CHARACTERIZATION AND MECHANISTIC INVESTIGATION OF (METHYL)LANTHIONINE STEREOCHEMISTRY

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Lanthipeptides, a class of bioactive RiPPs, contain lanthionine and methyllanthionine residues with diverse stereochemistry crucial to their function. We present a straightforward method for stereochemical analysis using advanced Marfey's approach with a non-chiral column system, which facilitates novel antimicrobial discovery. Additionally, this method supports mechanistic studies on lanthionine stereochemistry. Through co-crystallography, in vitro kinetics, and enzyme variant analysis of the lanthionine synthetase LanCL, we identified a previously uncharacterized residue that modulates enolate intermediate stabilization, influencing stereochemical outcomes. These findings pave the way for LanCL bioengineering to achieve precise stereochemical control in lanthipeptide biosynthesis.