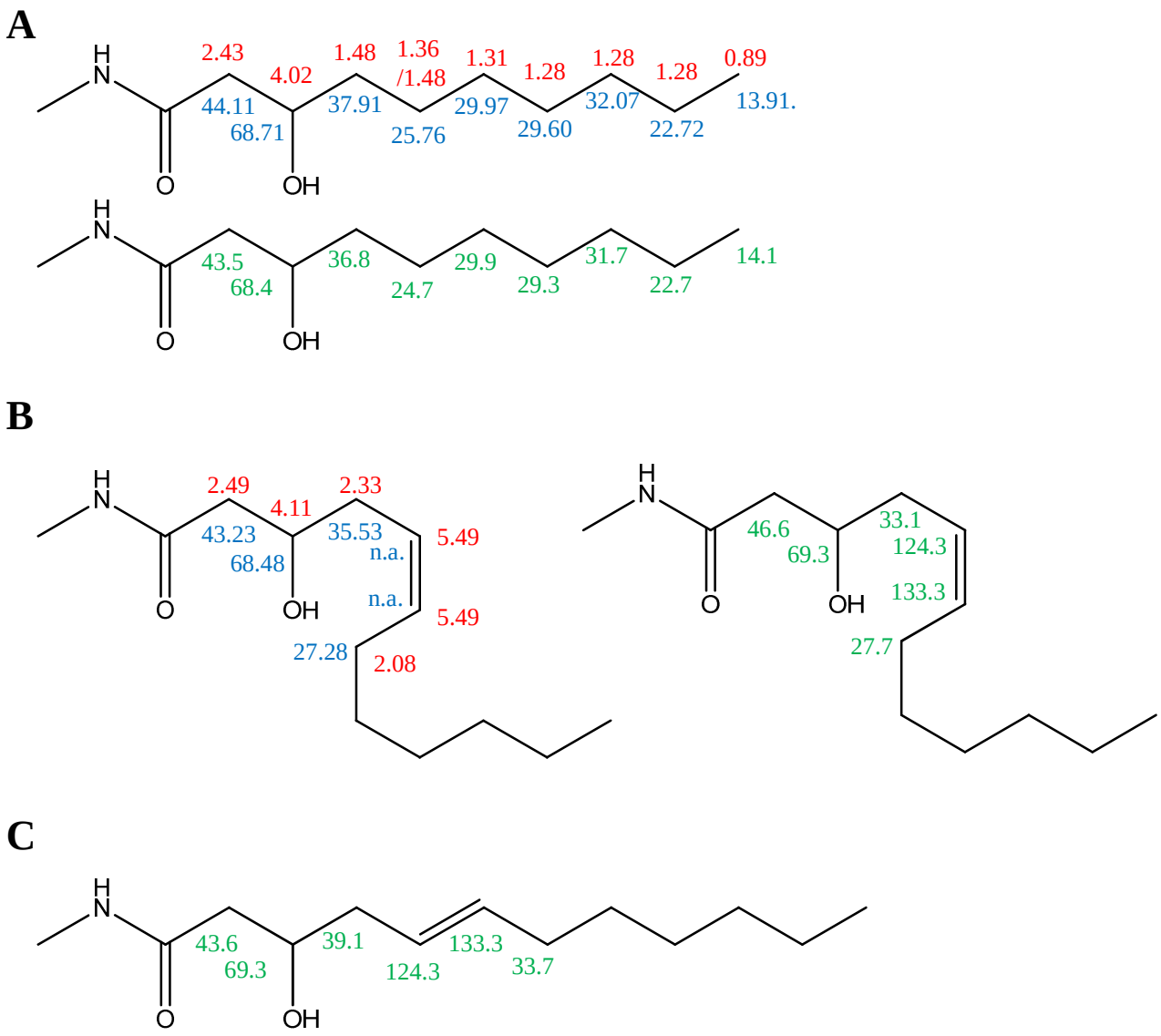

Figure S17. NMR analysis of fatty acid moiety in xantholysin C. ^{1}H and ^{13}C chemical shift comparison between the lipid tail of xantholysin A and variant 2. (A) Experimental ^{1}H (red) and ^{13}C (blue) chemical shifts of the xantholysin 3-hydroxydecanoic acid moiety, compared to predicted ^{13}C chemical shifts (green). (B) Experimental ^{1}H (red) and ^{13}C (blue) chemical shifts of the xantholysin variant 2 3-hydroxydodec-5-enoic acid moiety, compared to predicted ^{13}C chemical shifts (green) assuming a *cis*-configuration. (C) Predicted ^{13}C (green) chemical shifts of the xantholysin variant 2 3-hydroxydodec-5-enoic acid moiety assuming a *trans*-configuration. ^{13}C chemical shift predictions were calculated using ChemNMR Pro 12.0 as implemented in the ChemDraw Ultra 12.0 software (*PerkinElmer, Inc.*).

In the case of *cis*-3-hydroxydodec-5-enoic acid, the predicted ^{13}C chemical shifts indicate a decrease in both the γ and ζ ^{13}C chemical shifts compared to the HDA moiety (36.8 ppm→33.1 ppm and 29.3 ppm→27.7 ppm respectively). In the case of the *trans*-3-hydroxydodec-5-enoic acid, an increase in these same chemical shifts is predicted (36.8 ppm→39.1 ppm and 29.3 ppm→33.7 ppm respectively). In the *cis* configuration, the van der Waals radii of the hydrogens of the two carbons

flanking the double bond are overlapping due to their spatial proximity, as opposed to the trans configuration. This steric compression causes an additional shielding and thus upfield shift of the associated carbons, which is not present in the case of the trans configuration (for reference, see Breitmaier E., Voelter E. (1987) Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry, 3rd edition. Weinheim: VCH Verlagsgesellschaft mbH. 515 p.). The experimental values of the 3-hydroxydodec-5-enoic acid moiety compared to those of the HDA moiety (37.91 ppm→35.53 ppm and 29.60 ppm→27.28 ppm for the γ and ζ ^{13}C nuclei respectively) show that the ^{13}C chemical shifts correspond significantly better to the case of the *cis*-configuration, indeed showing a decrease. Therefore, this provides a significant indication for the lipid tail in xantholysin variant 2 to be in the *cis*-configuration.