

Evaluation of 8-azido-Kdo incorporation in LPS in gram-negative bacteria

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Metabolic Oligosaccharide Engineering¹ coupled with click-chemistry is a powerful and increasingly applied method to investigate cell components, including carbohydrates and glycoconjugates. Within this area, the 8-azido derivative of the bacterial sugar 3-deoxy-d-*manno*-oct-2-ulosonic acid (8-N₃-Kdo) has gained value in selective labeling of lipopolysaccharides (LPS) that are a key component of the outer membrane of Gram-negative bacteria. Several studies have reported that 8-N₃-Kdo is successful in labeling LPS of several Gram-negatives such as non-pathogenic and pathogenic *Escherichia coli* strains, *Salmonella typhimurium, Legionella pneumophila* and *Myxococcus xanthus*.^{2,3}

Due to its increasing application in the investigation of LPS biosynthesis and cell surface labeling, we became interested in exploring the nature and efficiency of LPS labeling using 8-N₃-Kdo in a variety of Gramnegative bacteria. First, we optimized the synthesis of 8-N₃-Kdo, which was subsequently used to metabolically label Gram-negative bacteria, mainly focusing on non-pathogenic and pathogenic *E. coli* strains. Interestingly, different extents of labeling were observed, and the majority of labeled LPS appears to be the 'rough' LPS variant. In this communication, our optimized synthesis route of 8-N₃-Kdo, our findings on the extent of LPS labeling across species and a characterization of the labeled LPS structures will be presented.



