Lung Inflammatory Disease
Program of Excellence in Glycosciences

Module 1

Chemo-enzymatic synthesis of glycans

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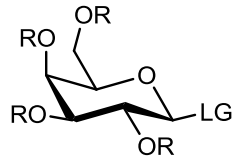
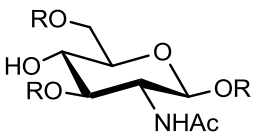
Carbohydrate Synthesis – The toolbox

Chemical synthesis



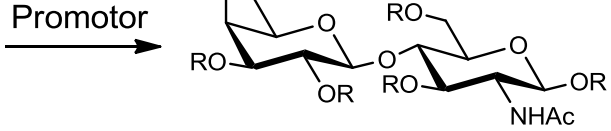
Glycosyl donor

2-3 steps from free sugars

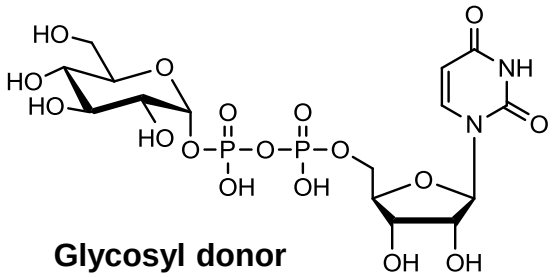


Glycosyl acceptor

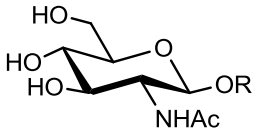
6-8 steps from free sugars



Deprotection

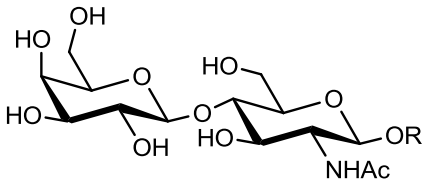


Glycosyl donor



Glycosyl acceptor

β4GalT-GalE



Enzymatic synthesis

Regioselectivity - reaction at one site (one hydroxyl group)

Stereoselectivity – formation of one stereoisomer over another

Enzymes allow formation of defined stereo- and regiospecific products

Glycosyltransferases

Biosynthesis of oligosaccharides

Formation of defined stereo- and regiospecific products with remarkable rate acceleration of the reaction

Leloir enzymes – glycosyltransferases which utilize activated glycosyl esters of nucleoside mono- or di-phosphates as glycosyl donors (Luis F. Leloir)

<u>Advantages:</u>

Formation of defined products

No protecting group chemistry

Mild conditions (Room temperature, aqueous conditions)

<u>Limitations for synthesis:</u>

Substrate specificity

High cost of glycosyl nucleotide donor

Limited enzyme availability.

Enzyme activity: moles of substrate converted per unit time

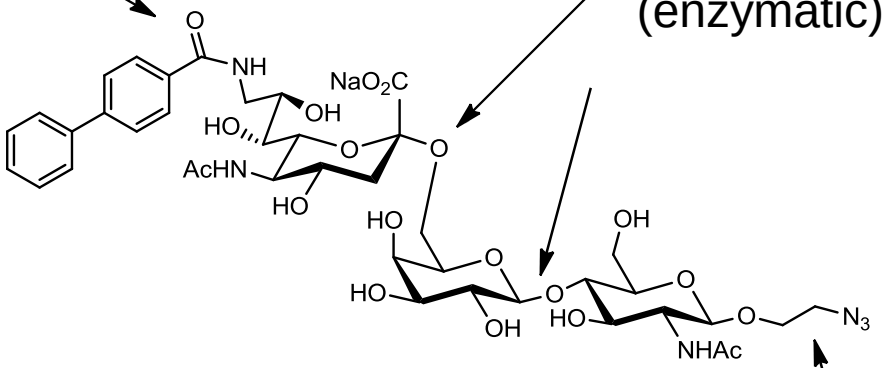
1 enzyme unit (U) = 1 μmol min^{-1}

Carbohydrate Synthesis

Chemo-enzymatic synthesis

Combination of both chemical and enzymatic methods

Functional group modifications
(chemical)

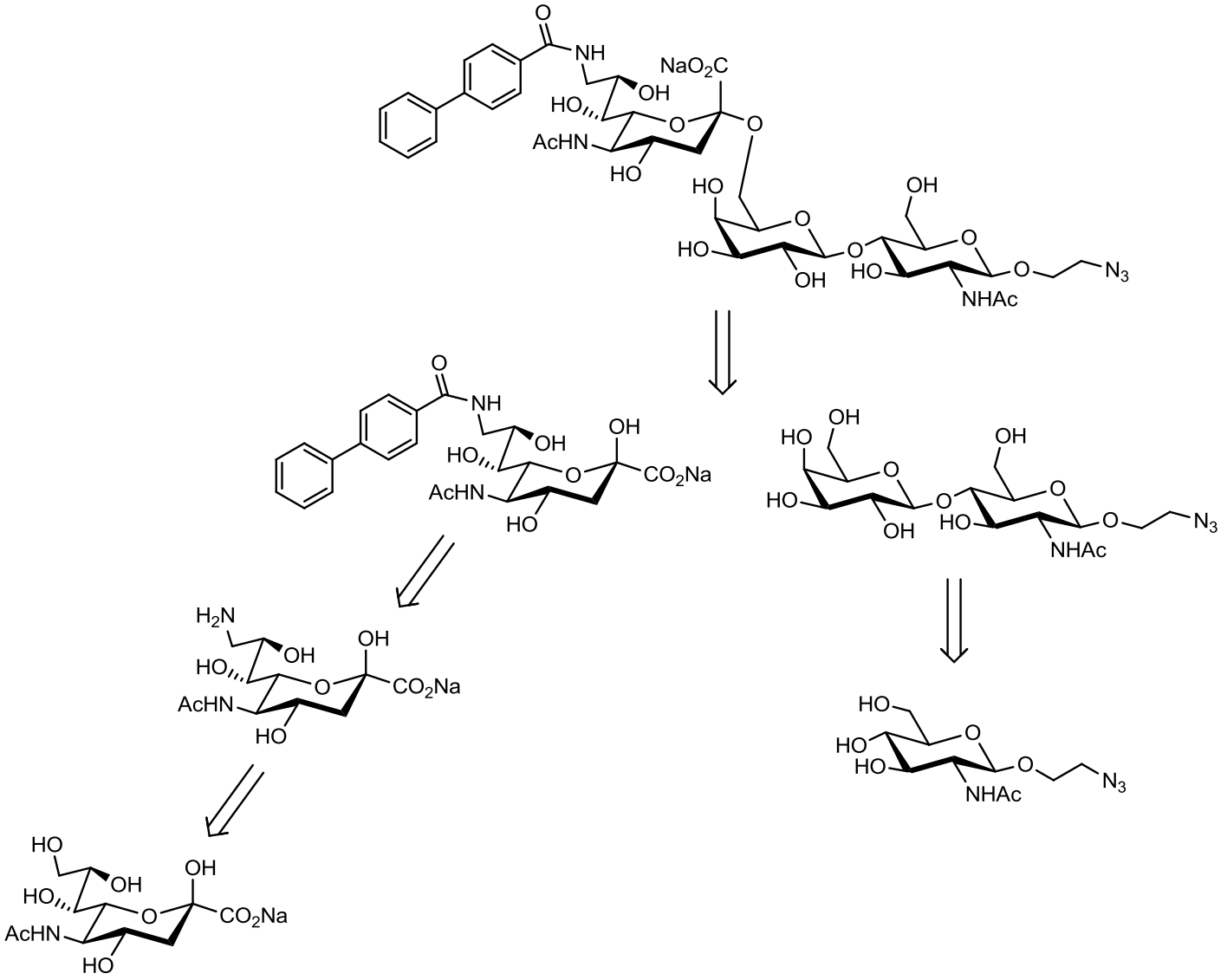


Where to start?

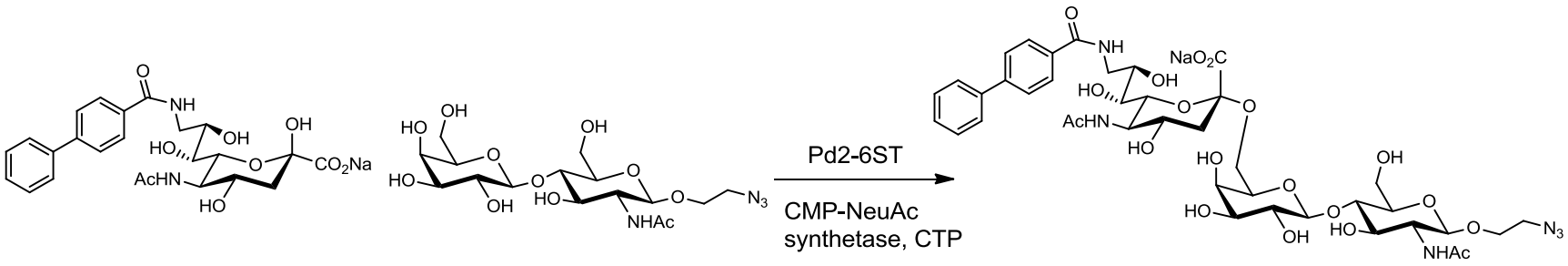
Installation of aglycone
(chemical)

Retro-Synthetic analysis of glycan structure

A technique for solving problems in the planning of organic syntheses (E.J. Corey).
The goal is structural simplification through bond disconnection.



Planning an enzymatic reaction



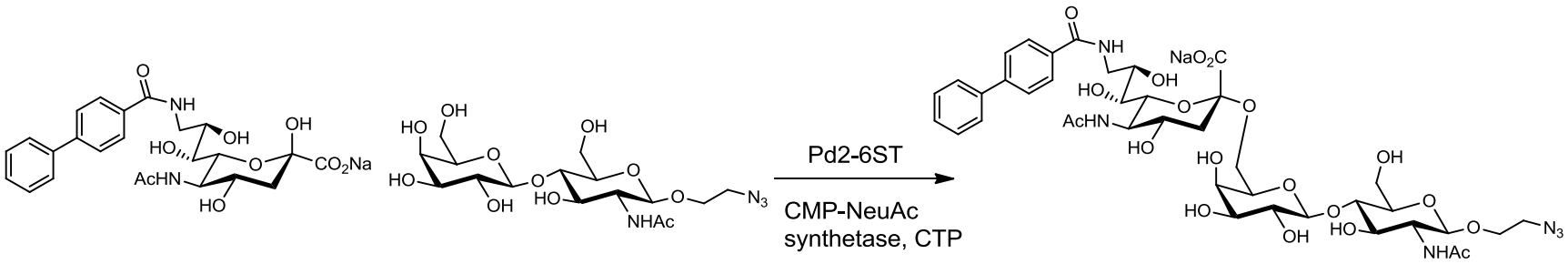
Reaction steps

1. Setup and initiation
2. Monitoring the reaction
3. Work-up and purification

Aim: Synthesis of 9-*N*-BPC-NeuAcα2-6-LacNAc-ethyl azide on a 2 mg scale .

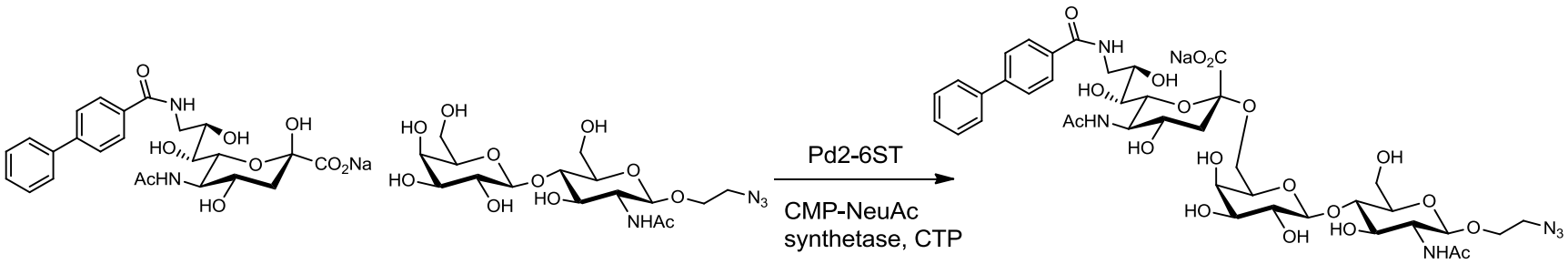
Purification of the product by C-18 reverse phase chromatography taking advantage of the hydrophobic BPC handle.

Setup and Initiation of the reaction

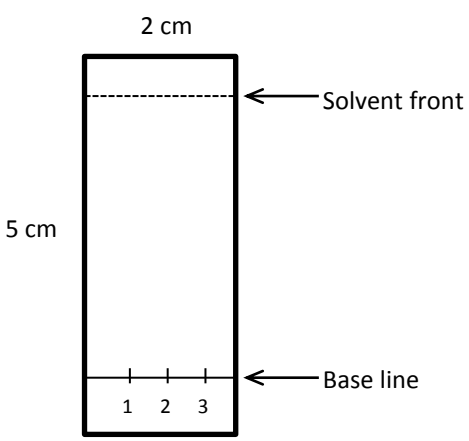


1. Determine the limiting reagent
2. Calculate the quantities of donor and acceptor required
3. Analyze reagents by thin layer chromatography (TLC)
4. Combine donor and acceptor in reaction vessel (eppendorf) in reaction buffer adjust pH (~ 8)
5. Calculate the quantity of enzyme required (reaction time ~ 1 h)
6. Add enzyme to reaction and mix at 37 °C

Monitoring the reaction



The progress of the reaction is assessed by TLC



1. Starting material
2. Co-spot of 1 and 3
3. Reaction mixture

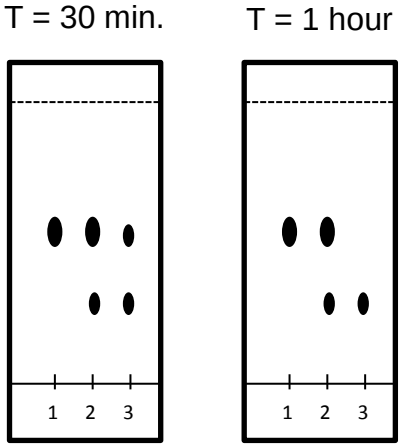
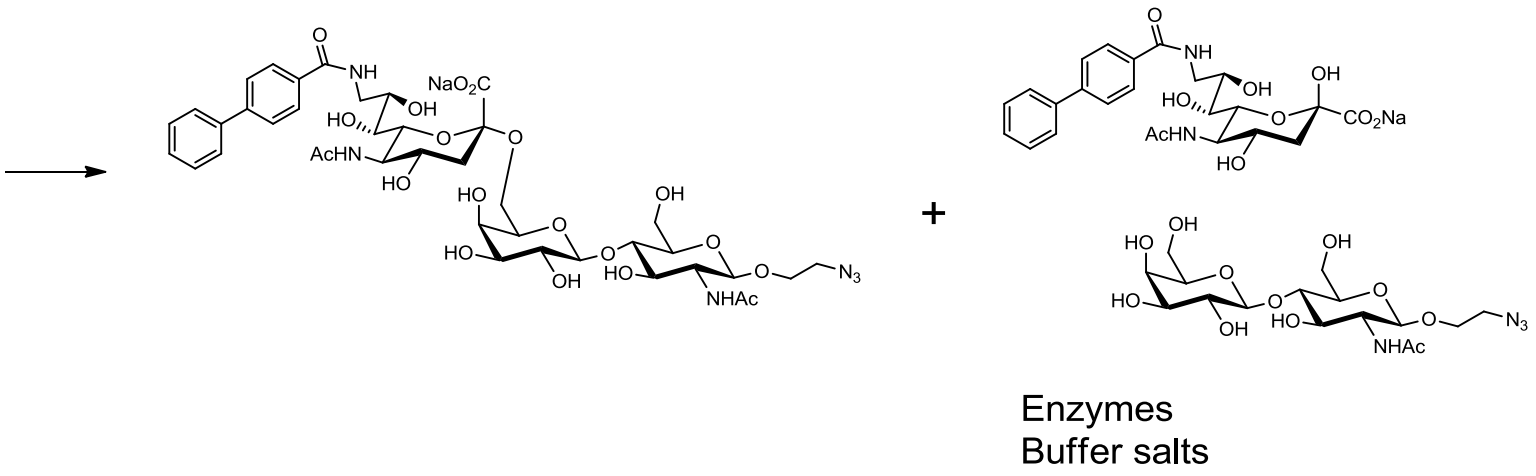


Figure 1.

Work-up and purification



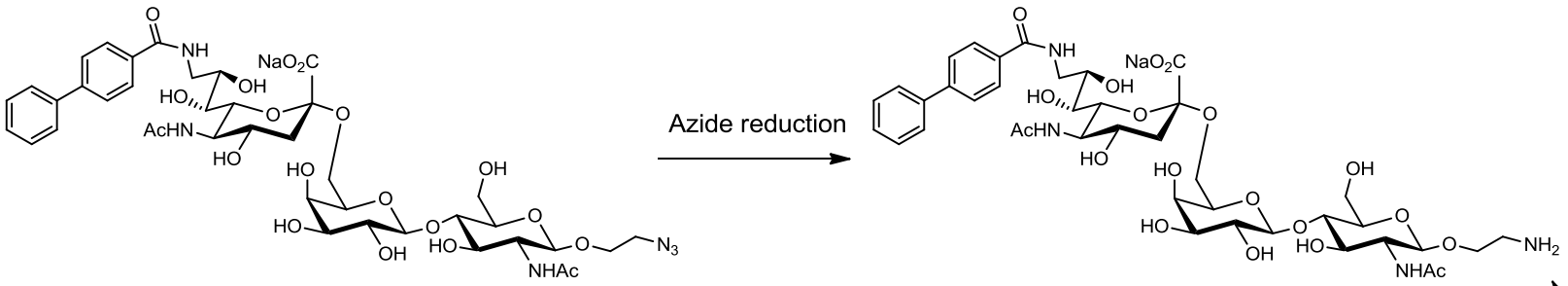
Purification of the product – removal of impurities

<u>Methods:</u>

C-18 Solid-phase extraction (polarity)

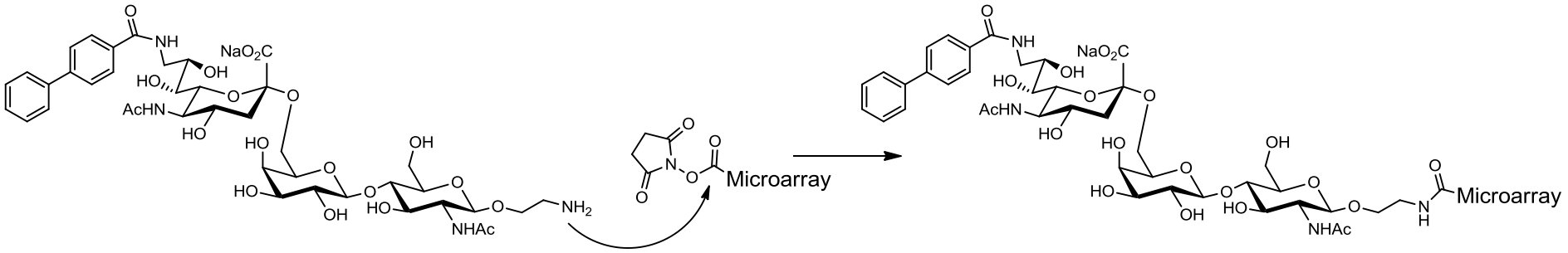
Gel Filtration (size exclusion) *Demonstration*

Preparation of glycans for microarray printing (Module 2)



Reactive amine…

for printing on NHS-activated slides:



Preparation of glyco-lipids for cell targeting (Module 3)

for preparation of glyco-lipids for cell targeting: