STEPHANIE FORGET
BSc (Chemistry), Dalhousie University, 2010

DEPARTMENT OF CHEMISTRY

TITLE OF THESIS:	EVALUATION OF SUGAR BIOSYNTHETIC ENZYMES AND STUDIES ON JADOMYCIN BIOSYNTHESIS
TIME/DATE:	9:30 am, Friday, November 10, 2017
PLACE:	Room 3107, The Mona Campbell Building, 1459 LeMarchant Street

EXAMINING COMMITTEE:

Dr. Mark Nitz, Department of Chemistry, University of Toronto (External Examiner)

Dr. Bruce Grindley, Department of Chemistry, Dalhousie University (Reader)

Dr. Stephen Bearne, Department of Chemistry and Department of Biochemistry and Molecular Biology, Dalhousie University (Reader)

Dr. Alison Thompson, Department of Chemistry, Dalhousie University (Reader)

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ABSTRACT

Carbohydrates are ubiquitous in biological systems, possessing diversity in terms of both structure and function. Studies pertaining to bacterial enzymes that recognize and process carbohydrates in cell wall biosynthesis and secondary metabolism are presented in this thesis.

L-rhamnose (Rha) is a carbohydrate monomer that serves as a building block in the cell wall-associated glycans of pathogenic bacteria. Gene products involved in Rha biosynthesis (RmlA-D) are essential for virulence and represent attractive targets for antibacterial development. These enzymes were evaluated with a series of substrate analogues to probe their substrate specificity. The RmlA/Cps2L enzyme was highly tolerant to substrate changes, whereas the RmlB-D enzymes were stringent in terms of their ability to turn over unnatural substrates. All enzymes in the Rml pathway turned over the phosphonate analogue of Glc 1-P enabling the chemo-enzymatic preparation of the phosphonate analogue of dTDP-Rha.

A number of bacterial secondary metabolites are glycosylated, including the jadomycins, a family of angucycline antibiotics produced by *Streptomyces venezuelae* ISP5230. Using precursor-directed biosynthesis, two jadomycin derivatives incorporating *N*ε-trifluoroacetyl-L-lysine (TFAL) were isolated, a jadomycin with the usual oxazolone ring and a 3a oxidized analogue. In the same production, two shunt products containing a furan B-ring were isolated, representing a new scaffold for angucyclines. A jadomycin decorated with D-glucose, in place of L-digitoxose, was isolated from a strain bearing a deletion of the biosynthetic 4,6-dehydratase gene. The glycosyltransferase JadS was identified as the catalyst responsible for appending the glucose moiety, demonstrating flexibility towards sugar donors. Studies towards the complementation of *S. venezuelae* with an iterative L-digitoxyltransferase from the kijanimicin gene cluster are described. Finally, the *S. venezuelae* GT1 family glycosyltransferase Sv0189 was characterized as a UDP-glycosyltransferase with a broad acceptor scope.

Overall, this work has characterized the substrate scope of several bacterial carbohydrate-recognizing enzymes that will serve as a basis for inhibitor design or for use in chemo-enzymatic applications.