OPTIMISED CHEMOENZYMATIC SYNTHESIS OF 3-*(EQ)*-*F*-PSE5AC7AC, A POTENTIAL PRODRUG INACTIVATOR OF PSEUDAMINYL TRANSFERASES, UTILISING RATIONALLY ENGINEERED MUTANTS OF PSEUDAMINIC ACID SYNTHASE PSEI

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5,7-di-*N*-acetyl-pseudaminic acid (Pse5Ac7Ac) is a non-mammalian nonulosonic acid sugar found on bacterial cell surfaces as well as directly *O*-glycosylated on flagella [1]. Notably Pse5Ac7Ac plays a key role in the virulence of various multidrug resistant bacteria including *Pseudomonas aeruginosa* and *Acinetobacter Baumannii* [2]. The discovery of the biosynthetic pathway of Pse5Ac7Ac in recent years has allowed for the chemoenzymatic synthesis of unnatural derivatives including potential inactivators of the pathway. Here we present a strategy, utilising rationally designed mutants of *Campylobacter jejuni* Pse5Ac7Ac synthase PseI, to increase the activity of PseI with unnatural cofactor 3-*F*-PEP to afford 3-(*eq*)-*F*-Pse5Ac7Ac **2** (Figure 1). Through motility assays, we have shown that 3-(*eq*)-*F*-Pse5Ac7Ac can decrease motility of *C. jejuni* 81116 which is tentatively predicted to be a result of *in vivo* metabolism of 3-(*eq*)-*F*-Pse5Ac7Ac **2** by PseF to active CMP-3-(*eq*)-*F*-Pse5Ac7Ac **3**. We propose that CMP-3-(*eq*)-*F*-Pse5Ac7Ac inactivates dedicated pseudaminyl transferases (PseTs) namely the motility associated factor proteins (Mafs) blocking transfer of Pse5Ac7Ac onto the flagella [3].

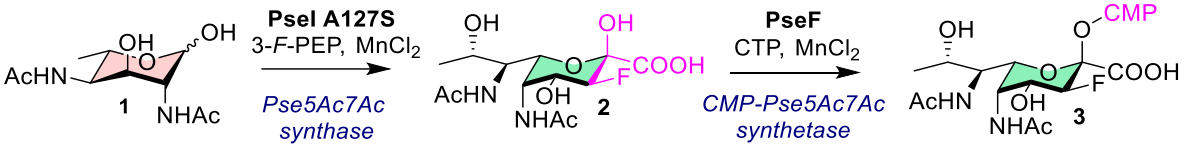

Figure 1. PseI A127S reacting 6-deoxy-Alt-diNAc **1** with 3-*F*-PEP to afford 3-(*eq*)-*F*-Pse5Ac7Ac **2** which in turn, through PseF, can react with CTP to give potential Maf inactivator CMP-3-(*eq*)-*F*-Pse5Ac7Ac **3**.

References:

1. Y. A. Knirel, *et al*. *Carbohydr.* Res. **1984**, *133*, C5–C8.
2. Walklett, A. J.; Flack, E. K. P, *et al*. *Angew. Chem. Weinheim Bergstr. Ger.* **2024**. https://doi.org/10.1002/ange.202318523.
3. Parker, J. L., *et al*. *Microbiologyopen* **2012**, *1* (2), 149–160.