Student Researcher: Aniqa M. Chowdhury, Class of 2026
Advisor: Danielle Dube
Bowdoin College
Biochemistry

is a gram-negative, spiral-shaped pathogenic bacterium that colonizes the human stomach. Infection can cause peptic ulcers and gastric cancer. While antibiotics have once been a powerful tool in the treatment of pathogenic bacteria, excess use of broad-spectrum antibiotics has led to an increase in antibiotic-resistant mechanisms. Broad-spectrum antibiotics also often disrupt the normal gut microbiome. Alternative methods are needed to mitigate negative side effects and selectively treat antibiotic resistant "priority pathogens" of concern, as categorized by the World Health Organization (WHO). The Dube lab focuses on utilizing bacterial glycans as therapeutic targets due to their distinctive monosaccharides and role in bacterial pathogenicity. Prior studies from the Dube Lab have shown substrate decoys O-glycosides and S-glycosides based on rare bacterial monosaccharides to have inhibitory effects on glycan biosynthesis and bacterial fitness (Williams, 2023; Quintana, 2023a). This summer, to strengthen our toolbox and discover agents with enhanced potency, I conducted parallel experiments on two classes of novel small molecule inhibitors of glycoprotein biosynthesis. The first inhibitor is protected Glc-1-C-phosphonate, termed C-glycoside 1, DT13 (Figure 1A). It is a glucose analog that contains a non-hydrolyzable carbon connection, effectively acting as a glycosyltransferase (GT) inhibitor. The second class of inhibitor includes novel inhibitors 3F-FucNHAc-OBn and 3F-FucNHAc-SBn, termed SSK-10 and SSK-11 respectively (Figure 1B, 1C). The SSK inhibitors were created to determine whether the precedented O-glycoside and S-glycoside substrate decoy inhibitors could be made more effective with the addition of a chain terminator (F-sugar). All inhibitors were found to inhibit glycoprotein biosynthesis, and C-glycoside 1, DT13 was found to elicit negative effects on bacterial fitness, thus impacting pathogenicity. My research in the Dube Lab aims to contribute to the development of necessary, novel therapeutics that target bacterial glycans in the face of increasing antibiotic resistance.

This research aims to assess the effectiveness of SSK-10, SSK-11, and C-glycoside 1,DT13 inhibitors in disrupting glycan biosynthesis in by measuring glycoprotein biosynthesis and changes in cell surface glycan architecture. Bacterial fitness assays were also utilized to determine the effects of inhibited glycoprotein biosynthesis from C-glycoside 1, DT13 on bacterial growth, motility, and biofilm formation.

To assess the effectiveness of 1, DT13, SSK-10, and SSK-11 as metabolic inhibitors in impeding glycosylation, experiments were run to measure glycoprotein biosynthesis via molecular weight using Metabolic Oligosaccharide Engineering (MOE) and Western Blot. Most of the experimental

nitrocellulose paper (Quintana, 2023a). Anti-FLAG-HRP was employed to visualize FLAG-tagged proteins via chemiluminescence (Quintana, 2023a).

Biorthogonal, complementary assays were then conducted to confirm glycoprotein inhibition by utilizing fluorescent carbohydrate-binding protein Alexa Fluor 488 Concanavalin A (ConA) to confirm changes in cell surface glycan architecture in whole-cell samples. Each experimental condition (reached)4(Qq0.000 untreated sample. All remaining samples included treated with either 0.025mM or 0.1mM of C-glycoside 1, DT13, or 0.5mM, 1.0mM, or 2.0mM of SSK-10 or SSK-11. The samples were left for 3 days at 37 °C with gentle shaking under microaerophilic conditions. They were then probed with ConA. As a negative control, ConA was preincubated with 400mM mannose (carbo-block) prior to binding to untreated i to ensure the use of functional lectins (Quintana, 2023a). Cells were analyzed by flow cytometry on a BD Accuri C6+ instrument, with 10,000 live cells gated for each replicate experiment (Quintana, 2023a). Data were analyzed by using FlowJo software.

After confirming glycoprotein inhibition, bacterial fitness assays were conducted to determine the impacts of inhibited glycan biosynthesis from C-glycoside 1, DT13 on fitness, specifically growth, biofilm formation, and motility. Bacterial growth was monitored during log phase until bacteria reached stationary phase - over the course of 8 days. Cells were initially inoculated into liquid media at a starting OD600 of 0.1 and cultured in the absence of inhibitors or the presence of either 0.025mM or 0.1mM of C-glycoside 1, DT13, or 0.5mM, 1.0mM, or 2udQq0.(toE)4of SSK

The results of the fluorescent carbohydrate-binding lectin assay acted as a complementary means to assess the effect of the inhibitors on impeding glycan biosynthesis. The fluorescent lectin Con A was used to bind to untreated versus i treated with varying concentrations of inhibitor. Results (Figure 2B) demonstrated that ConA binding was reduced by pretreatment of ConA with high concentrations of its monosaccharide ligand mannose (Quintana, 2023a). This confirmed that the lectins were functional as the monosaccharide ligand mannose did appreciably bind to ConA. Relative to untreated , cells that were treated with SSK-10 (Figure 2B, left) at 0.5mM, 1.0mM, and 2.0mM displayed an increase in ConA binding via increased fluorescence. The data not shown for SSK-11 contained nearly identical results. Similarly, relative to untreated , cells that were treated with C-glycoside 1, DT13 (Figure 2B, right) at 0.025mM and 0.1mM displayed an increase in ConA binding via increased fluorescence. The data confirm a change in cell surface glycan architecture, and it provides biorthogonal complementary evidence of glycoprotein biosynthesis inhibition from all compounds. This result is in line with previous reports of small molecules or genetic disruption of glycoprotein biosynthesis causing increased lectin binding (Quintana, 2023a).

Due to timing, bacterial fitness assays were run only on C-glycoside 1, DT13. Inhibition of glycan biosynthesis in at 0.1mM concentrations negatively impacted motility, growth, and biofilm formation at statistically significant levels (Figure 3A).

The Glc-1-C-phosphonate glycosyltransferase inhibitor

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