Carbohydrate Synthesis
Part 2: Solution and solid phase chemical synthesis. Chemoenzymatic synthesis.

“Essentials of Glycobiology”
3 June 2004

Michael VanNieuwenhze/Nathaniel Finney
Dept. of Chemistry and Biochemistry
UCSD
msv@chem.ucsd.edu
Lecture Outline

1. Iterative solution phase synthesis by Danishefsky’s glycal method.

2. Identity of glycosyl substituents alters the reactivity of glycosyl donors: Exploitation in Wong’s solution phase Optimer methodology.

3. Solid phase carbohydrate synthesis possesses many of the same advantages of solid phase peptide and oligonucleotide synthesis: Automated oligosaccharide synthesis.

4. Chemoenzymatic synthesis of oligosaccharides and glycoconjugates: Complementary to chemical methods; narrower is scope but more elegant and efficient in execution.
Iterative Solution Phase Synthesis with Glycals

Now that we’ve discussed the basics of chemical glycosylation, let’s look at more complex synthetic challenges. The Le$^\text{x}$-Le$^\text{y}$ nonasaccharide is not readily available from natural sources, but could be valuable for, e.g., developing anticancer vaccines.

Remarkably, this oligosaccharide can ultimately be prepared from just 3 glycal precursors.
Glycals for the Solution Phase Synthesis of Le\textsuperscript{x}-Le\textsuperscript{y}

The 3 glycals (in general form):

\begin{align*}
\text{A} & : RO \quad OP \quad RO \\
\text{B} & : HO \quad R^*O \quad OP \\
\text{C} & : HO \quad PO
\end{align*}

\begin{itemize}
\item P = protecting group
\item R = P or H
\item R' = nitrogen protecting group
\item R* = unique hydroxy protecting group and fucosylation site
\end{itemize}

Glycal method - quick reminder:

\begin{align*}
\text{BnO}^* \quad \text{Bn} \quad \text{OBn} & \quad \xrightarrow{\text{"DMDO"}} \quad \text{OBn} \quad \text{BnO}^* \quad \text{Bn} \quad \text{OBn} \\
\text{OBn} \quad \text{BnO}^* \quad \text{Bn} \quad \text{OBn} & \quad \xrightarrow{\text{ROH, ZnCl\textsubscript{2}}} \quad \text{OBn} \quad \text{BnO}^* \quad \text{Bn} \quad \text{OBn} \quad \text{OR}
\end{align*}
First Stage Glycal Couplings

A + B

A + C

1 step

2 steps

1 step

2 steps
Second Stage Glycal Couplings
Glycal Couplings - Summary

Pro: Synthesis of important and previously inaccessible oligosaccharide achieved.

Reaction conditions are mild and general.

Better than alternatives available at the time.

Con: Very labor intensive (~1.5 year to develop, 6 months to repeat).

Isolation, purification of intermediates difficult and time consuming.

Overall yield < 1%.
Protecting Groups Alter Glycosyl Donor Reactivity

For glycosylation with glycosyl cations, would predict that two of the things influencing the rate of glycosylation would be:

1. The rate at which the glycosyl cation is generated.

2. The reactivity of the glycosyl cation itself.

Specifically:

Variations in structure of X may alter rate of activation.

Protecting groups (R) may influence reactivity of X.

Protecting groups (R) will influence reactivity of cation.
More Specific Predictions of Reactivity

Focusing on the cation itself, would predict that electron donating protecting groups (Bn, PMB, TBS, e.g.) should stabilize a glycosyl cation, while electron withdrawing protecting groups (Ac, Bz, Piv - remember the carbonyl dipole, C=O ↔ +C–O⁻) should destabilize a glycosyl cation.

A glycosyl cation with electron withdrawing acetate protecting groups... ...should be less stable/more reactive than the benzyl protected analog.
Wong’s Optimer Method - Retrosynthesis

The Wong Group (Scripps Research Institute) has prepared *hundreds* of mono and disaccharides with different protecting group patterns to control glycosylation rate. This means that multiple components can be combined in the same reaction: the fastest component reacts first, then the next fastest, etc.

A computer program (Optimer) has been written that knows the relative reactivities of all of these potential reactants. It can carry out retrosynthetic analyses of complex carbohydrates and suggest a set of reactants that would allow synthesis of the oligosaccharide in one single reaction.

In addition to condensing multiple reactions into one, this *dramatically* simplifies the laborious and inefficient processes of isolation and purification.
Wong’s Optimer Method - Illustration

Optimer says that...

Globo H

...can be prepared from these three components.

(A) STol ~ SPh; NBz, Troc = similar to Ac; ClBn similar to Bn.)
Wong’s Optimer Method - Illustration

one pot
62 % yield (!)

DMTST
CH₂Cl₂
-78 °C to RT

A

B

C
Wong’s Optimer Method - Summary

Pro: Remarkably efficient and predictable assembly of complex carbohydrates in a single reaction. (Compare Globo H synthesis to that of Le\(^x\)-Le\(^y\) shown previously.)

Minimizes synthetic effort.

Dramatically reduces labor associated with isolation/purification.

Con: Requires access to a very large number of complex precursors.

Synthetic method still not accessible to non-chemists.
Solid Phase Synthesis of Oligomeric Biomolecules

Everyone probably knows solid phase deoxyoligo synthesis:
Advantages of Solid Phase Synthesis

Here are the most relevant advantages of solid phase peptide and oligonucleotide synthesis relative to solution-phase:

1. Improved yields and purities. Use of large excess of solution phase reagents can drive reactions to completion, as can repetition of reaction cycle.

2. Simplified isolation and purification. Multiple reactions require only a single isolation and purification, at the very end. (Of course, this means the chemistry has to be very efficient....)

3. No problems with solubility/precipitation. Material is site isolated (no chance for aggregation) on an insoluble solid support.

4. Automation!
Solid Phase Oligosaccharide Synthesis

Synthetic chemistry has finally advanced to the point that solid phase oligosaccharide synthesis is also feasible, and can now be automated.

Protected Leishmania antigen. Automated synthesis provides 42% in 9 hours!
Solid Phase Oligosaccharide Synthesis - Summary

Pro: Remarkably efficient synthesis of complex oligosaccharides.

Automated solid phase synthesis faster (often by > 10x) and higher yielding than corresponding solution phase syntheses.

Automation makes synthetic methodology accessible to non-chemists.

Con: Many glycosidic linkages of interest still inaccessible. (This is true of all chemical glycosylation approaches.)

Even with automation, a bewildering number of specialized reactants are still required. Not clear how this will be solved, although it must be if synthesizers are to become commercially available/viable.
Chemoenzymatic Synthesis of Oligosaccharides

As an alternative to chemical synthesis, many biochemists and bioorganic chemists have explored the use of glycosidases and glycosyltransferases in the synthesis of oligosaccharides and glycoconjugates.

The appeal of this approach is obvious: Nature has already figured out how to make all of the naturally occurring oligosaccharides, and if we could borrow from Her toolbox we’d save a lot of time and effort.

The use of glycosyltransferases and glycosidases have strengths and weaknesses that are in many ways complementary to those of chemical synthesis. Let’s look at a few examples before we discuss the Pros and Cons of the approach.
Synthetic Application of Glycosyltransferases

A comparison of the enzymatic and chemical synthesis of tetra- and pentasaccharide cell-surface epitopes from *Neisseria meningitidis* (a causative agent of meningitis) is instructive.

Chemoenzymatic synthesis of meningitis epitope:

\[
\text{GlcNAc transferase}\quad \text{UDP-GlcNAc}\quad 96\%\ (28\ mg)
\]

Chemical synthesis of meningitis epitope:

3 steps, 55%
Application of Glycosyltransferases

Chemoenzymatic synthesis of meningitis epitope (cont.):

A

UDP-Glc epimerase-Gal transferase fusion
UDP-Glc

96% (24 mg)

CMP-Neu5Ac synthase-transferase fusion
CTP, Neu5Ac

97% (30 mg)

Chemical synthesis of meningitis epitope (cont.):

B

1 step, 40% (260 mg)

9 steps from lactose

2 steps, 66% (165 mg)
Application of Glycosyltransferases - Summary

Pros:

Excellent yield with complete regio- and stereoselectivity.
No protecting groups needed.
Sialyltransferases allow facile sialylation. (This is the hardest glycosylation to carry out by chemical methods.)
Reactions can be carried out in water.

Cons:

Most requisite enzymes are not readily available, and those that are available are expensive.
Regio/stereoselectivity means that substrate scope is limited and a unique enzyme is needed for almost every reaction.
Nucleotide sugar donors are very expensive and/or unstable.
Scale is often limited by enzyme availability/volumetric productivity. (A large amount of enzyme produces only a small amount of sugar by weight.)
Application of Glycosyltransferases - Final Note

Although issues of enzyme cost/availability/substrate scope are likely to remain unsolved for some time, the issues of scale and nucleotide donor cost/availability can sometimes be overcome:

![Chemical structure](image)

59 g (as monohydrate)

59 g (as monohydrate)

68 g, 68 % yield
Synthetic Application of Glycosidases

Another chemoenzymatic approach is to employ glycosidase. While these enzymes normally cleave glycosidic linkages, they can be coerced to “run backwards” (to some extent) in the presence of an appropriate glycosyl donor.

\[
\begin{align*}
\text{Gal-β-PNP} & \quad \xrightarrow{\beta\text{-galactosidase}} \quad \text{PNP} \\
\end{align*}
\]
**Application of Glycosidases**

The synthesis of 2 Tn-antigen epitopes is illustrative:

\[ \text{Gal-β-PNP} + \text{PNP} \xrightarrow{β\text{-galactosidase}} \text{PNP} \]

\[ \text{Neu5Ac-α-PNP} \xrightarrow{\text{sialidase}} \text{PNP} \]

\[ \text{12\% (5 mg)} \quad \text{15\% (5 mg)} \]
Application of Glycosidases - Summary

Pros:

Many glycosidases available (esp. in comparison to glycosyltransferases).
Glycosidases are less expensive and more stable than transferases.
Specificity is generally relaxed rel. to transferases, allowing broader substrate scope.
Generally good regio- and stereoselectivity.
No protecting groups.

Cons:

Enzymatic glycosylations still less scalable than chemical reactions.
Volumetric productivity still low.
Yields much lower than with transferases - glycosidase activity (which degrades the product) competes with glycosylation.
Chemical v. Chemoenzymatic Synthesis - Summary

The use of glycosyltransferases and glycosidases have strengths and weaknesses that are in many ways complementary to those of chemical synthesis. Chemical synthesis provides flexibility that allows the preparation of diverse natural and unnatural structures, but requires the extensive use of protecting groups and preparation of specialized precursor compounds. In contrast, enzymes are typically much less flexible and/or available, but do not require the use of protecting groups or the preparation of elaborate precursors. In the near term it appears likely that both approaches will remain in use: indeed, a great many of the most successful applications of enzymes in oligosaccharide synthesis have been in “chemoenzymatic” syntheses, relying on a hybrid of chemical and enzymatic methods that typically begins with chemical synthesis and ends with enzymatic elaboration.