Peter Seeberger

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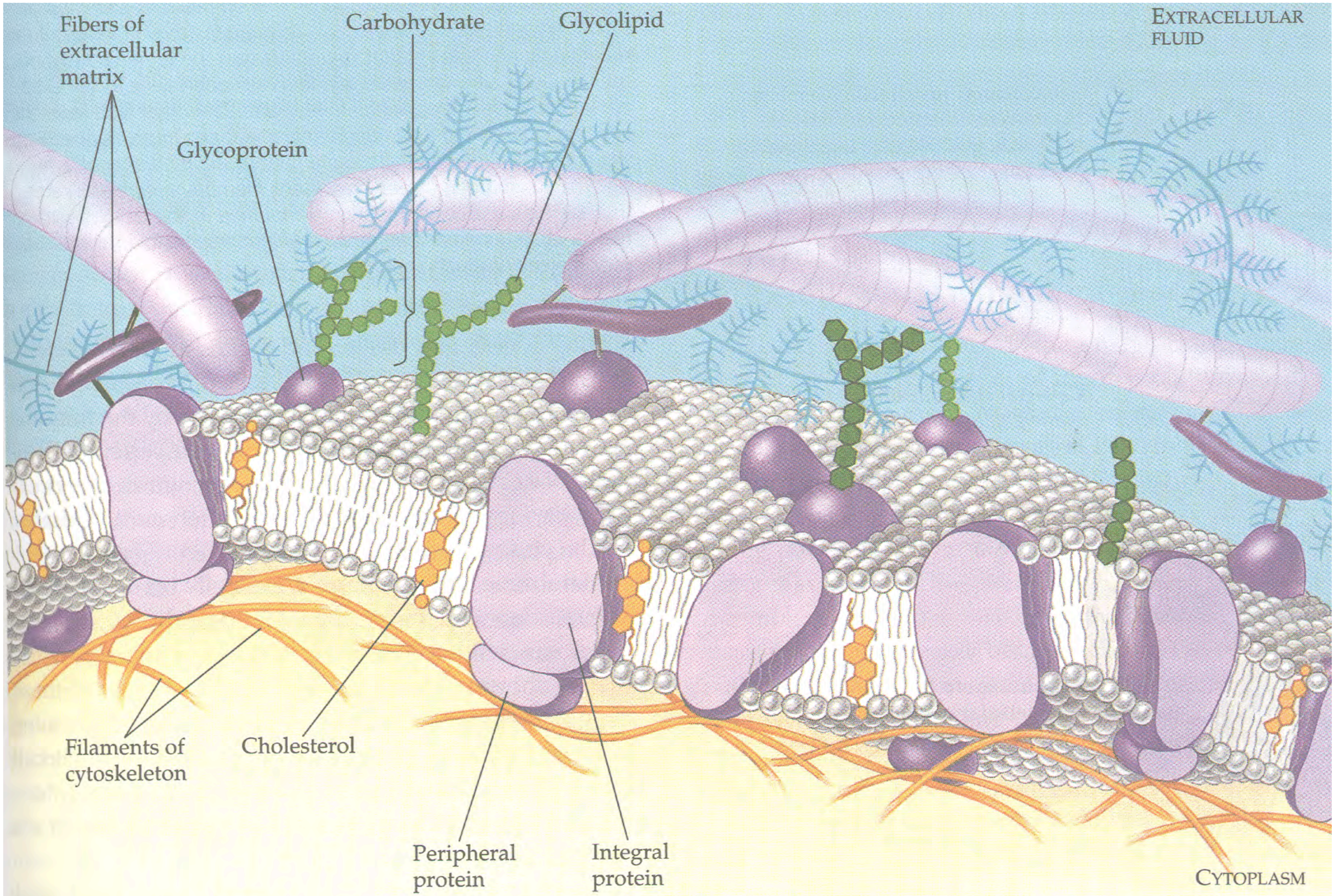
Cell-Surface Glycoconjugates



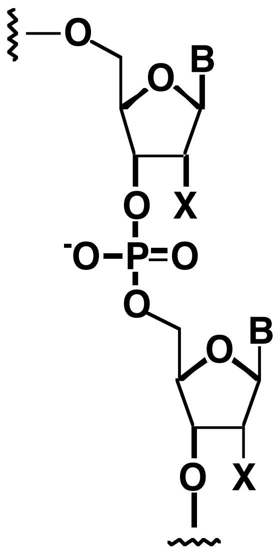
Voet and Voet

Biopolymers: Overview

Genomics

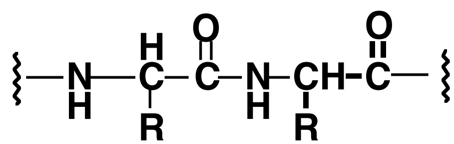
Proteomics

Glycomics

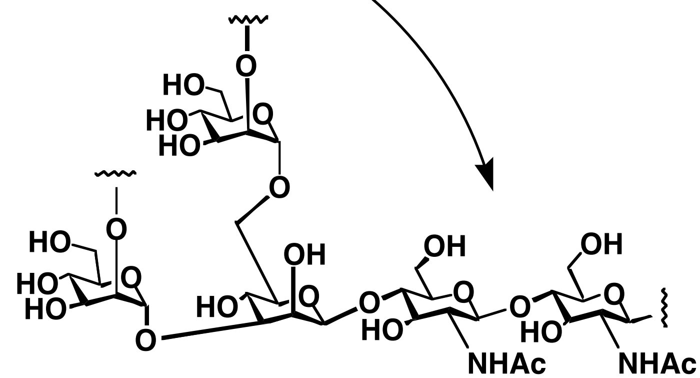


Transcription

Translation



Glycosyltransferases



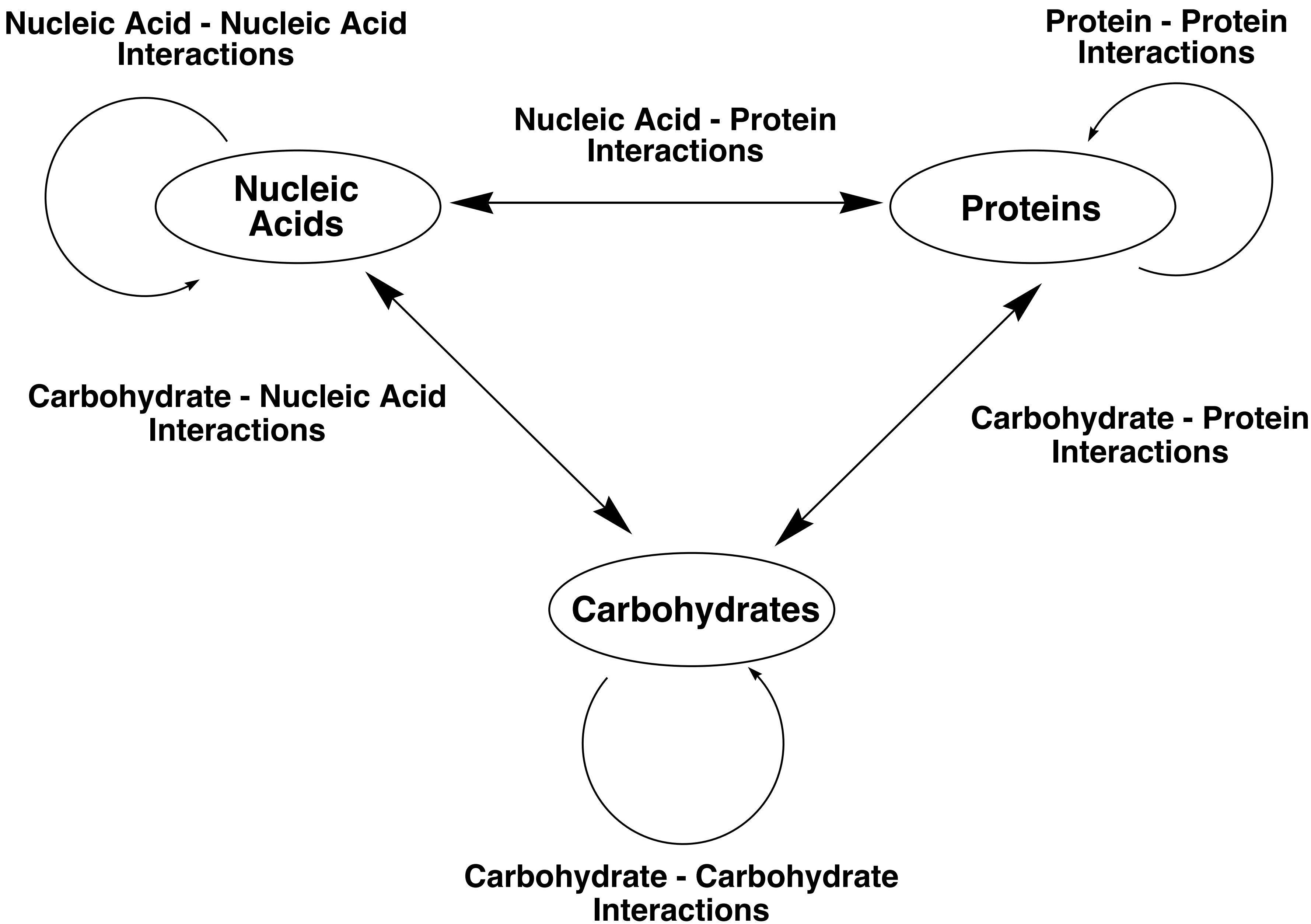
Replication

Nucleic Acids

Proteins

Oligosaccharides - Glycoconjugates

Biopolymer Interactions



Biopolymer Complexity

Oligomer size	Numbers of different oligomers		
	Nucleotides	Peptides	Carbohydrates
1	4	20	20
2	16	400	1 360
3	64	8 000	126 080
4	256	160 000	13 495 040
5	1 024	3 200 000	1 569 745 920
6	4 096	64 000 000	192 780 943 360

Synthesis Strategy

Acceptor-bound

Polymer Support

Polystyrene,
many others ...

Building Blocks

New protecting groups
JACS 2000, *122*, 7148

The Platform: Automated Solid-Phase Oligosaccharide Synthesis

Linkers

Octenediol,
Esters...
OrgLett 1999, *1*, 1811

Glycosylations

Glycosyl phosphates,
Trichloroacetimidates,
etc.
JACS 2001, *123*, 9545

Capping/Tagging

Fluorous Tags
Angew Chem 2001, *40*, 4433

Automation

Scale (5 mg-100 g)
Cycle Time
Temperature Control
Parallel Synthesis
Science 2001, *291*, 1523

"On-Resin" Analysis

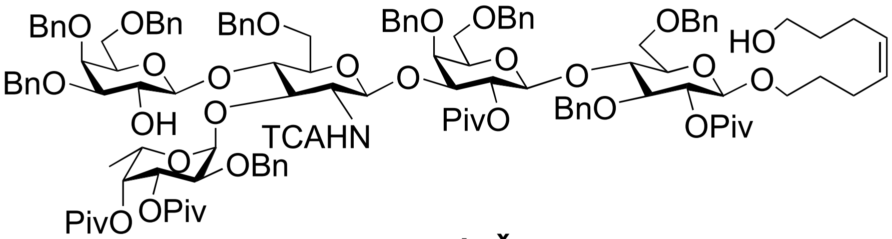
High resolution MAS NMR
AngewChem 1997, *36*, 491

Chem. Rev. 2000, *100*, 4349

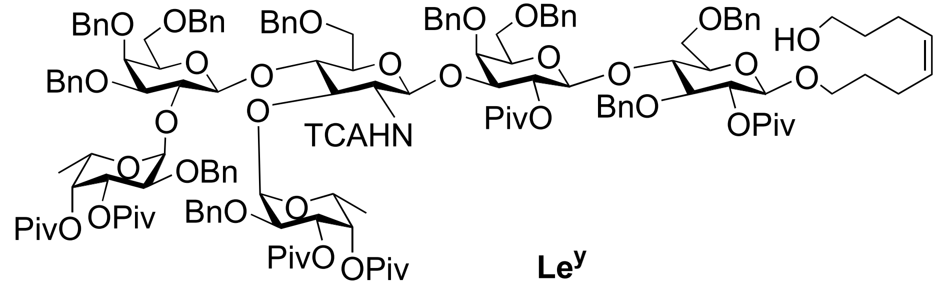
The Automated Oligosaccharide Synthesizer

Science 2001, *291*, 1523

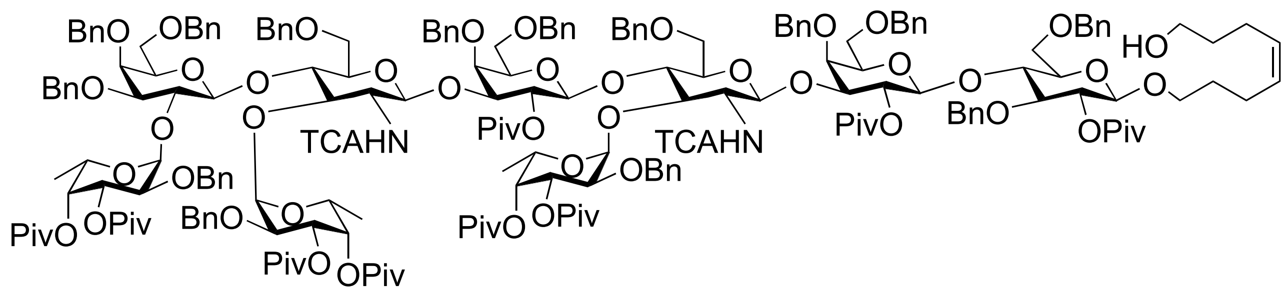
Blood Group Determinants and Tumor Associated Antigens



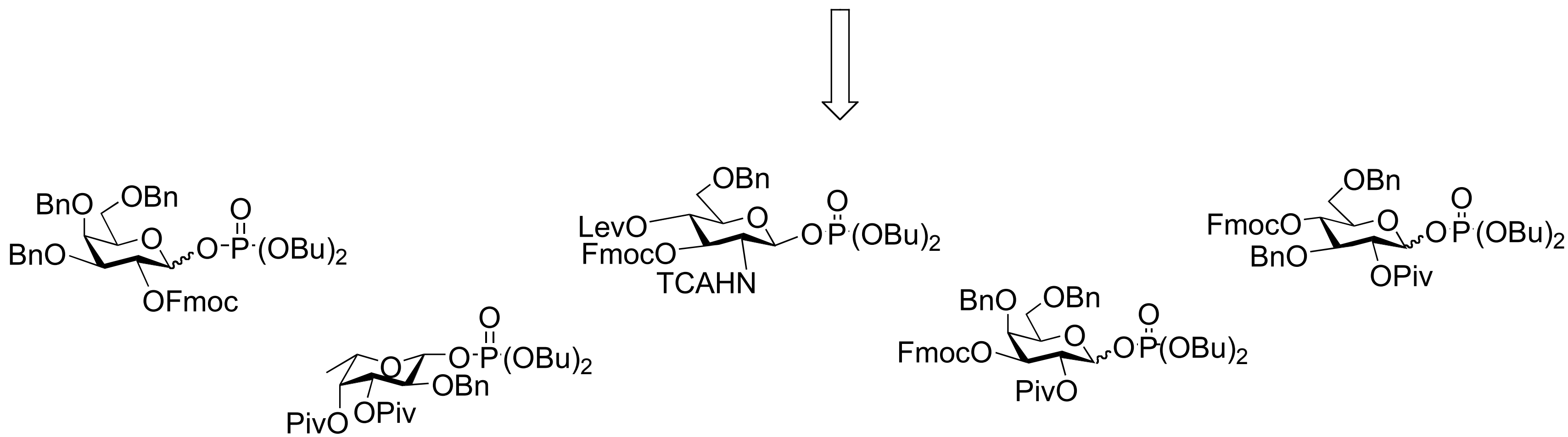
Le^{x}



Le^{y}

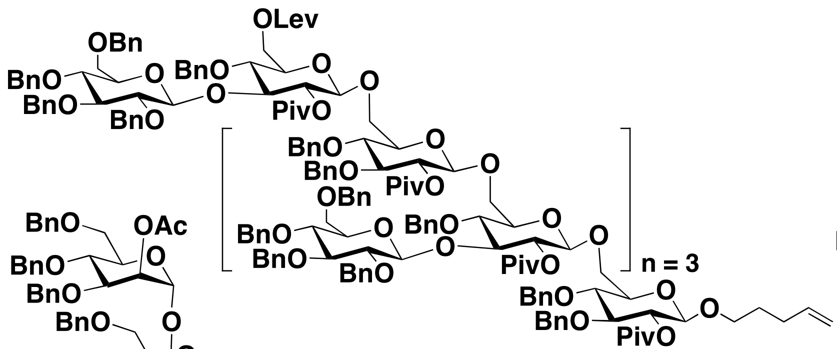


Le^{y}Le^{x}

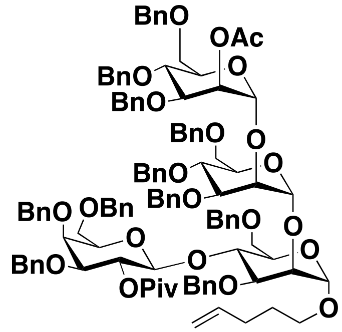


***Angew. Chem. Int. Ed.* 2004, *43*, 602**

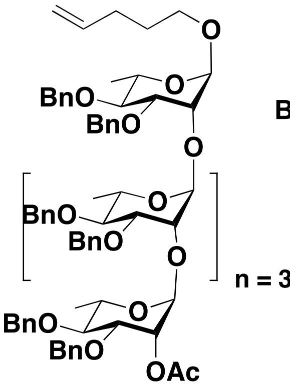
Automated Synthesis of Complex Structures



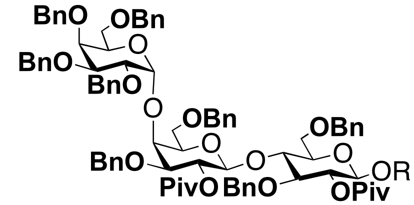
Phytoalexin Elicitor - 16 h



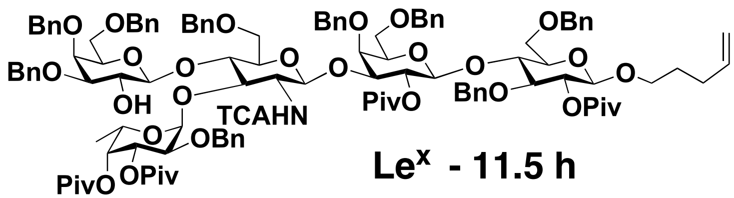
Leishmania Cap - 9 h



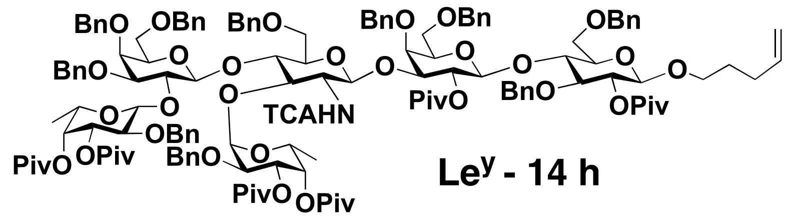
Rhamnose Pentamer
10 h



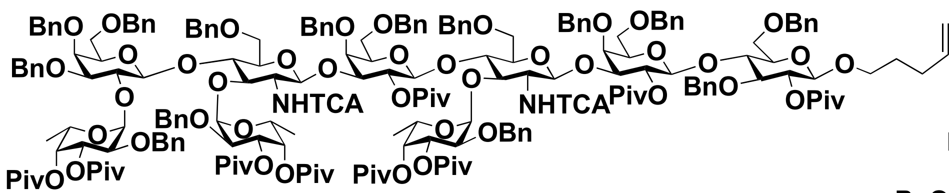
Gb3 - 6.5 h



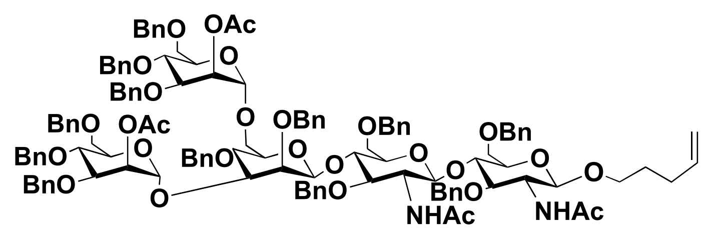
Le^x - 11.5 h



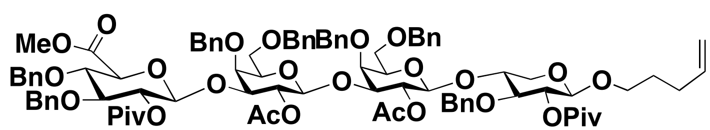
Le^y - 14 h



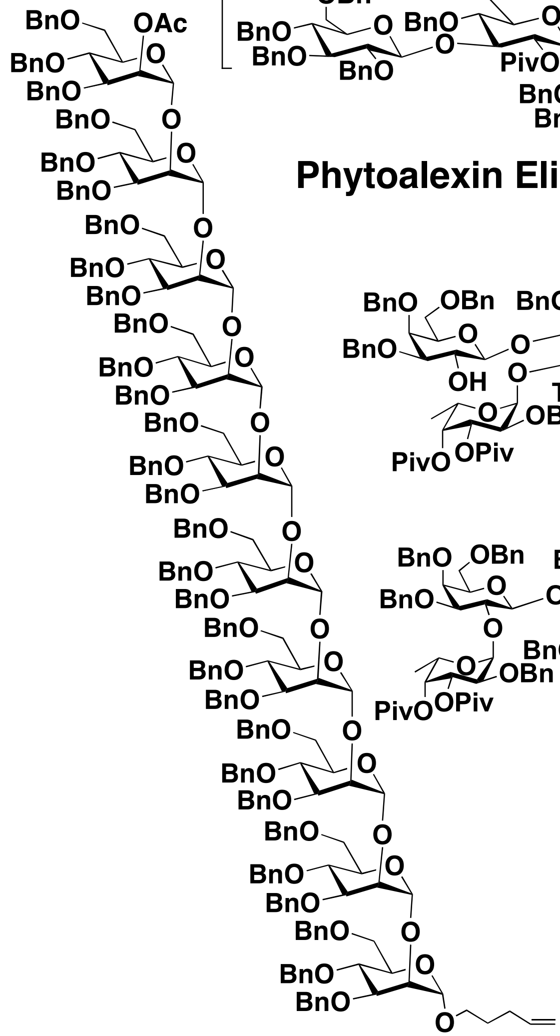
Le^y-Le^x - 23 h



N-Linked Core Region - 9 h



Proteoglycan Linkage Region - 9 h

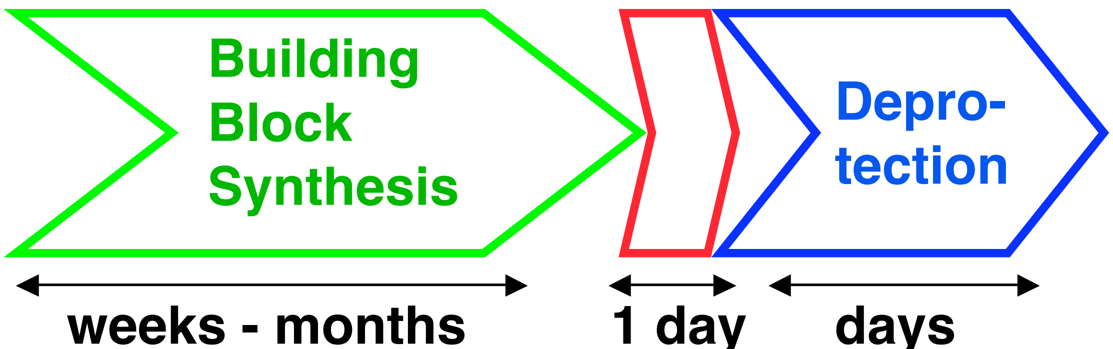


Mannose Oligomer - 23 h

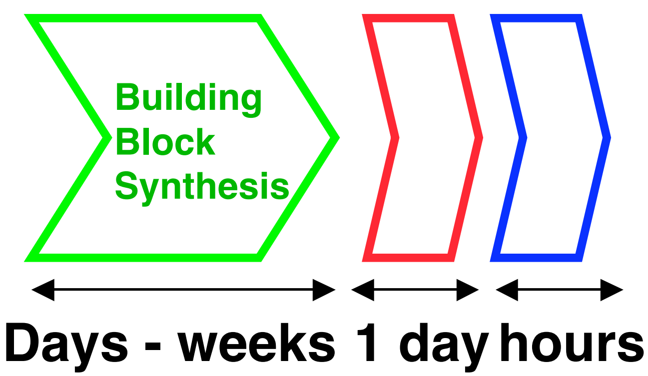
Time Allocation During Oligosaccharide Synthesis

Solution Phase

Automated Solid Phase (currently)

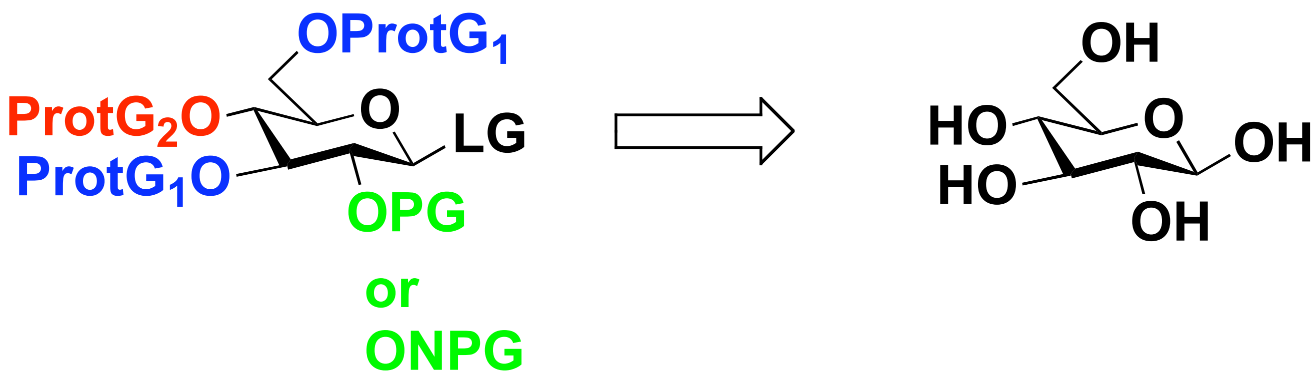


Automated Solid Phase (soon)



Feeding the Automated Synthesizer:

Short *De Novo* Synthesis of Fully Functionalized Uronic Acid Monosaccharides



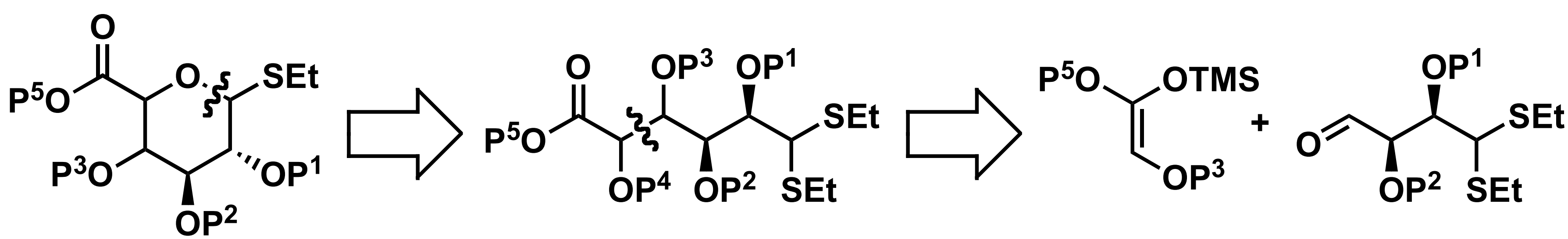
- Typically 6 to 20 steps

- Elaborate protecting group strategies

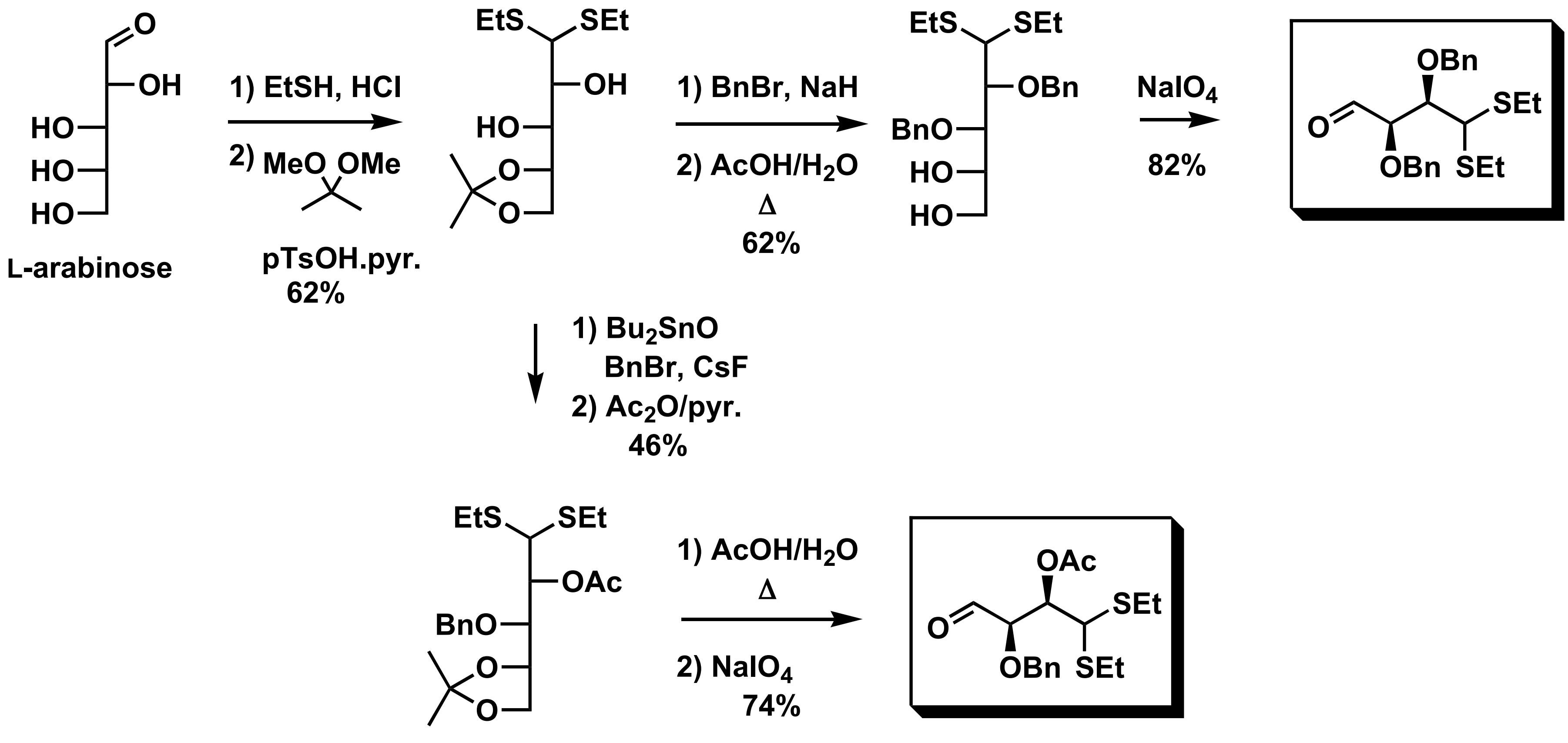
- Limiting factor for oligosaccharide synthesis

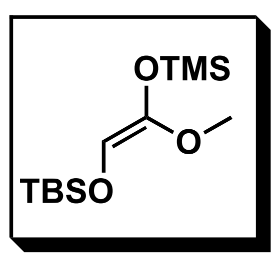
Angew.Chem.Int.Ed. 2005, *44*, 7605

Retrosynthesis of Hexose Thioglycosides

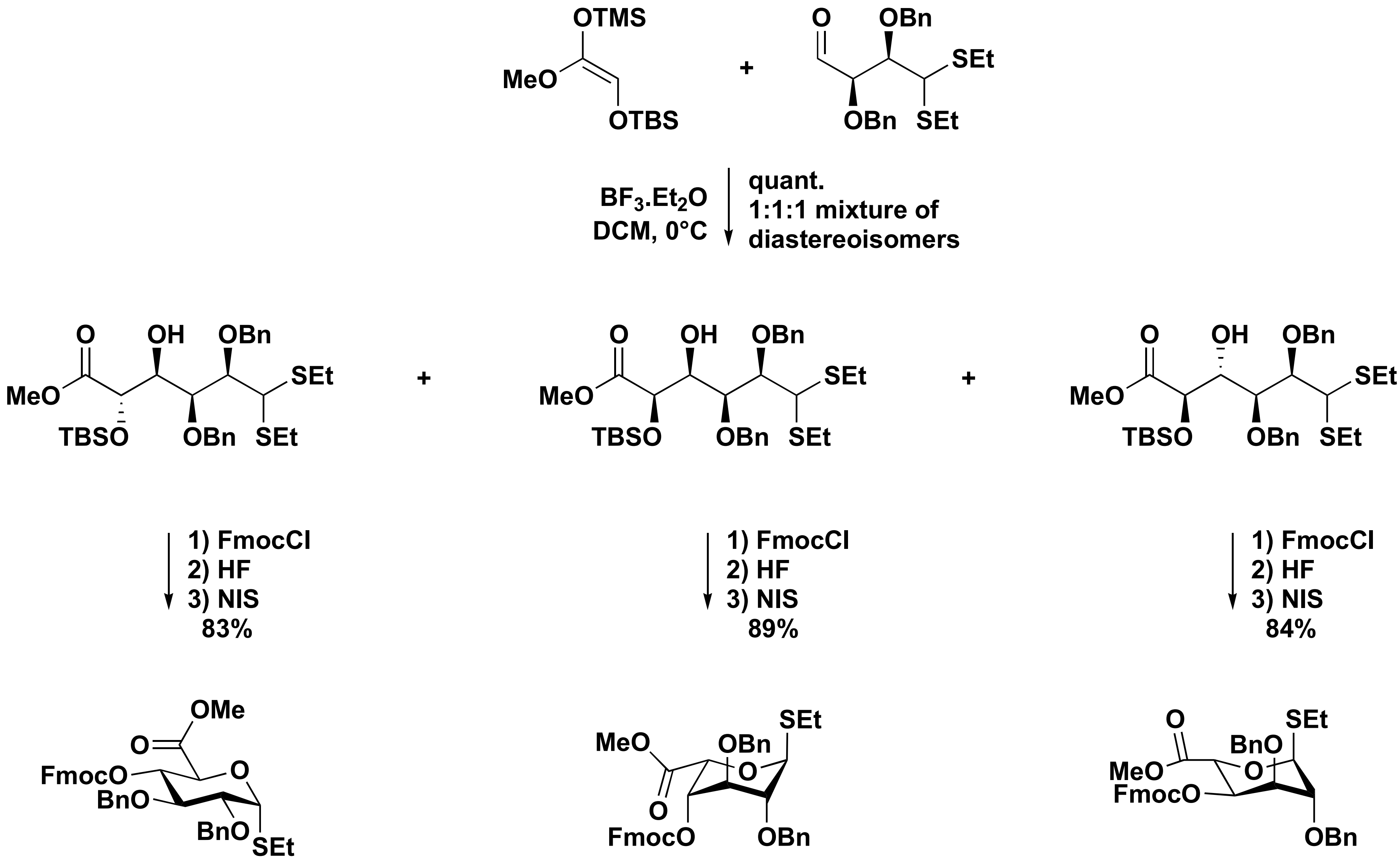


Synthesis of Precursors





Mukaiyama Aldol Reaction

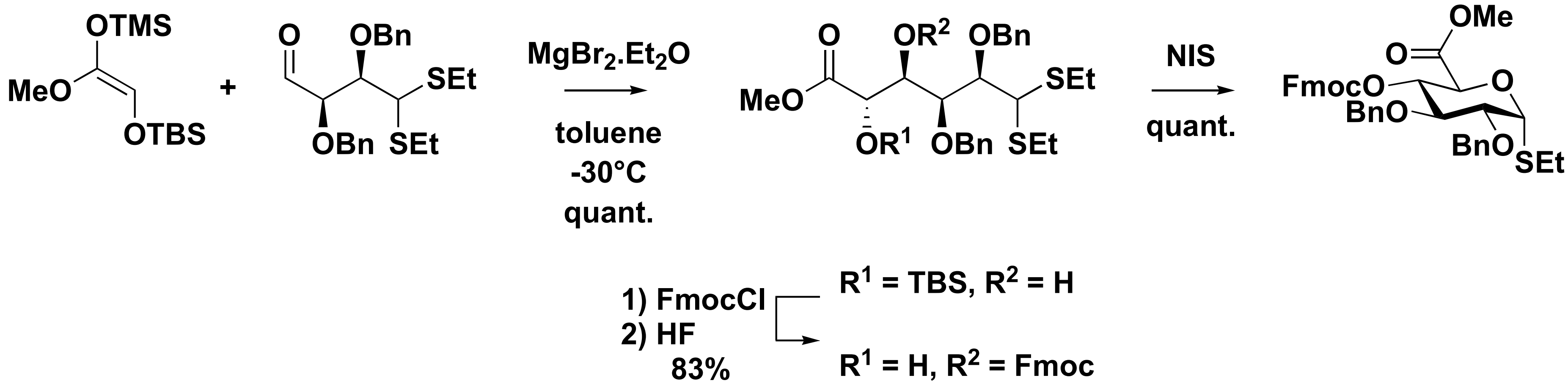


D-Glucuronic acid

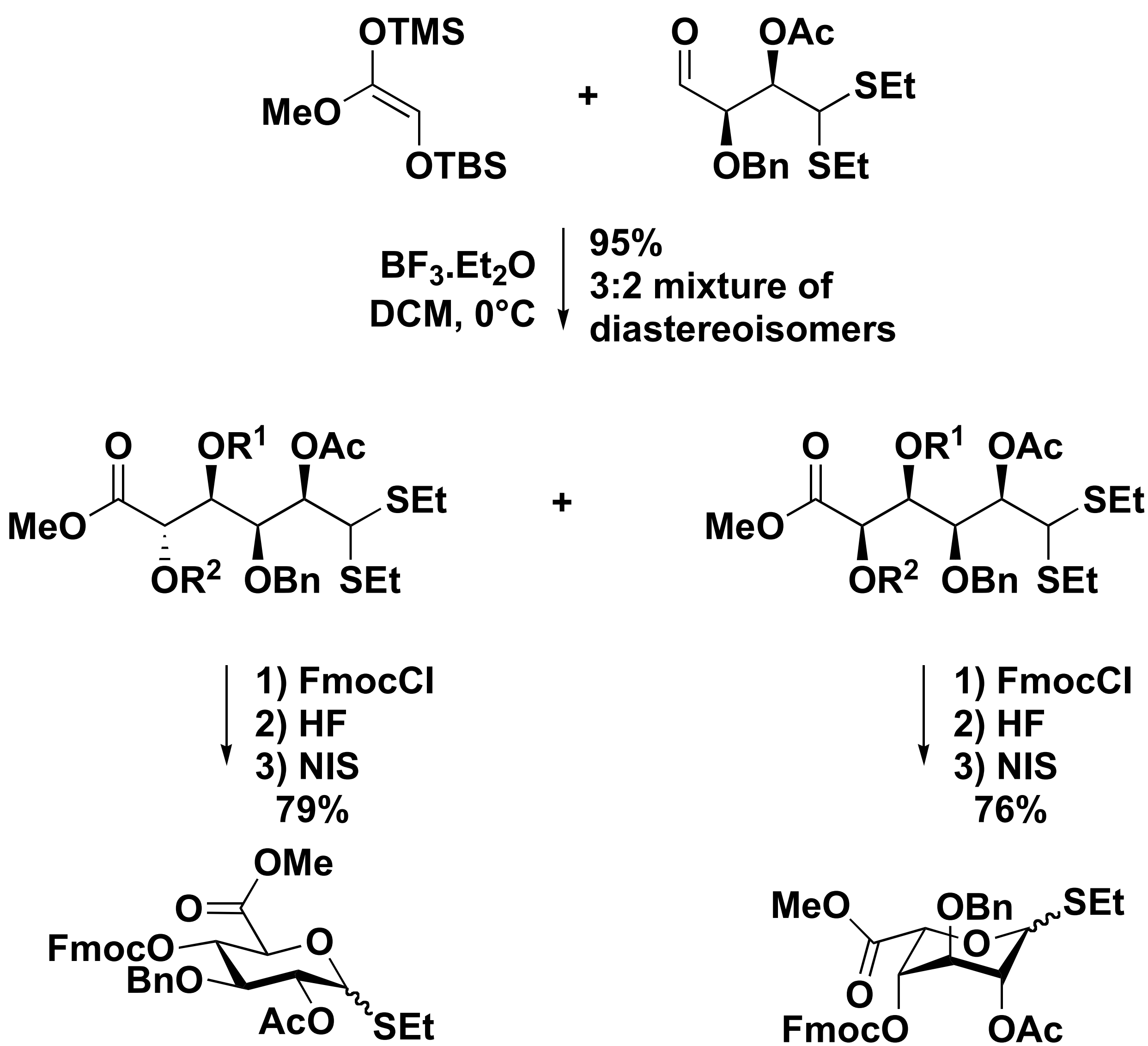
L-Iduronic acid

L-Altruronic acid

Selective Mukaiyama Aldol Reaction



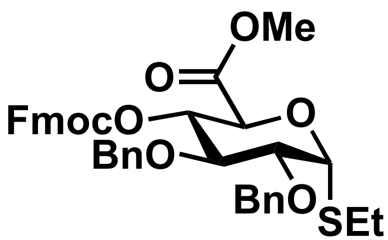
Orthogonal Protected Building Blocks



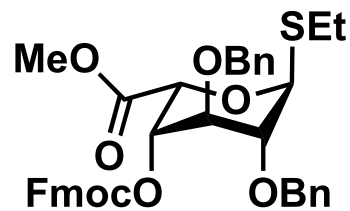
D-Glucuronic acid

L-Iduronic acid

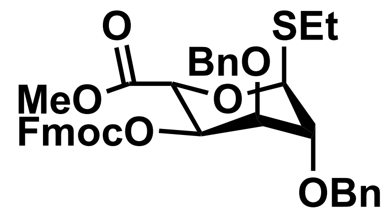
Uronic Acid Thioglycoside Building Blocks Prepared to Date



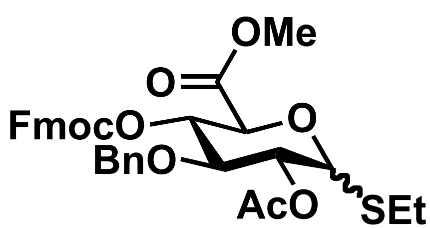
D-Glucuronic acid



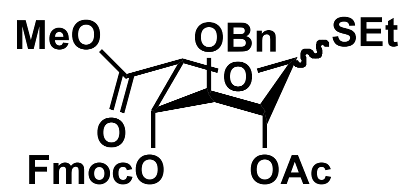
L-Iduronic acid



L-Altruronic acid

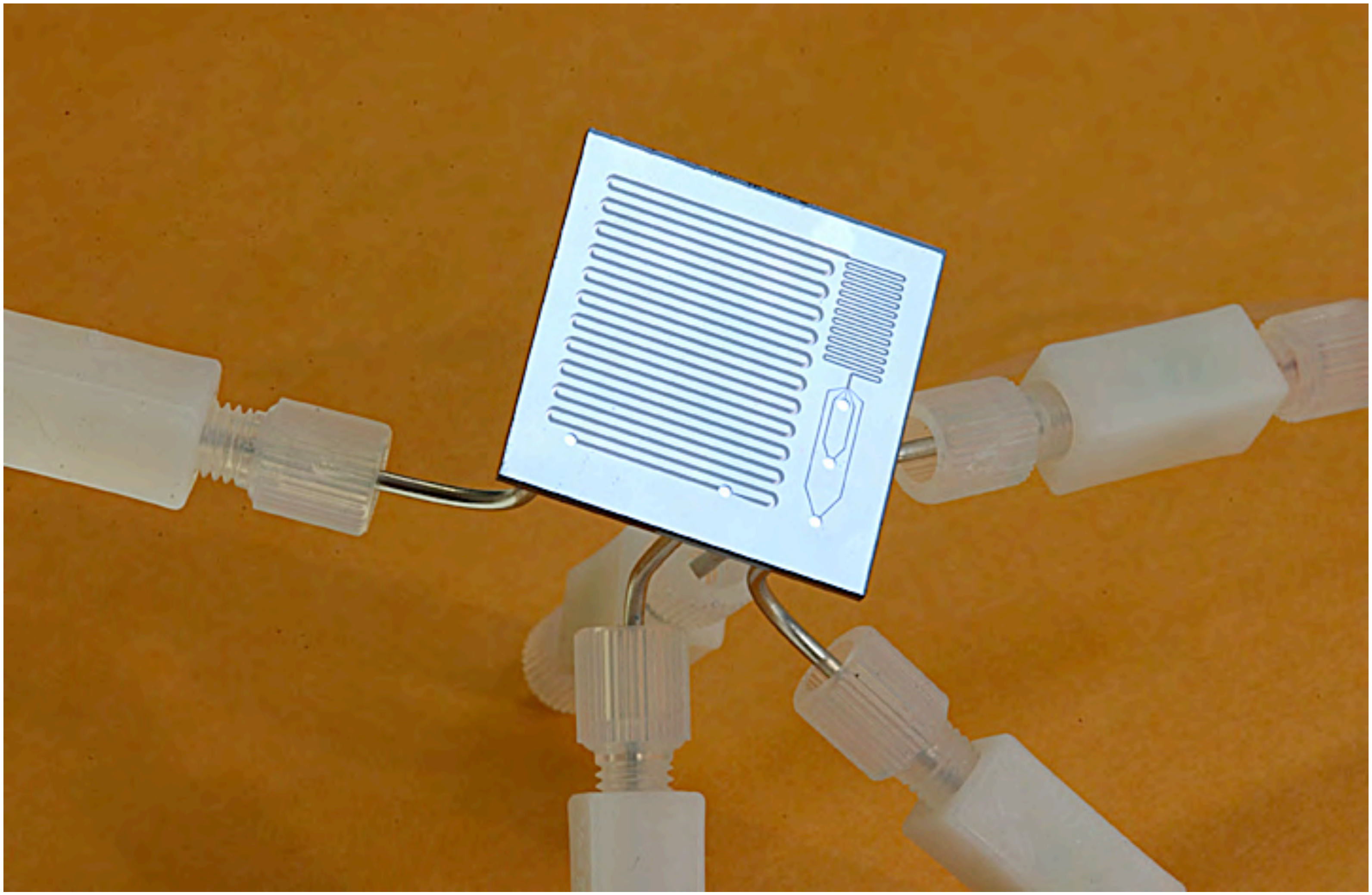


D-Glucuronic acid



L-Iduronic acid

Interlude:
Microreactors in Organic Synthesis



Geyer, K.; Codee, J.; Seeberger, P.H. *Chem. Eur. J.* 2006 in press.

From the Discovery Lab to the Production Plant

Discovery Chemistry

- make many different compounds
- speed is key
- 4 reactions per chemist\day
- all chemistries possible
- 10 mg - 500 mg scale
- material for biological (other) assays

Process Chemistry

- make one (or some) compounds
- efficiency is key
- months for one process
- only some chemistries possible
- 1 g - 100s of tons/year
- clean product for sale

Flow Trough Reactors in Chemistry

Discovery Chemistry	Process Chemistry	Production
Run 2 minutes	Run continuously	Run continuously Many in parallel

Same hardware

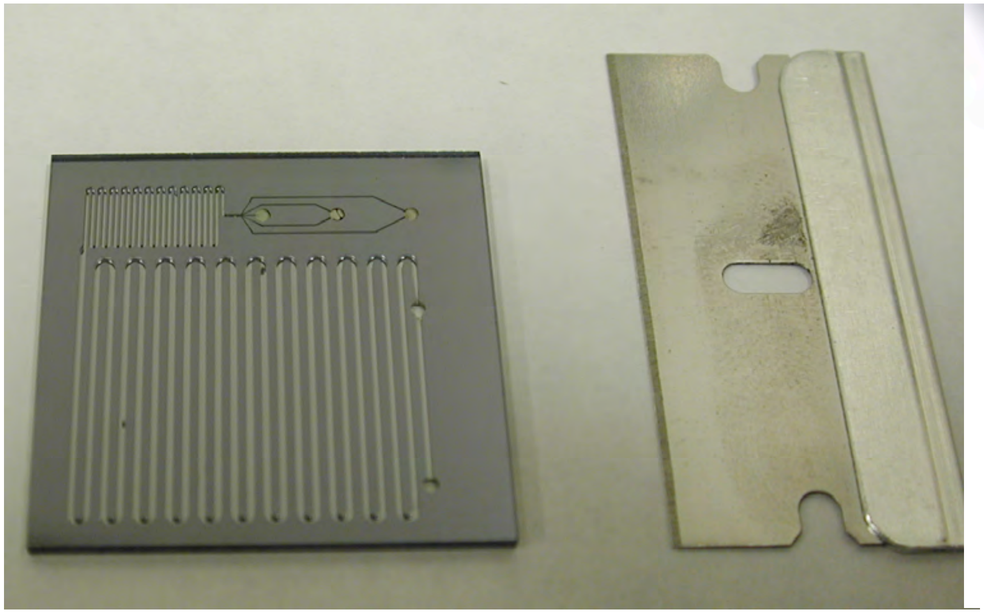
Same flexibility

Same safety

No change of protocols

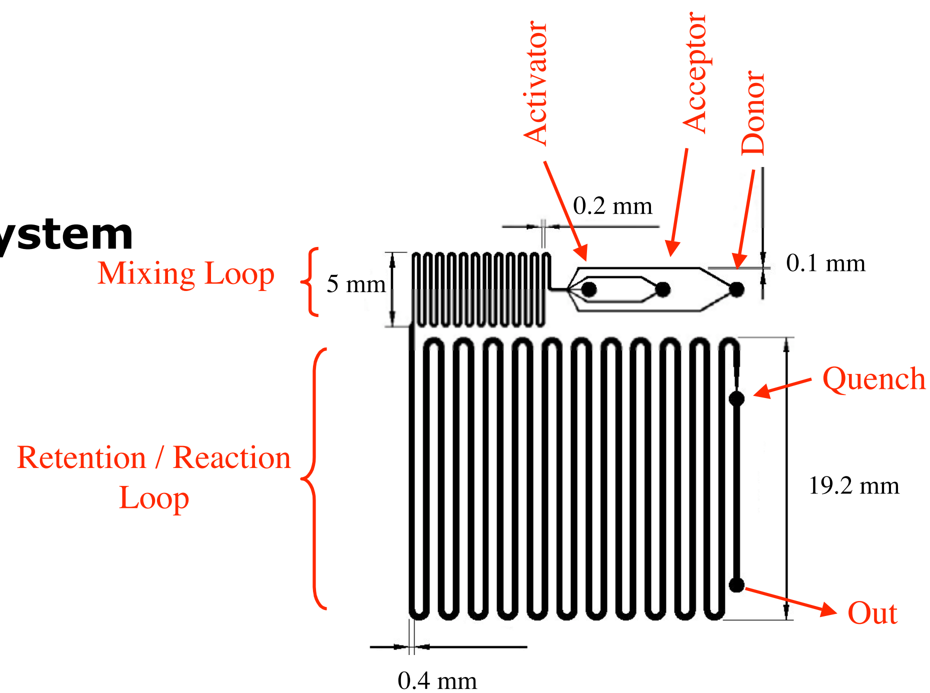
No cleaning times/costs

The Etched Silicon Microreactor

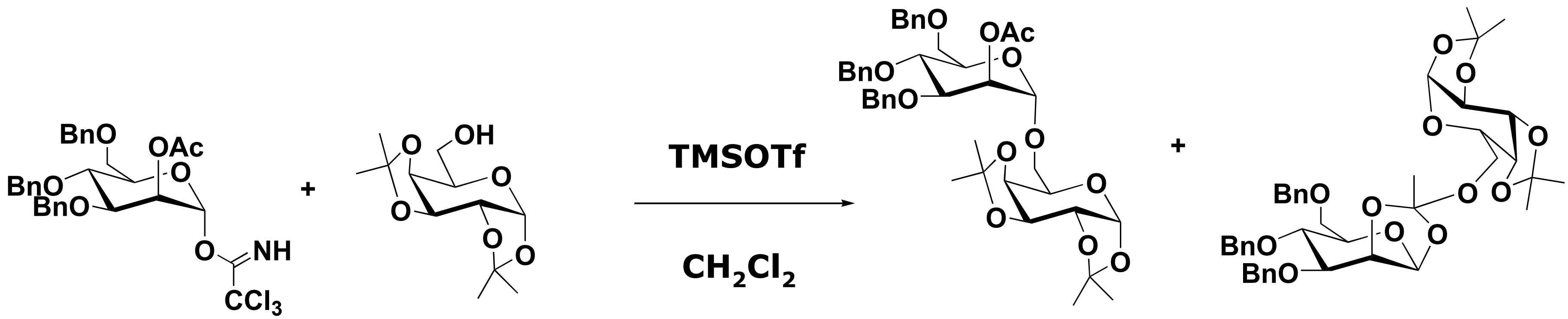


- Fabricated from etched silicon
- Reactor Volume ~78 μl
- Inert under most conditions; closed system
- Temperature range -70 °C to +170 °C
- Time range 13 sec to ~10 min
- From milligram to multi-gram scale

with Prof. K. Jensen (MIT)



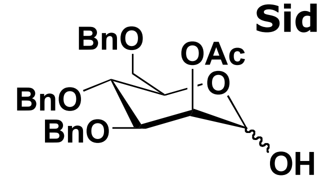
Synthesis of a Disaccharide in a Microreactor



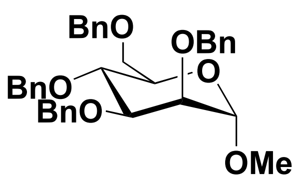
Donor

Acceptor

Orthoester Side Product



Hydrolyzed Donor Lactol

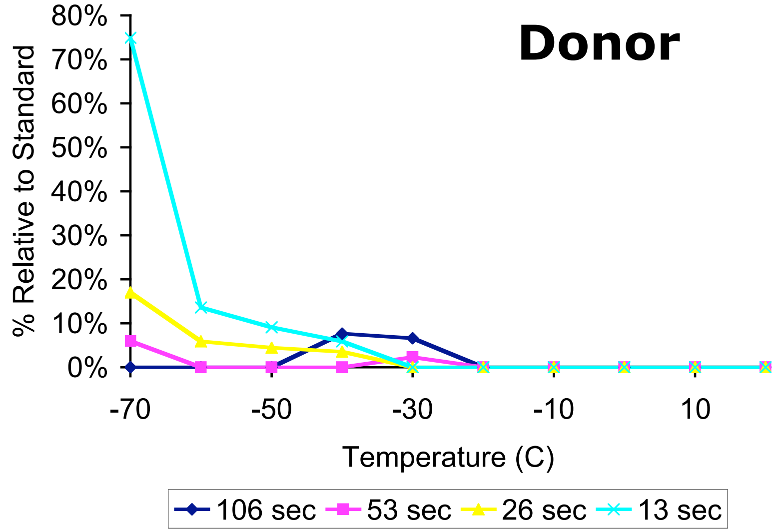


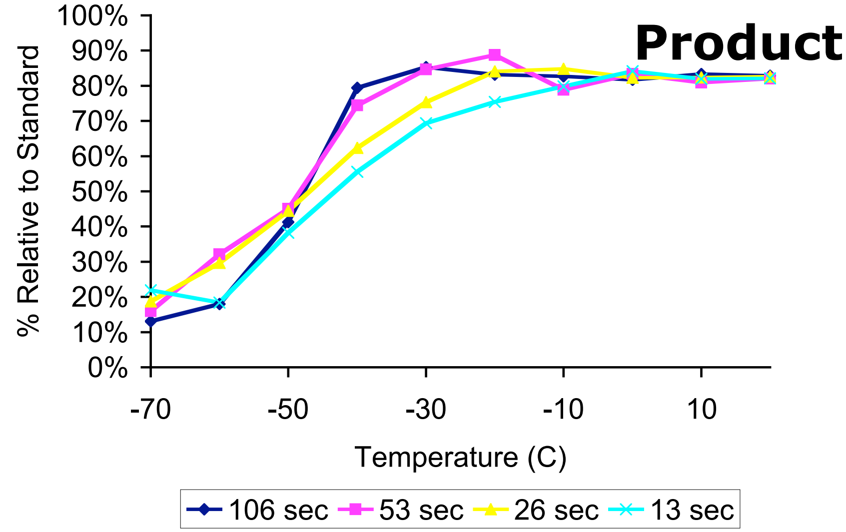
HPLC Reference Standard Included in Quench

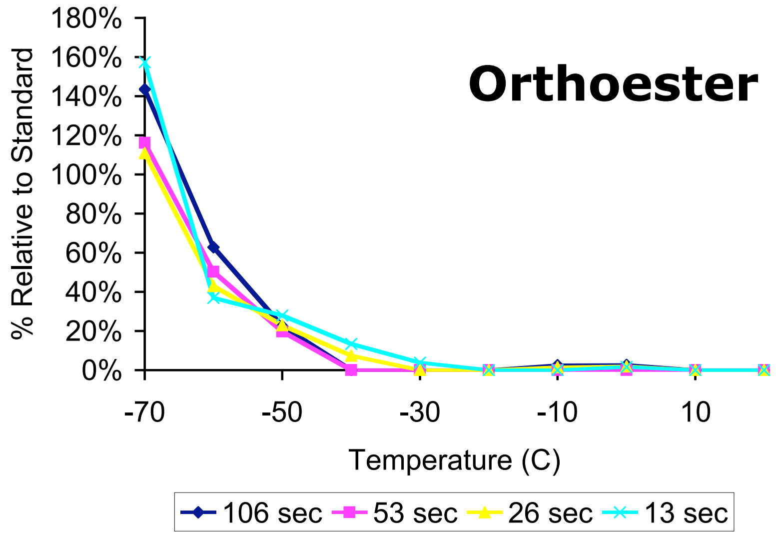
Variables:

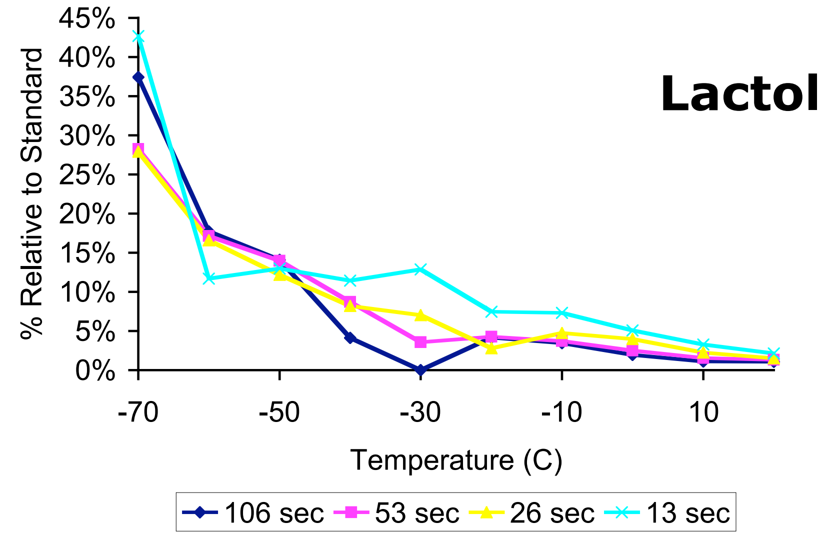
- *Reaction Time*
- *Reaction Temp.*
- *Donor Conc.*
- *Acceptor Conc.*

Glycosylation Reaction Profile: Reaction Time and Temp. vs. Yield









***Chem. Comm.* 2005, 571**

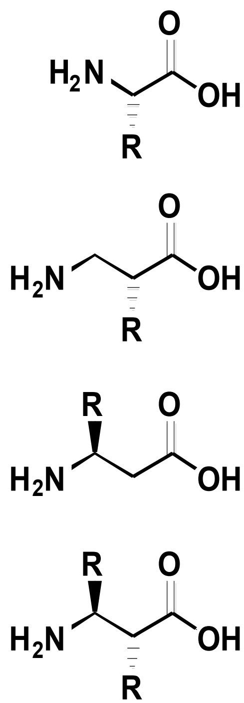
β-Peptides:
Novel Polymers With Interesting Structures

α

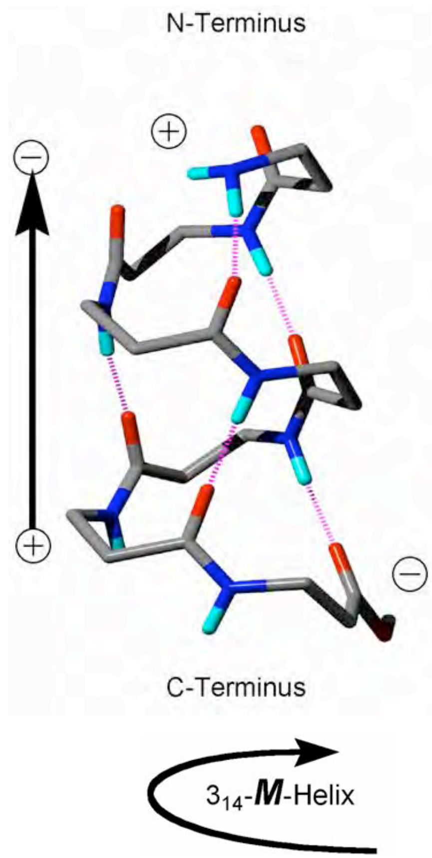
β^2

β^3

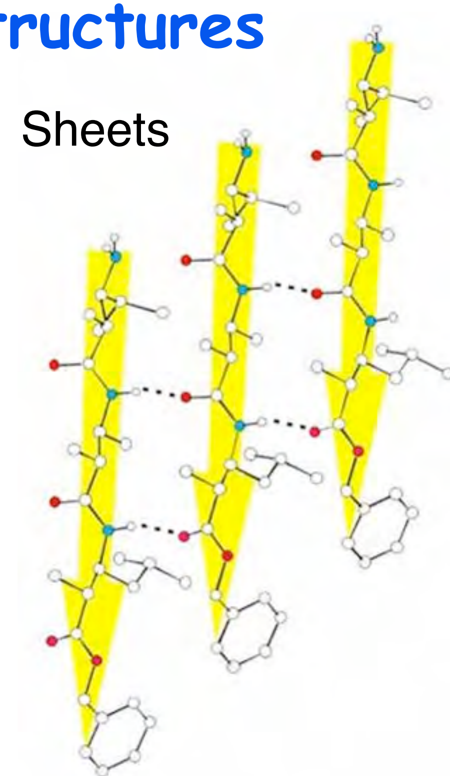
$\beta^{2,3}$



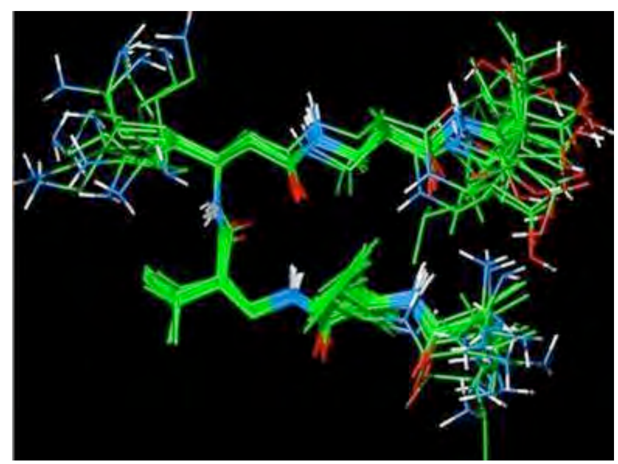
Helixes



Sheets



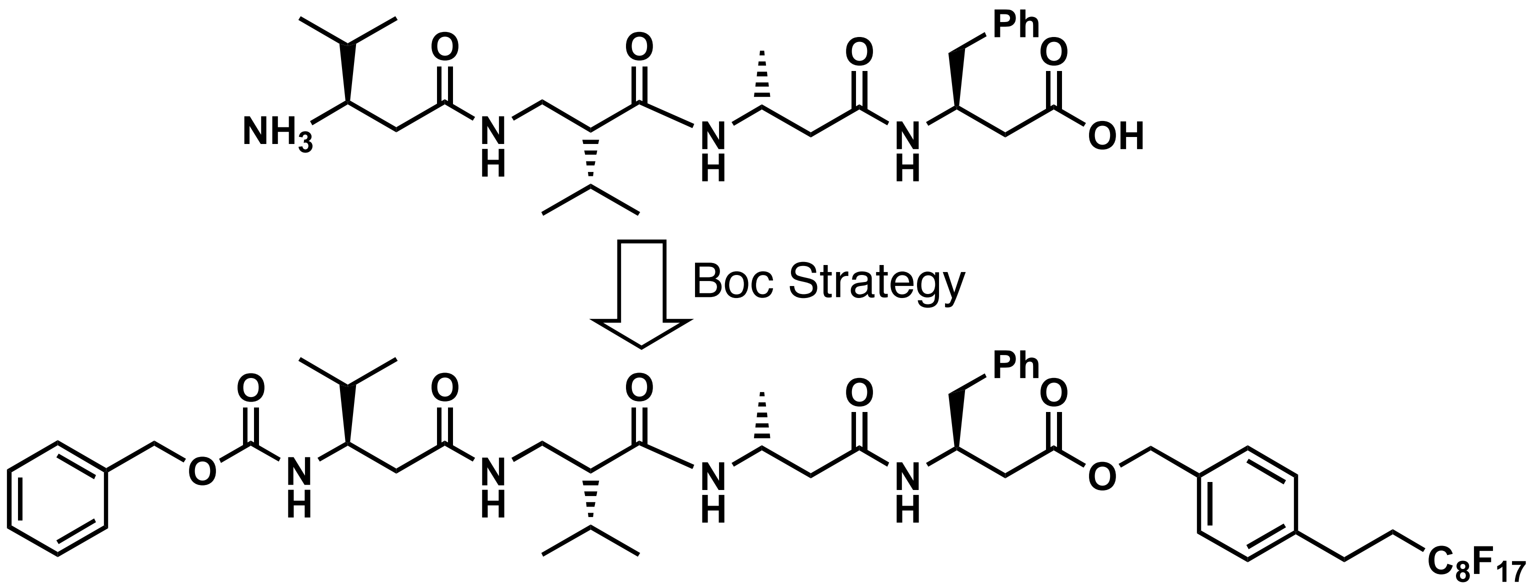
Hairpins



- Naturally occurring
- Metabolic stability

Seebach, Gellman

Synthesis of a Tetrapeptide



- β^2, β^3 Turn Inducing Element

    Formation of Secondary Structures?

    Lack of Solubility

    Coupling at High Temperatures → Microreactor

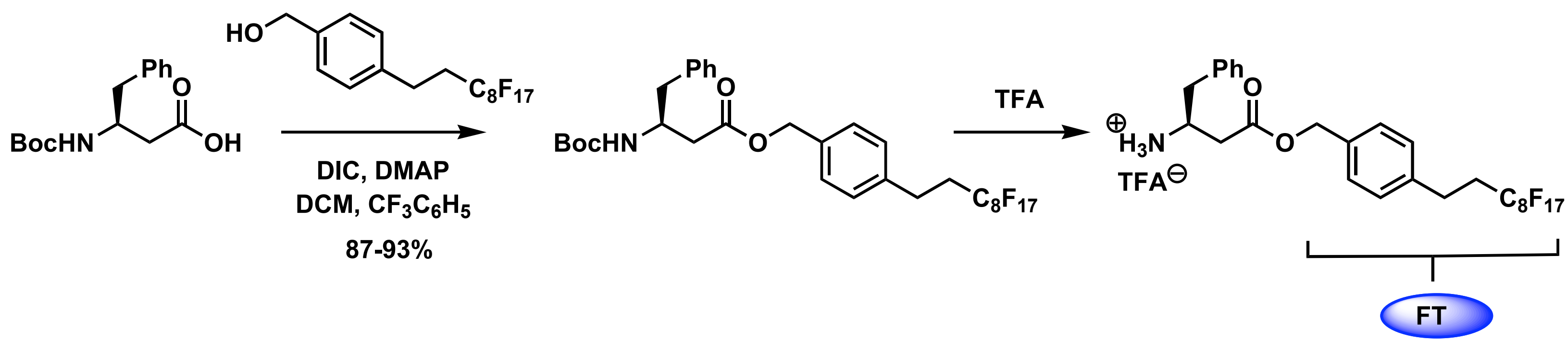
    Need Easy Method of Purification → Fluorous Tag

- Investigate structure and catalytic acitivity

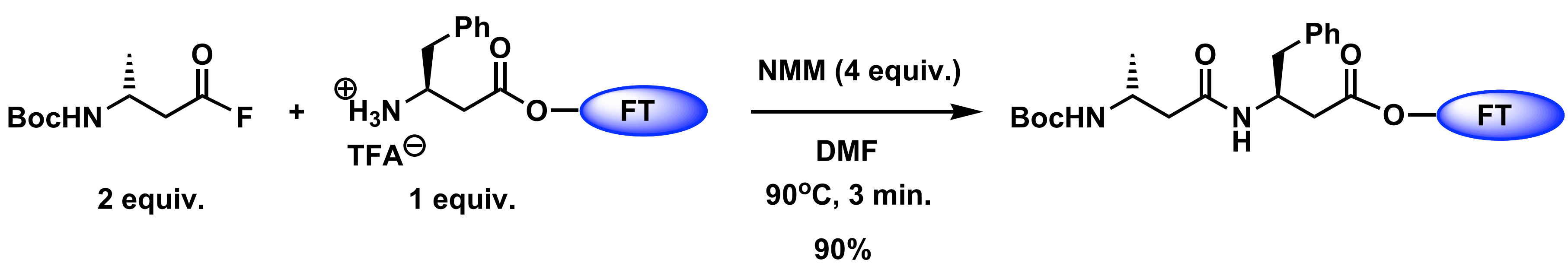
    Larger quantities

Synthesis of a Tetrapeptide

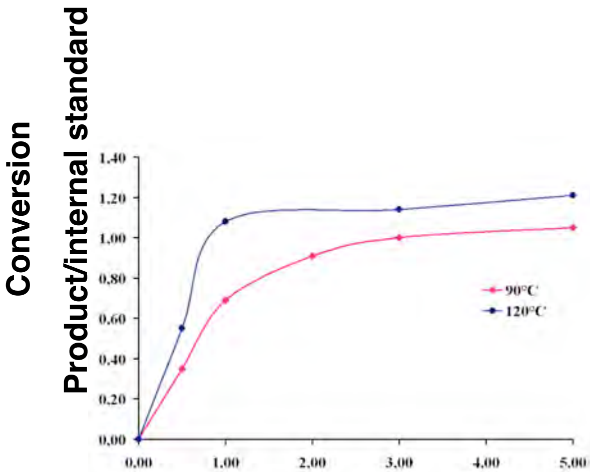
Preparation of tagged building block:

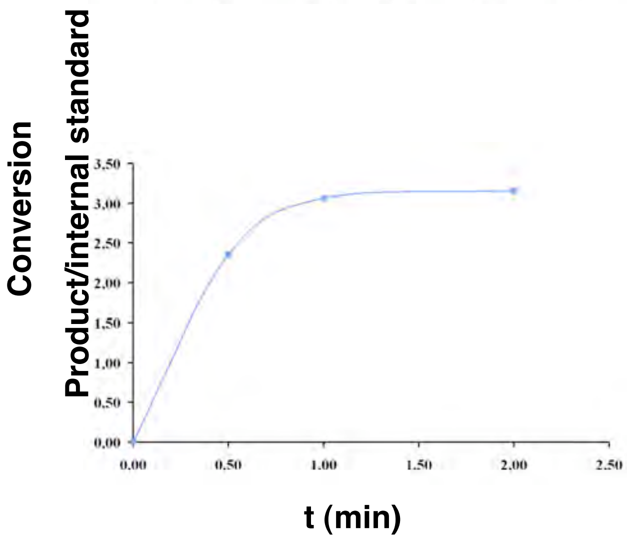


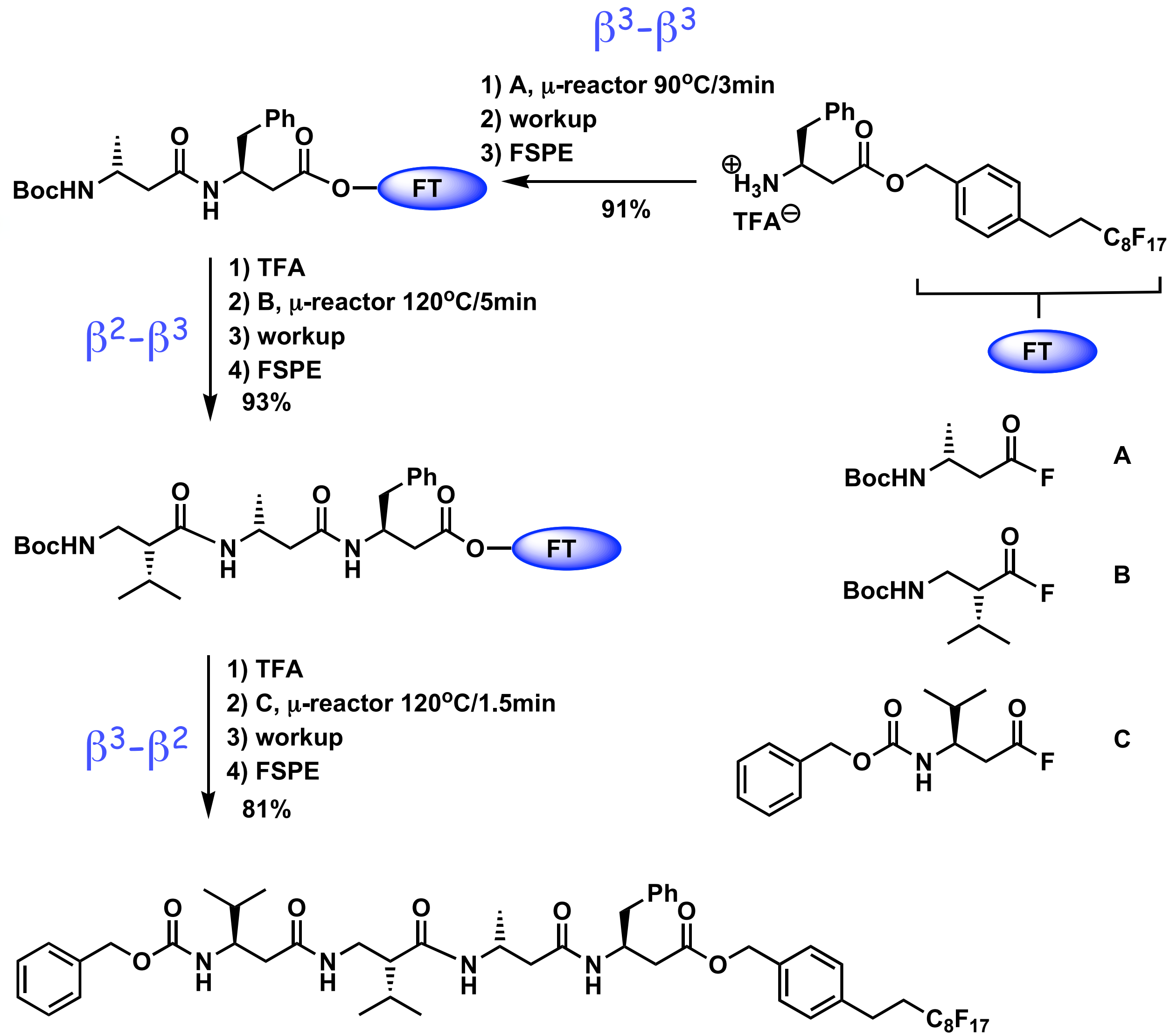
1st coupling ("optimized" conditions):



β-Tetrapeptide: Summary







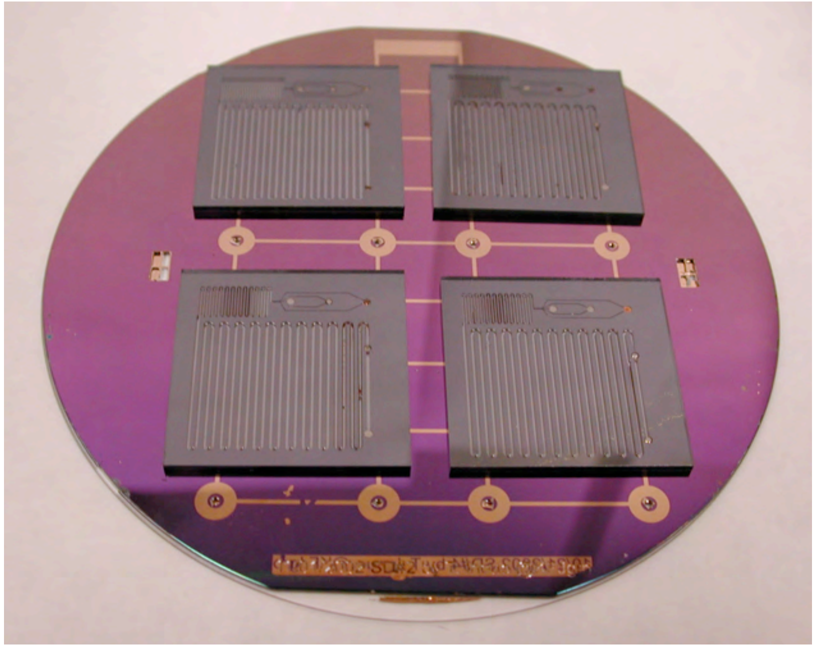
Codée, Flögel, Seebach, Seeberger, *Angw. Chem. Int. Ed. Engl. 2006 in press.*

Current Microreactor Work

- **Multiplexed reactors**
  - **–9 reactors in parallel**
  - **–Single wafer design**

- **Improved reactor design**
  - **–Faster mixing**
  - **–Wider range of reaction times**

- **Integrated detection**
  - **–UV/Vis, IR**
  - **–LC/MS**

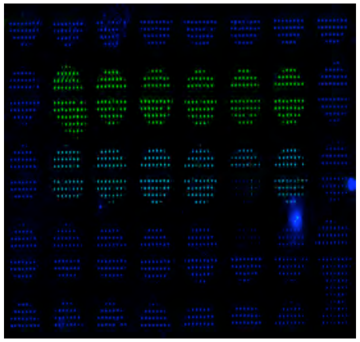


- **Additional reactor design for specific chemistries**
  - **–Chemistry-dependent**
  - **–Multiphase**

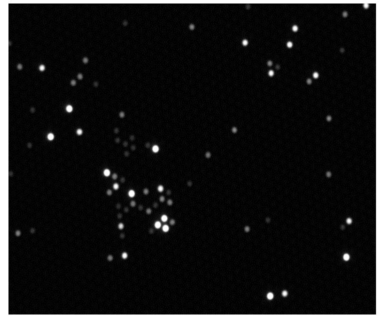
Automated Oligosaccharide Synthesis - Applications

- ***Carbohydrate-Protein Interactions***
    - **HIV gp120 - cyanovirin-N interactions**
    - **Bacterial cell adhesion**
    - **Innate immune response**
    - **Heparin - protein interactions**
    - **Glycolipid signaling**
- ***Nucleic Acid - Carbohydrate Interactions***
    - **Aminoglycoside - RNA binding**
- ***Synthetic Carbohydrate Vaccines***
    - **Tropical diseases (Malaria; Leishmania)**
    - **Bacterial Infections (*B. anthracis*; *M. tuberculosis*)**
    - **Viral Infections (HIV; Avian flu)**
    - **Cancer**
- ***Detection of Pathogenic Bacteria***

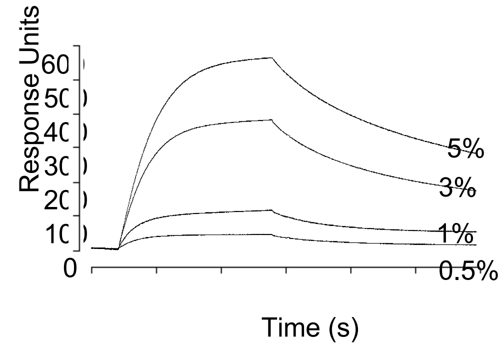
Tools for Glycobiology



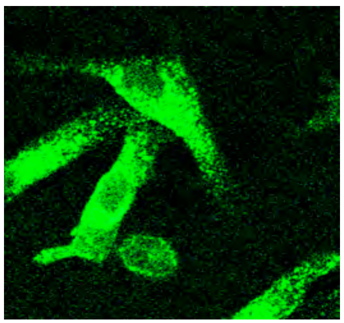
Carbohydrate arrays



Randomly ordered fiberoptic
carbohydrate microarrays



Carbohydrates on gold
surfaces for SPR

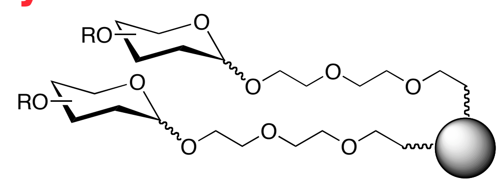


Labeled carbohydrates
For confocal microscopy

Identify ligands

Determine binding affinity

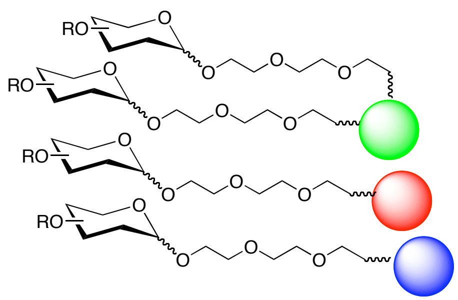
Synthetic/Isolated Oligosaccharides



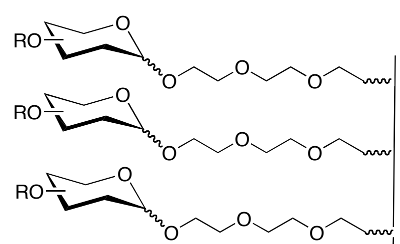
Carbohydrates
on magnetic beads

Imaging

Isolate carbohydrate binding proteins



Carbohydrates on quantum dots
for in vitro imaging



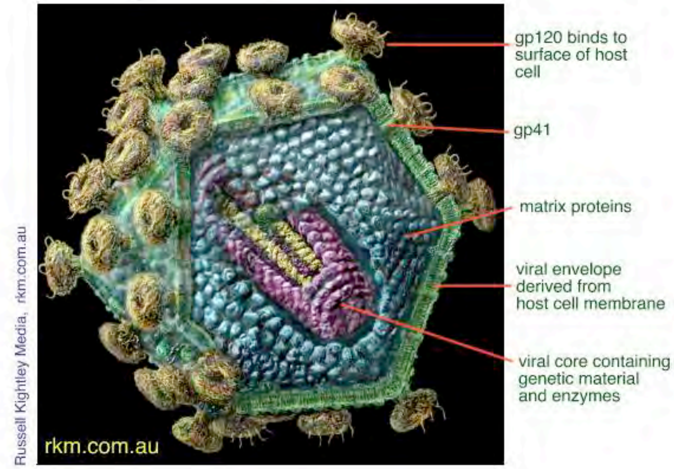
Carbohydrate
affinity columns

ChemBioChem 2004
5, 1375.

Carbohydrate Arrays - Applications

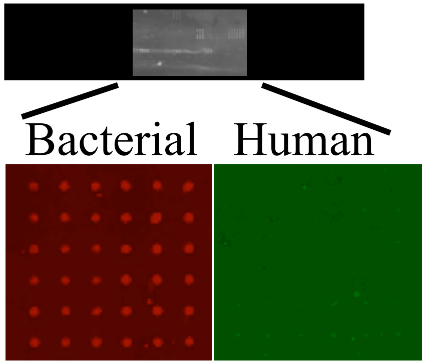
- Incubate with proteins, antibodies
  - Determine binding fingerprints
  - Identify new lectins
  - Screen blood for disease patterns
  - Lead structures for vaccine design

    Angew. Chem. Int. Ed. 2003, 42, 5317
    ChemBioChem. 2004, *5*, 379.



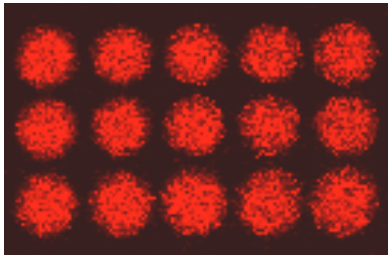
- Incubate with RNA
  - Aminoglycoside antibiotic selectivity
  - Resistance mechanisms and toxicity

    Angew. Chem. Int. Ed. 2004, *43*, 1591
    Chem. Eur. J. 2004, *10*, 3308

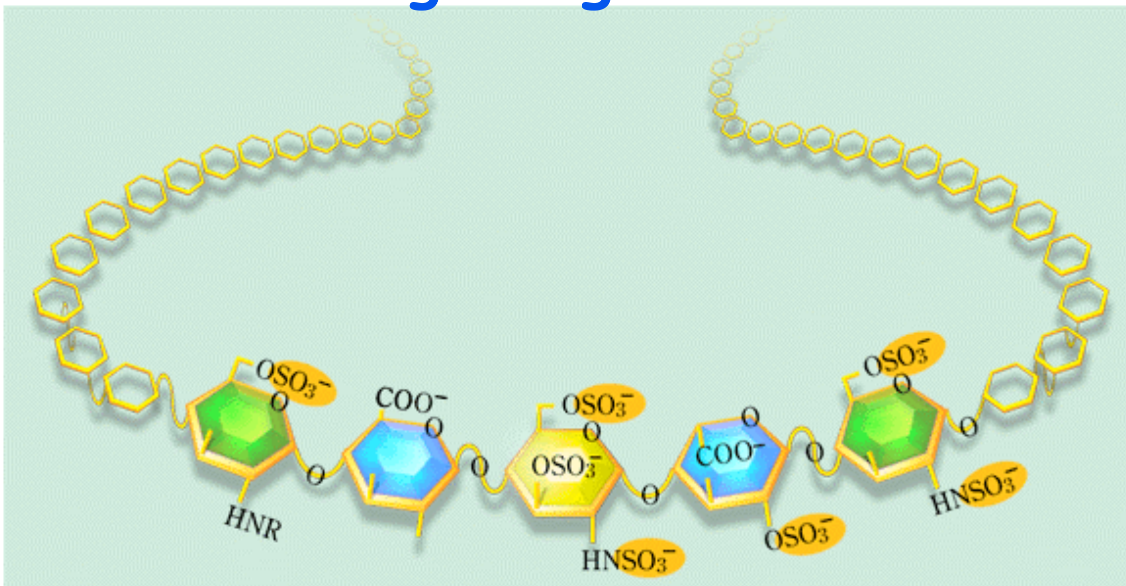


- Incubate with cells
  - Detect bacteria in biological samples
  - Identify cellular interactions

    Chem. Biol. 2004, *11*, 1701



Glycosaminoglycans:
Key Mediators of Signaling at the Plasma Membrane

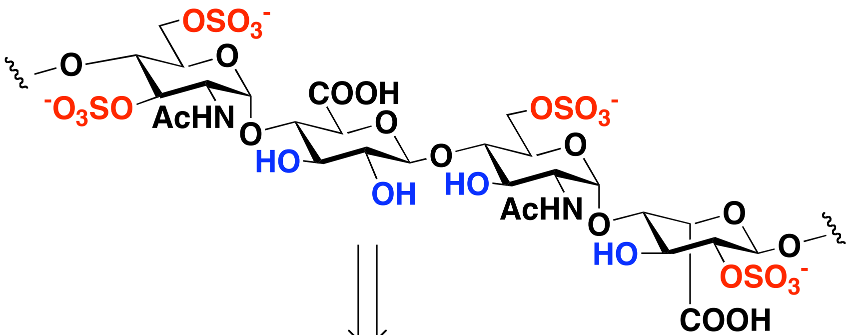


Growth Factor Interactions	Blood Coagulation	Receptor Interactions	Plaque Formation
⇩	⇩	⇩	⇩
Cancer	**Heart Disease**	**Viral Infections**	**Neurodegenerative Diseases**

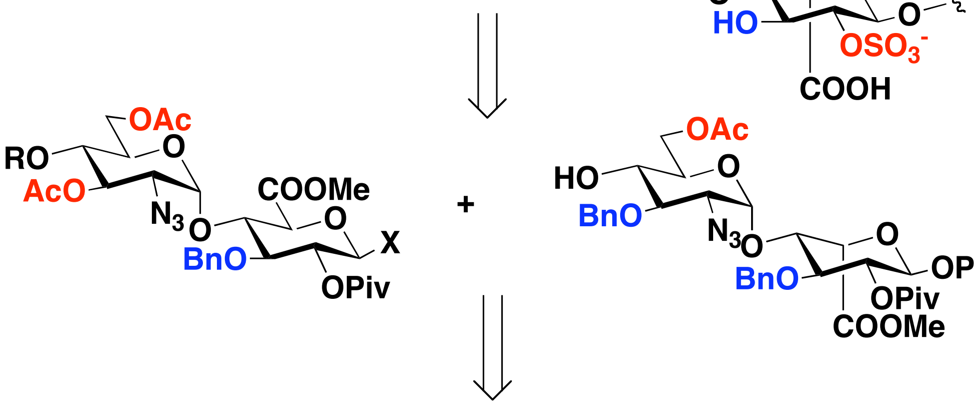
37 !! Major Disease Areas

Can We Identify Small Molecule Inhibitors of Heparin-Protein Interactions?

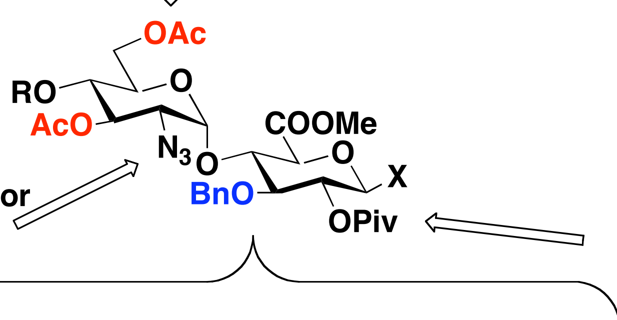
Modular Synthesis of Heparin-like Glycosaminoglycans



255 Million Decasaccharides
5.3 Million Octasaccharides
110,592 Hexasaccharides



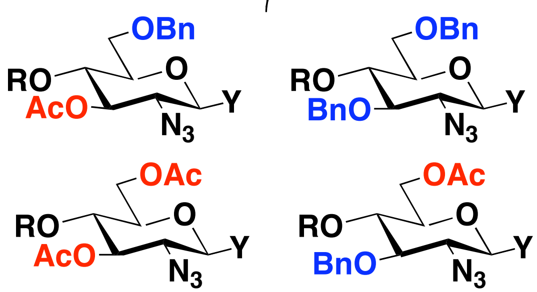
48 Modified Disaccharide Building Blocks



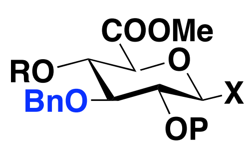
8 Disaccharide Building Blocks

Three 2-Amine modifications for *N*-Sulfate, *N*-Acetate, Amine

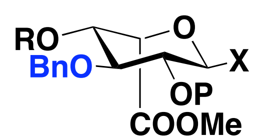
C2-Hydroxy or C2-Sulfate



4 Glucosamine Building Blocks



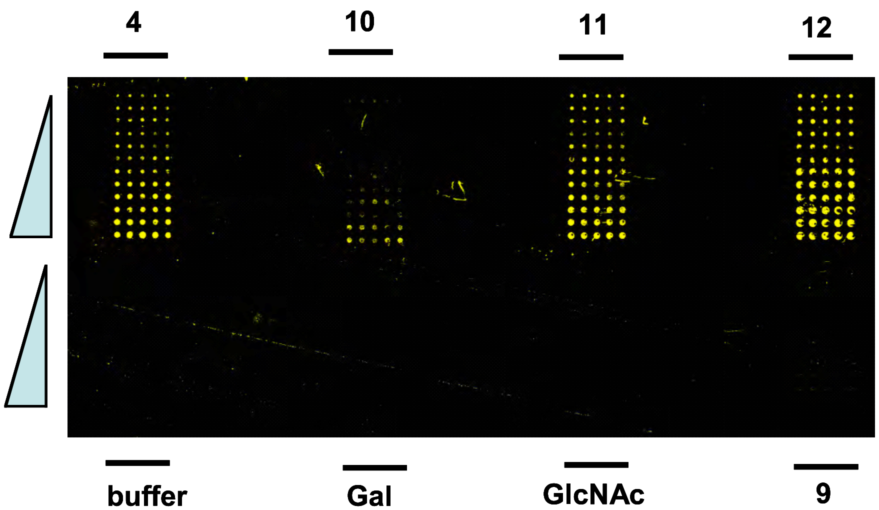
1 Glucuronic Acid Building Block

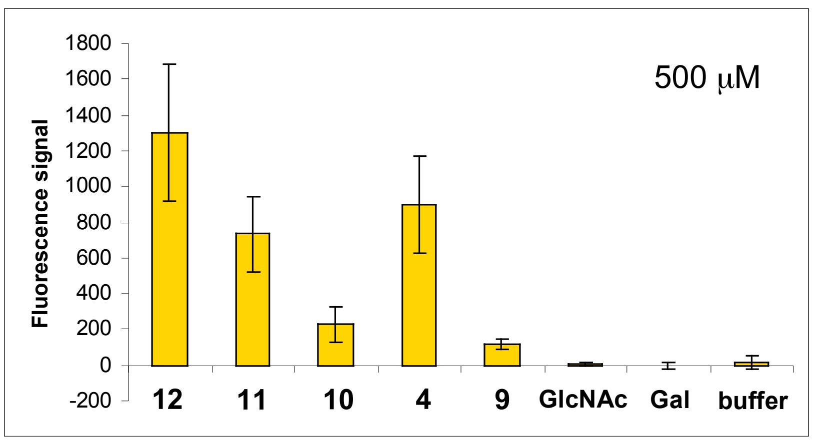


1 Iduronic Acid Building Block

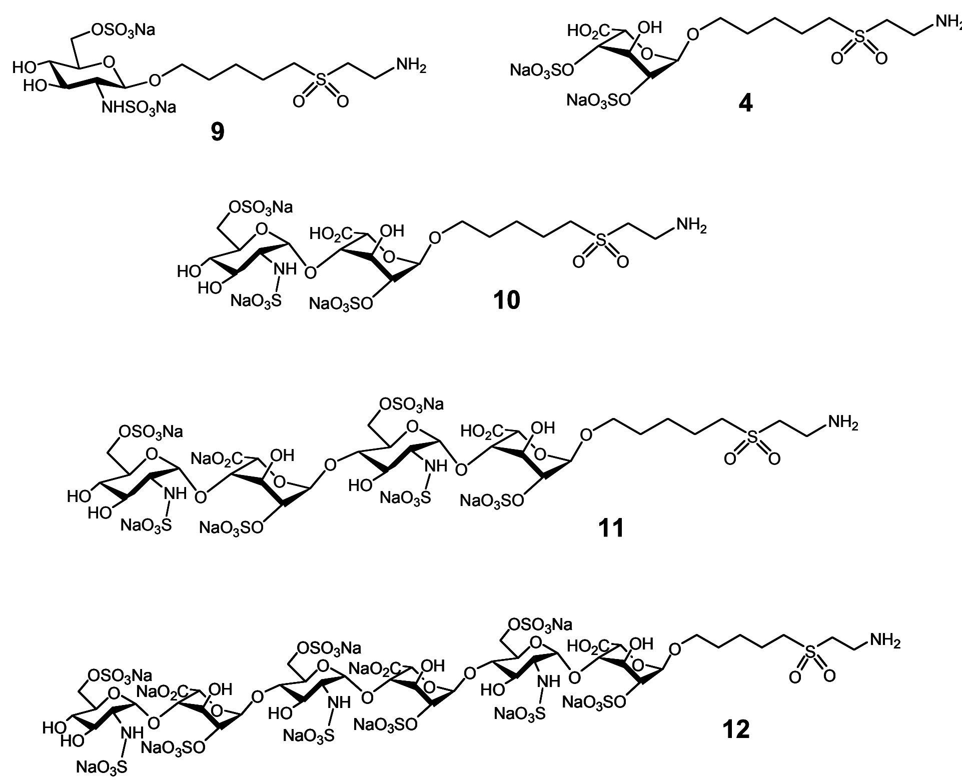
Angew. Chem. Int. Ed. 2002, *41*, 2128; *Chem. Eur. J.* 2003, *9,* 140

Microarray Incubation with FGF-1





4 concentrations: 2, 1, 0.5, 0.25 mM



J. Am. Chem. Soc. 2006, *128*, 2766

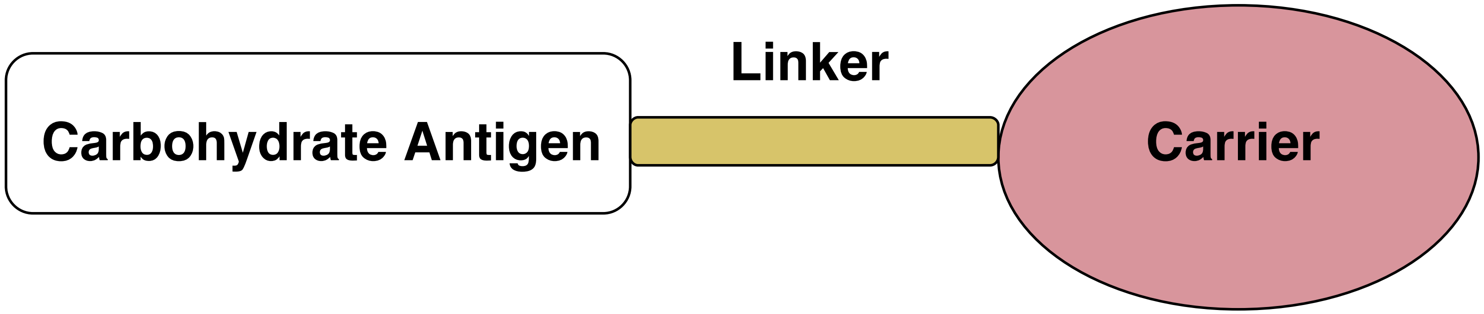
Heparin Chemistry and Biology: Current Work

- Synthesis of defined structures
- Automated synthesis in progress
- Define heparin sequences for different proteins
  (chemokines, viral receptors)
- Screen for small molecule inhibitors of heparin-protein interactions
- Synthesis and arraying of other glycosaminoglycans
  (hyaluronic acid,chondroitin sulfate etc.)

Synthetic Carbohydrate Antigens:

Development of Vaccine Candidates Against Parasites, Bacteria and Cancer

Carbohydrate Vaccine Architecture



1) Carbohydrate Antigen
   - Isolated polysaccharide
   - Synthetic oligosaccharide

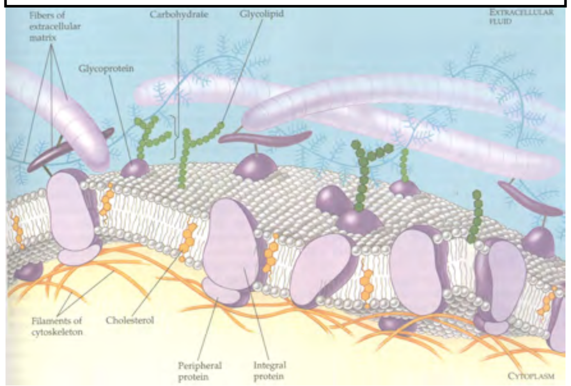
2) Linker

3) Carrier
   - Protein
   - Virosome

4) Adjuvant
   - Alumn
   - Experimental adjuvants

Carbohydrate Vaccine Development Path

Identify unique antigen



Synthesize antigen

Avian flu
Bacterial antigens

Conjugate and formulate

M. tuberculosis

Test Immunogenicity

Cancer antigens

Challenge animal model

Leishmania, HIV

B. anthracis

Preclinical Development

Malaria

ancora

Clinical Development

Anthrax Vaccine Candidate

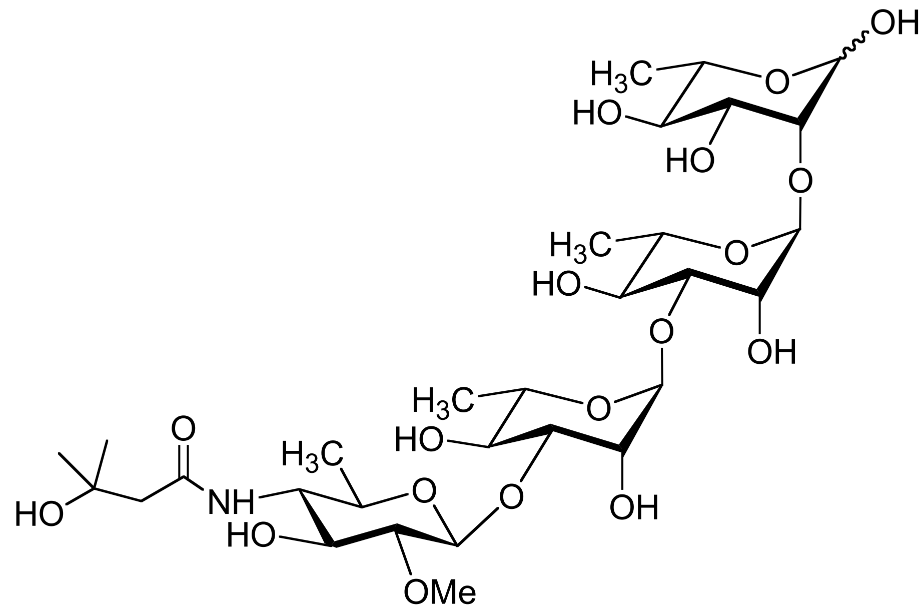
US, Autumn 2001

- **4 dead**
- **Millions terrorized**
- **US postal service at brink of collapse**

Angew. Chem. Int. Ed. **2005, 44, 6315.**

An Anthrax Vaccine Candidate

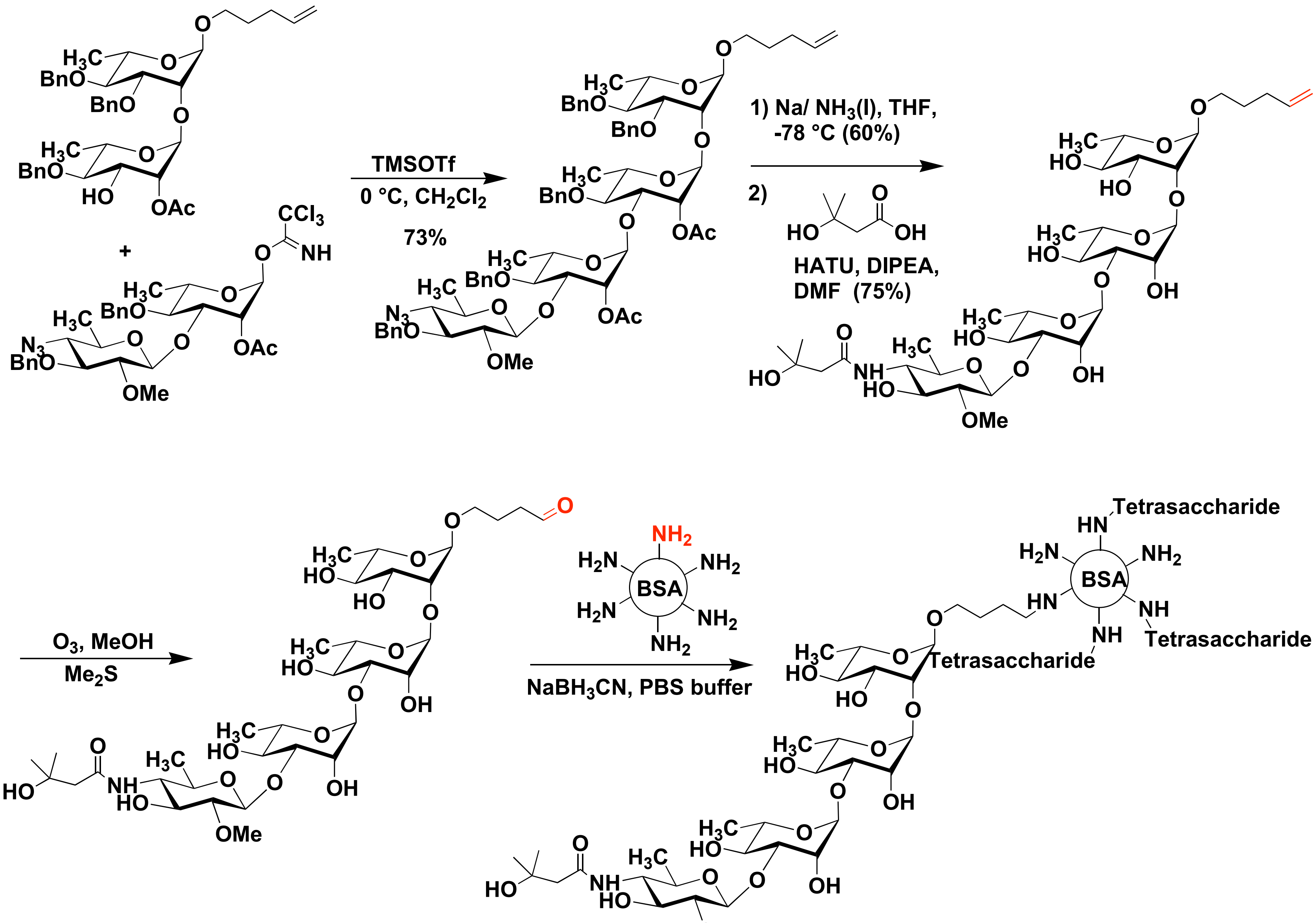
Bacillus anthracis **with spores**



Unique tetrasaccharide antigen

J. M. Daubenspeck *et al.*, *J. Biol. Chem.* 2004, *279*, 30945.

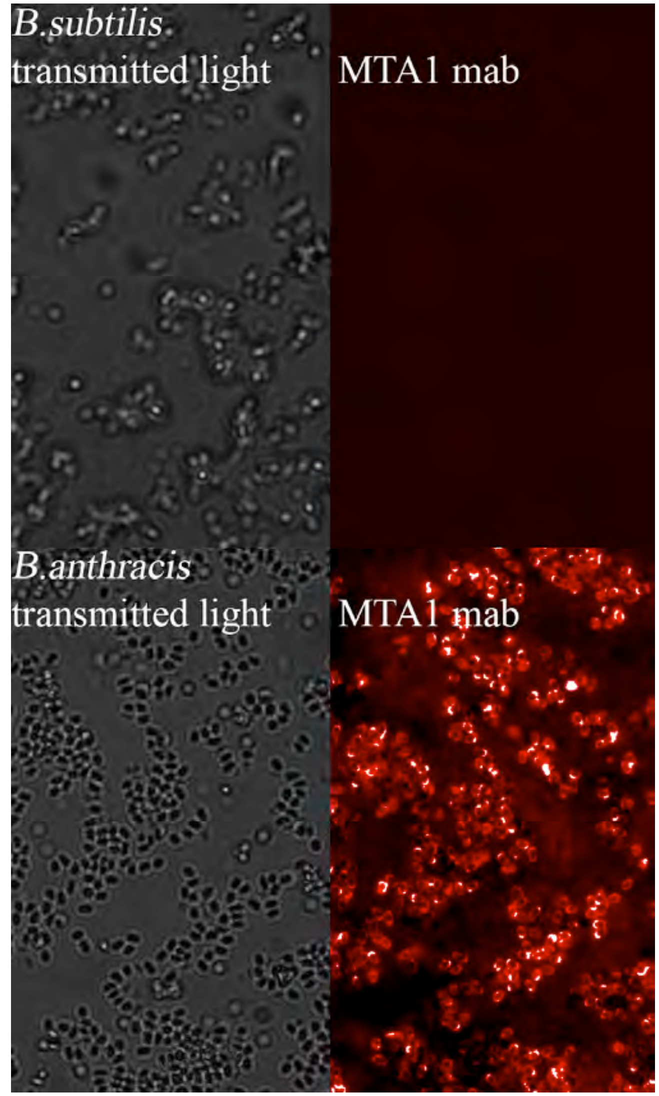
Total Synthesis and Conjugation



Angew. Chem. Int. Ed. 2005, 44, 6315.

Selective Anthrax Detection

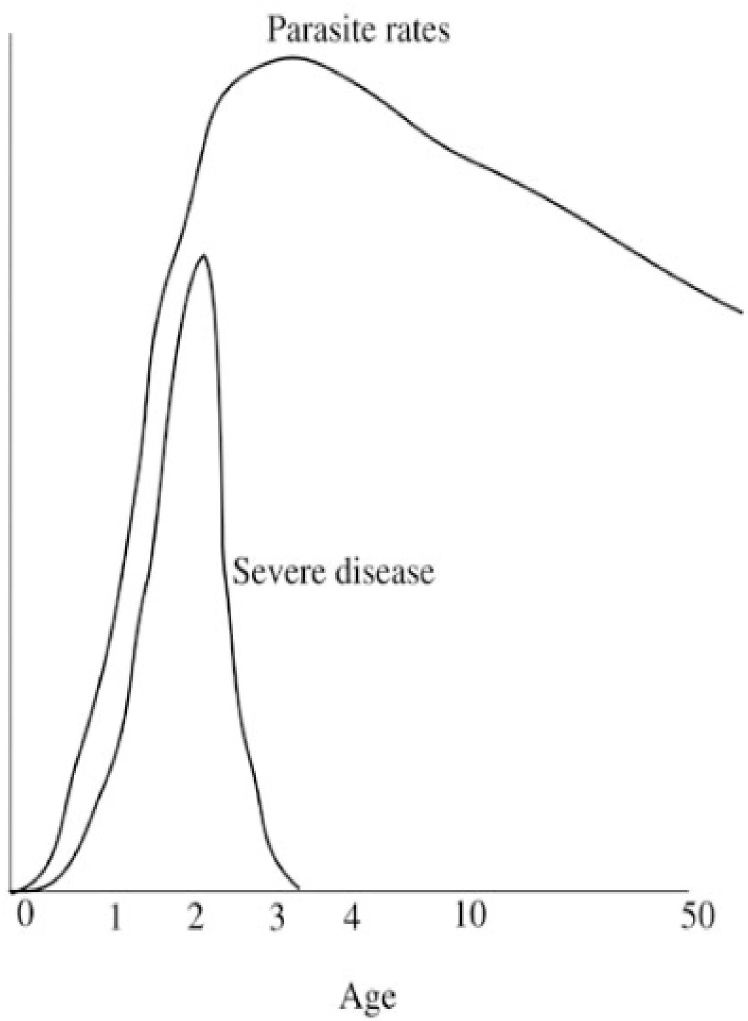
- **Excellent immune response in mice**
- **Hybridomas generated**
- **Monoclonal antibodies purified**
- **Superb specificity**
- **Test kit development in progress**



Tamborrini, M.; Werz, D.B.; Pluschke, G.; Seeberger, P.H.; *Angew. Chem. Int. Ed. Engl.*, 2006, in press.

An Anti-Toxin Malaria Vaccine

Clinical and Anti-parasite Immunity to Malaria



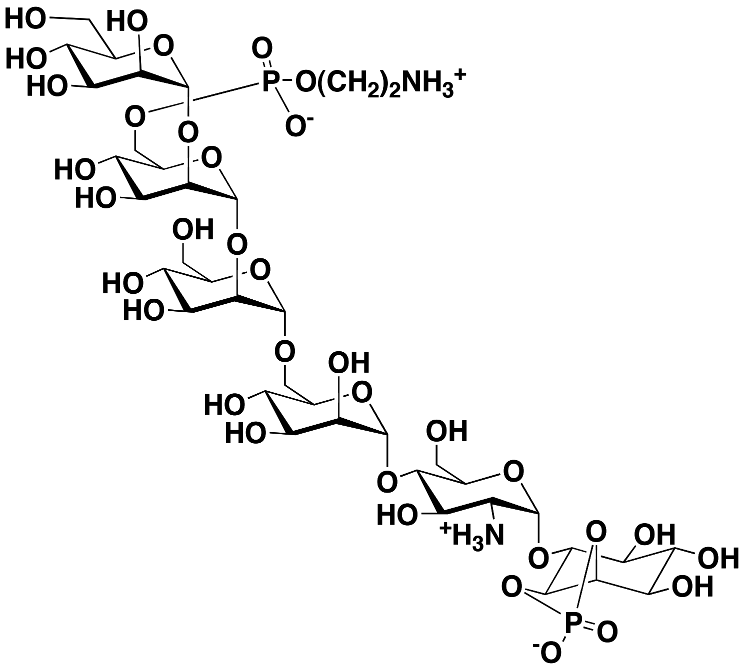
- many protein-based vaccines explored
- carbohydrate-based vaccines very successful against other diseases

Malaria Statistics (1994 WHO Estimate)

- 40% of world population at risk
- 5% infected (300 million people)
- 100 million clinical cases
- 2-3 million deaths (1% of cases fatal (predominantly children < 5 years)

1896
Golgi Postulates Malaria Toxin

2002
Toxin Identified



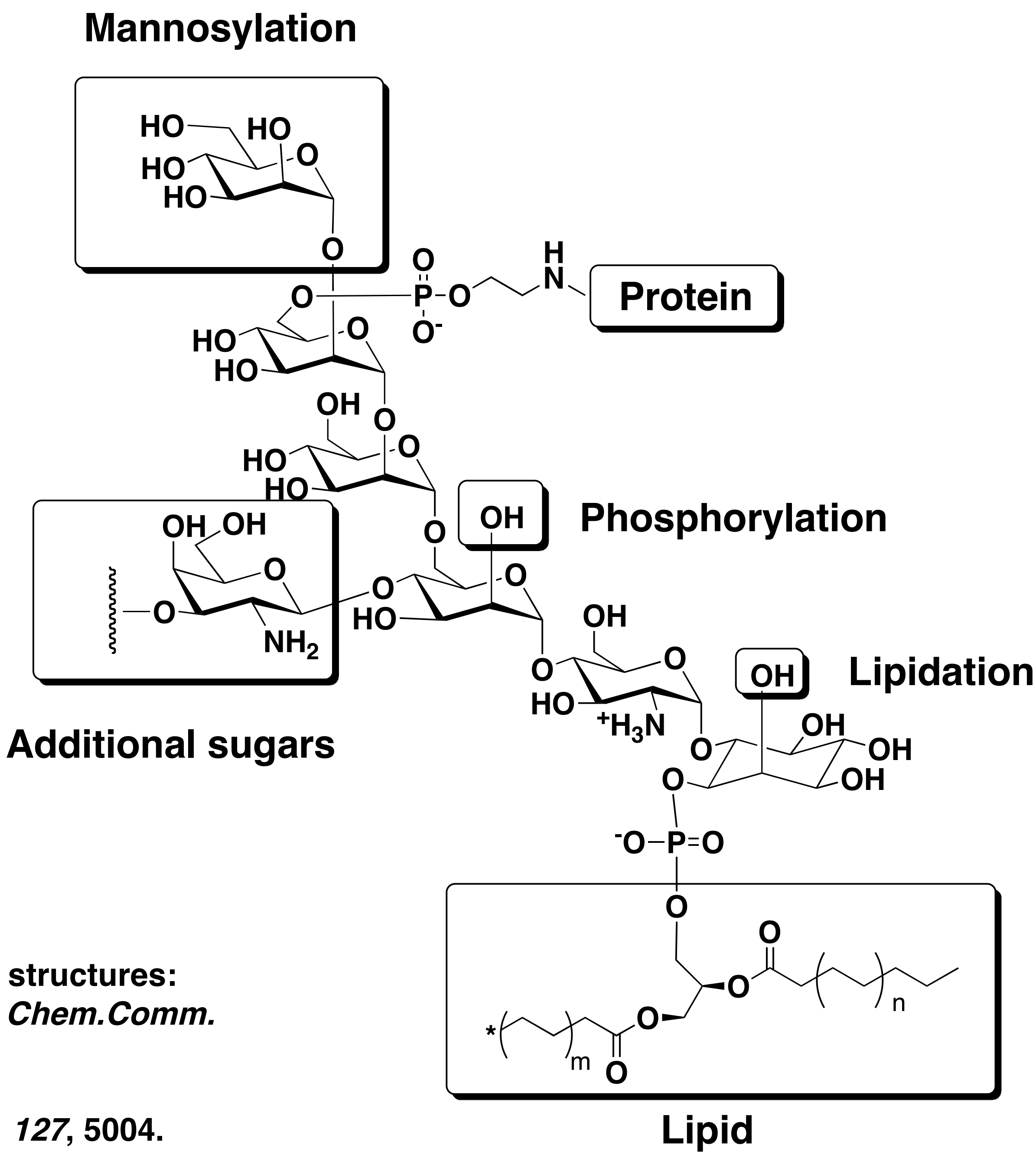
1) Substance isolated from *P. falciparum* - structure postulated

2) Synthesis of structure to confirm assignment

3) Use synthetic molecule as anti-toxin vaccine candidate

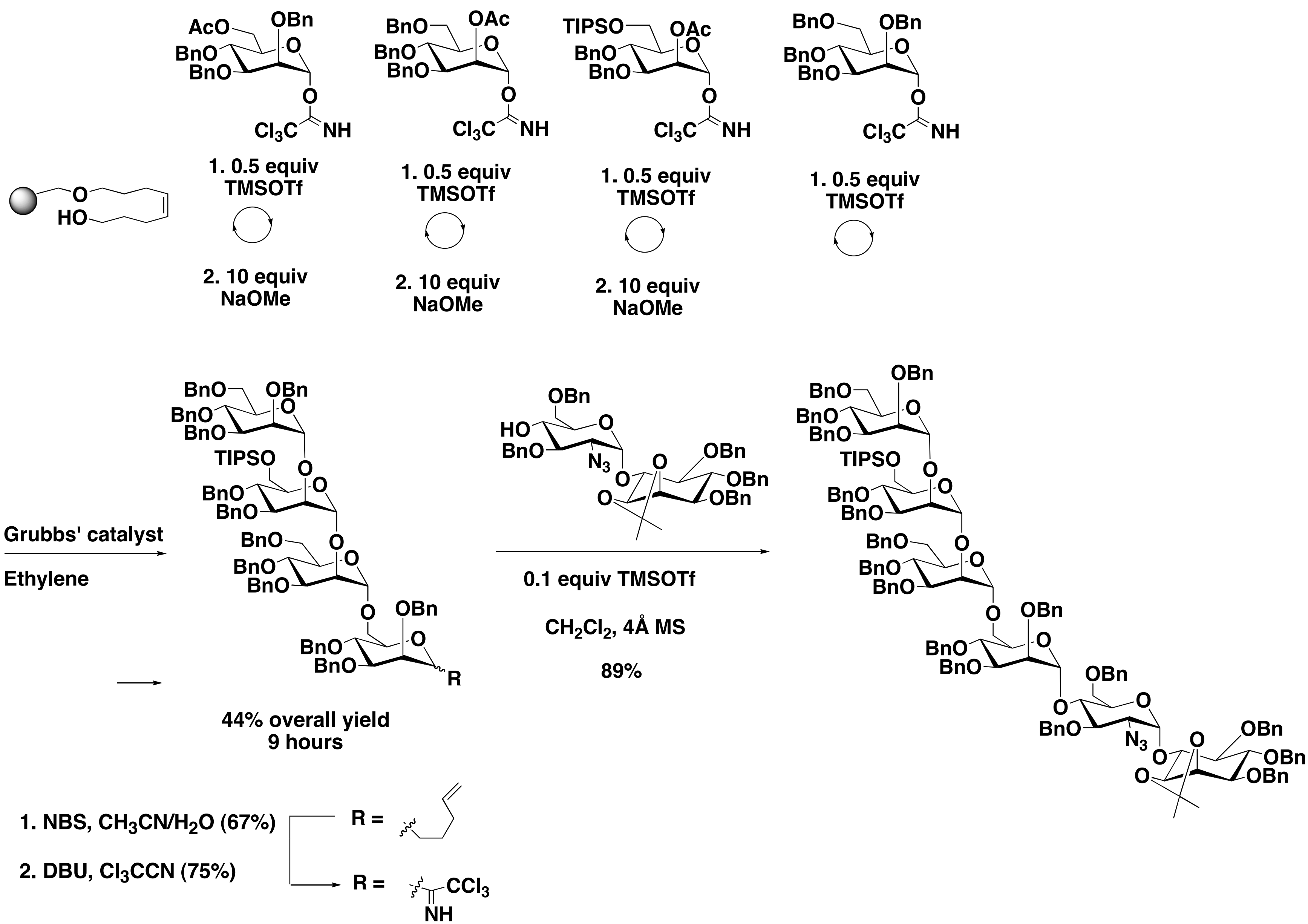
Nature, 2002, *418*, 785

Structural Diversity of GPIs



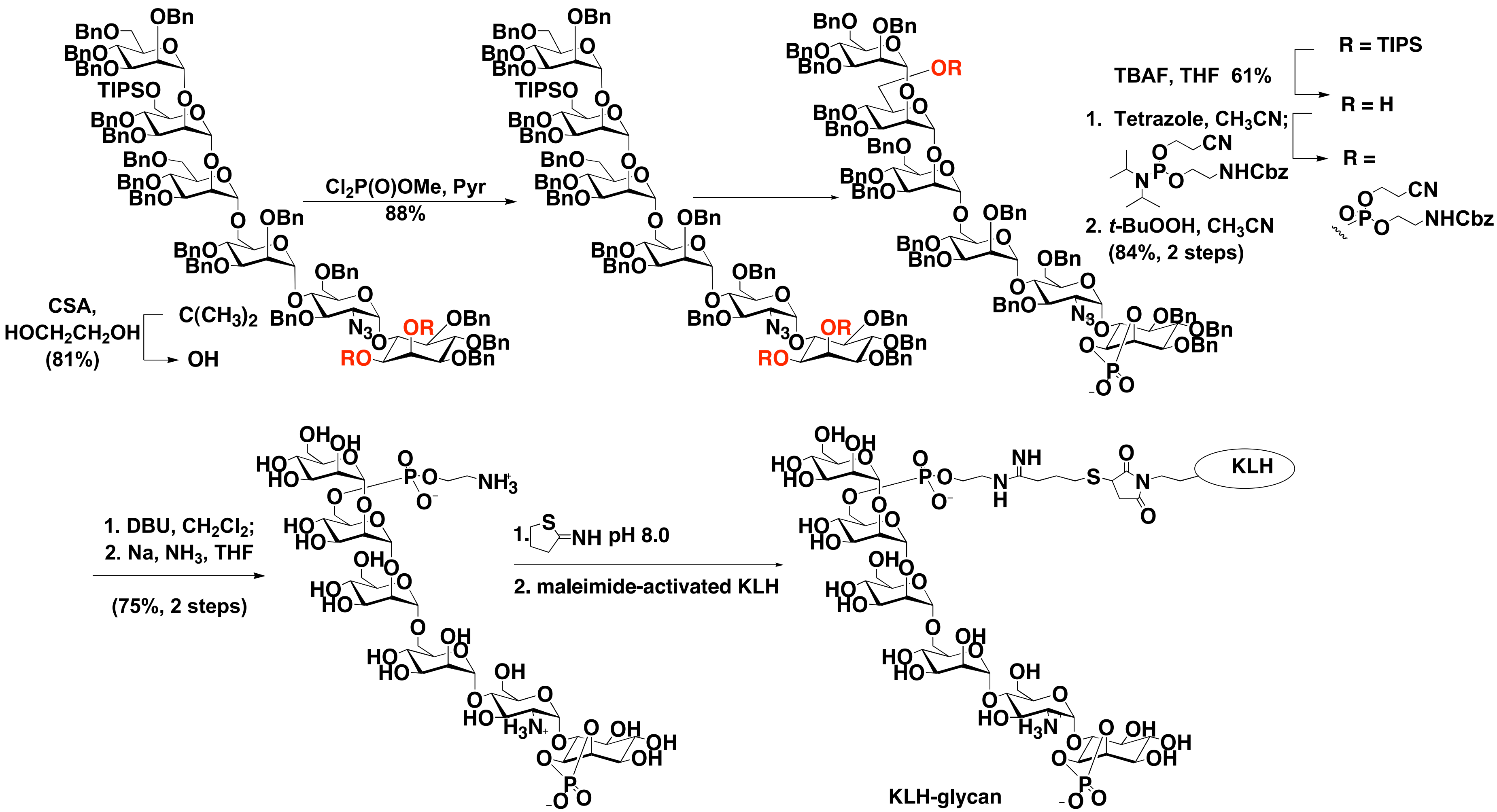
Syntheses of lipidated structures:
Kwon, Liu, Seeberger, *Chem.Comm.* 2005, 2280.
Liu, Kwon, Seeberger, *J.Am.Chem.Soc.* 2005, *127*, 5004.

Semi-Automated Assembly of the GPI Glycan



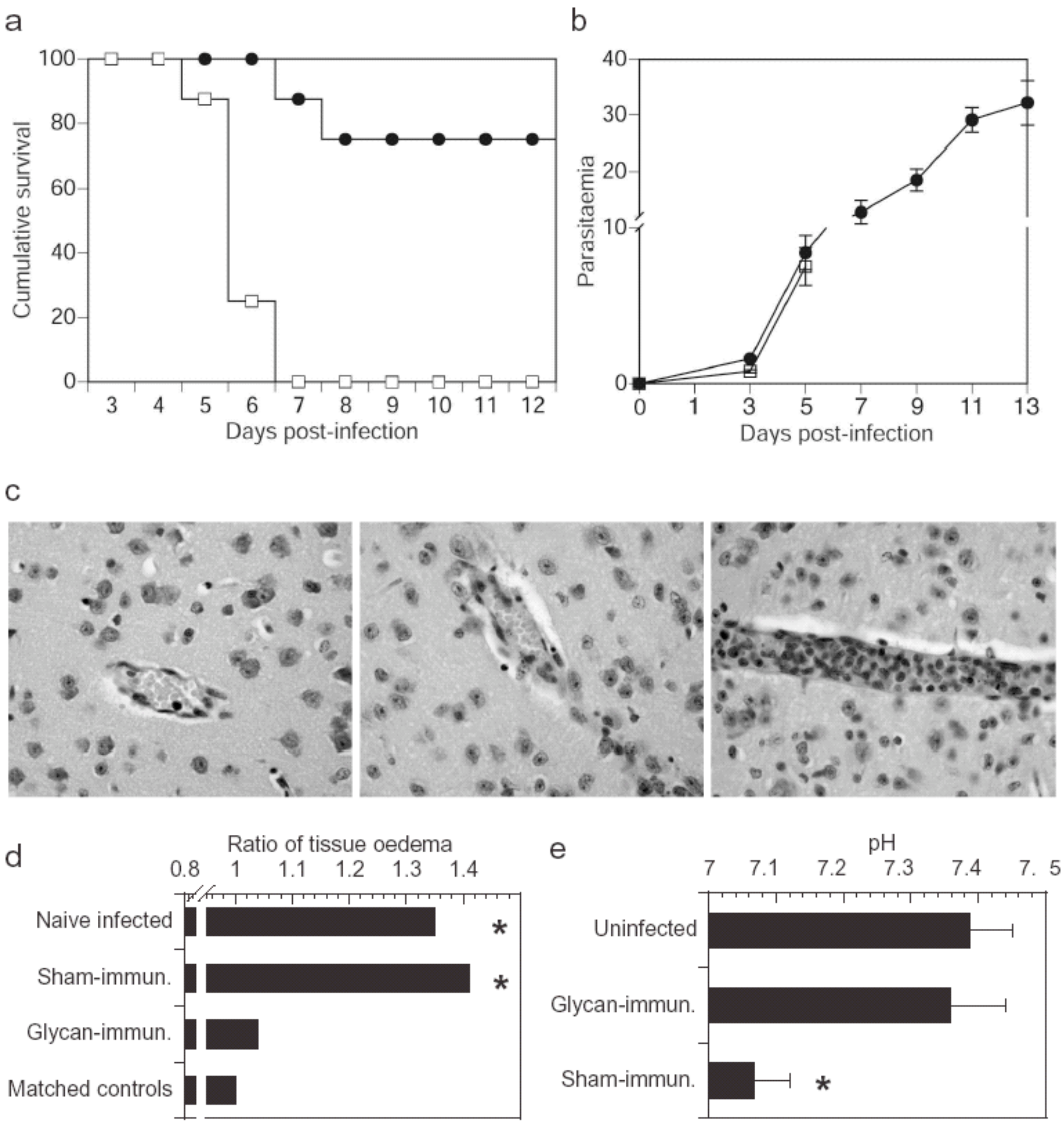
J. Am. Chem. Soc. **2002, *124*, 13434.**

Synthesis of a Malaria Vaccine Candidate



KLH-cysteine

Nature, 2002, *418*, 785



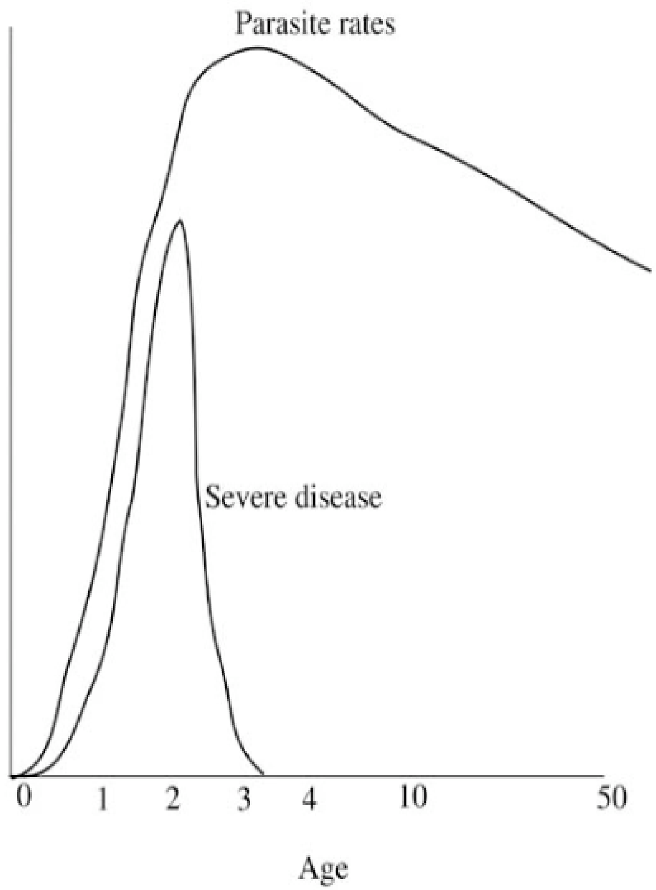
Vaccinees vs Controls

Survival and parasitaemia

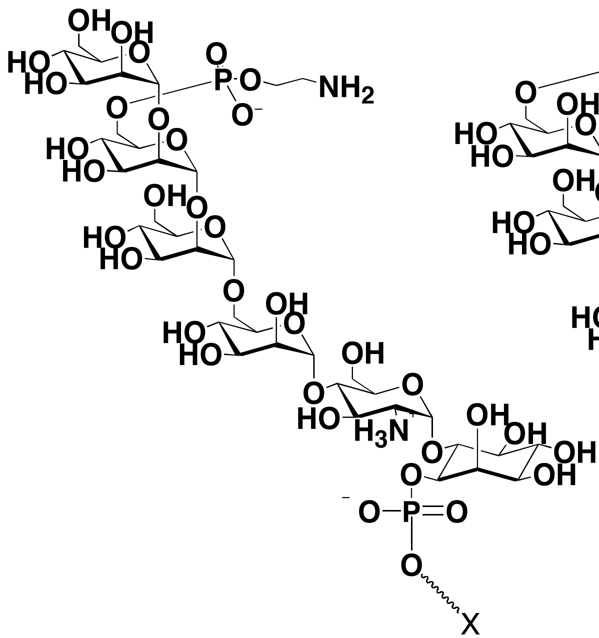
Cerebral Histology

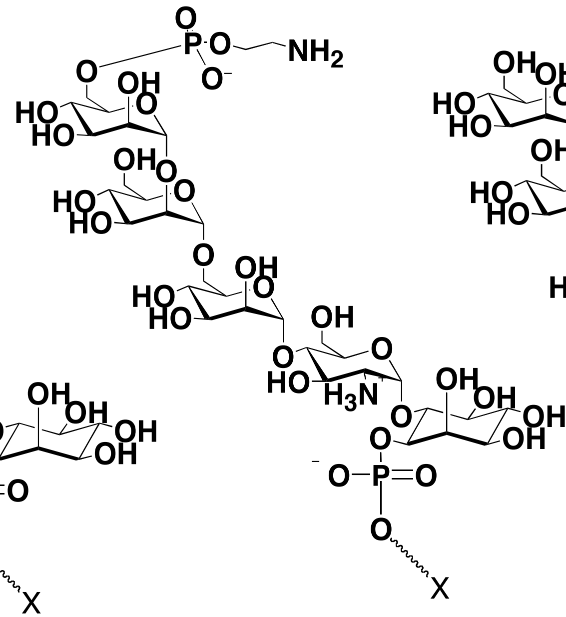
Systemic Pathology

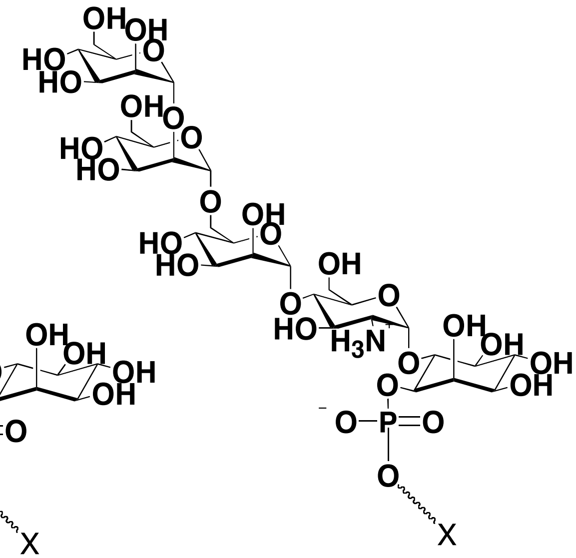
Does an Anti-GPI Response Protect from Malaria Mortality?

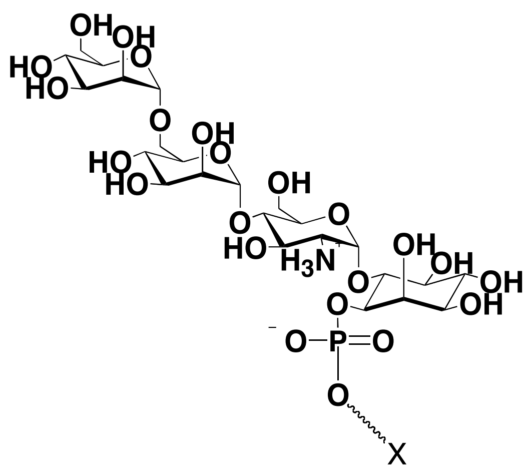


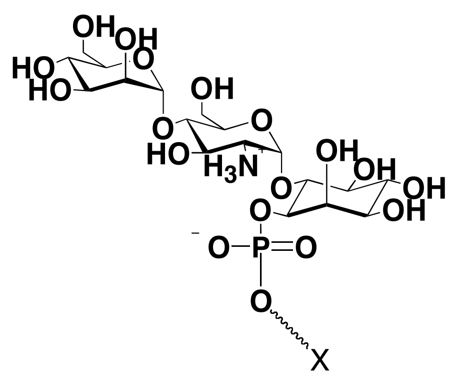
Tools for Epitope Mapping and Biosynthesis Investigations

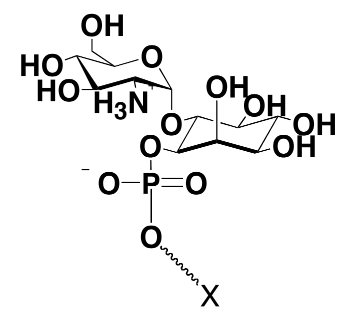








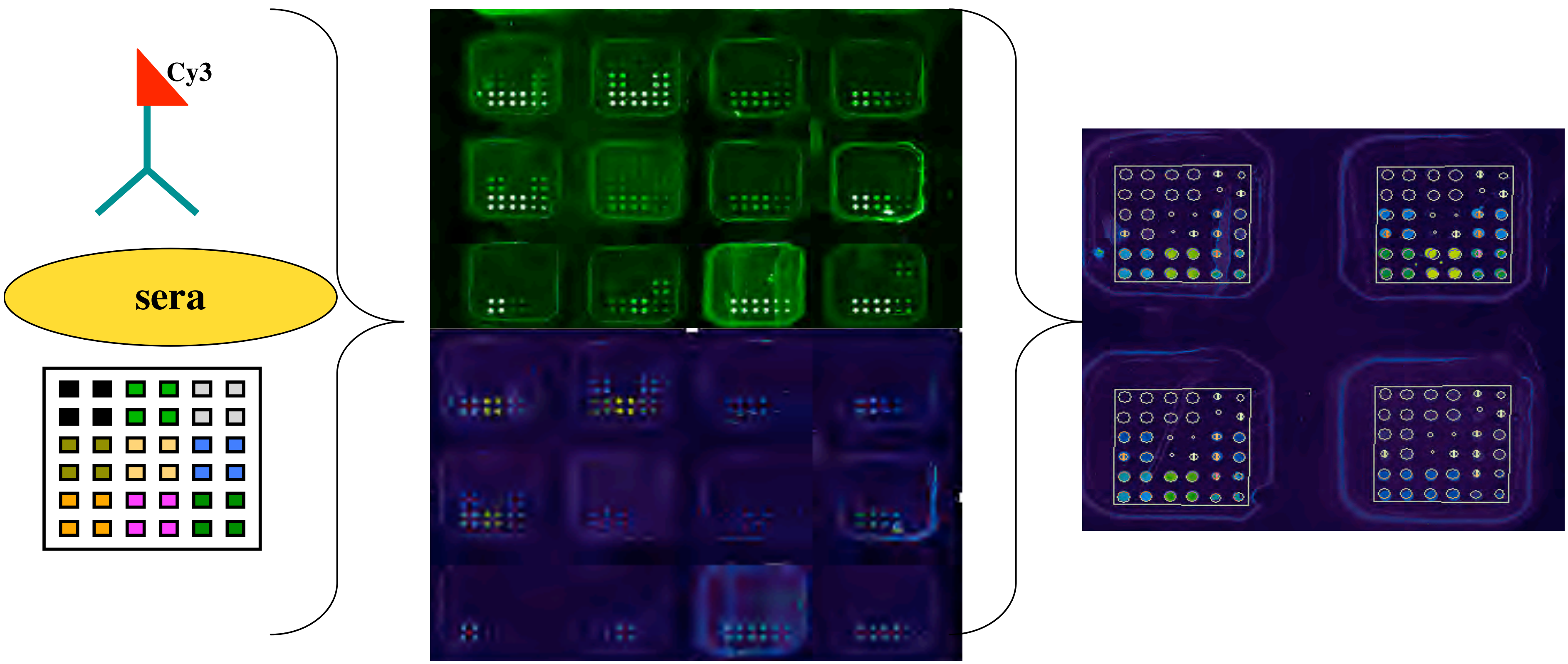




Chem. Eur. J. **2005, *11*, 2493.**

High Throughput Detection of Anti-GPI Antibodies on Microarrays

Incubation | Read out | Quantitation



Kamena, J.F.; Tamborrini, M.; Liu, X.; Pluschke, G.; Seeberger, P.H. in preparation

GPI Microarray Results - Summary

- **Fine specificities and titers differ between exposed and naive populations**
- **Children of mothers with specific antibodies have no antibodies**
- **Disease specific antibodies decline in migrants to about 40% in three years**

Specific GPI Antibodies Protect Adults in Endemic Areas from Severe Disease

↓

Induction of GPI-specific Antibodies Should Protect Naive Individuals and Small Children from Severe Disease

Development of an Anti-Toxin Malaria Vaccine

1) Vaccination experiments in mice using additional synthetic antigens

2) Scale-up and process development for synthetic antigen by Ancora

Synthesis	Total Yield	Linear Steps	Yield/Step	Scale
Seeberger Lab	0.26	26	79.5	10 -100 mg
Initial Ancora	2.70	27	87.5	1 - 100 g
Current Ancora	???	???	???	100 g - 5 kg

3) Conjugation and formulation agreement with major vaccine manufacturer

4) Toxicology and preclinical studies

5) Selection of sites for active and passive immunization trials

GPI Biology - Current Work

- Epitope mapping - disease outcome correlation
- SAR for GPI - inflammatory response and other diseases
- Proteomics attack at receptors interacting with GPIs
- Studies of GPI biosynthesis
- Comparison of GPIs with PIMs and LMs

Current and Future Work

1) **Innovate and improve all aspects of carbohydrate synthesis** **(common set of building blocks, better syntheses, difficult linkages, automation, work-up and purification, quality control)**

2) **Synthesize ever more complex targets from all classes of glycoconjugates in solution and on solid support (libraries of heparin oligos, glycolipids, glycooproteins, etc.)**

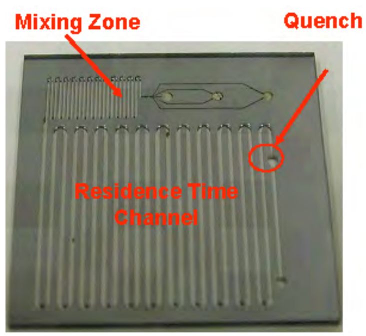
3) **Utilize carbohydrate arrays to study biomolecular interactions relevant to disease - identify small molecule inhibitors**

4) **Disect signaling pathways that include glycolipids**

5) **Advance vaccine programs (parasites, bacteria, viral, cancer)**

6) **The role of carbohydrates in autoimmune diseases**

7) **Microreactors - The Roundbottom Flask of the 21st Century**



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