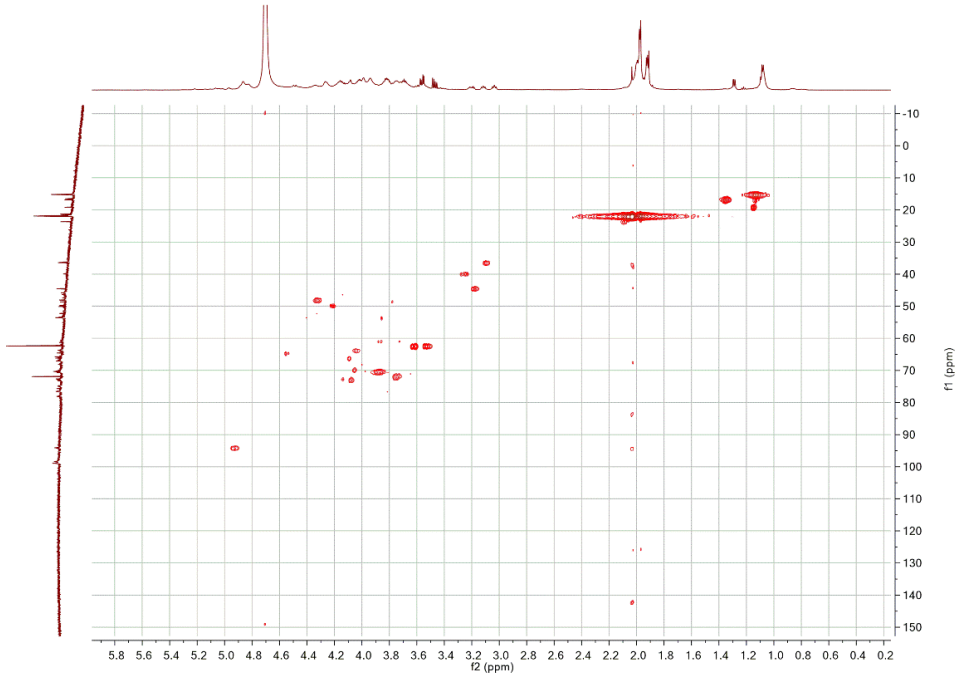
2B



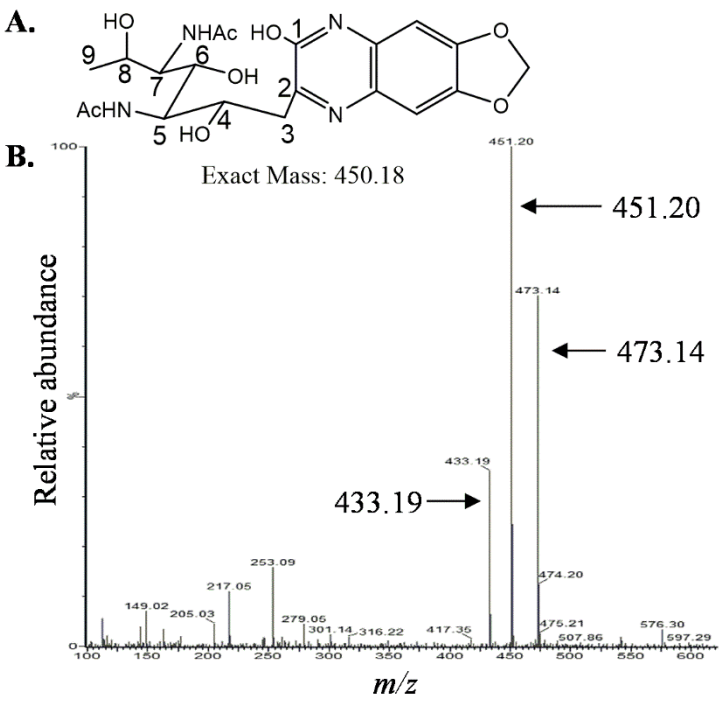
2C



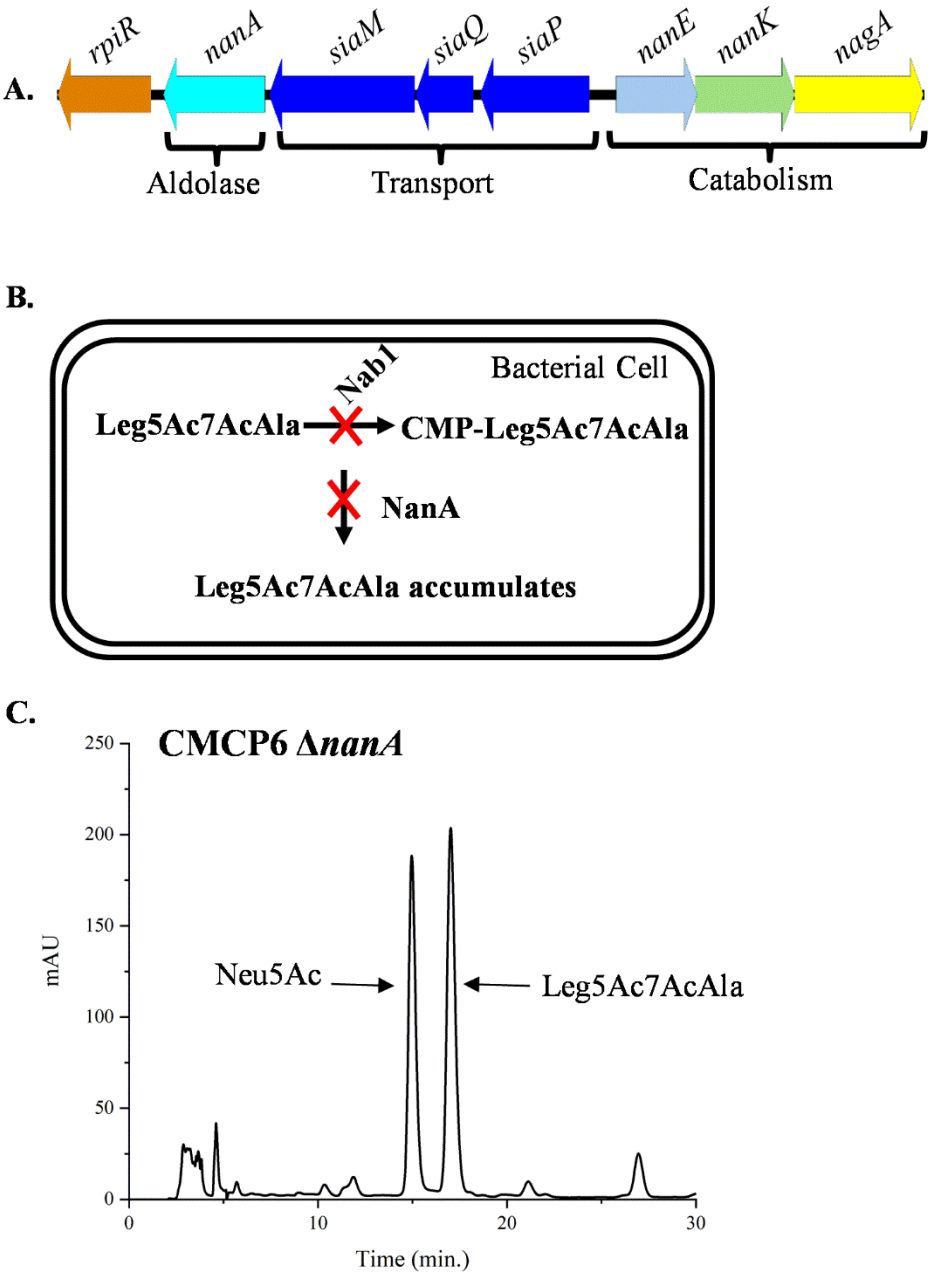
2D



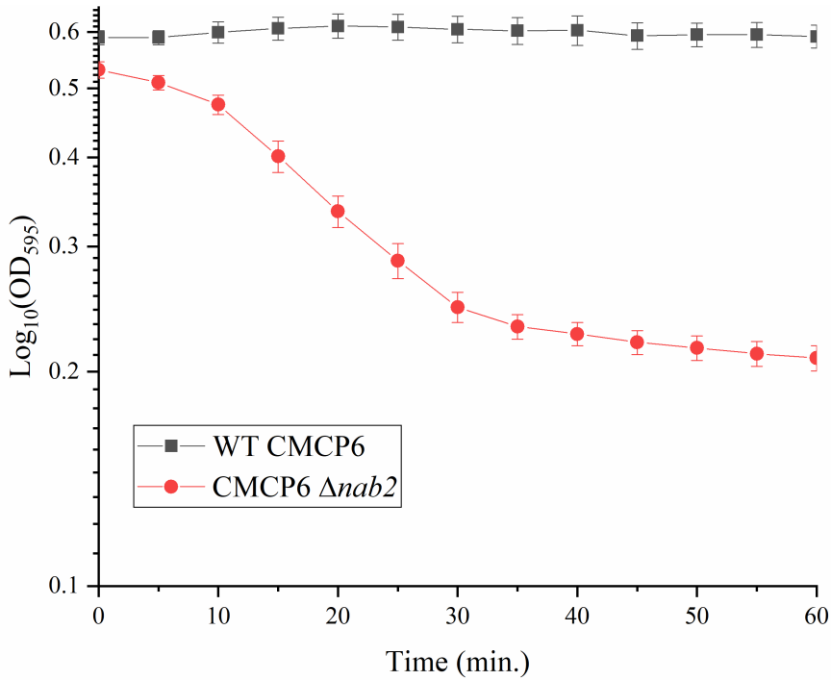
Supporting information Figure S2: Nuclear Magnetic Resonance (NMR) Characterization Spectra of **Leg5Ac7AcAla**. (A). Proton ^{1}HNMR analysis in D_2O (water suppression bbfo_zgpr at 4.70ppm) with zoomed in field offset in left corner (1-4.5ppm). (B) Carbon ^{13}CNMR analysis in D_2O. (C) 2-D Correlation Spectroscopy (COSY) and (D) 2-D Heteronuclear Single Quantum Correlation (HSQC) NMR spectra without water suppression.



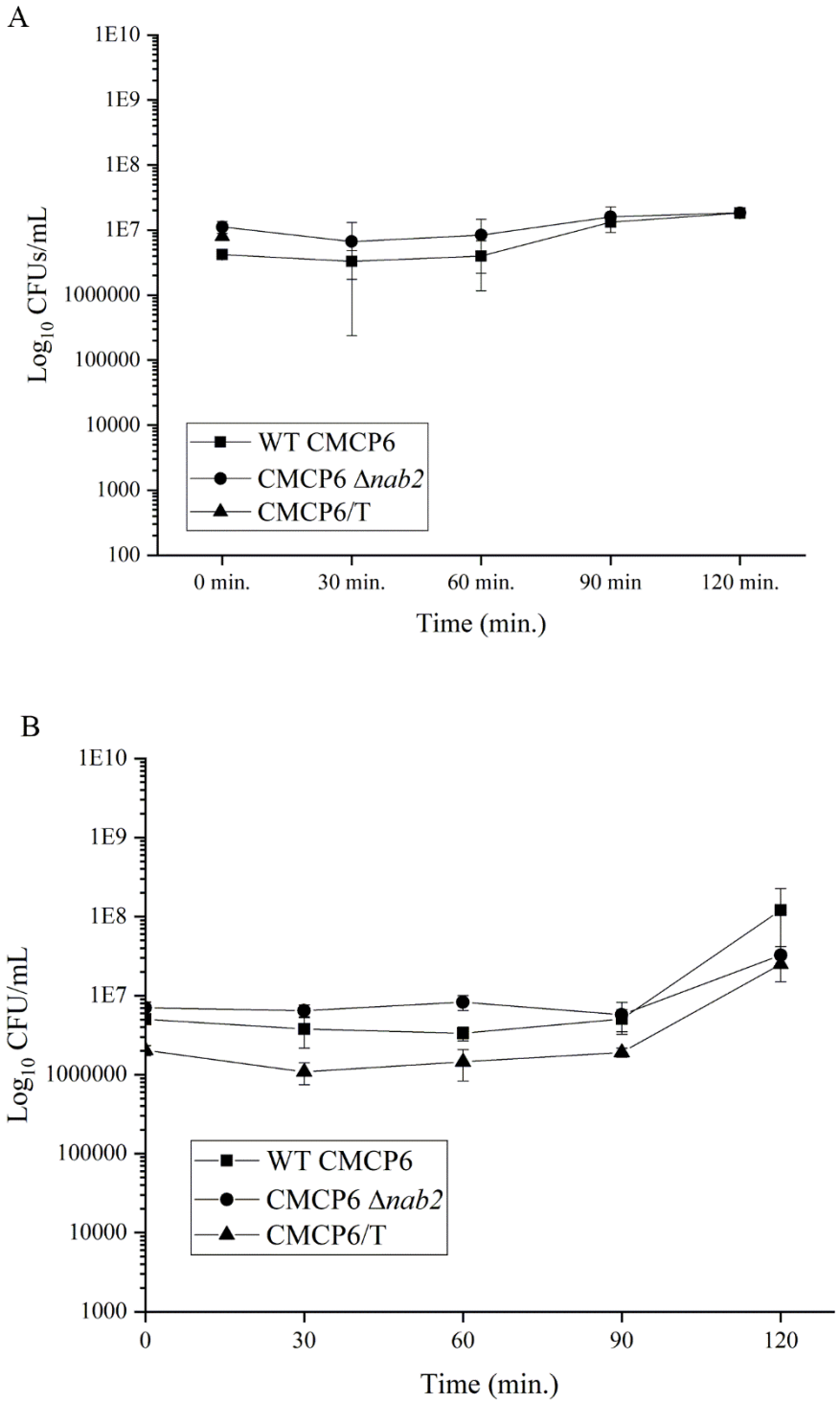
Supporting information Figure S3: Mass spectrometry analysis of *V. vulnificus* YJ016 Δ*nab1* nonulosonic acid. A) DMB-derivatized NulO collected from YJ016 Δ*nab1* which **B)** has a $[M+H]^+$ of 451.20.



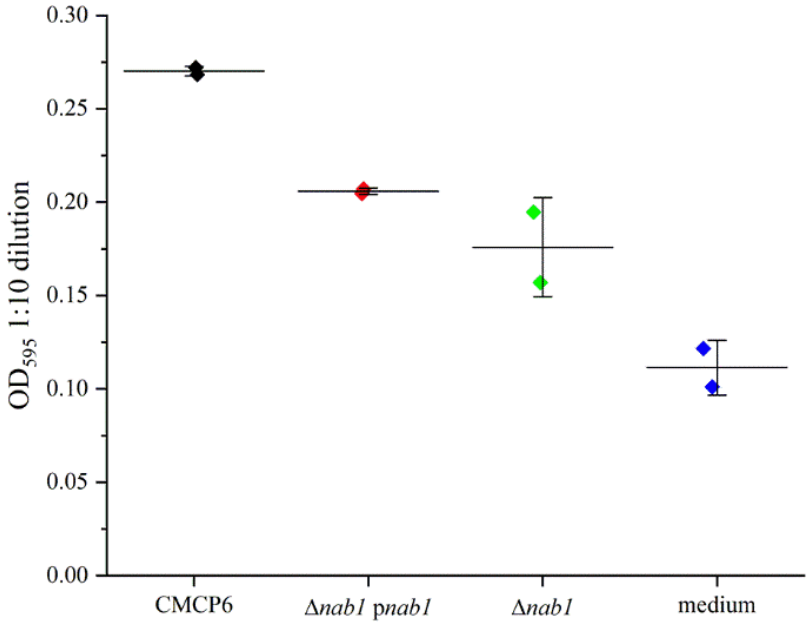
Supporting information Figure S4: Predicted catabolism of Leg5Ac7AcAla in *V. vulnificus* CMCP6. A) Genomic arrangement of sialic acid transport and catabolism cluster in *V. vulnificus* and **B**) schematic diagram of the predicted accumulation of **Leg5Ac7AcAla** upon mutation of Δ*nanA*/Δ*nab1* mutant. C) HPLC analysis of DMB-derivatized cellular fractions from CMCP6 Δ*nanA* revealing accumulation of sialic acid from the media as well as WT levels of **Leg5Ac7AcAla.**



Supporting information Figure S5: NulO mutant exhibit increased sensitivity towards the antimicrobial peptide polymyxin B (PmB). Wild-type CMCP6 and the Δ*nab2* deletion mutant were grown to mid-exponential phase and resuspended in PBS containing 80 µg/ml of PmB. Cell lysis was monitored as a decreased in optical density measured every 5 minutes over the course of 60 minutes. Error bars represent standard deviation (SD). Experiments performed in triplicate and two biological replicates.



Supporting information Figure S6: *V. vulnificus* survival in human serum. A) WT CMCP6 the Δ*nab2* deletion mutant and a capsule negative strain CMCP6/T were exposed to active normal human serum or B) heat inactivated serum. The CFUs were enumerated immediately following exposure to serum and in 30 min. intervals for 2 h. Experiment was conducted in duplicate with three technical replicates. Error bars represent SD.



Supporting information Figure S7: Biofilm formation *V. vulnificus nab1* complement strain. The strains were grown statically for 24 h in Marine Broth at RT. Biofilms were stained with 0.1% crystal violet resuspended in DMSO and the OD measured. Individual points represent independent experiments consisting of six technical replicates. Lines indicate mean and +/- SD.